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Impact of secondary salinisation on the structure and diversity of oligochaete communities

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Abstract – Secondary salinisation has become one of the most important factors responsible for changes in the aquatic biota. Earlier research has focused on macroinvertebrates including oligochaetes in anthropogenically saline rivers and streams, but studies on oligochaetes in anthropogenically saline stagnant waters remain scarce. Therefore, this study was conducted to assess changes in the species composition as well as the abundance and biomass of oligochaete communities along a large salinity gradient in the anthropogenic inland water bodies located in the Upper Silesian Coal Basin (Southern Poland), which is one of the largest coal basins in Europe. Herein, a total of 27 oligochaete species including five alien species were assessed, namely, *Potamothrix bavaricus*, *Potamothrix hammoniensis*, *Potamothrix moldaviensis*, *Psammoryctides albicola,* and *Psammoryctides barbatus*. The results confirmed that the freshwater oligochaetes could tolerate elevated water salinity and showed highest densities and taxa richness in intermediate salinity. Moreover, the waters with the highest salinity had an extremely low number of oligochaete species. A salinity level above 2800 mg L^{-1} led to significant loss of diversity of the oligochaetes, and consequently, these habitats were colonized by halotolerant species, especially *Paranais litoralis*, whose abundance increased with increasing salinity gradient.

Keywords: Oligochaeta / salinity gradient / anthropogenic water bodies / biodiversity

Résumé – Impact de la salinisation secondaire sur la structure et la diversité des communautés d'oligochètes. La salinisation secondaire est devenue l'un des facteurs les plus importants responsables des changements dans le biote aquatique. Des recherches antérieures se sont concentrées sur les macroinvertébrés, y compris les oligochètes dans les rivières et ruisseaux anthropiquement salins, mais les études sur les oligochètes dans les eaux stagnantes salines restent rares. Par conséquent, cette étude a été menée pour évaluer les changements dans la composition des espèces ainsi que l'abondance et la biomasse des communautés d'oligochètes le long d'un grand gradient de salinité dans les plans d'eau intérieurs artificiels situés dans le bassin houiller de la Silésie supérieure (sud de la Pologne), qui est l'un des les plus grands bassins houillers d'Europe. Ici, un total de 27 espèces d'oligochètes, dont cinq espèces exotiques, ont été recensées, à savoir *Potamothrix bavaricus*, *Potamothrix hammoniensis*, *Potamothrix moldaviensis*, *Psammoryctides albicola* et *Psammoryctides barbatus*. Les résultats ont confirmé que les oligochètes d'eau douce pouvaient tolérer une salinité de l'eau élevée et présentaient des densités et une richesse en taxons plus élevées en salinité intermédiaire. De plus, les eaux avec la salinité la plus élevée avaient un nombre extrêmement faible d'espèces d'oligochètes. Un niveau de salinité supérieur à 2800 mg L^{-1} entraîne une perte importante de diversité des oligochètes et, par conséquent, ces habitats sont colonisés par des espèces halotolérantes, en particulier *Paranais litoralis*, dont l'abondance augmente avec l'augmentation du gradient de salinité.

Mots-clés : Oligochète / gradient de salinité / plans d'eau anthropiques / biodiversité

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1 Introduction

Natural (so-called primary) salinisation of inland waters occurs most commonly in regions with arid, semi-arid, and Mediterranean climate, such as parts of Australia, southwestern North America, South America, Central Asia, and the Middle East (Williams, 2001). This process takes place in natural salt lakes, marshes, streams, and rivers (Williams, 1998; Brock *et al.,* 2005; Moreno *et al.,* 2010). The primary causes of natural salinisation are erosion of sediments due to microbial activity or weathering, saline groundwater, and rainwater from the atmosphere owing to seawater evaporation. All these processes contribute salt to the water bodies. The degree of salinization mainly depends on the distance from the sea as well as regional factors such as local geology, topography, current and past climatic conditions, geological basin, and vegetation (Nielsen *et al.,* 2003; Zinchenko and Golovatyuk, 2013).

Human activities can increase the dissolved ion concentration, measured as total dissolved solids (TDS), in inland aquatic ecosystems. Depending on the type of anthropogenic disturbance (*i.e.*, the discharge of highly saline mine water, saline agricultural wastewater, sewage from chemical industries, improper use of fertilizers in the soil, or use of salt for road de-icing in winter), the concentrations and compositions of ions can vary and, consequently, lead to secondary salinisation (Mount *et al.,* 1997; Johnson *et al.,* 2014). The concentration of salinity is reported as a small increase from freshwater to brackish water (up to $5\,g\,L^{-1}$) and sometimes even above the seawater level ($35\,g\,L^{-1}$) (Nielsen *et al.,* 2003, 2008; Brock *et al.,* 2005). Anthropogenic salinisation is common in industrial and urban areas (Rzętała, 2008; Machowski, 2010; Molenda, 2011; Cañedo-Argüelles *et al.,* 2013), especially apparent in small water bodies that receive effluents of coal mining (Jankowski and Rzętała, 1999; Harat and Grmela, 2008).

Currently, secondary salinisation has become one of the most important factors responsible for changes in the aquatic biota (Bäthe and Coring, 2011; Kang and King, 2012; Arle and Wagner, 2013). It leads to direct changes in the community structure of invertebrates, including the functioning of the ecosystem (Nielsen *et al.,* 2003; Brock *et al.,* 2005; Cañedo-Argüelles *et al.,* 2014, 2015). Increasing salinity results in the elimination of freshwater taxa and the replacement of salt-sensitive species by eurytopic species and species that are resistant to high salt concentration (Williams *et al.,* 1990; Piscart *et al.,* 2005; Boets *et al.,* 2012; Kefford *et al.,* 2012; Arle and Wagner, 2013; Szöcs *et al.,* 2014). Salt-sensitive species begin to disappear because of the osmoregulatory stress, behavioral drift, loss of food resources, or developmental failure (Johnson *et al.,* 2014). Moreover, the death or loss of functions of salt-sensitive species may free up resources for the more salt-tolerant species (Kefford *et al.,* 2016). Therefore, high water salinity may be the cause for the colonisation and establishment of alien species in aquatic ecosystems (Piscart *et al.,* 2005, 2011).

Oligochaetes are significant component of macrozoobenthos and play an important role in aquatic ecosystems. As active bioturbators, these organisms influence the microbial activities and biogeochemical processes occurring in sediments. Their movement in sediments contributes to the oxygenation of the bottom layer of the water body, the release of accumulated nutrients from sediments into water, and their re-entry into the aquatic ecosystem (Saaltink *et al.,* 2019). Oligochaetes play a significant role in the self-purification of water and therefore are used in municipal and industrial sewage treatment plants. They can be used as indicator organisms of water quality, sediment pollution, and trophy (*e.g.*, Lang, 1997; Prygiel *et al.,* 2000; van Haaren, 2002; Lang, 2006; Lv *et al.,* 2009; Vivien *et al.,* 2014; Krodkiewska and Kostecki, 2015). Many oligochaetes are resistant to oxygen deficits in the water, and therefore, they can inhabit highly polluted waters, where they can constitute to up to 100% of the total number and biomass of macrozoobenthos (Rodriguez, 1999; Timm *et al.,* 2001; Wolf *et al.,* 2009; Ferreira *et al.,* 2011; Chiu *et al.,* 2012). Despite the importance of oligochaetes in aquatic environments, they have not received much attention in many hydrobiology studies. They are either omitted or treated together as a class or a family, which has thus created a gap in knowledge in literature (Sambugar, 2007; Soors *et al.,* 2013). Earlier research has primarily focused on oligochaetes in the polluted waters of rivers and streams, including anthropogenically saline waters (*e.g.*, Rodriguez, 1999; Lin and Yo, 2008; Ferreira *et al.,* 2011; Chiu *et al.,* 2012; Frizzera and Alves, 2012; Jabłońska, 2014; Rosa *et al.,* 2014). However, there are scarce data on the oligochaete communities in secondary salinised inland water bodies located in urban areas. Therefore, the aim of this study was to assess any changes in the species composition as well as the abundance and biomass of the oligochaete communities along a large salinity gradient in the anthropogenically saline inland water bodies associated with underground coal mining.

2 Materials and methods

2.1 Study area

The research was carried out in the Upper Silesian Coal Basin (Southern Poland), which is the largest coal basins in Europe, where the entire area is strongly affected by underground coal mining and where there are no natural water bodies and lakes. Most of the water bodies in the study area were created as a result of human activities, and owing to the high number of water bodies (approximately 4773 water bodies of various origins), this region has been named the Upper Silesian Anthropogenic Lake District (Rzętała and Jagus, 2012). The present study was conducted in nine inland water bodies with different degrees of salinity. Three ponds (ponds 7, 8, and 9) were used to retain underground water and had a high salt content because of the constant flow of water from coal mines. The other water bodies originated as mining subsidence ponds, and they were used mainly for fishing and recreation. All the investigated water bodies were created in the 1970s (Tab. 1).

2.2 Sampling and laboratory procedure

Samples of the oligochaetes were collected once a month in 2016 (from June to October – in all ponds) and 2017 (in June, August, and October – in ponds 1, 3, 4, 5, and 8–and in July, August, and November – in ponds 2, 6, 7, and 9). Quantitative

Table 1. Characteristics of the studied ponds.

Water bodies		Geographic coordinates	Area (ha)	Year of creation	Management	Type of water body
Freshwater	1	50°12.978' N 18°42.896' E	0.2	1977	Fishing, wildfowl	Sinkhole pond
	2	50°11.825' N 18°37.355' E	21	1971	Stocked with fish, recreation, wildfowl	Sinkhole pond
	3	50°12.670' N 18°39.469' E	6.3	1974	Stocked with fish, recreation, wildfowl	Sinkhole pond
Subhaline	4	50°13.156' N 18°41.318' E	26	1974	Stocked with fish, recreation, wildfowl	Sinkhole pond
	5	50°12.902' N 18°41.972' E	22.8	1977	Stocked with fish, wildfowl	Sinkhole pond
	6	50°12.125' N 18°38.073' E	04.9	1971	Stocked with fish, wildfowl	Sinkhole pond
Hypohaline	7	50°11.387' N 18°38.073' E	0.6	1973	Mining use, fishing, wildfowl	Settling pond
	8	50°21.865' N 18°67.026' E	0.7	1974	Mining use, wildfowl	Settling pond
	9	50°13.346' N 18°37.251' E	1.5	1974	Mining use	Settling pond

samples were taken from two microhabitats – unvegetated bottom sediments and sediments that had been overgrown by macrophytes – using a 0.23-mm mesh net bounded by a square frame (25 cm × 25 cm × 50 cm). The frame was placed randomly at three sites in all the ponds at each of the microhabitats. A total of 143 samples of oligochaetes were taken during the study period. In the laboratory, the sediments were sieved through a 0.23-mm sieve. The oligochaetes were sorted under a stereoscopic microscope, preserved in 80% ethanol, and mounted in Amman's lactophenol. The oligochaetes were identified to the lowest possible taxonomic level using Timm's key (2009). The collected specimens were counted and weighed on laboratory scales with an accuracy of 0.001 g (wet mass).

The biological data of the oligochaete communities were processed according to density, Shannon–Wiener index (H'), constancy (C%), and dominance (D%). According to Górny and Grüm (1981), the following dominance classes were used: eudominants: $D > 10\%$, dominants: $D = 5.1–10\%$, subdominants: $D = 2.1–5.0\%$, recedents: $D < 2.0\%$, and subrecedents: $D \leq 1.0\%$ of sample.

Water samples were collected monthly from all the ponds. Parameters such as conductivity, TDS, dissolved oxygen, pH, and temperature were estimated in the field using Hanna Instruments and WTW portable meters, while the contents of chloride, potassium, sulphates, calcium, magnesium, iron, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, phosphates and alkalinity were measured in the laboratory using Hanna Instruments and Merck meters according to the standard methods of Hermanowicz *et al.* (1999).

Additionally, samples of bottom sediments were also taken for analyses. The grain size composition of the bottom sediments was determined using the sieve method. The total content of heavy metals such as Cd, Cu, Zn, and Pb in the sediments was determined by homogenisation and mineralisation with aqua regia (nitric acid and hydrochloric acid at a molar ratio of 1:3), followed by determination by inductively coupled plasma-optical emission spectroscopy (ICP OES), whereas the fractional composition of these heavy metals was determined by inductively coupled plasma-mass spectrometry (ICP MS) according to Tessier's procedures (Tessier *et al.*, 1979). The total content of organic matter (%) in each type of sediment was determined using the loss-on-ignition technique by combusting them at 550 °C for 4 h (Myslińska, 2001).

2.3 Data analyses

The water variables and the content of organic matter in the sediments of the various ponds with different water salinity levels were calculated using Kruskal-Wallis analysis of variance (ANOVA) and multiple comparisons post hoc test because the data showed non-normal distribution (determined using the Kolmogorov–Smirnov test for normality). These analyses were performed using Statistica (ver. 13.1).

Variation in the structure of the oligochaete communities along the salinity gradient was evaluated using principal coordinates analysis (PCO) based on the Bray–Curtis distance measure. Nonmetric multidimensional scaling (NMDS) on log (x+1)-transformed oligochaete abundance data and the Bray–Curtis distance measure were used to assess whether the substrate types (unvegetated bottom sediments and sediments that had been overgrown by macrophytes) were grouped. The relationship between the composition of the oligochaete communities and the environmental variables was determined using canonical correspondence analysis (CCA). A unimodal analysis was selected because of the large gradient (3.534, as determined using detrended correspondence analysis on 26 segments with only the species data). Before performing the CCA, the forward selection method was applied to environmental variables (conductivity, TDS, pH, alkalinity, oxygen, temperature, chloride, sulphates, potassium, calcium, magnesium, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, phosphates, iron, organic matter in sediments, type of substrate, grain size composition in bottom sediments) using the Monte Carlo permutation test (499 runs) to determine the variables that best explained the composition of the oligochaete communities. Pearson product–moment correlations were then calculated among the selected environmental variables to check for redundancy. Rare taxa (those that occurred in only one sample) were removed from the analysis to reduce the noise in the data set (Gauch, 1982). After removing the rare taxa and redundancy among the significant environmental variables (conductivity, chloride, sulphates, potassium, calcium, and magnesium were excluded from the analysis because they correlated with TDS), eight environmental variables and 22 species were used in the final CCA. The analysis was performed on log (x+1)-transformed taxa and environmental data. All these analyses were performed using the Canoco, ver. 5.0, software package (ter Braak and Šmilauer, 2012).

Multiple regression analysis (stepwise backward variable elimination) was used to assess the relationship of the environmental variables with oligochaete density and taxa richness. The analysis was performed on log (x+1)-transformed taxa and environmental data using Statistica, ver. 13.1.

Cluster analysis using the Bray–Curtis distance measure and the unweighted pair-group method with the arithmetic mean (UPGMA) linkage method was used to assess similarity among the oligochaete communities in the studied ponds. Species that occurred in only one sample were excluded. The analysis was performed on log (x+1)-transformed data using MVSP software (Kovach Computing Services, ver. 3.13p).

3 Results

3.1 Environmental conditions

The studied water bodies were ranked in terms of increasing salinity on the basis of TDS values. According to the classification of Hammer *et al.* (1990), ponds 1, 2, and 3 were freshwater (TDS <500 mg L^{-1}); ponds 4, 5, and 6 were subhaline (TDS 500–3000 mg L^{-1}); and ponds 7, 8, and 9 were hypohaline (TDS 3000–20,000 mg L^{-1}). In one of the hypohaline water bodies, a TDS value of 21,100 mg L^{-1} was found during one of the sampling months, which corresponds to the level in mesohaline water. The minimum and maximum values of the analysed physicochemical variables of the water in each type of pond are given in Table 2. There was a significant difference between the freshwater and subhaline water bodies in the median value of pH (Kruskal–Wallis ANOVA test H = 11.98829, p = 0.0025) and between the freshwater and hypohaline ponds in the median value of alkalinity (Kruskal–Wallis ANOVA test H = 17.79463, p < 0.001). The Kruskal–Wallis ANOVA test revealed statistically significant differences in the median value of conductivity (H = 63.13141, p < 0.0001) and the median concentration of TDS (H = 63.13141, p < 0.0001), chloride (H = 60.95674, p < 0.0001), sulphates (H = 45.23897, p < 0.0001), potassium (H = 37.00591, p < 0.0001), and iron (H = 28.45855, p < 0.0001) among the hypohaline, subhaline, and freshwater ponds. The Kruskal–Wallis ANOVA test also showed significant differences in the median concentration of nitrite nitrogen (H = 24.35032, p < 0.0001) between the

Table 2. The physical and chemical parameters of the water in the investigated ponds.

Parameter	Conductivity (µS cm^{-1})	TDS (mg L^{-1})	Chlorides (mg L^{-1})	Potassium (mg L^{-1})	Sulphates (mg L^{-1})	Nitrate nitrogen (mg L^{-1})	Nitrite nitrogen (mg L^{-1})	Ammonium nitrogen (mg L^{-1})	Phosphates (mg L^{-1})	Iron (mg L^{-1})	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	Alkalinity (mg L^{-1})
Water bodies														
Freshwater n = 24	220–910	100–450	8–167	1–10	23–147	0–13.6	0–0.2	0.1–9.3	0–2.2	0.1–2.8	7.9–27.1	2.5–15.3	6.4–9.6	40–250
Subhaline n = 24	1130–5200	560–2590	111–1090	4–48	132–720	0–8.0	0–0.04	0.03–0.7	0–0.2	0.01–1.0	6.8–26.2	4.9–12.9	7.1–8.7	110–300
Hypohaline n = 24	7750–42,400	2800–21,100	920–19,000	30–92	750–3600	0.06–10.5	0.001–1.8	0.3–5.7	0.001–1.2	0.06–1.6	10.7–25.6	6.8–17.6	7.1–8.5	150–445

Table 3. Selected heavy metal content in the bottom sediments of the studied ponds.

	Water bodies								
	Freshwater			Subhaline			Hypohaline		
Heavy metals	1	2	3	4	5	6	7	8	9
Ca bioavailable ($\mu g\,kg^{-1}$)	790.9	385.71	97.25	493.3	567.87	248.4	484.7	360.8	1445.1
Cd total ($\mu g\,kg^{-1}$)	813.6	385.71	97.25	510	633.77	304	557.2	377.8	1472.2
Cu bioavailable ($\mu g\,kg^{-1}$)	7236	1789.8	1788.9	9385.1	12,634	20,197	12,671	21,156	12,370
Cu total ($\mu g\,kg^{-1}$)	23,736	2632.8	2798.9	12,755.1	17,604	32,547	29,871	31,956	23,670
Pb bioavailable ($\mu g\,kg^{-1}$)	21,783.3	1726.04	5947.8	44,396	41,175.7	3715.6	19,289.3	3068	5959
Pb total ($\mu g\,kg^{-1}$)	29,853.3	2966.04	6651.8	55,046	46,385.7	11,785.6	39,689.3	13,268	16,059
Zn bioavailable ($\mu g\,kg^{-1}$)	104,626	37,452.6	15,278	122,282	122,495	19,447.31	65,182.2	63,421	75,971
Zn total ($\mu g\,kg^{-1}$)	168,926	42,882.6	26,478	168,982	160,695	61,547.31	131,682.2	80,521	108,571

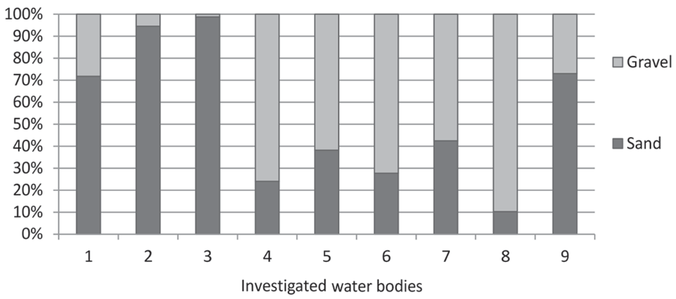

Fig. 1. Grain size composition of the bottom sediments in the studied ponds.

hypohaline ponds and the other types of water bodies and in the median concentration of ammonium nitrogen ($H = 22.62095$, $p < 0.0001$) between the subhaline ponds and the other types of water bodies.

The values of the concentration of heavy metals in the bottom sediments of each of the investigated water bodies are listed in Table 3. The bottom sediments of ponds 4–8 were mainly built of gravel, whereas the bottom sediments of the freshwater ponds and one hypohaline pond (pond 9) had predominantly sand (Fig. 1). The organic matter content in the bottom sediments ranged from 0.4 to 54.0% in the freshwater ponds, 1.2 to 34.4% in the subhaline ponds, and 2.2 to 35.4% in the hypohaline ponds. The Kruskal–Wallis ANOVA test showed statistically significant differences in the median content of organic matter in the sediments ($H = 68.30246$, $p < 0.0001$) between all the types of water bodies.

3.2 Composition of the oligochaete communities

A total of 17,192 oligochaetes belonging to the families Naididae, according to Erséus *et al.* (2008), Pristinidae, Lumbriculidae, and Enchytraeidae were collected. The species of Naididae accounted for approximately 99% of the oligochaete fauna (Tubificinae: 55%, Naidinae: 44%). The maximum mean densities of the oligochaetes were recorded in the order subhaline water body (pond 6), *i.e.*, 1477 ind./m^2; hypohaline water body (pond 9), *i.e.*, 1083 ind./m^2; and freshwater pond (pond 1), *i.e.*, 998 ind./m^2, whereas the lowest mean density was recorded in hypohaline (pond 8), *i.e.*, 110 ind./m^2. The lowest mean wet biomass ($g\,m^{-2}$) was recorded in the hypohaline water body, *i.e.*, 0.419 $g\,m^{-2}$, while the highest value was recorded in the freshwater, *i.e.*, 0.916 $g\,m^{-2}$ (Tab. 4).

During the study period, 27 species of oligochaetes were found in the water bodies with contrasting values of salinity (Tab. 4). Among them, five alien species (Dumnicka, 2016) were recorded, namely, *Potamothrix bavaricus* (Oschmann, 1913), *Potamothrix hammoniensis* (Michaelsen, 1901), *Potamothrix moldaviensis* (Vejdovský & Mrázek, 1903), *Psammoryctides albicola* (Michaelsen, 1901), and *Psammoryctides barbatus* (Grube, 1891). Species diversity was the highest in the freshwater, with 25 oligochaete species, and the lowest in the hypohaline waters, with only four species. *Limnodrilus hoffmeisteri* Claparède, 1862, *Dero digitata* Müller, 1774, and *Nais communis* Piguet, 1906, were eudominants, and *Limnodrilus claparedeanus* Ratzel, 1868, was the dominant species in the freshwater ponds. Six species were only present in the freshwater ponds (*Aulodrilus pluriseta* Bretscher, 1899; *Chaetogaster diaphanus* Gruithuisen, 1828; *Vejdovskyella comata* Vejdovský, 1884; *Uncinais uncinata* Oersted, 1842; *Pristina longiseta* Ehrenberg, 1828; and *Pristina aequiseta* Bourne, 1891). *Limnodrilus hoffmeisteri, Stylaria lacustris* Linnaeus, 1767, and *Ophidonais serpentina* Müller, 1774, were the dominant species in the subhaline waters, whereas *P. litoralis* Müller, 1784, only occurred in the hypohaline water bodies, in which it was the most frequent and the most abundant species (Tab. 4).

The diversity of the oligochaete communities, which was measured by the mean values of the Shannon–Wiener index, was the highest in the subhaline ponds ($H' = 2.042$) and lowest in the hypohaline waters ($H' = 0.879$) (Tab. 4).

3.3 Environmental variables and oligochaete communities

The results of NMDS analysis of log ($x+1$)-transformed abundance data did not indicate the grouping of the sampling sites with different substrate types (Fig. 2).

The PCO plot (Fig. 3) showed that the structure of the oligochaete communities in ponds with higher salinity was different from that of the species in the freshwater ponds and the water bodies with only slightly increased salinity. In addition, there were no seasonal changes in the communities.

Table 4. Values of the dominance (*D*%) and constancy (*C*%) indices that were calculated for the oligochaete communities in the studied ponds.

Taxon	Water bodies					
	Freshwater		Subhaline		Hypohaline	
	D (%)	C (%)	D (%)	C (%)	D (%)	C (%)
Limnodrilus hoffmeisteri Claparède, 1862	10.3	97.9	7.4	100.0	1.5	27.1
Limnodrilus claparedeanus Ratzel, 1868	5.3	76.6	3.7	93.8	0.02	2.1
Limnodrilus udekemianus Claparède, 1862	2.1	57.4	1.8	68.8		
Tubifex tubifex (Müller, 1774)	0.9	53.2	0.5	39.6		
Potamothrix bavaricus (Oschmann, 1913)			1.7	70.8	0.2	16.7
Potamothrix moldaviensis Vejdovský & Mrázek, 1903	2.1	66.0	2.3	70.8		
Potamothrix hammoniensis (Michaelsen, 1901)	0.7	48.9	1.0	64.6		
Psammoryctides barbatus (Grube, 1891)	0.7	48.9	0.7	45.8		
Psammoryctides albicola (Michaelsen, 1901)	0.5	31.9	0.8	66.7		
Ilyodrilus templetoni (Southern, 1909)	0.4	36.2	0.7	58.3		
Aulodrilus pluriseta Bretscher, 1899	0.9	29.8				
Tubificinae gen. spp. juv.	30.2	97.9	55.8	100.0	24.6	68.8
Paranais litoralis (Müller, 1784)					73.5	75.0
Chaetogaster diaphanus (Gruithuisen, 1828)	1.1	25.5				
Stylaria lacustris (Linnaeus, 1767)	2.6	53.2	5.1	39.6		
Ophidonais serpentina (Müller, 1774)	4.8	51.1	5.3	56.3		
Dero digitata (Müller, 1774)	12.2	68.1	4.3	77.1		
Specaria josinae (Veydovský, 1884)	0.9	36.2	1.1	39.6		
Nais communis Piguet, 1906	12.5	48.9	1.5	47.9		
Nais pardalis Piguet, 1906	3.5	40.4	0.9	35.4		
Nais barbata Müller, 1774	1.5	40.4	0.7	33.3		
Nais simplex Piguet, 1906	2.1	48.9	0.8	41.7		
Nais elinguis Müller, 1774	0.5	38.3	3.2	64.6		
Vejdovskyella comata (Vejdovský, 1884)	0.6	19.1				
Uncinais uncinata (Ørsted, 1842)	0.4	12.8				
Pristina longiseta Ehrenberg, 1828	0.5	19.1				
Pristina aequiseta Bourne,1891	0.6	19.1				
Lumbriculus variegatus (Müller, 1774)	1.8	48.9	0.4	27.1		
Enchytraeidae	0.2	19.1	0.3	16.7		
Total individuals	5648		6569		4975	
Density (ind./m^2) (range)	0-2709		144-5083		0-7808	
Mean density (ind./m^2)	641		730		553	
Mean wet weight (g m^{-2})	0.916		0.617		0.419	
Total number of species	25		20		4	
Mean number of species	11		11		1	
Mean values of the Shannon-Wiener index	1.640		2.042		0.879	

The CCA, performed by forward selection of environmental variables showed that TDS, alkalinity, nitrite nitrogen, ammonium nitrogen, phosphate, organic matter content in the sediments, and type of substrate (unvegetated bottom sediments and sediments overgrown with macrophytes) best explained the variance in the distribution of the oligochaete species in the studied ponds. The first two axes explained 35.8% of the variance in the taxa data and 88.0% of the variance in the relationship between the taxa and the environmental variables. *Paranais litoralis* was associated with a high content of TDS, whereas the other species were found in waters with lower TDS values (Fig. 4). *Stylaria lacustris* and *Lumbriculus variegatus* (Müller, 1774) were more abundant in the bottom sediments overgrown with macrophytes, whereas *Nais elinguis* Piguet, 1906, and *P. bavaricus* were associated with a higher content of ammonium nitrogen in the water and sediments without macrophytes (Fig. 4). The relationship between the composition of the oligochaete species and the environmental variables was significant (Monte Carlo test of significance of the first canonical axis [eigenvalue = 0.604], F ratio = 56.605, p = 0.002; test of significance of all the canonical axes [trace = 0.794], F ratio = 12.365, p = 0.002).

The results indicated that *P. litoralis*, *L. hoffmeisteri*, *P. bavaricus,* and *L. claparedeanus* were the species most resistant to saline water. Furthermore, *V. comata* was the most salt-sensitive species and present in waters with a TDS value of up to 130 mg L^{-1} (Fig. 5).

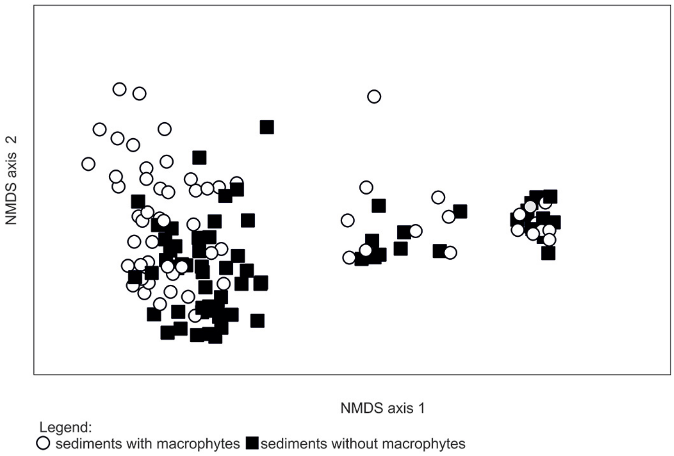

Fig. 2. Nonmetric multidimensional scaling (NMDS) plot based on log $(x+1)$-transformed abundance of oligochaetes in the studied substrate types.

Regression analysis did not reveal any relationship between the total oligochaete density and the environmental parameters ($p > 0.05$). Taxa richness was negatively related to the TDS (adj. $R^2 = 0.779$, $p < 0.0001$; Fig. 6).

Cluster analysis, which was based on the structure of the oligochaete communities, separated the hypohaline water ponds (ponds 7, 8, and 9) into distinct groups in relation to the freshwater and subhaline ponds (ponds 1–6) (Fig. 7).

4 Discussion

The oligochaete communities present in the studied water bodies were mainly represented by the eurytopic species that commonly occur in flowing and stagnant waters (*e.g.*, Dumnicka and Koszałka, 2005; Krodkiewska, 2006, 2010; Timm, 2013; Krodkiewska *et al.*, 2016; Yildiz, 2016). Only three species, *L. hoffmeisteri, L. claparedeanus,* and *L. udekemianus*, occurred in all the types of the investigated ponds.

The oligochaete fauna in the presented research were characterised by a similar species diversity as that in other

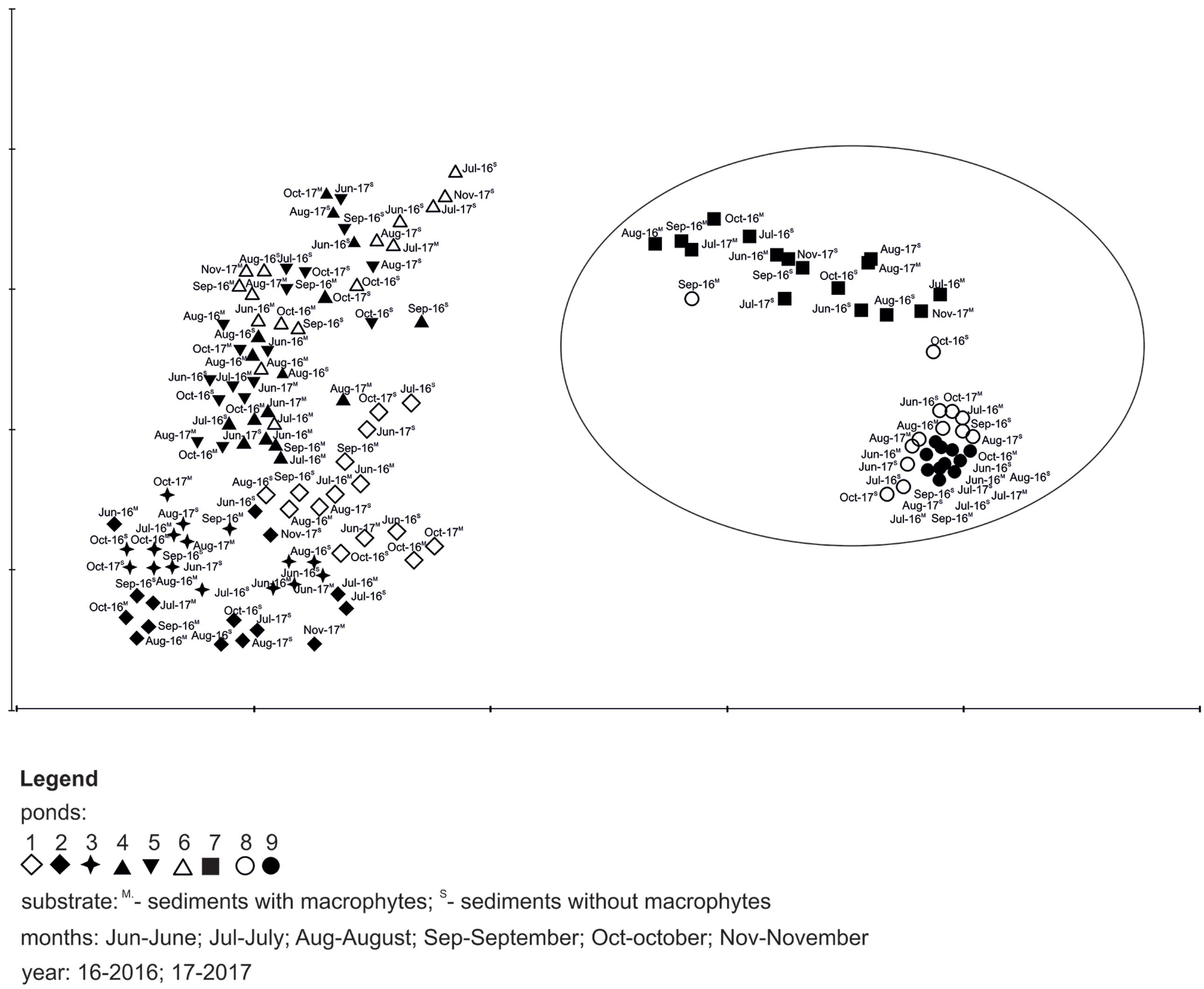

Fig. 3. Principal coordinates ordination (PCO) plot of the oligochaete communities based on the Bray-Curtis distance measure.

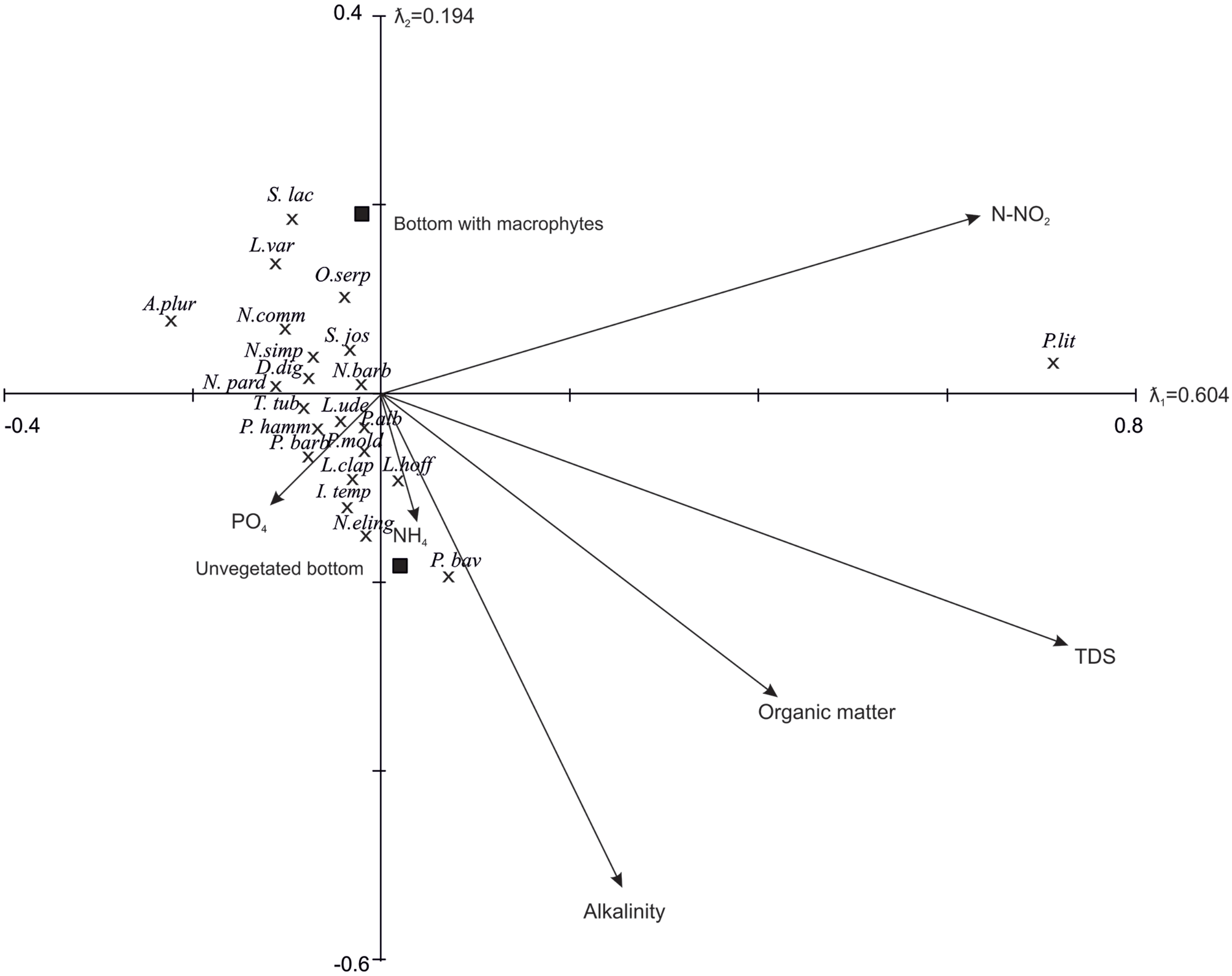

Fig. 4. Ordination diagram based on the canonical correspondence analysis of the species abundance data and the best explanatory variables. Abbreviations: *A. plur* – *Aulodrilus pluriseta*, *D. dig* – *Dero digitata*, *I. temp* – *Ilyodrilus templetoni*, *L. clap.* – *Limnodrilus claparedeanus*, *L. hoff* – *Limnodrilus hoffmeisteri*, *L. ude* – *Limnodrilus udekemianus*, *L. var* – *Lumbriculus variegatus, N. barb* – *Nais barbatus*, *N. comm* – *Nais communis*, *N. elin* – *Nais elinguis*, *N. pard* – *Nais pardalis*, *N. simp* – *Nais simplex*, *O. serp* – *Ophidonais serpentina*, *P. lit* – *Paranais litoralis, P. bav* – *Potamothrix bavaricus*, *P. hamm* – *Potamothrix hammoniensis*, *P. mold* – *Potamothrix moldaviensis*, *P. alb* – *Psammorictides albicola*, *P. bar* – *Psammoryctides barbatus, S. jos* – *Specaria josinae*, *S. lac* – *Stylaria lacustris*, *T. tub* – *Tubifex tubifex*.

anthropogenic habitats such as dam reservoirs, drainage ditches, navigable canals, sand-pit, clay-pit, or subsidence ponds (*e.g.*, Celik, 2002; Dumnicka and Krodkiewska, 2003; Heatherly *et al.,* 2005; Krodkiewska, 2006; Dumnicka, 2007; Krodkiewska, 2010; Krodkiewska and Królczyk, 2011; Krodkiewska *et al.,* 2016). Nevertheless, in the studied water bodies, taxa richness was significantly lower than that in natural aquatic habitats and naturally saline environments. For example, Timm *et al.* (1996) recorded 59 species of oligochaetes in Lake Peipsi-Pikhva in Estonia, Collado *et al.* (1999) found 49 species in German lakes, and Schenková and Helešic (2006) recorded 44 species in the Rokytná River in the Czech Republic, whereas Maximov (2015) indicated the presence of 66 species in the Gulf of Finland and Potyutko (2015) recorded 40 oligochaete species in the Curonian Lagoon of the Baltic Sea.

Our survey results confirmed that freshwater oligochaetes can tolerate elevated water salinity (Wolfram *et al.,* 1999; Berezina, 2003; Krodkiewska, 2010) and showed highest densities and species diversity in intermediate water salinity as shown in previous studies (*e.g.*, Hammer *et al.,* 1990; Piscart *et al.,* 2005; Cañedo-Argüelles *et al.,* 2014; Botwe *et al.,* 2018). Williams *et al.* (1990) and Piscart *et al.* (2006) stated that this relationship results from the broad range of salinity tolerance of different species under these conditions. The experiment of Chapman and Brinkhurst (1987) demonstrated that naidids are more tolerant to water salinity than tubificids. According to Hart *et al.* (1991), most species of oligochaetes prefer waters with a mineralisation level of 0.28–1 g L^{-1}. In turn, Berezina (2003) indicated that the upper limit of salinity tolerance was 6.3 g L^{-1} for *Tubifex tubifex* and *L. hoffmeisteri*, 4.2 g L^{-1} for *L. variegatus,* and 8.1 g L^{-1} for *S. lacustris*. In addition, a study conducted in Australia showed that the majority of the oligochaetes occur only below 5 g L^{-1} (Rutherford and Kefford, 2005). However, Zinchenko and Golovatyuk (2013) recorded the high abundance of

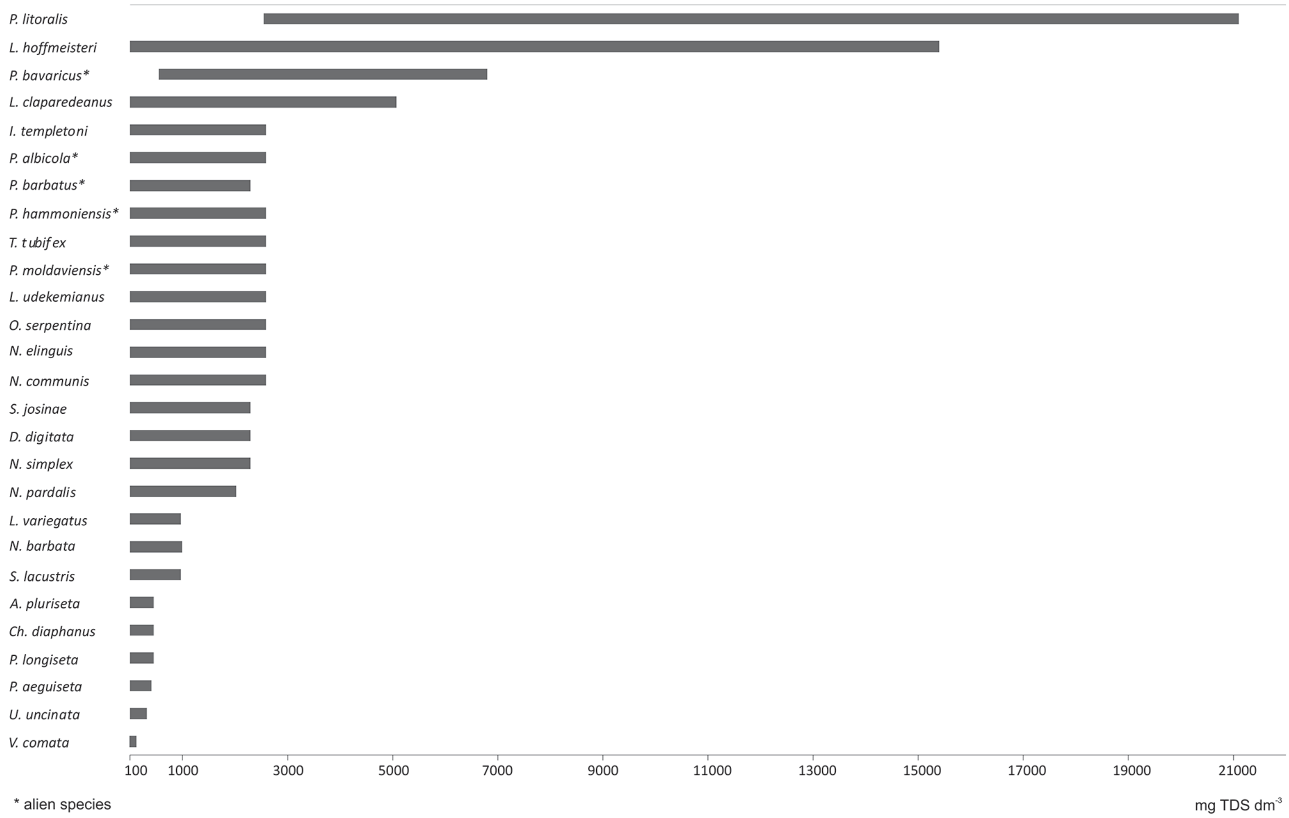

Fig. 5. Occurrence of oligochaete species along the salinity gradient.

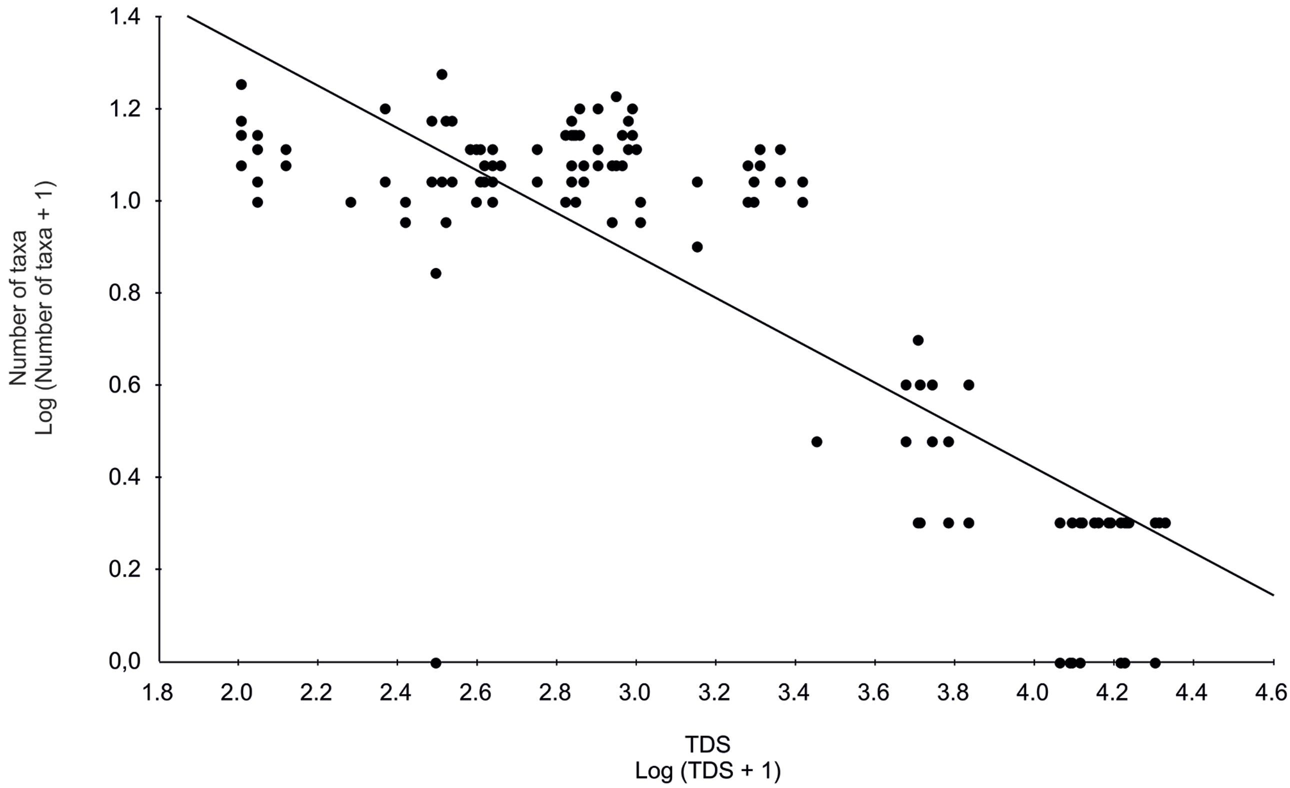

Fig. 6. Taxa richness as a function of the total dissolved solids (TDS) in the studied ponds. Multiple linear regression of the taxa richness on the TDS yielded the equation: $Y = 2.264 – 0.461*\text{TDS}$.

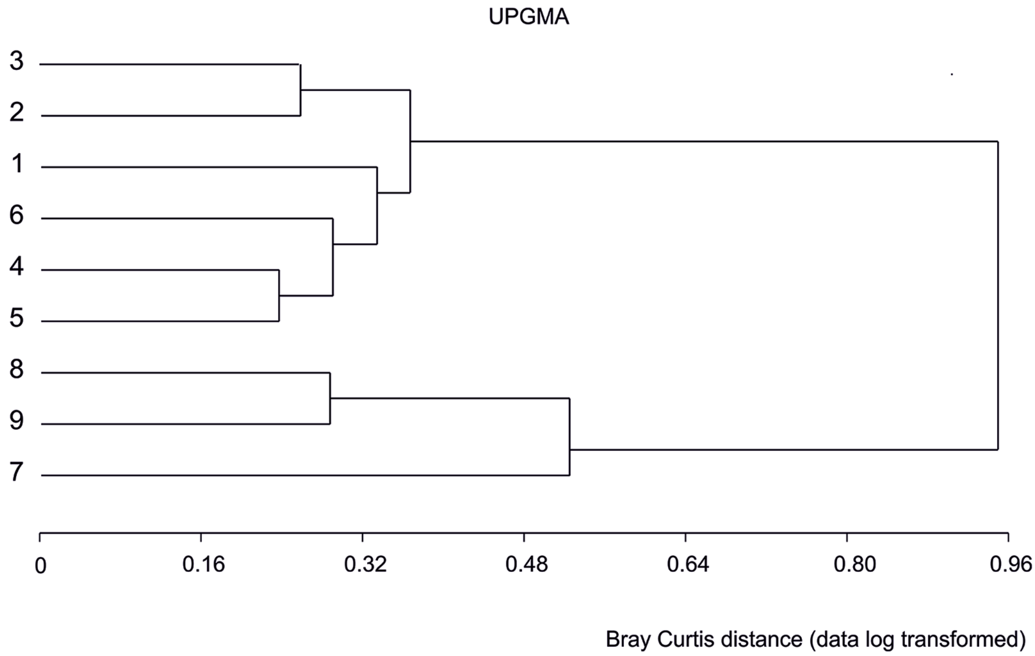

Fig. 7. Diagram of the faunal similarities of the studied ponds using the Bray-Curtis distance measure and unweighted pair-group methods with the arithmetic mean (UPGMA) linkage method.

Enchytraeus issykkulensis in rivers at mineralisations of 12.6–25.7 g L^{-1}, while the presence of *Paranais simplex* was found at salinities up to 25 g L^{-1}.

Similar to the findings of Braukmann and Böhme (2011), we found that the waters with the highest salt concentrations had an extremely low number of oligochaete species. In the hypohaline ponds, we observed the presence of three brackish water species *P. bavaricus*, *L. hoffmeisteri,* and *P. litoralis*, which can inhabit littoral sediments in the transitional zone between the freshwater and brackish habitats near seas (Balik *et al.,* 2004; Wolf *et al.,* 2009; Dumnicka *et al.,* 2014; Potyutko, 2015); on the contrary, in the most saline water body (pond 9 with a TDS content of 12,210–21,100 mg L^{-1} and conductivity in the range of 24,400–42,400 μS cm^{-1}), only *P. litoralis* occurred. Gillett *et al.* (2007) recorded only this species in the Malind and Okatie Creeks (South Carolina, USA). Similar as in the investigated hypohaline water bodies (ponds 8 and 9), *P. litoralis* was the dominant oligochaete species or commonly found in naturally saline environments such as estuaries, salt marshes, spring streams, rivers, mangrove biotopes, and tidal flats (*e.g.*, Sardà *et al.,* 1996; Timm, 1999; Kolbe and Michaelis, 2001; Erséus, 2003; Moseman *et al.,* 2004; Giere, 2006; Fujii, 2007; Gillett *et al.,* 2007; Moreno *et al.,* 2010; Capítulo *et al.,* 2014) as well as in the Gostynka River, which is strongly contaminated with salt (Dumnicka *et al.,* 2018); the Bolina River (Halabowski *et al.,* 2019); and in salt marsh clay pits (Vöge *et al.,* 2008). Therefore, these findings suggest that euryhaline *P. litoralis* could be considered as a good indicator of anthropogenically saline aquatic habitats.

The alien oligochaete species of Ponto–Caspian origin are the most abundant ones in Europe (Timm, 2013), *e.g.*, *P. bavaricus,* which is widely distributed in Europe but considered as rare in Poland (Krodkiewska, 2007, 2010; Dumnicka, 2016). It is a constant element among the oligochaetes in the Upper Silesian Coal Basin (Krodkiewska, 2006, 2007, 2010; Krodkiewska *et al.,* 2016). Our results are consistent with previous research, demonstrating that *P. bavaricus* can settle in environments that are unsuitable for other species of Oligochaeta and that it prefers waters with an elevated level of mineralisation, wherein there is a high concentration of nutrients and heavy metals, and with location in urban areas (Pascar-Gluzman and Dimentman, 1984; Erséus *et al.,* 1998; Krodkiewska, 2007; Krodkiewska *et al.,* 2016). The presence of this organism in the studied saline water bodies confirms that secondary salinisation may promote the establishment of non-native species (Piscart *et al.,* 2011). Two other alien species from the genus *Potamothrix* (*P. hammoniensis* and *P. moldaviensis*), which are commonly found in European water bodies (Wolfram *et al.,* 1999; Milbrink and Timm, 2001; Jabłońska-Barna *et al.,* 2013; Timm, 2013; Dumnicka *et al.,* 2014; Maximov, 2015; Potyutko, 2015), were also noted in our research (freshwater and subhaline ponds). It is worth adding that the increasing salinity of aquatic environments contributes to the spread of non-native annelid species. For example, Halabowski *et al.* (2019) observed the presence of the Asian oligochaete species *Monopylephorus limosus* (Hatai, 1898) in the Bolina River (the Upper Silesian Coal Basin, Poland), which is highly contaminated with saline water associated with underground mining, and Pabis *et al.* (2017) reported the occurrence of the alien polychaete species *Laonome calida* Capa, 2007 (now considered to be a new species *L. xeprovala* Bick & Bastrop (Bick *et al.*, 2018)), and *Hypania invalida* (Grube, 1860) in the brackish waters of the Odra River.

The results of the present study suggest that in addition to anthropogenic salinisation, the structure of the oligochaete assemblages was also affected by the content of nutrients in the water. This is consistent with the results of many previous surveys, which have confirmed that aquatic oligochaetes are good bioindicators of water trophy (*e.g.*, Timm *et al.*, 2001; Nijboer *et al.*, 2004; Schenková and Helešic, 2006; Krodkiewska and Michalik-Kucharz, 2009; Lv *et al.*, 2009; Krodkiewska, 2010; Jabłońska, 2014; Krodkiewska and Kostecki, 2015).

Presented research proved that species of Tubificinae occur in very high densities in anthropogenic environments (Bis *et al.*, 2000; Frizzera and Alves, 2012; Jabłońska, 2014). In the study of Krodkiewska *et al.* (2016), the proportion of the tubificid species *T. tubifex, P. bavaricus, L. claparedeanus,* and *L. hoffmeisteri* was much higher in urban ponds than in woodland ponds. Many earlier studies (*e.g.*, Timm *et al.*, 2001; Rodriquez *et al.*, 2006; Lin and Yo, 2008; Wolf *et al.*, 2009) demonstrated the mass occurrence of *L. hoffmeisteri* and *T. tubifex* in strongly polluted waters, among others, those with municipal sewage and heavy metals. *Limnodrilus hoffmeisteri* can adapt to increasing concentrations of heavy metals in sediments through the production of metallothionein-like proteins (MT) and metal-rich granules (MRG) for the storage and detoxification of metals (Klerks and Bartholomew, 1991).

Oligochaetes occur abundantly in the coastal zone water bodies, which are overgrown by macrophytes – on the bottom in decaying debris, on the surface of macrophytes, or at the bottom among their roots. Naidine and pristinine worms are particularly numerous in such habitats because of their feeding behavior. Many of these worms consume detritus with bacteria and epiphytic algae (Timm *et al.*, 2001; Verdonschot, 2006; Lin and Yo, 2008; Ohtaka *et al.*, 2011; Timm, 2012; Potyutko, 2015; Yildiz, 2016; Ohtaka, 2018). The present research confirms that bottom sediments that are overgrown by macrophytes are important habitats for many naidid species (*O. serpentina*, *Specaria josinae*, *S. lacustris,* and all the species in the *Nais* genus except for *N. elinguis*, which is associated with unvegetated bottom sediments). Our findings are consistent with the results of the study by Dumnicka (2007), who found a relationship between *L. variegatus* and macrophytes. Our results also showed that *N. elinguis* had the highest preferences for a high content of organic matter in the bottom sediments. Moreover, Levinton and Kelaher (2004) showed that detritus stimulates the distribution of *P. litoralis*.

The present research highlights the fact that a high anthropogenic salinity (TDS above $2800\,\mathrm{mg\,L^{-1}}$) causes significant loss of the diversity of freshwater oligochaete communities and contributes to colonisation by halotolerant species, especially *P. litoralis*, whose abundance increased along with an increasing salinity gradient. The increasing number of new records of non-native oligochaete species means that further studies of saline aquatic habitats are necessary. Brackish anthropogenic water bodies should be monitored because oligochaetes have developed many strategies to migrate and colonise new environments, which are unfavorable to other invertebrates. They spread through active movement (upstream migration as well as movement up from within the substrate or by drift) and passive transport by water birds, fish, and humans. Moreover, some species demonstrate adaptations to subterranean life and hence may migrate to the surface waters, especially during the early spring when the ground water level is high and the temperature of the surface waters is low (Lafont and Malard, 2001; van Haaren and Soors, 2013). Thus, the oligochaetes could have migrated by underground connections between the catchments areas of the largest rivers in Central Europe (Dumnicka, 2014). In addition, some species (*T. tubifex* and some Lumbriculidae and Aelosomatidae) form cysts, which enable them to be passively transported by the wind. Moreover, many oligochaete worms can survive dry periods by forming cysts or burrowing in the moist substratum (Milbrink and Timm, 2001; Otermin *et al.*, 2002; Montalto and Marchese, 2005).

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