



Response of the mollusc communities to environmental factors along an anthropogenic salinity gradient

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Abstract

Anthropogenic salinisation of freshwater ecosystems is frequent across the world. The scale of this phenomenon remains unrecognised, and therefore, monitoring and management of such ecosystems is very important. We conducted a study on the mollusc communities in inland anthropogenic ponds covering a large gradient of salinity located in an area of underground coal mining activity. A total of 14 gastropod and 6 bivalve species were noted. No molluscs were found in waters with total dissolved solids (TDS) higher than 17.1 g L⁻¹. The share of alien species in the communities was very high in waters with elevated salinity and significantly lower in the freshwaters. Canonical correspondence analysis (CCA) showed that TDS, pH, alkalinity, nitrate nitrogen, ammonium nitrogen, iron, the content of organic matter in sediments, the type of substrate and the content of sand and gravel in sediments were the variables that were significantly associated with the distribution of molluscs. The regression analysis revealed that total mollusc density was positively related to alkalinity and negatively related to nitrate nitrogen. The taxa richness was negatively related to TDS, which is consistent with previous studies which indicated that a high salinity level is a significant threat to freshwater malacofauna, causing a loss of biodiversity and contributing to the colonisation and establishment of alien species in aquatic ecosystems.

Keywords Malacofauna · Salinisation · Anthropogenic ponds · Alien species · Mining activity

Introduction

The anthropogenic salinisation of inland waters is manifested by an increase in the total dissolved solids (TDS) which among others is caused by mining activity (Pond et al. 2008; Cañedo-Argüelles et al. 2013; Ciparis et al. 2015). On a global scale, there are no regulations regarding the introduction of salt loads into the aquatic environment. For example, the European Union Water Framework Directive has no regulations of the limits on the introduction of major ions into the environment by member countries (Schuler et al. 2018). It is often unknown how significant is salinity in comparison to other environmental stressors, how it may interact with these

stressors, how important the different drivers of salinisation are and how water salinity might influence aquatic ecosystems in the future. Research that answers these questions are necessary for an effective ecosystem management (Dunlop et al. 2007; Cañedo-Argüelles et al. 2018). Schuler et al. (2018) point out that to reduce anthropogenic salt pollution management strategies must include multi-stakeholder and co-adaptive management strategies. It is also very important to monitor rivers and streams whose salty water can enter into stagnant freshwater ecosystems or flow from saline water bodies and bring additional salt into lotic ecosystems (Williams 2001). Monitoring can be used to predict the direction of the dispersion of alien species and would contribute to the more effective and precise prevention of biological invasions and their deleterious effects (Petruček and Stöffler 2011; Piscart et al. 2011; Kefford et al. 2012) as well as to predict changes in water chemistry due to the fact that, according to scientist (e.g. Cañedo-Argüelles et al. 2013; Dugan et al. 2017), salinisation of freshwater ecosystems is more and more frequent not only in arid, semi-arid and Mediterranean climates but also in temperate and cold regions of the world. Moreover, freshwater salinisation will continue to increase, due to

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climate change and numerous anthropogenic pressures that have not changed in their intensity or geographical extent (Cañedo-Argüelles et al. 2013, 2018; Le et al. 2019; Olson 2019).

Bivalves and gastropods have a wide global distribution and perform an important role in the functioning of aquatic ecosystems. Anthropogenic habitats, especially water bodies located in urban, industrial and mining areas, may create environments that are inhabited by a diverse malacofauna and that can be refuges for rare and threatened species (Kiviat and MacDonald 2004; Lewin et al. 2015; Spyra 2017). In clay pit ponds, Lewin and Smoliński (2006) showed the presence of rare and protected species of the gastropods *Anisus vorticulus* (Troschel, 1834) and *Valvata naticina* (Menke, 1845), which is one of the rarest species and is critically endangered with extinction. *Stagnicola turricula* (Held, 1836), which is another very rare and disappearing species in Germany and Austria, was also noted in anthropogenic ponds (Lewin and Cebula 2003; Skowrońska-Ochmann et al. 2012). Sulikowska-Drozd (2009) recorded *Gyraulus rosmaessleri* (Auerswald, 1852), which is rare in Europe, and the protected mussel *Anodonta cygnea* (Linnaeus, 1758) in city park ponds. This species was also noted in mine subsidence reservoirs with elevated water salinity in coal mining areas (e.g. Lewin 2012; Kašovská et al. 2014; Lewin et al. 2015).

The creation of water bodies and anthropogenic disturbances of the aquatic environment (e.g. drainage, pollutions, hydrotechnical structures) contribute to the introduction and dispersion of alien gastropod species, among them *Potamopyrgus antipodarum* (Gray, 1843), *Physa acuta* (Draparnaud, 1805) and *Ferrissia fragilis* (Tryon, 1863), whose abundances are currently increasing (Meier-Brook 2002; Havel et al. 2005; Lockwood et al. 2007; Spyra 2008; Früh et al. 2012; Spyra and Strzelec 2014). Alien species are able to tolerate a wide range of environmental conditions, and their evolutionary potential allows them to adapt to new conditions (Winterbourn 1969; Hylleberg and Siegismund 1987; Strzelec 1999; Hänfling and Kollmann 2002; Dunlop et al. 2007; Van Leeuwen et al. 2013). Species that are introduced into rivers and estuaries such as the invasive New Zealand mud snail *P. antipodarum*, which was brought into Europe through commercial shipping, can easily get into inland waters. It is assumed that its high tolerance to salinisation is one of the factors that enable it to colonise new areas (Piscart et al. 2011). Anthropogenic water bodies also create 'open niches' for the invasion of the Chinese mussel *Sinanodonta woodiana* (Lea, 1834), which is rapidly spreading in European countries (e.g. Beran 2008; Mouthon 2008; Adam 2010; Douda et al. 2011; Spyra et al. 2012). The success of invaders is also promoted by the disappearance of native freshwater species from environments with anthropogenic high salinity. Freshwater species can be excluded by salinity stress, which causes them

to fail in the competition for food and habitat when they occupy the same ecological niche as alien species (Piscart et al. 2005; Alonso and Castro-Díez 2012). This often enables the invader species to become the dominant groups in non-native regions (Alonso and Castro-Díez 2008; Ba et al. 2010; Bätke and Coring 2011; Braukmann and Böhme 2011). Survival in a new territory is also dependent on the degree of environmental similarity between the donor and recipient regions (Ba et al. 2010). There is still no detailed data on the resistance mechanisms of alien species to the salinity of water. There are also cases in which exotic aquatic species have colonised and become well developed in freshwaters. Piscart et al. (2011) showed that many non-native species have a salinity tolerance that is similar to native species. This also proves that other biotic and abiotic factors such as temperature, waste water pollution, increases in sedimentation, nutrition, microhabitat structure, hydromorphological degradation and different biological interactions are important for a successful invasion and that these factors should be considered together (Velasco et al. 2006; Bätke and Coring 2011).

To date, the impact of anthropogenic salinity on a whole benthic macroinvertebrate fauna has primarily been studied in running waters (e.g. Ziemann 1997; Kefford et al. 2003; Piscart et al. 2005; Piscart et al. 2006a, b; Bätke and Coring 2011; Kefford et al. 2011; Piscart et al. 2011; Cañedo-Argüelles et al. 2013) and in laboratory experiments (e.g. Kefford et al. 2004; Carver et al. 2009; Cañedo-Argüelles et al. 2014; Hintz et al. 2017; Schuler et al. 2017). Meanwhile, research that focuses only on malacocoenosis and on anthropogenic saline water bodies (e.g. Kašovská et al. 2014) with a large gradient of anthropogenic salinity is scarce. Such studies were conducted only in natural ecosystems such as lakes (e.g. Timms 1981, 1983; Hammer et al. 1990; Williams et al. 1990; Wollheim and Lovvorn 1995, 1996; Anufrieva and Shadrin 2018) and wetlands (e.g. Brock and Shiel 1983; Pinder et al. 2005). Therefore, the objectives of the presented study were to determine the composition and structure of the mollusc communities along the anthropogenic salinity gradient; to assess the impact of other environmental factors on the biodiversity, abundance and biomass of the molluscs in the anthropogenic water bodies that are located in an area of underground coal mining and to identify species that might be useful in monitoring of water bodies contaminated with salt.

Materials and methods

Study area

The research was conducted once a month from June to October in 2016 and in June, July, August, October and

November in 2017 in nine anthropogenic water bodies with different salinity degrees. All of the ponds are situated in one of the most industrialised and urbanised regions in Europe and one of the largest coal basins in the world—the Silesian Region (southern Poland) (Fig. 1). The entire area has strongly been affected by underground coal mining. It has almost no natural water bodies, and it is characterised by a large concentration of anthropogenic reservoirs, including mining subsidence ponds and settling ponds (Jaruchiewicz 2014). The investigated ponds were created as a result of land subsidence over exploited hard coal seams and were used by coal mines to retain saline underground waters (Table 1). According to the classification of Hammer et al. (1990), three freshwater ponds (numbers 1, 2, and 3) ($\text{TDS} < 0.5 \text{ g L}^{-1}$), three subhaline (numbers 4, 5, and 6) ($\text{TDS} = 0.5\text{--}3.0 \text{ g L}^{-1}$) and three hypohaline water bodies (numbers 7, 8, and 9) ($\text{TDS} = 3.0\text{--}20.0 \text{ g L}^{-1}$) were selected for the study. Their characteristics are presented in Table 1.

Field surveys and laboratory analysis

In each pond, samples of molluscs were taken by the quantitative method using a $25 \times 25 \text{ cm}$ frame that was placed randomly at three sites in two habitats, i.e. unvegetated bottom sediments and sediments that had been overgrown by macrophytes. A total of 143 samples were taken during the study period. The samples of molluscs were transported to the laboratory in plastic containers and then sieved with a 0.4-mm diameter mesh, sorted and preserved in 80% ethanol. The molluscs were identified to the species level based on their morphological and anatomical features according to Glöer and Meier-Brook (1998), Glöer (2002) and Jackiewicz (2000). The nomenclature follows Piechocki and Wawrzyniak-Wydrowska (2016). The collected specimens were counted and weighed (wet mass) on laboratory scales that have an accuracy of 0.001g. The density of individuals per 1 m^2 was estimated.

The zoocenological analysis of the communities of molluscs was based on the following indices:

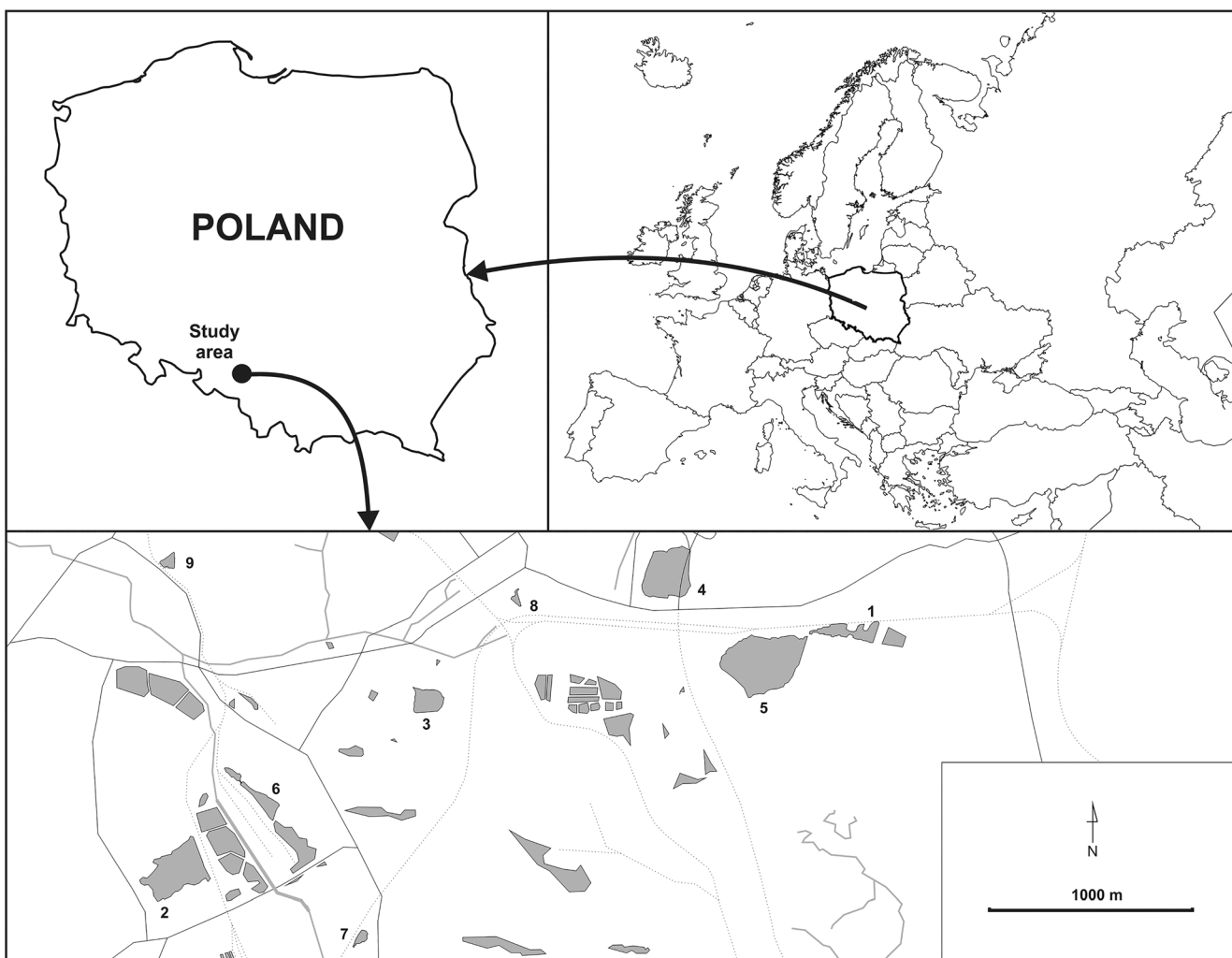


Fig. 1 Location of the anthropogenic ponds that were studied (1–3 freshwater, 4–6 subhaline, 7–9 hypohaline)

Table 1 Characteristics of the investigated anthropogenic water bodies with different salinity levels (1–3: freshwater ponds, 4–6: subhaline ponds, 7–9: hypohaline ponds)

Characteristic	Freshwater ponds			Subhaline ponds			Hypohaline ponds		
	1	2	3	4	5	6	7	8	9
Geographic coordinates	50° 12.978' N 18° 42.896' E	50° 11.825' N 18° 37.355' E	50° 12.670' N 18° 39.469' E	50° 13.156' N 18° 41.318' E	50° 12.902' N 18° 41.972' E	50° 12.125' N 18° 38.073' E	50° 11.387' N 18° 38.888' E	50° 21.865' N 18° 67.026' E	50° 13.346' N 18° 37.251' E
Year of creation	1977	1971	1974	1974	1977	1971	1973	1974	1974
Type of water body	Sinkhole pond	Sinkhole pond	Sinkhole pond	Sinkhole pond	Sinkhole pond	Sinkhole pond	Sinkhole pond	Settling pond	Settling pond
Surface area (ha)	0.2	21	6.3	26	22.8	4.9	0.6	0.7	1.5
Bottom type	Sand	Sand	Sand	Gravel	Gravel	Gravel	Gravel	Gravel	Sand
Management	Fishing, wildfowl	Stocked with fish, recreation, wildfowl	Stocked with fish, recreation, wildfowl	Stocked with fish, recreation, wildfowl	Stocked with fish, wildfowl	Stocked with fish, wildfowl	Mining use, fishing, wildfowl	Mining use, wildfowl	Mining use
Surroundings of the pond	Farmlands, wasteland, railway embankment	Mine dump, forest, garden plots	Forest	Wasteland, highway, garden plots	Wasteland, railway embankment, forest	Mine dump, garden plots	Mine dump, railway embankment	Wasteland, carpark	Forest, wasteland

1. Dominance index (D): $D = k_a/K \times 100$, where k is the number of individuals of a species and a and K are the total number of individuals in a sample. The following dominance classes were used according to Górný and Grüm 1981—eudominants: $D > 10\%$, dominants: $D = 5.1–10\%$, subdominants: $D = 2.1–5.0\%$, recedents: $D < 2.0\%$ and subrecedents: $\leq 1.0\%$ of sample.

2. Constancy (C): $C = n/N \times 100$, where n is the number of samples in which a given species occurs and N represents the total number of samples.

3. The Shannon-Wiener index (Hauer and Lamberti 2006): $H' = -\sum (P_i)(\log_2 P_i)$, where $P_i = N_i/N$ – the proportion of individuals of species i .

During the sampling of the molluscs, samples of the water were also taken from each pond once a month. Water variables, i.e. conductivity, pH, total dissolved solids (TDS), temperature and dissolved oxygen, were measured in the field using Hanna Instruments and WTW portable metres. Other variables: alkalinity, iron, chlorides, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, phosphates, calcium, magnesium, potassium and sulphates, were analysed in the laboratory using metres and reagents by Hanna Instruments and Merck according to the standard methods of Hermanowicz et al. (1999).

Additionally, samples of bottom sediments were collected in order to determine the content of organic matter and selected heavy metals and the grain size composition of bottom sediments in all of the water bodies. The total organic matter content (%) from the habitats that had been overgrown by macrophytes and from unvegetated bottom sediments was determined using the loss-on-ignition (LOI) method, which measures weight loss in sediment samples after combusting them at 550 °C according to PN-88/B04481 (Myślińska 2001). The total content of heavy metals (Cd, Cu, Zn, Pb) in the bottom sediments was determined after homogenisation and mineralisation with aqua regia (nitric acid and hydrochloric acid in a molar ratio of 1:3) using an inductively coupled plasma-optical emission spectroscope (ICP OES), while their fractional composition was determined using an inductively coupled plasma-mass spectrometry (ICP MS) according to Tessier’s procedures (Tessier et al. 1979) and were partitioned into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual. The grain size composition of the bottom sediments was determined using the sieve method.

Recorded macrophytes were identified to the species or genus level according to Szafer et al. (1986) at the sampling sites.

Data analyses

The significance of differences in the water variables among the ponds with different water salinity levels (according to the

classification of Hammer et al. 1990) was calculated using the ANOVA Kruskal-Wallis and multiple comparisons post hoc test because the data did not have a normal distribution (using the Kolmogorov–Smirnov test for normality). The analyses were performed using Statistica (version 13.1).

A non-metric multidimensional scaling (NMDS) on $\log(x + 1)$ -transformed mollusc abundance data and the Bray-Curtis distance measure was used for testing the grouping of the examined substrate types (unvegetated bottom sediments and sediment overgrown by macrophytes). Variation in mollusc community structure was visualised graphically using a principal coordinates ordination (PCO) on the basis of Bray-Curtis distance measure. All these analyses were performed using the Canoco ver. 5.0 package.

The relationship between the composition of the mollusc communities and the environmental variables was determined using canonical correspondence analysis (CCA) (CANOCO 5.0). A unimodal analysis was chosen because the length of the gradient was long (3.541 as determined using a detrended correspondence analysis on 26 segments with only the species data). Prior to CCA, the forward selection method for environmental variables (conductivity, TDS, pH, alkalinity, oxygen, temperature, chlorides, calcium, magnesium, sulphates, potassium, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, phosphates, iron, organic matter in sediments, type of substrate, grain size composition of bottom sediments) was applied using the Monte Carlo permutation test (499 runs) to find the variables that best explained the composition of the mollusc fauna. The 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 in order to reduce the noise in a data set (Gauch 1982). After removing the rare taxa and redundancy among the significant environmental variables (conductivity and the major ions, i.e. chlorides, sulphates, potassium, calcium and magnesium were excluded from the analysis because they correlated with TDS as a measure of salinity—Fig. 2), 11 environmental variables and 15 species were used in the final CCA. The analysis was performed on $\log(x + 1)$ -transformed taxa and environmental data. The results were displayed graphically in ordination diagrams using CanoDraw (version 4.12).

Multiple regression analyses (stepwise backward variable elimination) were used to assess relationships between environmental variables and mollusc density and the taxa richness. With each step in a regression, the environmental variable with the lowest partial effect indicated by the highest p value was removed until only environmental variables related ($p \leq 0.05$) to the mollusc variables remained. These analyses were performed using Statistica ver. 13.1.

Cluster analysis using the Bray-Curtis distance measure and the unweighted pair-group method with arithmetic mean (UPGMA) linkage method was used to assess the similarity of

the mollusc fauna among the studied ponds. Species that occurred in only one sample were excluded. The analysis was computed using MVSP software (Kovach Computing Services, version 3.13p).

Results

Habitat characteristics of the investigated ponds

In total, 35 macrophyte taxa were recorded in the investigated water bodies. *Mougeotia* sp., *Cladophora* sp., *Zanichella palustris* (L.), *Najas marina* (L.) and *Ruppia maritima* (L.), which can tolerate elevated water salinity (Podbielkowski and Tomaszewicz 1996), occurred only in the subhaline and hypohaline water bodies. *Trapa natans* (L.), which is legally protected in Poland (Dz U 2014), occurred in one of the freshwater ponds. Five taxa of macroalgae were found during the study period (Table 2). Management of all of the freshwater ponds (1 to 3) and subhaline ponds (4 to 6) includes the removal of macrophytes.

Data summarising the physical and chemical variables of the water in the investigated water bodies are given in Table 3. A relatively high concentration of dissolved oxygen was found in all of the ponds (maximum: from 12.9 mg dm⁻³ in the subhaline ponds to 17.6 in the hypohaline ponds). The content of nutrients (nitrate nitrogen, nitrite nitrogen, ammonium nitrogen and phosphates) was the lowest in the subhaline water bodies (Table 3). Percent ionic composition of the studied ponds determined from concentration in mEq L⁻¹ are shown in Table 4. In the 1, 2 and 3 ponds (freshwaters), the highest contribution of calcium was observed, while chlorides dominated in the subhaline and hypohaline water bodies (numbers 5, 6, 8, and 9). In the ponds 4 and 7, the highest percentage share had sulphates (Table 4). 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 total dissolved solids ($H = 63.13141$, $p < 0.0001$), chlorides ($H = 60.95674$, $p < 0.0001$), sulphates ($H = 45.23897$, $p < 0.0001$), potassium ($H = 37.00591$, $p < 0.0001$), magnesium ($H = 53.37663$, $p < 0.0001$) and iron ($H = 28.45855$, $p < 0.0001$) between the hypohaline, subhaline and freshwater ponds. The Kruskal-Wallis ANOVA test also showed significant differences in the median concentration of nitrite nitrogen ($H = 24.35032$, $p < 0.0001$) and calcium ($H = 52.13484$, $p < 0.0001$) between the 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 and other types of ponds. The subhaline and freshwater ponds differed significantly in the median value of the water pH and the hypohaline and freshwater water bodies in the median value of alkalinity.

The organic matter content in the bottom sediments of the studied anthropogenic water bodies ranged from 0.4 to 54.0%. The Kruskal-Wallis ANOVA test revealed statistically significant differences in the median content of organic matter in the sediments ($H = 68.30246$, $p < 0.0001$) between all of the types of ponds. The highest concentration of bioavailable Cd and Cu were found in the hypohaline water bodies, whereas bioavailable Zn and Pb were found in the subhaline water bodies (Table 3). The bottom sediments of the freshwater ponds and

one hypohaline pond (9) were built mainly of sand, whereas gravel predominated in the sediments in the other water bodies (4–8) (Table 1).

Mollusc communities

In total, 20 mollusc species were recorded in the investigated water bodies—14 gastropod species and 6 bivalve species (Table 5). Among them, three alien

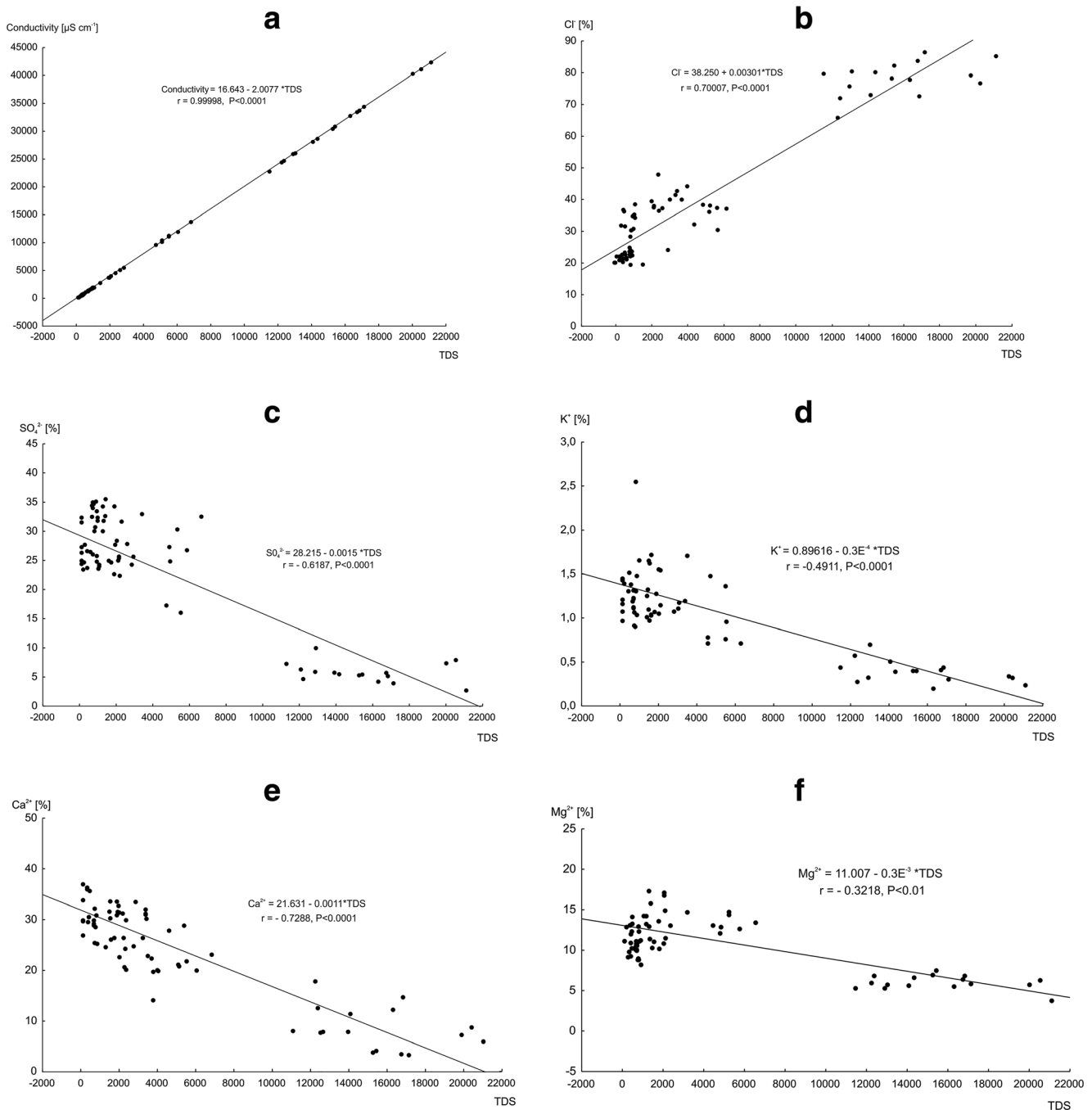


Fig. 2 Linear regression equations for the conductivity (a), chlorides (b), sulphates (c), potassium (d), calcium (e) and magnesium (f) as the function of the total dissolved solids (TDS); r : the Pearson coefficient, P : level of significance

gastropods were recorded, i.e. *P. antipodarum*, *P. acuta* and *F. fragilis*. *Potamopyrgus antipodarum* only occurred in the hypohaline ponds. The share of bivalves in the total number of collected molluscs was low. The highest density and biomass of Mollusca were noted in the subhaline water bodies (Table 5).

Mollusc communities in relation to environmental factors

The NMDS analysis based on log (x + 1) transformed abundance data did not indicate the grouping of the sampling sites with different substrate types (Fig. 3).

Table 2 The occurrence of macrophytes at the sampling sites in the investigated freshwater (1–3), subhaline (4–6) and hypohaline (7–9) water bodies

Taxa	Ponds								
	Freshwater			Subhaline			Hypohaline		
	1	2	3	4	5	6	7	8	9
<i>Eleocharis palustris</i> (L.) Roem. & Schult.		x	x						
<i>Phragmites australis</i> (Cav.) Trin. Ex. Steud.	x	x	x	x	x	x	x	x	x
<i>Persicaria amphibia</i> L.		x							
<i>Myriophyllum spicatum</i> L.		x			x	x			
<i>Typha angustifolia</i> L.		x	x		x	x			
<i>Typha latifolia</i> L.	x	x	x	x			x		
<i>Najas marina</i> L.	x	x		x	x	x			
<i>Ceratophyllum demersum</i> L. S. Str.				x	x				
<i>Potamogeton crispus</i> L.		x	x	x	x	x			
<i>Scirpus sylvaticus</i> L.		x	x		x				
<i>Lycopus europaeus</i> L.	x		x						
<i>Lythrum salicaria</i> L.		x							
<i>Stuckenia pectinata</i> (L.) Börner		x							
<i>Ranunculus aquatilis</i> L.		x							
<i>Ranunculus circinatus</i> Sibht.				x		x			
<i>Sparganium erectum</i> L.				x					
<i>Lemna minor</i> L.	x								
<i>Alisma plantago-aquatica</i> L.	x		x		x				
<i>Schoenoplectus lacustris</i> (L.) Palla				x					
<i>Trapa natans</i> L.		x							
<i>Solanum dulcamara</i> L.			x						
<i>Juncus conglomeratus</i> L.			x						
<i>Juncus bulbosus</i> L.		x							
<i>Iris sibirica</i> L.			x						
<i>Ruppia maritima</i> L.								x	
<i>Mentha aquatica</i> L.		x							
<i>Potamogeton natans</i> L.			x						
<i>Galium palustre</i> L.			x						
<i>Zannichellia palustris</i> L.					x				
<i>Chara vulgaris</i> L.					x				
<i>Enteromorpha</i> sp.				x	x	x			
<i>Cladophora</i> sp.		x			x	x			
<i>Mougeotia</i> sp.									x
<i>Spirogyra</i> sp.			x						
<i>Lyngbya</i> sp.			x						
No. of taxa in water body	6	16	15	8	11	8	2	2	2
No. of taxa in salinity type of water bodies	26			16			4		

The PCO plot (Fig. 4) showed that the structure of the mollusc communities changed: *P. antipodarum* occurs abundantly in the more saline ponds, while *P. acuta* and *F. fragilis* were more common in the freshwater ponds. On the other hand, there were no seasonal changes in the communities.

The CCA with a forward selection of environmental variables showed that TDS, pH, alkalinity, nitrate nitrogen, ammonium nitrogen, iron, the content of organic matter in the sediments, type of substrate (unvegetated bottom sediments and sediments that had been overgrown by macrophytes), the content of sand and gravel in the bottom sediments best explained the variance in the distribution of mollusc species in the studied ponds. The first two axes explained 21.2% of the variance in the taxa data and 64.9% of the variance in the relationship between the taxa and environmental variables. *Potamopyrgus antipodarum* was associated with a high content of TDS, whereas the other species were found in waters with lower TDS values (Fig. 5). *Segmentina nitida* (Müller, 1774) and *F. fragilis* were more abundant on the bottoms that had been overgrown by macrophytes and those with a higher

content of sand in the sediments. *Physa acuta* and *Radix balthica* Linnaeus, 1758 were associated with a higher content of ammonium nitrogen in the water, whereas *R. auricularia* (Linnaeus, 1758), *U. pictorum*, *Gyraulus alba* (Müller, 1774), *G. crista*, *Hippeutis complanatus* (Linnaeus, 1758), *Planorbarius corneus* (Linnaeus, 1758) and *Lymnaea stagnalis* (Linnaeus, 1758) were associated with a higher content of iron in the water (Fig. 5). The relationship between the composition of mollusc species and the environmental variables was significant (Monte Carlo test of significance of the first canonical axis [eigenvalue = 0.358], F ratio = 14.147, p = 0.002; test of significance of all of the canonical axes [trace = 0.933], F ratio = 4.501, p = 0.002).

Potamopyrgus antipodarum was the only species that was recorded in the hypohaline waters with a salinity of up to 17.1 g L⁻¹. *Physa acuta*, *F. fragilis*, *L. stagnalis*, *G. crista*, *R. balthica*, *A. anatina* and *U. pictorum* were noted in waters in which the salinity did not exceed 2.59 g L⁻¹. However, *Aplexa hypnorum* (Linnaeus, 1758), *P. corneus*, *Bathymophalus contortus* (Linnaeus, 1758) and *Anisus*

Table 3 The physical and chemical variables of the water, organic matter content and selected heavy metal content in the bottom sediments in the investigated types of water bodies

Parameter	Ponds		
	Freshwater (ranges)	Subhaline (ranges)	Hypohaline (ranges)
Water			
Temperature (°C)	7.9–27.1	6.8–26.2	10.7–25.6
Dissolved oxygen (mg L ⁻¹)	2.5–15.3	4.9–12.9	6.8–17.6
pH	6.4–9.6	7.1–8.7	7.1–8.5
Alkalinity (mg L ⁻¹)	40–250	110–300	150–445
Conductivity (μS cm ⁻¹)	220–910	1130–5200	7750–42,400
Total dissolved solids (mg L ⁻¹)	100–450	560–2590	3860–21,100
Chlorides (mg L ⁻¹)	8–167	111–1090	920–19,000
Potassium (mg L ⁻¹)	1–10	4–48	30–92
Sulphates (mg L ⁻¹)	23–147	132–720	750–3600
Calcium (mg L ⁻¹)	10–124	64–135	200–995
Magnesium (mg L ⁻¹)	1.2–28	0.7–51	180–340
Nitrate nitrogen (mg L ⁻¹)	0–13.6	0–8.0	0.06–10.5
Nitrite nitrogen (mg L ⁻¹)	0–0.2	0–0.04	0.001–1.8
Ammonium nitrogen (mg L ⁻¹)	0.1–9.3	0.03–0.7	0.3–5.7
Phosphates (mg L ⁻¹)	0–2.2	0–0.2	0.001–1.2
Iron (mg L ⁻¹)	0.1–2.8	0.01–1.0	0.06–1.6
Sediments			
Organic matter (%)	0.4–54.0	1.2–34.4	2.2–35.4
Cadmium bioavailable (μg kg ⁻¹)	97–791	248–568	361–1445
Cadmium total (μg kg ⁻¹)	97–814	304–634	378–1472
Copper bioavailable (μg kg ⁻¹)	1789–7236	9385–20,197	12,370–21,156
Copper total (μg kg ⁻¹)	2633–23,736	12,755–32,547	23,670–31,956
Lead bioavailable (μg kg ⁻¹)	5948–21,783	3716–44,396	3068–19,289
Lead total (μg kg ⁻¹)	2966–29,853	11,786–55,046	13,268–39,689
Zinc bioavailable (μg kg ⁻¹)	15,278–104,626	19,447–122,495	63,421–75,971
Zinc total (μg kg ⁻¹)	26,478–168,926	61,547–168,982	80,521–131,682

spirorbis (Linnaeus, 1758) were only present in freshwaters with TDS of up to 0.45 g L⁻¹ (Fig. 6).

A regression analysis revealed that total mollusc density was positively related to alkalinity and negatively related to nitrate nitrogen (adj. R² = 0.205, p = 0.024 and p = 0.029). The taxa richness was negatively related to TDS (adj. R² = 0.325, p = 0.00048).

A cluster analysis, which was based on the structure of the mollusc communities, separated the hypohaline and subhaline ponds (i.e. ponds 4 to 9) in a distinct group in relation to the freshwater ponds (ponds 1 to 3) (Fig. 7).

Discussion

Many previous studies have shown that increasing salinity results in the elimination of sensitive taxa and their replacement by eurytopic species (e.g. Williams et al. 1990; Boets et al. 2012; Kefford et al. 2012; Arle and Wagner 2013; Szöcs et al. 2014; Patnode et al. 2015). Salt-tolerant species including alien species usually become greatly abundant as a result of a lack of competition (an additional effect of a decrease in species richness) and then communities became monospecific (Williams et al. 1990; James et al. 2003; Carver et al. 2009; Bäche and Coring 2011). Our survey results are consistent with this hypothesis—only *P. antipodarum* occurred in the hypohaline water bodies (TDS above 3.9 g L⁻¹). Thus, the study may confirm that salinisation may be a factor contributing to the success of alien species in freshwater environments. This finding is consistent with the result of previous research by Piscart et al. (2005), Velasco et al. (2006), Piscart et al. (2011) and Arle and Wagner (2013). Kašovská et al. (2014) recorded the highest share of *P. antipodarum* and *P. acuta*, while Piscart et al. (2005, 2006a) observed *P. antipodarum*, *Corbicula fluminalis* (O.F. Müller, 1774) and *D. polymorpha* in waters with a high content of salt. In turn, Braukmann and

Böhme (2011) noted that *P. antipodarum* reached 99.8% of the total number of all specimens over all of the recorded taxa. Kašovská et al. (2014) stated that the New Zealand mud snail could be a potential bioindicator of a high amount of salt in inland waters. The results of the presented study are consistent with this statement. The recorded density of *P. antipodarum* in this survey was relatively high compared to the studies mentioned above and reached up to 12 150 individuals m⁻². Even a single snail that is introduced into a new habitat can start a population in a short period of time in various types of aquatic ecosystems—from freshwater to saltwater and from lotic to lentic ecosystems (Wallace 1985; Ponder 1988; Økland 1990; Gangloff 1998; Jensen et al. 2001; Duft et al. 2003; Lewin 2012). To date, the majority of studies on the impact of water salinity on the New Zealand mud snail have been focused on its tolerance (Hoy et al. 2012), growth rate (Herbst et al. 2008) and the effects of water salinity on its fecundity under field conditions (Gérard et al. 2003; McKenzie et al. 2013) or in laboratory experiments (Jacobsen and Forbes 1997; Drown et al. 2011; Vazquez et al. 2016). The research has shown the broad salinity tolerance of *P. antipodarum*. It can live in salinity up to 64‰, but the species is able to reproduce only in water with salinity up to 18‰ (Duncan and Klekowski 1967).

Previous studies in the anthropogenic water bodies in the area that was studied by us (e.g. Strzelec et al. 2006; Lewin 2012; Spyra and Strzelec 2014; Strzelec et al. 2014; Lewin et al. 2015; Spyra and Strzelec 2015; Spyra 2017) indicated that *P. acuta* and *F. fragilis* are becoming a more and more common and abundant in such habitats. The presented results confirmed that alien *P. acuta* and *F. fragilis* are associated with waters that are rich in nutrients. Moreover, *F. fragilis* occurred most frequently at the sites that had been overgrown by macrophytes. Spyra and Strzelec (2015) determined that *F. fragilis* prefers floating *Nuphar lutea* (L.) Sibth. & Sm. leaves as a substrate for life, and its abundance was the lowest on *P. australis*. In the presented study, the highest share of *F. fragilis* was recorded in the freshwater ponds, which had the highest richness of macrophytes. This may be related to the fact that vascular plants are a food source for grazers, provide dead organic matter for detritivorous and create a favourable habitat for the growth of the periphyton that snails eat and where they can breed and hide from predators (Storey 1971; Thomaz and Ribeiro da Cunha 2010; Spyra and Strzelec 2015). It appears that the numerous occurrences of macrophytes in water bodies with low salinities were associated with a high content of nutrients in the water.

Clements et al. (2006) and Garg et al. (2009) noted that the species richness and distribution of Mollusca is affected by the cumulative effect of macrophytes, a high content of calcium in the water and pH. In turn, the laboratory experiment of Berezina (2003) demonstrated that the development of molluscs is primarily associated with the concentrations of sodium, magnesium and potassium in the water. The survey of Pip

Table 4 Percent ionic composition of the investigated freshwater (1–3), subhaline (4–6) and hypohaline (7–9) water bodies

Ponds	Ca ²⁺	Mg ²⁺	K ⁺	SO ₄ ²⁻	Cl ⁻	TDS
1	49.8	13.4	2.0	20.9	13.9	382
2	36.4	8.9	1.9	19.6	33.2	325
3	35.0	24.0	1.2	25.7	14.1	108
4	26.7	12.1	1.1	35.9	24.2	691
5	21.5	9.5	0.8	31.8	36.4	892
6	16.8	3.5	1.1	29.5	49.1	1716
7	22.8	17.4	0.9	34.9	24.0	5184
8	3.6	6.2	0.4	5.5	84.2	14,519
9	12.9	5.9	0.4	4.9	76.0	16,669

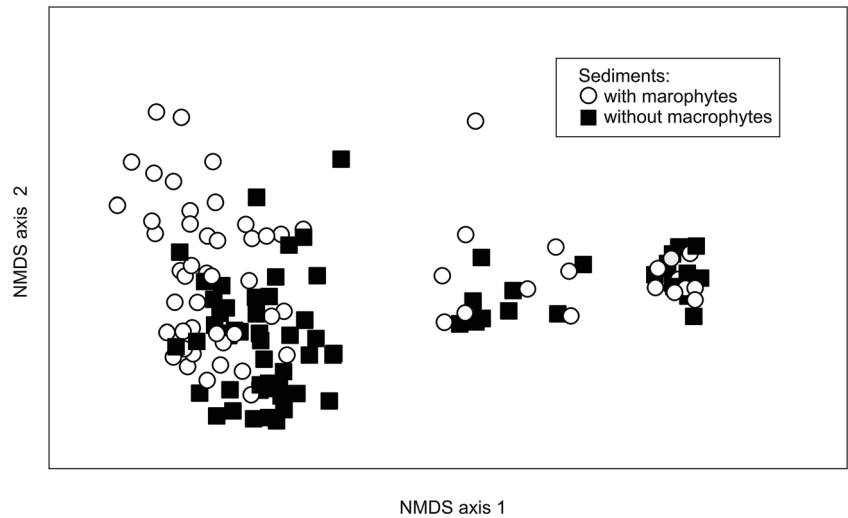
(1986) showed that the densities of co-occurring mollusc species, the presence of parasites and predators and the availability of food have a significant influence on malacofauna. The presented research showed that the salinity affected the malacofauna in the studied anthropogenic water bodies the most. Water salinity above 17.1 g L^{-1} TDS created conditions that prevented the survival of molluscs. Many studies in saline ecosystems have shown a direct relationship between the species richness of macroinvertebrates and salinisation (e.g. Timms 1983; Hammer et al. 1990; Williams and Williams 1998; Piscart, et al. 2005; Carver et al. 2009; Uwadiae 2009; Bätthe and Coring 2011; CCME 2011). In general, biodiversity decreases with an increasing salinity. Braukmann and Böhme (2011) noted that waters with the highest salt concentration were extremely poor in species. Almost all of the benthic

groups had disappeared. Our research indicated that the highest density, biomass and biodiversity (expressed by the Shannon-Wiener index H') of mollusc communities were recorded in the subhaline water bodies, which had a medium content of TDS: $0.56\text{--}2.6 \text{ g L}^{-1}$. Some authors have also observed that aquatic macroinvertebrates had the highest biomass and diversity in intermediate salinities (e.g. Hammer et al. 1990; Williams et al. 1990; Cañedo-Argüelles et al. 2014). Timms (1983) related this pattern to increased primary production in aquatic ecosystems with an intermediate salinity, whereas Williams et al. (1990) related it to the broad range of salinity tolerance of species in such conditions. Moreover, Piscart et al. (2006b) considered that the medium content of salt in water creates favourable conditions for salt-tolerant and salt-sensitive species.

Table 5 Values of the dominance (D%) and constancy (C%) indices that were calculated for the mollusc communities in the water bodies with different salinity levels

Taxa	Ponds					
	Freshwater		Subhaline		Hypohaline	
	D (%)	C (%)	D (%)	C (%)	D (%)	C (%)
<i>Potamopyrgus antipodarum</i> (Gray, 1843)	3.7	12.8	90.5	100	100	43.8
<i>Physa acuta</i> (Draparnaud, 1805)	49.7	42.6	5.9	75		
<i>Lymnaea</i> sp. (Linnaeus, 1758)	5.2	6.4	1.6	18.8		
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)	1.2	10.6				
<i>Radix auricularia</i> (Linnaeus, 1758)	1.5	12.8	0.1	10.4		
<i>Radix balthica</i> Linnaeus, 1758			0.1	12.5		
<i>Aplexa hypnorum</i> (Linnaeus, 1758)	0.3	2.1				
<i>Anisus spirorbis</i> (Linnaeus, 1758)	0.2	2.1				
<i>Bathyomphalus contortus</i> (Linnaeus, 1758)	0.2	2.1				
<i>Ferrissia fragilis</i> (Tryon, 1863)	17.9	17	0.7	22.9		
<i>Gyraulus albus</i> (O. F. Müller, 1774)	3.0	10.6	0.05	6.3		
<i>Gyraulus crista</i> (Linnaeus, 1758)	9.3	8.5	0.2	25		
<i>Hippeutis complanatus</i> (Linnaeus, 1758)	3.4	8.5	0.03	6.3		
<i>Segmentina nitida</i> (O. F. Müller, 1774)			0.03	4.2		
<i>Planorbarius corneus</i> (Linnaeus, 1758)	1.3	6.4				
<i>Anodonta anatina</i> (Linnaeus, 1758)	1.7	6.4	0.7	45.8		
<i>Unio pictorum</i> (Linnaeus, 1758)	1.3	6.4	0.02	6.3		
<i>Unio tumidus</i> Philipsson, 1788			0.01	2.1		
<i>Pisidium henslowanum</i> (Sheppard, 1823)			0.06	8.3		
<i>Pisidium subtruncatum</i> Malm, 1855			0.1	14.6		
<i>Musculium lacustre</i> (O. F. Müller, 1774)			0.01	2.1		
Total individuals	593		14,009		6099	
Density (individuals m^{-2}) (range)	0–624		21–13,168		0–5952	
Mean density (individuals m^{-2})	67		1557		678	
Biomass (g m^{-2}) (range)	0–1105.42		0.15–1736.76		0–17.62	
Mean biomass (g m^{-2})	46.52		273.23		2.32	
Total number of species	15		16		1	
Number of species (range)	0–9		1–7		0–1	
Shannon-Wiener index H' (range)	0–1.78		0–1.48		0	
Mean Shannon-Wiener index H'	0.31		0.52		0	

Fig. 3 Result of the non-metric multidimensional scaling (NMDS) ordination (based on log-transformed mollusc abundance data) showing the location of sampling sites with different substrate types



Zinchenko and Golovatyuk (2013) recorded the presence of molluscs in rivers with salinities that were no higher than 6.8 g L^{-1} . The survey of Piscart et al. (2005) indicated that the most favourable values of water mineralisation is $0.46\text{--}2.6 \text{ g}$

L^{-1} for the development of *D. polymorpha*, *C. fluminalis*, *P. corneus*, *Physa* sp., *Gyraulus* sp. and *Radix* sp. According to Metzeling (1986), the highest densities of molluscs are observed in water with a salinity of less than 1 g L^{-1} , whereas

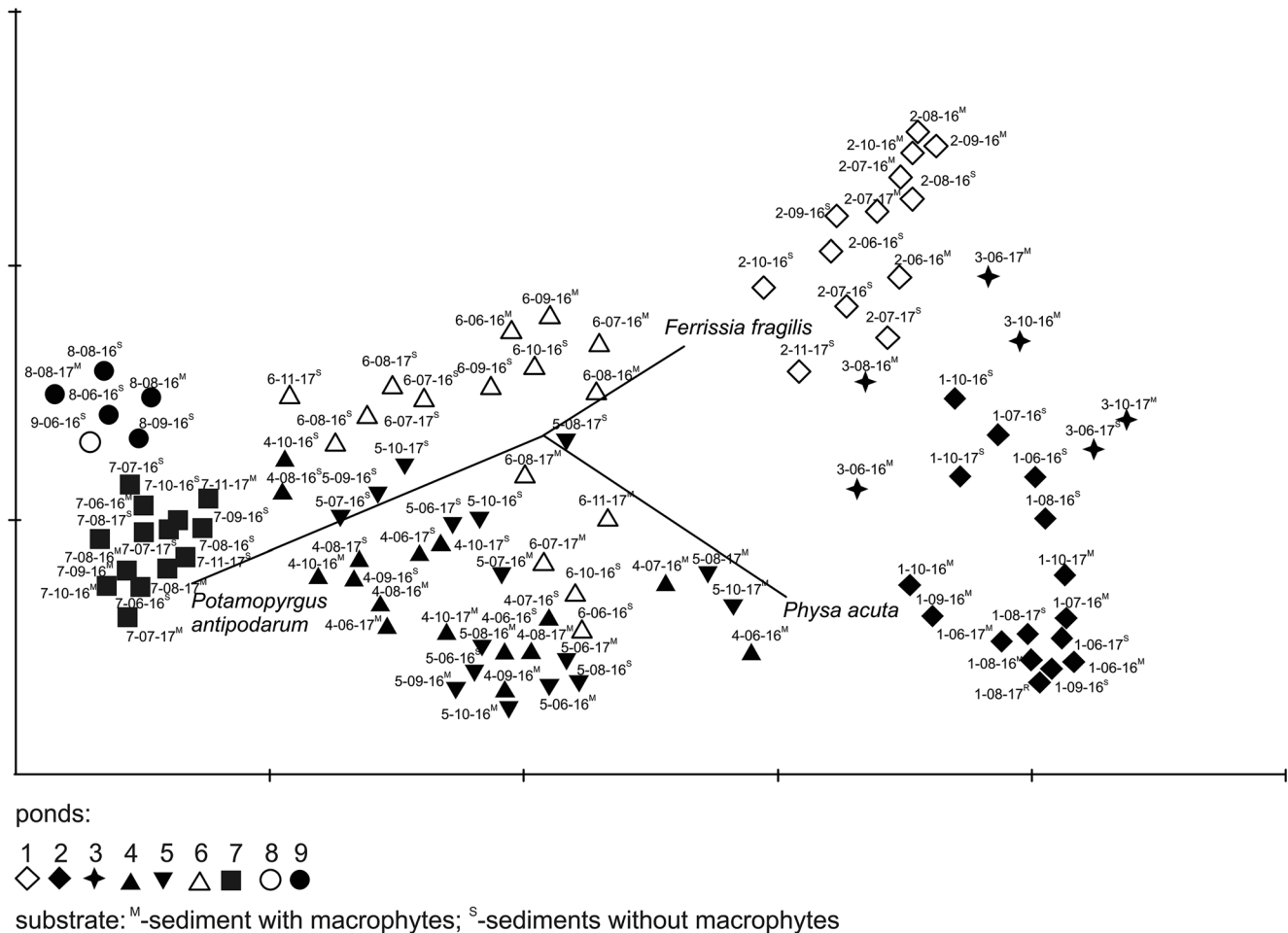


Fig. 4 Principal coordinates analysis (PCO) plot of mollusc communities at freshwater (1–3), subhaline (4–6) and hypohaline (7–9) ponds on the basis of Bray-Curtis distance measure of the log-transformed data. Sample dates are given as follows: day-month-year

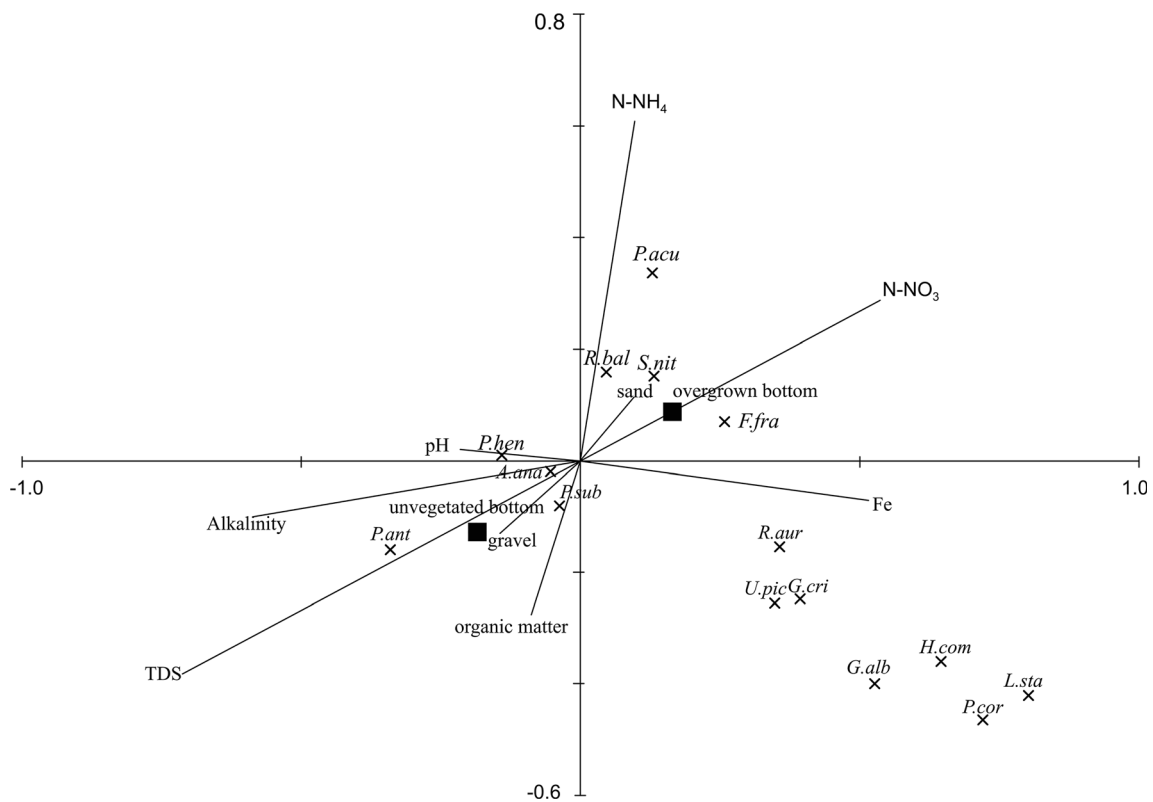


Fig. 5 Result of the canonical correspondence analysis (CCA): a ordination diagram of the best explanatory variables and mollusc species abundance data. Abbreviations: A.ana: *Anodonta anatina*, F.fra: *Ferrissia fragilis*, G.alb: *Gyraulus albus*, G.cri: *Gyraulus crista*, H.com: *Hippeutis complanatus*, L.sta: *Lymnaea stagnalis*, P.acu: *Physa acuta*, P.

ant: *Potamopyrgus antipodarum*, P.cor: *Planorbarius corneus*, P.hen: *Pisidium henslowanum*, P.sub: *Pisidium subtruncatum*, R.aur: *Radix auricularia*, R.bal: *Radix balthica*, S.nit: *Segmentina nitida*, U.pic: *Unio pictorum*

Kefford et al. (2011) found the maximum abundance of Mollusca in salinities that ranged from 0.64 to 1.0 g L⁻¹.

Similar results were recorded by Kašovská et al. (2014) and in the laboratory experiments of Berezina (2003). It is worth

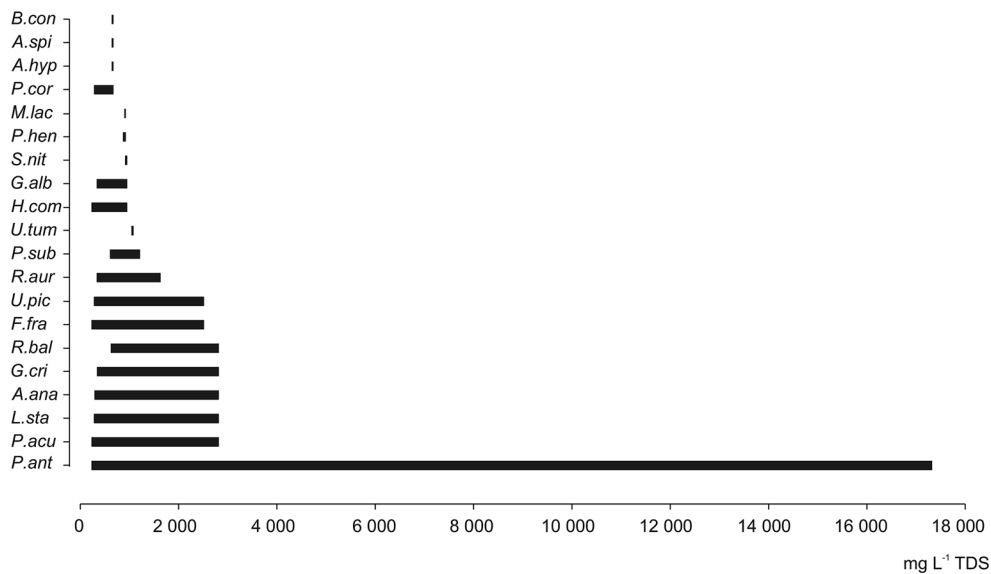


Fig. 6 Distribution of mollusc species along the salinity gradient (B.con: *Bathyomphalus contortus*, A.spi: *Anisus spirorbis*, A.hyp: *Aplexa hypnorum*, P.cor: *Planorbarius corneus*, M.lac: *Musculium lacustre*, P.hen: *Pisidium henslowanum*, S.nit: *Segmentina nitida*, G.alb: *Gyraulus albus*, H.com: *Hippeutis complanatus*, U.tum: *Unio tumidus*,

P.sub: *Pisidium subtruncatum*, R.aur: *Radix auricularia*, U.pic: *Unio pictorum*, F.fra: *Ferrissia fragilis*, R.bal: *Radix balthica*, G.cri: *Gyraulus crista*, A.ana: *Anodonta anatina*, L.sta: *Lymnaea stagnalis*, P.acu: *Physa acuta*, P.ant: *Potamopyrgus antipodarum*)

adding that many scientists (e.g. Ziemann 1997; Blasius and Merritt 2002; Kefford et al. 2004; Zalizniak et al. 2009b; Van Dam et al. 2010; CCME 2011; Johnson et al. 2014; Cañedo-Argüelles et al. 2016; Dunlop et al. 2015, 2016; Kefford et al. 2016) have shown that the toxic effects of salinisation on macroinvertebrates are due to the osmotic effect not only of the total salt concentration in the water but also of the composition of the major ions as well as of the proportion of these ions, thus some species that are tolerant of one set of ionic proportions may be sensitive to another; in addition, some of the ions may increase or decrease the adverse effect of salinity.

Similar to Piscart et al. (2006b), this study indicated the highest occurrence of mussels in waters with an intermediate salinity, especially *A. anatina*, which has recently disappeared from several parts of Europe (Beggel and Geist 2015). This could be explained by the fact that the subhaline ponds were stocked with the fish that are required for the development of its glochidia and are a good vector for its dispersion (Pip 1986). All of the life stages of unionid mussels are sensitive to an elevated content of chlorides (Bringolf et al. 2007), but glochidia are particularly sensitive to acute exposure (Gills 2011; Echols et al. 2012; Patnode et al. 2015). Beggel and Geist (2015) found that a chloride concentration above 5962 mg L⁻¹ caused the death of *A. anatina* glochidium in laboratory conditions, whereas according to Canadian Water Quality Guidelines for the Protection of Aquatic Life (2011), the short-term exposures to chloride levels above 640 mg L⁻¹ may pose the greatest toxic effects on glochidia of certain freshwater mussel species (CCME 2011).

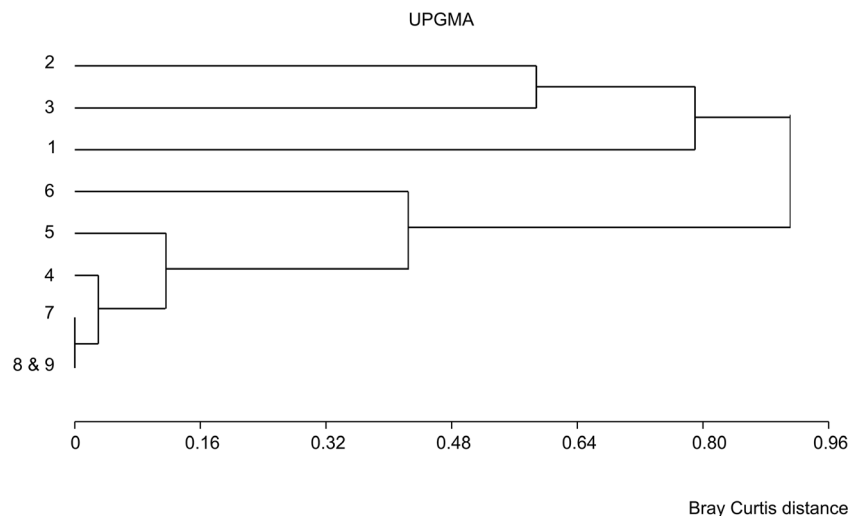
The results of our study indicated that in addition to water salinity (expressed by the content of TDS), the distribution of mollusc species was affected among others by alkalinity and pH. Previous studies (e.g. Bendell and McNicol 1993; Skowrońska-Ochmann et al. 2012) showed that molluscs are the most sensitive group of aquatic biota to acidification; thus, they are greatly influenced by pH of the water. According to

Hoverman et al. (2011), gastropod richness should be high in alkaline and large habitats. The densities and number of gastropod species increase at a higher pH (especially above pH 7.0) and decrease below pH 6.0 (e.g. Hall et al. 1980; Økland 1990, 1992; Heino 2000; Spyra 2010, 2017). Økland (1983) stated that at a low pH of water, calcium ions are hardly available for freshwater snails. Briers (2003) and Lewin et al. (2015) found that species such as *P. planorbis*, *P. corneus* and *L. stagnalis* were associated with a high concentration of calcium because they are considered to be calciphiles. Vinogradov et al. (1987) stated that in waters with a low salinity, the concentration of calcium that is needed for shell building is insufficient for most of molluscs. However, Berezina (2003) showed that for gastropods of the genus *Planorbis* and *Lymnaea*, a low content of calcium was favourable and that they were the most tolerant to a decrease in water salinity. The high share of these snails in the mollusc communities in the studied freshwater ponds may be due to the low calcium content compared to the other water bodies. The laboratory experiments of Zalizniak et al. (2006, 2009a, b) also indicated that low pH may affect a tolerance on the water salinity with low calcium concentrations of some invertebrates, especially *P. acuta*.

Results of our research in ponds with different water salinity located in the one of the largest coal basins in the world indicated the important role of freshwater and medium-saline anthropogenic ponds as substitute habitats for malacofauna. In these water bodies, we noted 17 species that are included on the European red list of non-marine molluscs as Least Concern (LC) (Cuttelod et al. 2011). Therefore, such ponds should be protected against pollution, including salinisation, because, as our research has shown, the high water salinity is a significant threat to freshwater malacofauna and contributes to the colonisation and establishment of alien species.

Monitoring and management of mining wastewater is necessary. We inclined to introduce the regulations proposed by

Fig. 7 Diagram of the faunal similarities of the studied freshwater (1–3), subhaline (4–6) and hypohaline (7–9) ponds using the Bray-Curtis distance measure and unweighted pair-group methods with the arithmetic mean (UPGMA) linkage method.



Lach et al. (2006), which include a system for continuous monitoring of the quantity and quality of underground mine waters as well as the performance of water tests below the point of water discharge. These measurements should primarily concern electrical conductivity and major ions. Ion-specific regulations should be included in the regional, national and global legislation. This would allow for permanent control of mining activity, to limit contamination and capture of exceedances of pollutants introduced into the aquatic habitats, as well as for a real valuation of environmental losses (Bogart et al. 2018; Cañedo-Argüelles et al. 2018; Schuler et al. 2018). Moreover, we promote the proposal of Schuler et al. (2018) that desalination technologies could be used by coal mines, focusing on removing specific ions from mining waters and its phytostabilisation. In order to protect aquatic life from secondary salinisation, cooperation between scientists, entrepreneurs, politicians and the local community is also necessary.

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