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IMPACT OF THE SALINITY GRADIENT ON THE MOLLUSC FAUNA IN FLOODED MINE SUBSIDENCES (KARVINA, CZECH REPUBLIC)

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Abstract: This paper presents the impact of salinisation on the aquatic mollusc fauna in flooded mine subsidences in the Karvina region (Czech Republic). The results of the previous research on salinity in flooded mine subsidences show that some of them contain a high content of dissolved inorganic substances (above 1000 $mg \cdot l^{-1}$). These substances can affect the vegetation and animals occurring in the water and the surrounding area. The phylum of *Mollusca* was selected as a model group for the fieldwork as it includes species with the proven bioindication potential.

The occurrence of aquatic mollusc species was studied at 10 sites. The sites were selected based on the content of dissolved substances (the salinity gradient from <500 to >1000 $mg \cdot l^{-1}$. A total of 12 aquatic mollusc species were found, including one species identified as a potential bioindicator of the negative effect of salinisation on aquatic biota.

The analysis showed statistically significant positive correlations between the content of dissolved inorganic substances and the presence of alien species *Potamopyrgus antipodarum* (J.E. Gray, 1843). The gradient of salinity significantly affects the species composition of the mollusc fauna in flooded mine subsidences and may affect the biodiversity of this group.

INTRODUCTION

The increasing salinity (as a concentration of dissolved inorganic ions, further referred to as TDS) in surface freshwater ecosystems is becoming a serious problem worldwide [38, 52, 21, 22], including the Czech Republic, especially in post-industrial structures, such as flooded mine subsidences [25, 43]. Salinity is also regarded as one of the most important environmental contamination types occurring in freshwater. Although dissolved salts are natural components of freshwater and some aquatic systems have naturally high salinity levels the impact of excessive concentrations of dissolved salts derived from the human activity may have profound and measurable effects on freshwater aquatic ecosystems [15, 50, 19, 21, 31, 13, 14, 27]. Human activities, such as removal of vegetation [51, 40], irrigation, mining [45] and industrial discharges [36, 37, 38, 39] may lead to a salinity increase.

As evidenced by the previous research, the impact exerted by the increased salinity on freshwater organisms [7, 10, 23, 31] is currently a worldwide environmental issue, including in particular the protection of biodiversity of freshwater organisms. Although the study by Kefford et al. [22] also proved that the tolerance of freshwater organisms to salinity does not differ significantly, it is necessary to verify this statement and compare it on a global scale. The comparison should account for different measurement methods (and hence different units), as well as different approaches to this concept in different parts of the world.

Several studies have been recently conducted in the field of lethal effects of different salinity levels on molluscs and other macroinvertebrate species [21, 23, 52, 53], as well as field research performed in wetlands, riverine ecosystems and marine biotopes [13, 14, 15, 31]. Macroinvertebrates (also molluscs) are able to respond to changes in the water conditions and thus they may act as indicators of the biotope health. Their presence or absence can indicate the extent of pollution. From a logistic perspective, they are good research objects, because they are abundant, easily surveyed and taxonomically rich [11]. The diversity and abundance of molluscs can provide accurate information about the overall health of the freshwater biotope. The present research focused on several physico-chemical properties of water and the composition of the aquatic malacocoenosis [48].

The available data indicate that salinity above 1000 $mg \cdot l^{-1}$ [34, 33] may have an adverse effect on aquatic biota. According to the aforementioned studies and the study by Stalmachová et al. [46], water in the flooded mine subsidences is characterised by an increased content of chlorides and sulphates (caused by leaching of tailings – mining waste rock) and consequently the water salinity is above 1000 $mg \cdot l^{-1}$.

There have been no prior field research, neither on mollusc communities in flooded mine subsidences in the Czech Republic, nor on their response to salinity. The required data from Central Europe (Czech Republic, Poland) is missing and it is necessary to fill this gap.

This study evaluates whether the increasing salinity affected the aquatic mollusc fauna in the flooded mine subsidences and compares the results with field studies from Poland [48] and laboratory studies (ecotoxicity) from Australia and Africa. The second objectives of this study was to identify species that are potentially useful for bioindication of the increasing salinity in the flooded mine subsidences.

MATERIALS AND METHODS

Study area

The study area is located in Northeastern Moravia, the Czech Republic, the Karvina region (Fig. 1). The entire region is strongly affected by underground coal mining. The coal mining influences the vertical movement of the geological beds above the working area. The character and strength of this movement depend on the thickness of the coal strata, the depth of their dipping and the hydrology [41]. This results in the ground sinking above the coal excavation [44]. After a certain period of time, subsidence hollows are filled with surface and ground water. Consequently, mining subsidence reservoirs are created and eventually colonised by macrophytes, invertebrates (insects, molluscs etc.), amphibians and waterfowl.

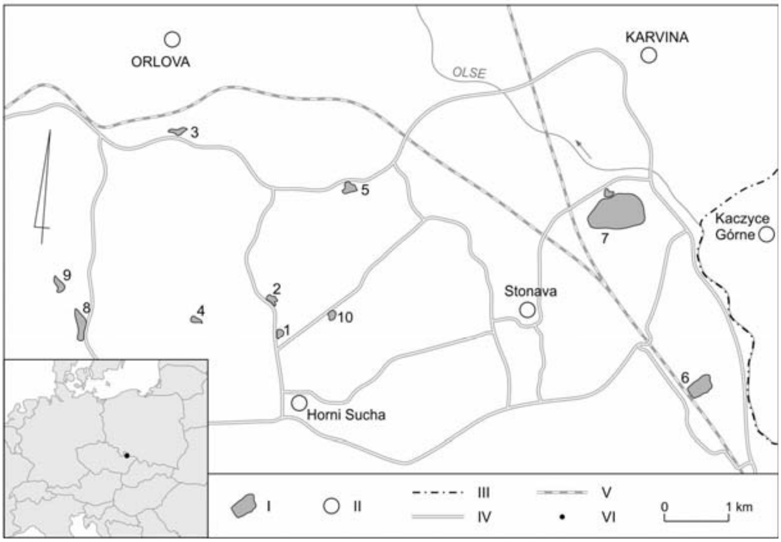

Fig. 1. Location of the study area: I – reservoirs: 1 – U cesty, 2 – Barbora,3 – U Obalovny, 4 – František skládka, 5 – U Kostela, 6 – Louky, 7 – Darkov, 8 – Bartošůvka, 9 – Pod lessem, 10 – Solecká; II – towns; III – border; IV – roads; V – railways; VI – location of study area

This type of reservoirs is characterised by a relatively shallow, regular shape, gentle slopes of the shores, a flat bottom and, in most cases, a depth of 6.5 m [20]. Waters in these reservoirs are stirred several times throughout the year (polymictic reservoirs). Intensive mixing of the water column causes the transfer of bottom sediments, which reduces the water transparency [9]. This flow results in oxygenated water and consequently in rapid aerobic decomposition of suspended organic matter, and hence an increase in available nutrients [35].

The vegetation is very similar to wetlands and consists of emergent species such as *Phragmites australis* (Cav.) Trin. Ex Steud), *Typha angustifolia* (L.) *T. latifolia* (L.), aquatic flora such as *Batrachium aquatile* (L.) Dumort., *Myriophyllum verticillatum* (L.) and woody species from the genera *Betula sp.*, *Populus sp.* or *Salix sp.* along the shores, and in reclaimed parts – mostly *Fagus sylvatica* (L.), *Carpinus betulus* (L.), *Alnus glutinosa* (L.) Gaertn. Flooded mine subsidences are also good refugia for amphibians, reptiles and bird species.

The research on water salinity in the flooded mine subsidences was conducted at 10 sites located in the Karvina region. The sites were selected based on the previous research performed by VŠB-Technical University of Ostrava. The detailed description of the sites is presented in Table 1. The sites were divided into two groups according to their size and depth: sink lakes (100 m^2 in area and 2 m deep) and slide pools [42].

Table 1. Description of the research objects

Number	Name	Dissolved components (mg·l^{-1})	Location	Effect of soil overburden, tailings presence	Characteristics
1	U cesty	> 1000	Horní Suchá 49°48'40" 18°28'49"	tailings remediation from the shore	Primary subsidence lake
2	Barbora	> 1000	Horní Suchá 49°48'57" 18°28'41"	N, E and partly S made of PT, in the E part – reclaimed soil	Primary subsidence lake
3	U Obalovny	> 1000	Karviná – Doly 49°50'26" 18°27'05"	NE tailings material	Primary subsidence lake
4	František skládka	500–1000	Horní Suchá 49°48'50" 18°27'32"	S part with tailings	Primary subsidence lake
5	U Kostela	500–1000	Karviná – Doly 49°50'04" 18°29'26"	reclamation since 1999	Primary subsidence lake
6	Louky	500–1000	Karviná – Louky 49°48'37" 18°34'17"	reclamation 1996–2008	Secondary large subsidence lake
7	Darkov	500–1000	Karviná – Darkov 49°50'06" 18°33'03"	N and NE part with tailings, reclamation 1997–2014	Primary subsidence lake, reclaimed banks
8	Bartošůvka	< 500	Havířov – Suchá 49°48'43" 18°26'04"	NE part with tailings	Primary subsidence lake, reclaimed banks
9	Pod lesem	< 500	Havířov – Suchá 49°49'01" 18°25'44"	reclaimed 2003–2010	Primary subsidence lake
10	Solecká	< 500	Horní Suchá 49°49'21" 18°29'02"	NW part with tailings, penetrating to the bottom	Primary subsidence lake

Description of mollusc communities in flooded mine subsidences

Aquatic species were obtained using a metal sieve with a diameter of 20 cm (mesh size 0.8 × 0.8 mm) by washing out the aquatic vegetation and bottom substrate; in addition, some direct collection from objects submerged in water (fallen logs, litter) was performed. Harvesting was carried out for 1 hour (in the research sites) around the selected water reservoir. A more detailed description of the methodology can be found in the following papers [3, 4, 5, 6]. This methodology is commonly used by the staff of the Agency for Nature Conservation and Landscape to map the occurrence of aquatic mollusc species. The nomenclature follows Horsák et al. [16]. The material is deposited in Kašovská collection (Ostrava – Poruba). Some individuals were returned to their original habitat immediately after the identification.

Ecoelements are presented according to [30, 26]. The tenth group consists of aquatic molluscs, which are further divided into several basic and intermediate groups. The basic type, i.e. the group PD (PALUDICOLAE), is known as overgrown swamps and marshes, a group of associated PDT (periodic, overgrown marshy grounds) recurring types of wetlands. Other ecoelements occurring at the site are SG (STAGNICOLAE – species of larger and permanent lentic water bodies) and transient groups SG (RV) (RIVICOLAE − types of lotic water), SGRV, SG – PD, RV (SG), which are intermediate between the two above-mentioned groups (the abbreviations listed by priority).

Threat: NT − near threatened, LC − least concerned, VU − vulnerable, EN − endangered according to IUCN [17].

The determination was performed by Beran [4], Horsák et al. [16], Ložek [29]. The nomenclature follows the work by Horsák et al. [16].

Water parameters

Water sampling was carried out in 2010, once a month during the growing season (May−October). At each site, permanent sampling stations were selected. Sampling stations were located in the open water areas at a distance greater than 50 m from any inflow or outflow. Samples were collected 2.5 m from the shore at a depth of 0–20 cm into the sampling bottles (Bürkle). Temperature and oxygen saturation were measured (by oxygen sensor Hach sension 6) in the collected samples. Before the analysis, the samples were stored at a temperature of 10°C. The determined hydrochemical parameters are presented in Table 2. To compare the salinity expressed in different units, the conversion procedure of Pawlowicz [28] was applied.

Statistical analysis

Basic statistics were applied to describe the differences in the species richness and their relationship to abiotic factors (dissolved inorganic ions, pH), ecological groups and sites.

Table 2. Standards of physico-chemical analysis of water parameters

Parameter	Standard	Name
pH	ČSN ISO 10523	Determination of pH in the waters by potentiometry
total dissolved substances	ČSN EN 872	Determination of dissolved solids by gravimetric analysis
chlorides	ČSN EN ISO 10304-1	Water quality – Determination of dissolved anions. Part 1: Determination of bromide, chloride, fluoride, nitrates, nitrites and phosphates
calcium	ČSN ISO 6058	Water quality. Determination of calcium. Titrimetric method with EDTA

Cluster analysis produced hierarchical clusters of items based on the Euclidean distance measures. Cluster analysis was performed by the UPGMA methods from the software MVSP 3.12. The statistics were used to assess the similarity between the sites in terms of all analysed physico-chemical parameters of water.

Since the Shapiro-Wilk test indicated that the data have a normal distribution, we used a parametric test in statistical analysis. For the correlation test between the malacocoenosis characteristics and the environmental variables, we used Pearson's Correlation Coefficient. To investigate whether the largest portion of mollusc fauna variation is correlated with environmental factors, the PCA method [32] was applied (from the software Canoco for Windows 4.5) [49]. The number of environmental variables in the multivariate analysis was reduced to parameters that were most strongly correlated with the main directions of quantitative and qualitative variation in the analysed group (Pearson's coefficient).

RESULTS

Cluster analysis of hydrochemical similarities between the sites split the analysed locations into 2 groups. The most significant difference defines the group with locations U Obalovny, Barbora and U cesty. These are flooded mine subsidences characterised by the values of salinity above 1000 $mg \cdot l^{-1}$ (Fig. 2).

The group of flooded mine subsidences with salinity higher than 1000 $mg \cdot l^{-1}$ (mentioned above) is characterised by significantly different abundance of *Potamopyrgus antipodarum* (J.E. Gray, 1843) (Fig. 3). There was no significant difference between the abundance of ecoelements, the number of species, the number of individuals and the Shannon and Evenness indices. The smallest number of species was found at the site with the highest and the lowest salinity (Solecká, Barbora, U Obalovny) (Table 3).

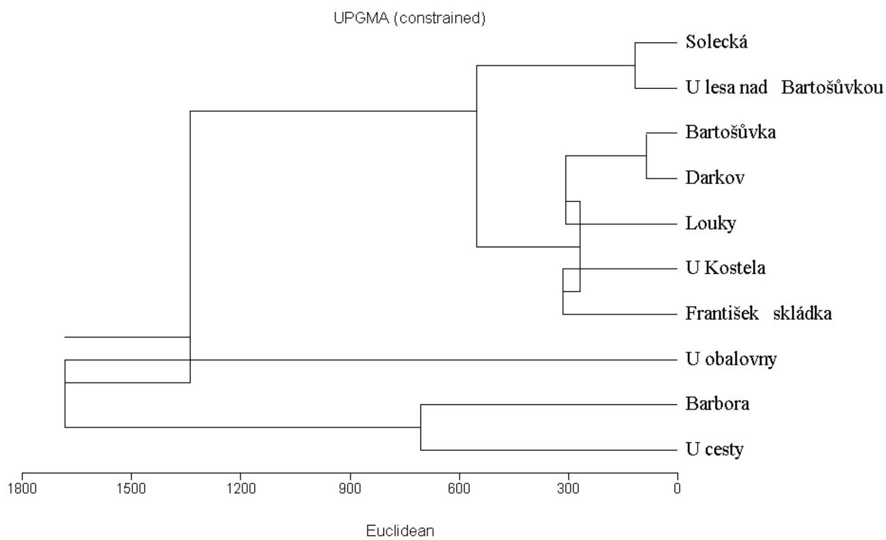

Fig. 2. Results of the cluster analysis of 10 research objects in respect all analysed physico-chemical parameters of water

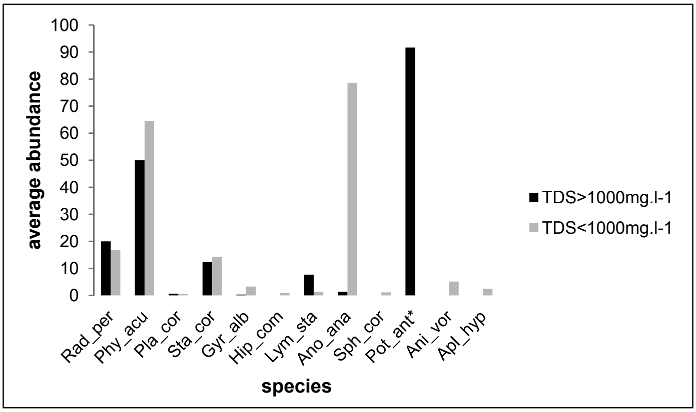

Fig. 3. Average abundance of species in the group of study plots with salinity higher than 1000 mg·l^{-1} (RL>1000 mg·l^{-1}) and in the group where salinity is lower (RL<1000 mg·l^{-1}). Species: Hip_com – *Hippeutis complanatus* (Linnaeus, 1758), Ana_ana – *Anodonta anatina* (Linnaeus, 1758), Ani_vor – *Anisus vortex* (Linnaeus, 1758), Sph_cor – *Sphaerium corneum* (Linnaeus, 1758), Apl_hyp – *Aplexa hypnorum* (Linnaeus, 1758), Pla_cor – *Planorbarius corneus* (Linnaeus, 1758), Lym_sta – *Lymnea stagnalis* (Linnaeues, 1758), Rad_per – *Radix peregra* (Linnaeus, 1758), Sta_cor – *Stagnicola corvus* (Gmelin, 1778), Phy_acu – *Physella cf. acuta* (Draparnaud, 1805), Gyr_alb – *Gyraulus albus* (O.F. Müller, 1774), Pot_ant – *Potamopyrgus antipodarum* (Gray, 1843).

* denotes species with significantly different abundance between these two groups of study objects (p <0.05)

Table 3. Relationship between TDS (mg·l^{-1}), Shannon diversity index (Index and Evenness), the number of species (Num. Spec) and the number of individuals (Num. Ind) at each search objects

	TDS (mg·l^{-1})	Index	Evenness	Num. Spec.	Num. Ind
Solecká	127	0.739	0.673	3	33
U lesa nad Bartošůvkou	237	1.381	0.771	6	363
Louky	432	1.456	0.748	7	81
Bartošůvka	684	0.457	0.255	6	523
Darkov	728	1.419	0.792	6	141
František skládka	731	0.537	0.488	3	23
U Kostela	911	0.379	0.273	4	207
U cesty	1068	1.29	0.663	7	88
Barbora	1701	1.19	0.858	4	197
U Obalovny	1982	0.623	0.899	2	273

The correlation test between the hydrochemical parameters and the characteristics of the analysed malacocoenosis shows a strong relationship between the water salinity and the concentration of chlorides and the abundance of *Potamopyrgus antipodarum* (J.E. Gray, 1843). A significant correlation was found between salinity, concentration of chlorides and alien species (Table 4).

Table 4. The result of correlation between environmental factors and malacocoenosis characteristics (*p <0.05)

	TDS	chlorides	Pot_ant	alien
TDS	1	0.69*	0.9*	0.65*
chlorides	0.89*	1	0.85*	0.58*
Pot_ant	0.85*	0.90*	1	0.72*
alien	0.58*	0.65*	0.72*	1

The results of PCA analysis (Fig. 4) indicated that the salinity and concentration of chloride are positively correlated with the second and third ordination axes, which together explain 22% of the variance (Table 5). There was no significant correlation with the first ordination axis, which explains 65.3% of the variance. The abundance of *Potamopyrgus antipodarum* (J.E. Gray, 1843) had the strongest correlation with these water parameters. The number of species was negatively correlated with environmental factors.

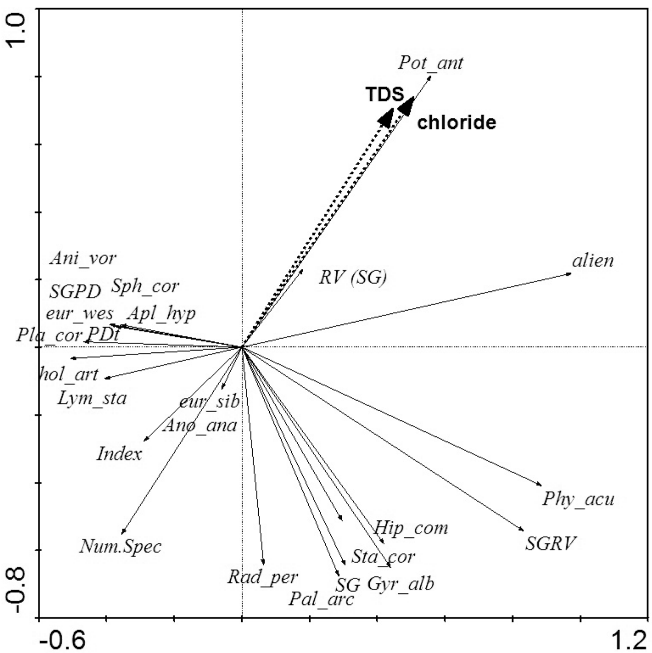

Fig. 4. PCA ordination graph − the correlation between patterns of malacocoenosis variation and concentration of chloride and water salinity (TDS). Species: Hip_com – *Hippeutis complanatus* (Linnæus, 1758), Ana_ana – *Anodonta anatina* (Linnaeus, 1758), Ani_vor – *Anisus vortex* (Linnaeus, 1758), Sph_cor – *Sphaerium corneum* (Linnaeus, 1758), Apl_hyp – *Aplexa hypnorum* (Linnaeus, 1758), Pla_cor – *Planorbarius corneus* (Linnaeus, 1758), Lym_sta – *Lymnea stagnalis* (Linnaeues, 1758), Rad_per – *Radix peregra* (Linnaeus, 1758), Sta_cor – *Stagnicola corvus* (Gmelin, 1778), Phy_acu – *Physella cf. acuta* (Draparnaud, 1805), Gyr_alb – *Gyraulus albus* (O.F.Müller, 1774), Pot_ant – *Potamopyrgus antipodarum* (Gray, 1843).

Ecoelement groups: SGPD, PDt, RV (SG), SGRV, SG (for explanations see Methods).

Table 5. Result of multivariate PCA analysis

Correlation	Axis 1	Axis 2	Axis 3	Axis 4	
chloride	-0.0186	0.5063	0.7387	-0.0954	
TDS	-0.0288	0.4465	0.7043	-0.1383	
Total variance					
Eigenvalues	0.653	0.22	0.098	0.022	1
Species-environment correlations	0.029	0.523	0.785	0.138	
Cumulative percentage variance					
of species data	65.3	87.3	97.1	99.3	
of species-environment relation	0.4	50	99.3	99.7	
Sum of all eigenvalues					1
Sum of all canonical eigenvalues					0.122

DISCUSSION

The fauna of aquatic molluscs in flooded mine subsidences is generally considered to be impoverished [2, 48]. Only 6 species were confirmed in England [1], 18 species in Upper Silesia (Poland) [47], although in the reservoirs located near the heaps − only 6 species were found (in Upper Silesia, Poland). Compared to natural, "species rich" aquatic reservoirs, more species were found for instance in the Bystřice – 26 [3].

As evidenced by the correlation analysis, the increasing salinity induced by the chloride concentration was responsible for the increasing abundance of alien species, including mostly *Potamopyrgus antipodarum* (J.E. Gray, 1843). This species has also significantly higher abundance in the group of flooded mine subsidences with the salinity above 1000 $mg \cdot l^{-1}$. The results of PCA analysis indicated that the salinity affected by chlorides is not the main environmental factor affecting the variability of the mollusc community but has a considerable impact on the malacocoenosis. The multivariate analysis revealed that the concentration of chloride could also affect the biodiversity (a decreasing number of species and the Shannon index).

The latest findings about the effect of salinity on the biodiversity is summarised in the study by Kefford et al. [24]. The maximum number of mollusc species was found in the water with salinity 640–998 $mg \cdot l^{-1}$ and it declined in a statistically significant way in the waters with higher and lower salinity. Kefford and Nugegoda [22] suggest that salinity of 337–670 $mg \cdot l^{-1}$ is conducive to species richness. Our study produced very similar results in terms of species abundance. Table 3 shows that there are different responses on the species level. There were no statistically significant differences between the range of salinity and the number of species, but a decreasing number of species was observed with the lowest and the highest value of salinity. According to these results, the optimum salinity for the species-rich malacocoenosis development is within the range of 200–1100 $mg \cdot l^{-1}$. Piscart et al. [38] suggested that the intermediate salinity levels may be conducive to both salt sensitive and salt tolerant species in accordance with the intermediate disturbance hypothesis of Connell [8]. This hypothesis assumes that in habitats with low salinity, salt tolerant species tend to be excluded from the competition. At a high salinity level, salt sensitive species would be excluded by salinity stress. While at the intermediate salinity levels, both salt sensitive and salt tolerant species can coexist. The minimum number of species was found at the site with the highest salinity (2 species in the U obalovny reservoir, cca 1900 $mg \cdot l^{-1}$). They were alien species (*Potamopyrgus antipodarum* (J.E. Gray, 1843), *Physella* cf. *acuta* (Draparnaud, 1805) spreading to newly created reservoirs in central Europe (such as former sandpits, mine subsidences etc.) These species have a wide ecological amplitude and presumably tend to be good competitors in higher salinity and they are inhibiting the development of other aquatic mollusc species. The absence of native species in this reservoir could also be caused by salinity stress.

Sublethal laboratory experiments [12, 16, 22] have demonstrated that freshwater gastropods have their physiological optimum at the intermediate salinity that reduced the growth both at lower and higher salinity, and which is reflected in the decreasing numbers of individuals. Our study has proved, however, that this also depends on mollusc species, because some of them, e.g. *Potamopyrgus antipodarum* (J.E. Gray, 1843) and *Physella* cf. *acuta* (Draparnaud, 1805) are able to adapt to these conditions and even prefer waters with a very high content of dissolved inorganic components (TDS). The presence of these species could explain why we did not observe a decreasing number of individuals with the increasing salinity. These species can be considered as potential bioindicators of the increasing salinisation and its negative effects on fresh water biota. The usefulness of *Potamopyrgus antipodarum* (J.E. Gray, 1843) as a bioindicator is confirmed by a significant correlation with the salinity and significantly higher abundance in the group of flooded mine subsidences with a broader salinity range.

We partly agree with the statements of Kefford et al. [21, 23, 24], but it is also important to allow for the fact that we cannot evaluate molluscs only as a group, but also as individuals.

CONCLUSIONS

This study shows that the increasing salinity affects the species composition of molluscs and may affect the biodiversity of this group. We conclude that the number of individuals per species with a wide ecological amplitude is increasing with the increasing salinity, and the abundance of *Potamopyrgus antipodarum* (J.E. Gray, 1843) can be considered as a potential bioindicator of the negative effect of salinisation on aquatic biota.

The data obtained during this study are, however, only the first step in recognising this problem in the Czech Republic.

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WPŁYW ZRÓŻNICOWANIA ZASOLENIA WÓD ZBIORNIKÓW W NIECKACH OSIADANIA NA FAUNĘ MIĘCZAKÓW (KARWINA, CZECHY)

W artykule przedstawiono reakcję wodnych gatunków mięczaków (*Mollusca*), jako grupy modelowej, na zasolenie wód zbiorników powstałych w nieckach osiadania rejonu Karwiny (Czechy). Analizę występowania wodnych gatunków mięczaków przeprowadzono w obrębie 10 obiektów. Wody badanych zbiorników tworzyły gradient zasolenia w zakresie od <500 do >1000 $mg \cdot l^{-1}$. W ich obrębie stwierdzono występowanie łącznie 12 gatunków wodnych mięczaków, w tym jednego gatunku, który uznano za potencjalny indykator znacznego zasolenia zbiorników. Wyniki analizy statystycznej wykazały istotną pozytywną zależność między zawartością rozpuszczonych substancji nieorganicznych i występowaniem gatunku *Potamopyrgus antipodarum* (J.E. Gray, 1843). Wykazano, że gradient zasolenia w istotny sposób wpływa na skład gatunkowy fauny mięczaków, kształtujących się w zbiornikach w nieckach osiadania oraz mieć wpływa na bioróżnorodność tej grupy.