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Freshwater alien species *Physella acuta* (Draparnaud, 1805) - A possible model for bioaccumulation of heavy metals

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ABSTRACT

In this study we focused on *Physella acuta*, an alien snail species in order to determine their ability of bioaccumulation of heavy metals in their shells, bodies, the difference in accumulation in relation to age classes, and the influence of ecological variables on the community composition and density. On the basis of the results of ecological, toxicological, and experimental analyses we aimed to study the potential invasive features of *P. acuta* in comparision with the native species *Stagnicola palustris*. The content of Cu and Zn in the substratum and ammonia in the water was strongly related to the patterns of distribution of *P. acuta*. The content of Cd, Pb, and Cu in the shell fraction was always significantly lower than in the body fraction. A comparison of accumulation with respect to the size classes of *P. acuta* indicated that the lowest metal concentration in the body was typical for the largest individuals, except for Zn. Metal content in the bodies of the native species did not differ from the content measured in their analogous group of the largest individuals of *P. acuta*. The lowest value of bioaccumulation factor (BAF) was found for the large class of specimens of this species for each metal. A distinct decrease in the value of BAF in relation to the size of snails was found for cadmium. A 100% hatching success found in masses collected from pond confirmed the high reproductive potential of *P. acuta* which can be a factor that promotes its invasive features following its ability to occur in very high densities, but not necessarily the ability of metal accumulation in the body. *Physella acuta* can be used as a model organism in the studies on the accumulation of heavy metals however, the extend of accumulation can differ among the age classes. Because of the high tolerance of *P. acuta* to heavy metal pollution, in the future this species can be found in significantly polluted habitats, inhabiting free ecological niches, and occurring in high densities in snail communities.

1. Introduction

Heavy metal pollution of freshwater environments is a subject of serious international concern since metals enter the food chain and can undergo bioaccumulation, thereby endangering human health (Hossain and Aditya, 2013). Industrial emissions and the inappropriate management of waste waters lead to the continuous pollution of the environment, and thus adversely affect the development of organisms (Cortet et al., 1999; Järup, 2003) and promote the accumulation of heavy metals (Du et al., 2011; Lefcort et al., 2015).

The mollusc species demonstrate different preferences for the uptake of various metals (Lefcort et al., 2015) and have been used to test the toxicity of pesticides (e.g. insecticides, and other contaminants (PHBs) (Wilbrink et al., 1992; Woin and Bronmark, 1992). These organisms exhibit differences in the accumulation of heavy metals in their body and shell (Khangarot and Ray, 1988; Petare and Waykar, 2018), as well as in relation to the age class or size. Depending on the environmental conditions (different values of pH and total hardness, sediments), their capabilities of metal uptake are differ (Karadede-Akin and Ünlü, 2007). Several investigations (Ibrahim, 2006; Moolman et al., 2007; Mahmoud and Abu Taleb, 2013) have confirmed that snails are suitable organisms for the identification of contaminated sites due to their ability to accumulate large quantities of metals, which is reflected in the values of bioaccumulation factor (BAF).

BAF belongs to a group of measures that are often used to assess the risks caused by pollutants in relation to the safety of animals and human in the event of exposure to the chemicals present in the environment. The factor compares the concentration of a pollutant in the food and/or medium (water or soil) and in the animal body and is calculated as the quotient of the chemical concentration in the body of the animal (or plant) and in its food (or medium) (Arnot and Gobas, 2006). According to this factor, environmental scientists often classify

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the animals into three groups: macro-, micro- or deconcentrators of a chemical substances if BAF > 2, 1 < BAF < 2 or BAF < 1, respectively (Dallinger, 1993). Both the elimination (as is in the case of deconcentrators) and the safe storage and neutralization (macroconcentrators) of potentially harmful chemicals require energy allocation and a trade-off between detoxification and growth, development or reproduction of an organism (Shirley and Sibly, 1999; Zhang et al., 2015). The efficiency of the trade-off may contribute to the potential invasiveness of an alien species colonizing the anthropogenic environments. A study on the terrestrial invertebrates, ants, indicated that environmental toxins may limit the developmental potential of both native and invasive species (De La Riva and Trumble, 2016). It is also stressed that plasticity, including the tolerance to pollutants, may enhance the invasiveness of an alien species (Novo et al., 2015). This was proven by Matthews et al. (2015) in the quagga mussel, a species that gained dominance over the zebra mussel. The estimation of metal concentrations in the soft tissues of both mussel species indicated that the concentrations were higher in the invasive quagga mussels than in the zebra mussel individuals. Matthews et al. (2015) did not explain the possible mechanisms underlying this difference but pointed out its ecological consequences. We cannot exclude the possibility that a tolerance to metals may contribute to the colonizing success of alien species in anthropogenically polluted environments.

Physella (Physa) acuta (Draparnaud, 1805) is an aquatic pulmonate snail originating from North America, which is notorious for its high invasive potential (Vinarski, 2017) and called as invasive in some research (Hossain and Aditya, 2013; Van Leuven et al., 2013; Saha et al., 2016). It is known to be a highly successful invader worldwide (Holomuzki and Biggs, 2012; Ebbs et al., 2018). Its invasive features are not so obvious in all the sites of its occurrence for example in Poland, it has the status of an alien species but not an invasive one. The very high expansiveness of this species permits its wide distribution on various continents probably by accidental dispersal mediated by humans during the transport of exotic plants to European botanical gardens (Vinarski, 2017). Their wide distribution could have also been caused by natural processes such as long-distance dispersal from the Americas to Europe.

Our study was focused on snails due to their high rates of metal accumulation, responsiveness to changes in metal exposure (Adewunmi et al., 1996), and different sensitivities to metal contaminants (Lefcort et al., 2015). They are widely distributed in nearly all types of water systems and are frequently very abundant (Elder and Collins, 1991). Pulmonate species of snails such as *P. acuta* and *Stagnicola palustris* are known and important for their abundant biomass in the littoral zone of freshwater bodies (Ravera and Mariani, 1966). As was reflected by Elder and Collins (1991), while some processes of toxicity have been studied in marine and freshwater animals they have been, rarely, if ever, demonstrated in alien freshwater snails along with the total environment (e.g. sediments, substrates, environmental influence and ecological importance).

The purpose of this study was to investigate the potential success of *P. acuta* to colonize a new environment by measuring the metal concentrations and other ecological parameters. The study also aimed to determine whether the alien snail species differ from the native species in their strategies of metal accumulation/elimination by analyzing the metal deposits in the shell and body and calculating the BAF of the tested metals, and whether the size class of the alien species is significant in metal accumulation. Furthermore, the study estimated the reproductive potential of an alien species, which is measured as hatching success. Additional information on the densities and dominance patterns and the probable influence of the environmental variables on the occurrence of snails are also given. Considering two sites as a source of food with different metal levels we used the alien species as a possible model for bioaccumulation and compared this parameter with that of the native species.

2. Methods

2.1. Analytical procedures and bioaccumulation analysis

For this study, *Physella acuta* and *Stagnicola palustris* were collected from exposed environments and their body and shell were subjected to chemical analyses to determine the bioaccumulation of heavy metals. As the native species, *S. palustris* was selected because it is found in a higher density than other snails and its size is similar to that of the alien *P. acuta*.

In the laboratory the collected snails were placed into glass aquaria containing filtered pond water for maintenance for 24 h. Following the removal of detritus and decomposing plants collected in the process, the snail populations (*S. palustris* and *P. acuta)* were segregated into different size classes based on the shell length (in millimeter) with an accuracy of 0.5 mm. For each snail, the shell length (from the apex to the tip of the last whorl in mm) and shell width (width of the last whorl) were measured. Snails were classified into three different size classes as follows: small specimens (juveniles), 1.0–6.9 mm (N = 20), medium specimens, 7.0–8.9 mm (N = 25) and large specimens (adults) 9.0–12.4 mm (N = 25). We used the same size separation scheme for the native species. The large snail specimens of both species were used to compare bioaccumulation.

The concentration of heavy metals (Cu, Pb, Cd, Zn) was analyzed in the snail samples (soft tissues and shells), leaf deposits, plant deposits, and bottom sediments. Plant materials (leaves, plants) and sediments were sampled qualitatively using a frame net and bottom scraper. The collected materials were placed into plastic containers and were transported to the laboratory. For analyzing the metal concentrations, the snails were anesthetized on ice and dissected to obtain the shells and body samples. These samples as well as the samples of leaves and plant remnants were dried by heating (approximately- 50 °C) to a constant mass, weighed to get the dry mass of the sample, slightly crushed and mineralized in quartz tubes in nitric acid (approx. 65%, Suprapur ®, Merck KGaA Darmstadt, Germany 0.5 ml of the acid was used per 50 μg of the sample) at 150 °C until the samples was light straw coloured and perfectly transparent. Then the samples were dissolved in 4 mL redistilled water. The digests were analyzed with a ThermoScientific ICE 3500 atomic absorption spectrophotometer in a graphite furnace for the Pb, Cd and Cu content and in an air–acetylene flame for determining the Zn content. The metal level was calculated based on similarly prepared Merck standards. The accuracy of the determination was checked using spiked samples with BRC-158 bovine liver as the reference material (IRMM Geel, Belgium). The percentage recovery of the spiked samples was high (93–96%) for the measured concentration of metals. The metal concentrations were expressed as μg Me/g dry mass of each sample. Samples of each kind of material (snail shells and bodies, plants) were prepared in six replicates. Mean values were calculated using the results of measurements of six replicates for each sample.

BAF was calculated for each metal separately according to the following equation: BAF = Me snail conc/Me plant conc, where: Me snail conc = mean (shell Me conc + soft tissue conc), Me plant conc = mean (leaf conc + Typha conc). Snails were classified into three groups as deconcentrators, microconcentrators or macroconcentrators if BAF < 1, 1 < BAF < 2 or BAF > 2, respectively.

2.2. Experimental study

For the experimental part of this study, specimens of *P. acuta* and *S. palustris* were collected from an anthropogenic forest pond to determine their eggs hatching success. The snails were successively collected during the period from 2016 to 2017 using qualitative hydrobiological methods (bottom scraper) together with plants and leaves from the trees. After collection, the snails were separated from the plant materials. Fifty individuals of each species were selected and transported to the laboratory where they were placed in aquaria under the same conditions of water and temperature until they laid eggs. In addition, the egg masses of *P. acuta* laid in the field were also collected. They were found at the surface of the water plant remains as well as on the leaves. All the masses (LM – laboratory masses, FM – field masses) were measured, and the eggs in the masses were counted. None masses of *Stagnicola* were observed in the field during the study period, or during the experimental study. Egg masses were monitored daily for the number of eggs hatched, and the time to the first hatching and the percentage of hatching were determined. Hatching was considered to have occurred when juvenile snails had emerged from both the egg and the egg mass. Observations were carried out for more than one month until the snails had laid the masses and juveniles had hatched. Finally, all the juvenile specimens were counted. The same procedure was followed for both types of masses (pond and laboratory). Hatching success was assessed for 29 field egg masses and 24 laboratory egg masses.

2.3. Ecological study

The field study of this research was carried out in an anthropogenic forest pond located in a mining area of southern Poland, 0.5 km from the Budryk coal mine (50°10′36.15″N, 18°46′04.60″E) to detect metal bioaccumulation. The mining activity results in a land depression, which after water filling leads to the creation of a new anthropogenic water body. In addition, the mining activity results in a high availability of metals. The study samples were collected from 2016 to 2017 (from May to November). To estimate the snail density, quantitative samples were taken from a bottom area of 0.5 m^2. Because the density of freshwater snails differs in different sediments, sampling was done in two sites that differed in the type of substrate – allochthonous plant matter (leaves from trees) and plant deposit (*Typha latifolia* remains). A square frame (0.25 × 0.25 m) was used for the quantitative sampling (Maqboul et al., 2014). It was digged into the bottom four times for each sample. After taken to the laboratory, the samples of leaf and plant remains together with the snails were washed using sieves (0.02 mm mesh size). The snails were sorted under a stereoscopic microscope and then preserved in 75% ethanol. We followed the identification method proposed by Glöer (2002) and Piechocki and Wawrzyniak-Wydrowska (2016). The snails were identified to the species rank based on their morphological features except Lymnaeidae - for the determination of which we also included the pigmentation (Jackiewicz, 2000). The density of the snails was expressed as individuals per square meter (ind/m^2).

The most commonly measured ecological variables, including community structure, and species diversity may reflect the stresses caused by the contaminants. Therefore, in the analysis of the gastropod community, we used the following indices: Dominance (D), the Shannon -Wiener index (H′), and the Simpson diversity index (S) (standardized version). The values of the Dominance index were considered within the ranges (four classes) according to Biesiadka and Kowalik (1980). The diversity indices were calculated using MVSP (3.13.p Kovach Computing Services).

The following parameters were analyzed in the field using a portable HI 9811-5 pH/EC/TDS/°C meter (Hanna Instruments, USA): conductivity (EC), total dissolved solids (TDS), temperature, and pH. Analysis of the other parameters was performed in the laboratory (titrimetric determination) using the reagents from Merck and Hanna Instruments.

2.4. Statistical analysis

Statistical comparison among the physico chemical parameters of the water and the densities of snails was performed using Student's t-test. To compare the mean measurements obtained for the *P. acuta* population, a one-way analysis of variance (ANOVA, F distribution) was performed. The data were found to be of a normal distribution. Before the comparison of the means, an inferential statistic - the Levene's test was used to assess the equality of variances. To determine the significant differences between the group means in the ANOVA, Scheffe's test, which is considered to be one of the most conservative post hoc tests (Winer et al., 1991), was adopted.

For the analyses of metal concentrations, the data were tested for normality using the Kolmogorov–Smirnov and Lilliefors tests, and then post hoc comparisons were performed using Tuckey's honestly significant differences (HSD) test (ANOVA for the plant samples with the plant type as a factor and multivariate MANOVA for the snail samples with the species/class and fraction as factors) to determine the differences between the groups. Differences were considered to be significant at $p \le 0.05$ (STATISTICA 13.0 package Dell Inc., 2016).

Multivariate analysis of the composition of freshwater snail was performed using Canoco Ver. 4.5. Detrended Correspondence analysis (DCA) showed that the species responded linearly to the gradient (0.264 SD). The redundancy analysis (RDA) ordination technique was used to test for any significant linear trend in the community composition and to develop a model to determine whether the measured habitat variables were significant in explaining any variation in snail density. The Monte Carlo permutation was used for testing the significance of the model. Details of the application of RDA and the Monte Carlo permutation in model-ecosystem experiments can be found elsewhere (Van Wijngaarden et al., 1995; Makarenkov and Legendre, 2002). Twenty one explanatory variables were used in the model. Before applying the RDA model, the environmental data set and the parameters were standardized to correct for the differences in the measuring range between the different parameters, which would make them equally mathematically important. The axes of the RDA diagram represent the percentage of the total sum of the canonical eigenvalues; thus, the higher these percentages, the better explained the observed variation. According to Ter Braak (1988), values of about 30–40% are quite common in ecological applications.

3. Results

3.1. Bioaccumulation study

The BAF values of metals calculated for *P. acuta* differed in relation to the size of the snail individuals (Table 1). The lowest BAF value of metals (except Zn) was found for the large class of specimens of this snail species. The BAF value of cadmium decreased with the size of the snails. For each size class and species the BAFCd and BAFPb were within the range of 0–2 and lower than the BAFCu and BAFZn, the values of which exceeded 2. Irrespective of the size class and species, the BAFCu was the highest among the BAF values of the analyzed metals.

The analysis of the metal concentration in the fractions of snail individuals revealed that the contents of Cd, Pb, and Cu in the shell fraction were always significantly lower than in the body fraction (Fig. 1). The concentration of the metals in the shells was less than 20% of the concentration found in the body fraction. For the Zn concentration, a similar significant difference was only found in the group of

Table 1
Bioaccumulation factor values for the alien (*P. acuta*, P.a) and native (*S. palustris*, S.p.) snails.

Snail species, size	metal			
	Cd	Pb	Cu	Zn
P.a. 5–7 mm (small)	1.35	1.73	7.39	2.45
P.a. 7.1–9 mm (medium)	0.73	1.87	9.43	1.85
P.a. 9.1–12.4 mm (large)	0.52	0.56	6.15	1.98
S.p. 16–28.5 mm (large)	0.63	0.6	7.13	2.96

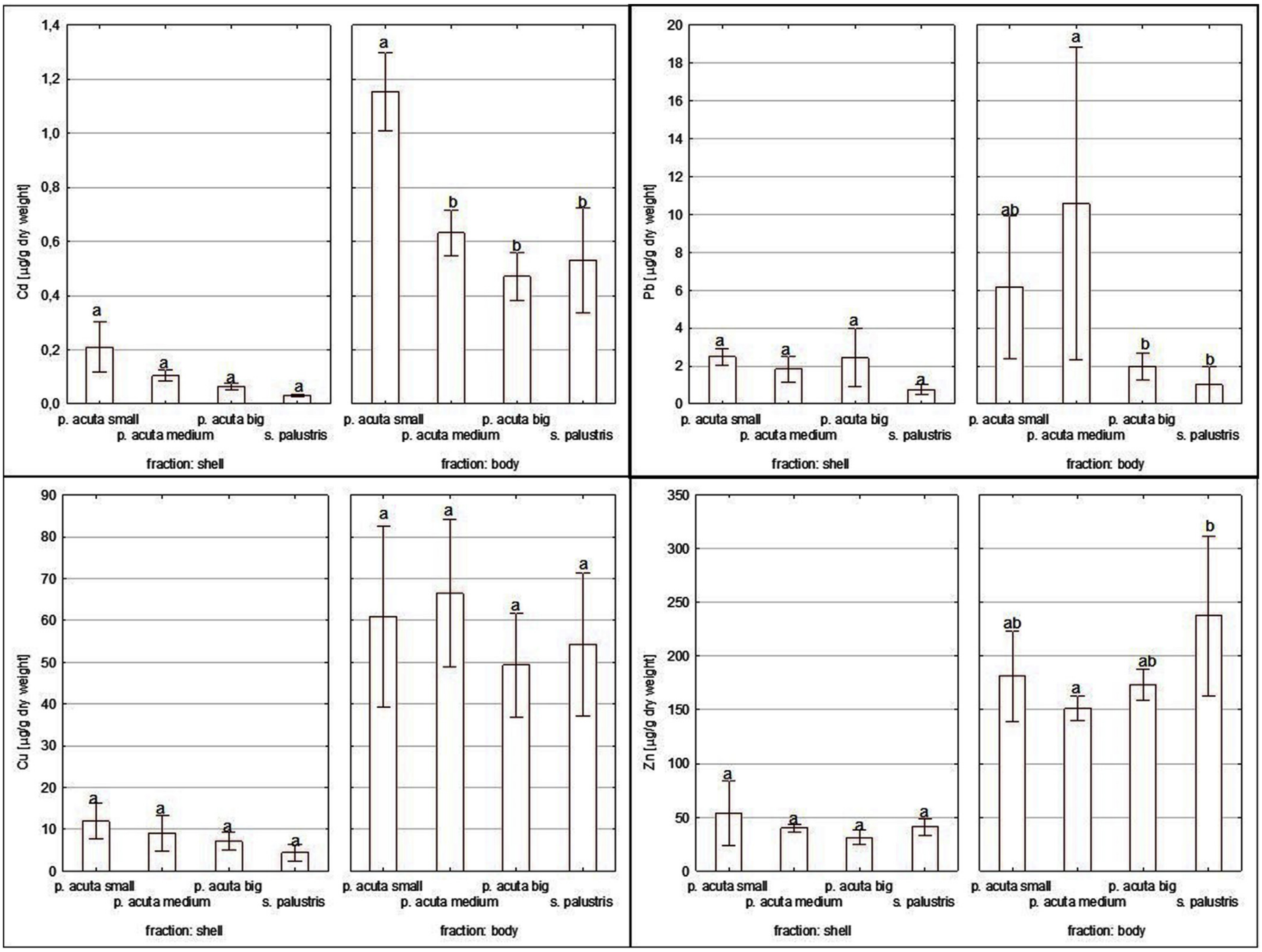

Fig. 1. Metal concentration [µg/g dry weight] (mean ± SD) in the shell and body fractions of two snail species *P. acuta* (alien, three size classes) and *S. palustris* (native). Different letters (a, b) indicate statistically significant differences between the groups within fraction (ANOVA, Tukey's HSD test, $p \leq 0.05$).

medium-sized *P. acuta* snails. There were no significant inter- and intraspecies differences in the metal concentrations in the shells. Except for Zn, the comparisons conducted within the size classes of *P. acuta* indicated that, in general, the lowest metal concentration in the body was typical for the largest individuals. The metal concentration in the body of the native species did not differ from the content measured in their analogous group of the largest individuals of *P. acuta* (Fig. 1).

The concentrations of Cd and Zn in the Typha plant were significantly higher than in the samples of leaves (Fig. 2). There were no significant differences in the concentrations of Cu and Pb between the two sampled groups of plant materials. For all the metals studied, the content in the sediments was higher compared to the organic matter deposits (Table 2).

3.2. *Experimental study*

Twenty nine egg masses of *P. acuta* were found in the studied pond (FM) (Table 3). The masses were attached to the plant remains, leaves, and other specimens of this species. They were transparent and deposited in a thick, stiff, and relatively strong external casing. The length of the masses reached up to 13,395 µm. After collection, they were found to contain from 29 to 77 eggs per cocoon. It was impossible to determine precisely when the masses were deposited, but after transferring to the laboratory they began to hatch after five days. After 14 days, all the eggs from the 29 masses had hatched (Table 3). Analysis of the process of the incubation and hatching of the eggs of *P. acuta* showed that after 5 days all the juvenile snails from 65% of the masses had hatched whereas after 14 days 100%.

The masses deposited in laboratory conditions were very delicate, transparent, and weaker than those deposited in the pond. There was variation in their size with LM being slighty larger than the FM (mean length – 8988.59 µm) (Table 3). The average number of eggs was similar in the LM and FM, but a greater number of empty eggs was found in the LM. A 100% hatching success was observed in the egg masses collected in the pond (FM) but the snails from the masses laid the laboratory were hatched after seven days (94.8%).

Hatching success was not determined for *S. palustris* because the collected specimens did not lay masses in any the replicate which we have done. Unfortunately, no egg masses of this species were found during the study period in the studied pond, and therefore, it was not possible to collect them.

3.3. *Ecological study*

Water chemistry. The values of the physico-chemical parameters of the water at both sampling sites were similar except for ammonia ($t = -2.77$, $p < 0.05$) and nitrates ($t = -2.45$, $p < 0.05$) (Table 4). The pH of the water was high (7.8–8.6), and the values of the salinity indicators were similar at both sampling sites. The water was soft with a low content of phosphates, nitrites, and chlorides.

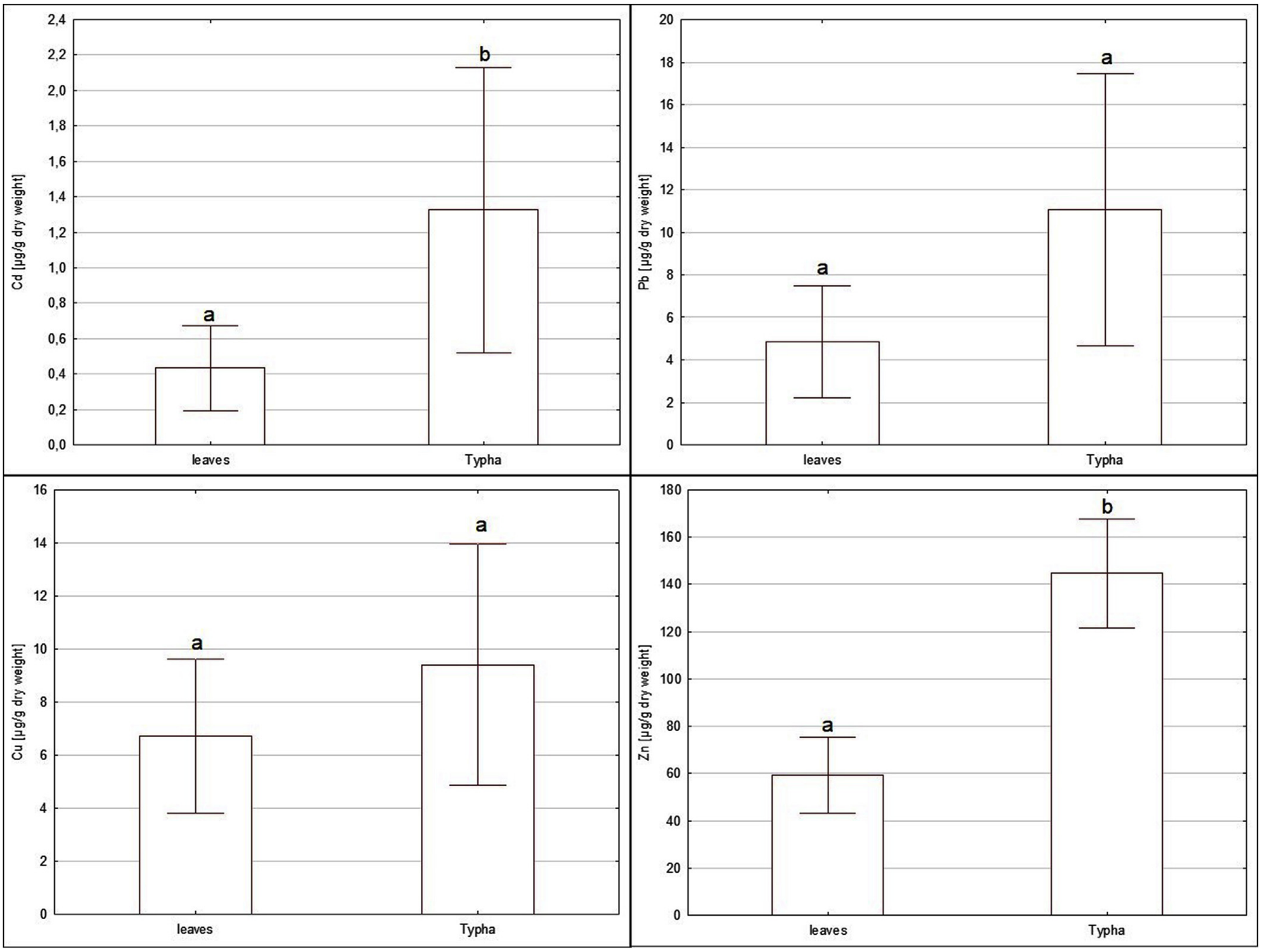

Fig. 2. Metal concentration [µg/g dry weight] (mean ± SD) in the deposit of leaves and Typha. Different letters (a, b) indicate statistically significant differences between the groups (ANOVA, Tukey's HSD test, $p \leq 0.05$).

Alien species in snail assemblages. *P. acuta* was the eudominant species ($D > 10\%$) at both sampling sites (Table 2). The densities in the assemblages varied across the sampling sites for *P. acuta* ($t = 3.44$, $p < 0.05$) but not for *S. palustris* ($t = -0.83$, $p > 0.64$). We found much higher densities of snails in the plant deposit but the values of the diversity indices were almost similar at both sampling sites due to the presence of a very high number of one species - *P. acuta*.

The native *S. palustris* was found to be in the subrecedent class in the leaf deposit and the recedent class in the plant remains. Their density at both sampling sites was greater compared to other snails (Table 2). The diversity of snails was low at sampling sites due to the extreme abundance of *P. acuta*.

RDA showed the significant relations of the snail communities with the environmental variables which were found to be statistically best explained by the variations (Fig. 3). The content of copper, zinc, and ammonia was most strongly related to the patterns of distribution of the alien *P. acuta*, and also of *Galba truncatula*, *Planorbarius corneus*, *Gyraulus crista,* whereas in the case of native *S. palustris* was related to a higher concentration of calcium in the water (the first and second axes explain almost 40% (species data) and almost 99% (species and environmental relationships) of the variance, respectively). The Monte Carlo test showed that the model was statistically significant ($p < 0.05$). Field samples showed that shell lengths differed among the three selected age classes in the case of *P. acuta* (one-way ANOVA: $F = 150.17$, $p < 0.05$ for shell height, $F = 124.42$, $p < 0.05$ for shell width).

4. Discussion

Freshwater ecosystems are known as the most valuable water resources which are affected by human influences. Pollution and degradation of habitats have a negative effect on the water quality and ecological conditions (Sahin and Zeybek, 2016); however, they also contributes to the appearance of alien and invasive species. The alien *P. acuta* snails can efficiently disperse via water (Van de Meutter et al., 2007) and have also been found to be carried with plant materials on boats between lakes (Albrecht et al., 2009). According to Van Leeuwen et al. (2013), the potential for aquatic snails such as *P. acuta* to exploit several dispersal vectors may contribute to their wide distribution on different continents and their successful invasion. This species inhabits a broad range of waters and can survive, for example in sewage treatment plants (Mácha, 1971; Hoffman, 1999; Turner and Montgomery, 2009), and sewage drains and the associated puddles (Saha et al., 2016). In the pond studied here, *P. acuta* was found to occur in high densities (maximal density of 5640 ind/m^2) and as the dominant species (D index near 94%) regardless of the substrate and sample; therefore, it is likely that these features can be considered are responsible for its potential invasiveness. The environmental context cannot be disregarded when investigating the interaction between the native and alien snails, and predicting the success and impact of the alien species

Table 2
The composition of the snail communities, the content of heavy metals in the two types of habitats. Leaf deposit - the deposits of leaves fallen from the trees.

		Leaf deposit		Plant deposit	
		D [%]	Mean density,	D [%]	Mean density
Physidae	*Physella acuta* (Draparnaud, 1805)	94.1	931 ± 584.4	93.8	4003 ± 2102.2
Lymnaeidae	*Radix balthica* (L., 1758)	0.3	5 ± 9.2	0.0	0.0
	R. auricularia (L., 1758)	0.6	19 ± 22.3	0.4	24 ± 34.9
	S. palustris (O.F.Müller, 1774)	2.0	32 ± 29.6	1.7	75 ± 65.2
	G. truncatula (O.F.Müller, 1774)	0.0	0.0	0.6	24 ± 41.6
Planorbidae	*G. crista* (L., 1758)	1.9	19 ± 32.3	0.5	21 ± 12.2
	P. corneus (L., 1758)	0.5	5 ± 4.6	0.2	11 ± 4.6
	A. vortex (L., 1758)	0.0	0.0	0.1	5 ± 9.2
	F. fragilis (Tryon, 1863)	0.8	8 ± 13.9	2.7	107 ± 184.8
Number of species in total		6		8	
Total individuals		2968		12,808	
Density of snails (individuals m − 2) (mean)		989		4269	
Density (individuals m − 2) (min-max)		400–1624		1745–6187	
Simpson diversity index		0.03–0.2		0.05–0.2	
Shannon-Wiener diversity index		0.1–0.6		0.2–0.57	
Content of heavy metals in deposits [μg/g]					
Mean (min-max)					
Cd		0.820 (0.06–2.75)		1.326 (0.56–2.42)	
Pb		10.367 (0.72–37.89)		12.574 (2.63–28.54)	
Cu		7.873 (0.86–19.47)		9.407 (4.89–15.20)	
Zn		83.742 (5.26–260.19)		150.552 (78.21–246.15)	
Content of heavy metals in the bottom sediments [μg/g]*					
Cd		3.28			
Pb		80.48			
Cu		28.16			
Zn		499.62			

Mean density is given ± 1SD (Standard deviation), * mean from the six measurements, D-dominance index, mean density is expressed in individuals/m.^{2}.

Table 3
Water chemistry at sampling sites.

	Leaf deposit		Plant deposit	
	Mean	SD	Mean	SD
Temperature ° C	20.3	2.7	21.0	3.2
pH	8.6	0.5	7.8	0.5
Conductivity μS/cm	1138.7	344.5	1030.0	296.1
TDS [mg/l]	566.7	180.1	610.0	195.2
Chlorides [mg/l]	138.0	54.1	148.7	92.9
Nitrates [mg/l] [a]	1.9	3.2	20.9	22.4
$N–NO_3$	2.0	2,3	9.0	8.2
Nitrites [mg/l]	0.1	0.2	0.1	0.1
$N–NO_2$	0.02	0.006	0.02	0.01
Ammonia [mg/l] [a]	0.3	0.2	0.7	0.2
Phosphates [mg/l]	0.8	1.2	0.1	0.1
Total hardness mg $CaCO_3$/l	156.0	10.4	134.3	45.2
Calcium [mg/l]	100.7	25.7	97.7	39.6
Iron [mg/l]	0.5	0.2	0.7	0.6
Alkalinity	206.6	80.9	198.3	16.07

[a] - statistically significant (T-test, $p < 0.05$).

(Früh et al., 2017).

The accumulation of metals in aquatic invertebrates exposed to pollution is well documented, and many ecological and ecophysiological studies suggest that molluscs respond to environmental stress and pollution (Rainbow, 1995). Our study revealed that the content of Cd, Pb, and Cu in the shell fraction was always significantly lower than in the body of the snails studied. Bioaccumulation analysis of freshwater snails is sometimes limited only to the soft tissues but their shell is also important in accumulation, which can continue over the lifetime of the organisms (Imlay, 1982). In some cases, the accumulation of heavy metals in shells can be substantial; however, some metals are accumulated more in the soft tissues than in the shells (e.g. lead in *Lymnaea (Radix) peregra*) (Fischer, 1983). In contrast to our result, Karadede-Akin and Ünlü (2007) showed very low BAF values for Cu and Zn accumulated in the body of *P. acuta* snails. They also did not detect Pb; however, in their research the mean concentrations followed the sequence: Cu > Zn. In our study, the distribution of metals in both the body and the shell of *P. acuta* showed an opposite trend: Cu < Zn. It is likely that the authors of the above study analyzed the snails without assigning them to age groups. By contrast, our research showed that the uptake of the studied metals differ completely depending on the life stage of the snails.

As was reflected by Boening (1997), Elder and Collins (1991), and Roméo et al. (2000), although the bioaccumulation of metals mainly depends on, for example the size, age, sex, feeding activity, and even genotype, it also relies on the environmental variables such as water hardness, temperature, and pH as well as site-specific conditions, which according to Fischer (1983) and Wuncheng (1987), can moderate metal toxicity considerably. Because both the studied species are grazers, which feed on macrophytes and epibenthic algae as a component of periphyton (Dillon et al., 2002), we aimed to compare their ability to bioaccumulate metals because they uptake metals from their food sources. Our study design did not allow us to assess or compare the extent of bioaccumulation and the BAF index in the three classes of the native *S. palustris* as all specimens were large, and therefore, we compared the individuals of both species from one size class. We found that the metal concentration in the body of the native species did not differ from the content measured in their analogous group of the largest individuals of *P. acuta*. Analysis of metal uptake in different age classes showed that the lowest metal concentration in the body was typical for the largest individuals, especially in case of Cd and Pb, but not Zn. In shells, this finding was similar, especially forthe accumulation of Cd and Cu. The BAF value is often used for pollution biomonitoring (Rosioru et al., 2016; Dirrigl et al., 2018). We found the lowest BAF value for each metal in the *P. acuta* snails from large sixe class A distinct decrease in the BAF values in relation to the size of the snails was found for cadmium (BAF = 0.52). Both species can be classified as macroconcentrators of Cu (BAF > 2). Lefcort et al. (2004) suggested that the ability of mining site snails to avoid heavy metals is due to both genetic and environmental factors, and snails are more sensitive to groups of metals than to individual metals. While Newman and Mcintosh (1982)

Table 4
Characteristics of the egg masses and the process of eggs hatching and the characteristics of *P. acuta* masses deposited in the field (29 masses) and under laboratory conditions (24 masses).

	FM				LM	
Length (μm) mean	9148.06				8988.59	
min- max	7210.74–13,395.18				5028.35–14,093.09	
Average number of eggs (min-max)	45 (29–119)				45 (13–106)	
Empty eggs	7 eggs from only one cocoon				45 eggs from 9 cocoons	
Hatching process	day of collection	after 5 days	after 14 days	after 20 days	day of layout	after 7 days
eggs per mass min – max	29–77	29–119	33–119	33–119	13–106	13–106
cocoons with all of the eggs hatched	–	21of 29	29	29	–	19 of 24
Total number of eggs in masses	1313	1592	1686	1686	1095	1130
Total number of empty eggs	–	–	–	–	40	45
Average number of eggs in masses	45	54	54	58	45	46 + 4 empty eggs

FM - Field masses: masses deposited and collected from the pond, LM - laboratory masses: masses deposited in the laboratory (after 20 and until 30 days, 16 eggs still remain unhatched.

pointed out that the concentrations of lead in molluscs reflected the degree of lead contamination at sites with different levels of Pb, they also concluded that the extent of habitat contamination only partially helped in determining the lead concentrations in the molluscs. In the context of our study, the content of Cu and Zn in the substratum and ammonia in the water was most strongly related to the patterns of distribution of *P. acuta* whereas *S. palustris* was associated with a higher concentration of calcium in the water. Balogh et al. (1988) found that the concentration of some toxic metals (Cr, Cd, Hg, Pb) showed a decreasing tendency with an increase in the body weight of snails that had originated from different biotopes. This confirm our results regarding the bioaccumulation of Cd and Pb but not biogenic metals (Cu, Zn), which in our study was also found decreased in the large specimens. Heavy metal concentrations found in snails reflect the differences in the extent of environmental pollution (Balogh et al., 1988); in our study, the metal concentration were always higher in plant deposits, especially Zn.

The ability to colonize anthropologically changed areas requires

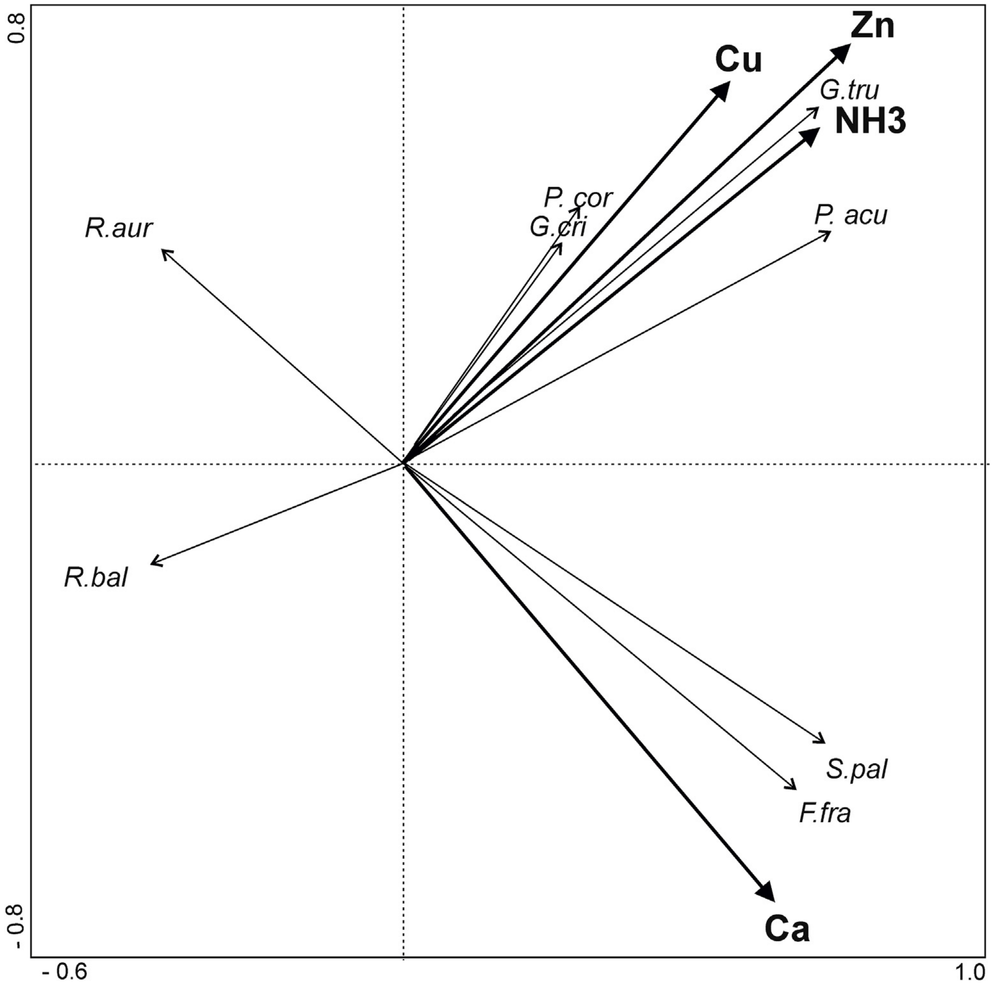

Fig. 3. RDA biplot of snail species in relation to the strongest environmental variables. Thick arrows represent the environmental variables, and thin ones represent the snail species.

efficient detoxifying or neutralizing mechanisms. The defensive mechanisms against metal pollution include, among others, the elimination of the excess of metals or the regulation of their concentration through, for example, lower assimilation (Laskowski et al., 2010; Ardestani et al., 2014; Zhang and Van Gestel, 2017), or in the case of accumulation, their storage in an inactive form in intercellular granules or sequestration with ligands (e.g. metal-binding proteins) (Bebianno and Langston, 1998; Wang and Rainbow, 2010).

Stable populations are created by those species whose individuals are characterized by either a wide tolerance to environmental pollution or efficient neutralization or detoxification strategies. This efficiency of a species to form a stale population depends on the trade-off and energy allocation between the parameters of life history and defensive mechanisms (Jones and Hopkin, 1998; Augustyniak et al., 2005). These phenomena seem especially interesting in the case of alien (and potentially invasive) species in anthropogenically polluted areas. Comparisons of the responses of the two mussel species (native: *Anodonta anatine*, exotic: *Corbicula fluminea*) to various mercury concentrations under laboratory conditions revealed that the individuals of the invasive species were less sensitive to this metal than the specimen of the native species (Oliveira et al., 2015). In this study the number of *P. acuta* individuals was much higher than the number of other native species in the snail community.

Data concerning the metal body burden of *P. acuta* are extremely scarce. Some information can be found for several metals in the study of Zaldibar et al. (2006). These data indicate that the metal concentrations in the soft tissues of *P. acuta* depend on the bioavailability of the metals and suggest that these snails are able to cope with the environmental pressure by accumulating metal ions in their body.

It is suggested that the presence of stable populations of *P. acuta* in spite of metal pollution is possible due to a kind of plastic adaptation (Zaldibar et al., 2006), which is characteristic for the invasive features of an alien species (Davies et al., 2015), especially when it enables them to dominate over the native ones. This phenomenon was experimentally confirmed in their study by Bielen et al. (2016), in which native and alien species of bivalve were exposed to various zinc concentrations and temperatures under laboratory conditions. Comparisons of the biomarker levels revealed that the alien (invasive) species appeared to be more tolerant to both unfavorable factors (Bielen et al., 2016). In the present study, the comparisons of metal accumulating abilities between the native and alien aquatic snails were done under field conditions. The concentrations Cd, Zn, Pb, and Cu in the body of the two snail species did not differ significantly within their respective age classes. Moreover, the BAF values for all the metals, except for Cu, were similar in both species, which suggests that the individuals of both species exhibit a similar strategy for metal neutralization: to some extent, the elements were accumulated in their bodies but since the BAF values did not significantly exceed 2, it seems that both species they probably regulate the metal content at a similar level. As for Cu, the increased body concentration and increased BAF value may be related to the fact that it is a biogenic metal and it increased accumulation may compensate for a possible deficiency of this element in the environment. Therefore, in anthropogenically changed environments, the environmental pressure does not seem to be a factor that promotes the occurrence of any of the species, unlike reported by Oliveira et al. (2015). Since all the individuals of *S. palustris* in the collected samples were of the largest (oldest) size class, individuals of the highest size class of *P. acuta* were included for the inter-species comparisons. In the intra-species analyses conducted for *P. acuta*, the BAF values for all the metals were in general higher in the youngest (smallest) snails compared to the older (bigger) ones. However, opposite results were shown in the study of Cubadda et al. (2001), which demonstrated a positive correlation between the metal concentration and the body size of four marine gastropods. The higher BAF value found in young individuals may be regarded as representing a trade-off between a developmental and growth priority and an investment in metal elimination.

Both storage in an inactive form and elimination in feces (Sá et al., 2008) or with exchanging gut epithelial cells (Pigino et al., 2006) require the initiation of the synthesis of additional metal-binding or enzymatic proteins, which is energy-consuming. On the other hand, a possible explanation for the relatively high BAF value in the youngest individuals may be connected with an increased food consumption due to the requirements connected with developmental processes compared to the adult snails. Calculations of hatching success, however, indicate that the reproductive functions in adult individuals were not disturbed.

P. acuta is a hermaphrodite with the ability to self-fertilize (Raković et al., 2016), and its development is direct, with no larval phase (Dillon et al., 2002). Fertilization is internal, and the eggs are laid within a gelatinous egg masses. The snails survive for one season and lay one clutch of up to 50 eggs (Gittenberger et al., 2004). Its ability to self-fertilize can further promote its rapid spread (Bousset et al., 2004). Analysis of the hatching of *P. acuta* showed a 100% hatching success after 14 days of incubation from the masses that had been laid in the water body but hutching occurred after seven days from the masses laid under laboratory conditions (94.8%). Previous studies have documented that fecundity is not seriously influenced by the low concentrations of heavy metals (Ravera, 1991). At a high concentration of Cd, the fecundity of *P. acuta* is completely abolished, while it may be depressed by lower concentrations such as 125 pg Cd/l (Agostini, 1983). The hatching success and shell length of *P. acuta* decreased with an increase in Cu concentration, thus indicating the genetic toxicity effect of Cu (Gao et al., 2017), and also displaying a typical threshold response to increased salinity that was high - up to 5000 (Paradise, 2009). According to Ravera (1991), studies on the reproduction and embryonic development provide useful informations that can be used to predict the survival probabilities of species living in polluted environments.

Summarizing, the ability of metal accumulation in the body does not seem to be the factor promoting the invasive character of *P. acuta*. Rather, its high reproductive potential (100% hatching success observed from the egg masses collected in the pond) and ability to occur in high densities (mean 4003 ind/m2) enable this species invasiveness in an anthropogenic aquatic ecosystems.

5. Concluding remarks

This comprehensive study showed the high densities of the alien snail species *P. acuta* in the studied sites. The content of ammonia, calcium, copper, and zinc were found to statistically best explain the variations in the distribution of snails. The concentrations of Cd, Pb, and Cu in the shell fraction were always significantly lower than in the body fraction. The comparison of metal concentrations within the size classes of *P. acuta* indicated that the lowest concentration in the body was typical for the largest individuals, except for Zn. Metal concentration in the body of the native species did not differ from the content measured in the largest individuals of *P. acuta*. The lowest BAF value for each metal was found in the large class of snails. A distinct decrease in the BAF value in relation to the size of the snails was found for cadmium. Moreover, the BAF values for all the metals, except for Cu, were similar in both species, which suggests that the individuals of both species represent a similar strategy for metal neutralization: to some extent, the elements were accumulated in their bodies but since the BAF values did not significantly exceed 2, it seems that both species probably regulate the metal content at a similar level. Thus, the high reproductive potential (100% hatching success observed from the egg masses collected in the pond) and ability to occur in high densities (mean 4003 ind/m^2), rather than the ability of metal accumulation in the body, seem to promote the invasiveness of *P. acuta*.

Conflicts of interest

The authors declare they have no conflict of interest.

Ethical standards

All experiments comply with the current Polish laws.

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