Resistance of great ramshorn *Planorbarius* (superspecies) *corneus* allospecies (Mollusca: Gastropoda: Pulmonata) from the Ukrainian river network to Mn^{2+} ions

Olena Uvaieva[1], Agnessa Stadnychenko[2], Yuliia Ikonnikova[3] and Tetiana Vakaliuk[4,5,6,7]

[1] Department of Earth Sciences, Zhytomyr Polytechnic State University, Zhytomyr, Ukraine
[2] Department of Zoology, Biological Monitoring and Nature Conservation, Zhytomyr Ivan Franko State University, Zhytomyr, Ukraine
[3] Department of Botany, Biological Resources and Conservation of Biological Diversity, Zhytomyr Ivan Franko State University, Zhytomyr, Ukraine
[4] Software Engineering Department, Zhytomyr Polytechnic State University, Zhytomyr, Ukraine
[5] Institute for Digitalisation of Education of the NAES of Ukraine, Kyiv, Ukraine
[6] Kryvyi Rih State Pedagogical University, Kryvyi Rih, Ukraine
[7] Academy of Cognitive and Natural Sciences, Kryvyi Rih, Ukraine

*E-mail: tetianavakaliuk@gmail.com

Abstract. This study examines the impact of Mn^{2+} ions at varying concentrations on ecotoxicological and physiological traits of two genetic allospecies of the great ramshorn *Planorbarius corneus* from distinct geographic zones in Ukraine: the Polissia Forest (Irsha river, Malyn) and the Forest-Steppe (Psel river, Balakliia). The two allospecies show significant differences in their toxicological indices (LC_0, LC_{50}, LC_{100}) and in the duration of intoxication processes, as well as in mean lethal time and lethality under different concentrations of manganese chloride ($p \leq 0.05$). The "western" allospecies from the cooler Polissia zone exhibited better survival and higher adaptation to manganese exposure compared to the "eastern" allospecies, which is more vulnerable in the warmer and drier Forest-Steppe region. The survival differences correlate with bioclimatic modeling, which shows significant ecological differentiation between the two allospecies' niches. This geographic separation of ranges is also evidenced by the spatial divide of the Dnipro River, which separates the "western" and "eastern" allospecies. The study highlights the need for ecological monitoring to protect these mollusks from the escalating impacts of global warming, which are threatening their populations and habitats. The findings suggest that the adaptation of the "western" allospecies to cooler conditions provides a critical insight into survival strategies under environmental stress.

Keywords: Mn^{2+}, great ramshorn, ecotoxicological and physiological responses, Ukrainian river network.

1. Introduction

One of the largest and most widespread gastropod mollusks in the freshwater bodies of the Ukrainian river network is the secondarily pulmonate great ramshorn *Planorbarius* (superspecies) *corneus* sensu lato. This species is represented in Ukrainian malacofauna by two genetically distinct allospecies: the "western" and "eastern" variants, which inhabit the Right-Bank and Left-Bank regions of Ukraine, respectively [1]. The genetic and karyological differences between these allospecies, established using genetic markers, result in clear distinctions in conchology, anatomy, physiology, and ecotoxicological responses to toxicants. The physiological and behavioral responses to similar concentrations of toxicants are more pronounced in the "eastern" allospecies, indicating its higher sensitivity and lower endurance compared to the "western" allospecies. This difference is likely due to the more challenging environmental conditions in the South-East Forest-Steppe zone, which is warmer and drier than the Polissia Forest zone [2].

Manganese is an essential microelement for mollusks, vital for the functioning of enzymes (prolidase, pyruvate carboxylase, imidopeptidase) as well as hormones and vitamins that support various physiological functions and enhance their immunological stability. However, excessive concentrations of Mn^{2+} ions are harmful, leading to gonadotoxic, mutagenic, and carcinogenic effects in these organisms [3, 4]. Since the early 21st century, anthropogenic contributions of various chemical elements to the Ukrainian hydrosphere have consistently exceeded natural levels, leading to water pollution by heavy metals and an increased negative impact on aquatic biota [1].

Global warming has profound effects on aquatic ecosystems, raising water temperatures and altering water chemistry, which negatively impacts aquatic organisms, including mollusks. Higher temperatures reduce dissolved oxygen levels, intensify metabolic stress, and increase the solubility of toxic substances such as heavy metals, exacerbating their harmful effects [4, 5, 6]. These environmental changes disrupt the delicate balance of aquatic ecosystems, reducing biodiversity and the resilience of hydrobiont populations [3, 7]. Heavy metals, particularly manganese (Mn^{2+}), cadmium, and lead, pose a significant threat to aquatic organisms due to their toxic and bioaccumulative properties. These metals interfere with physiological processes, inducing oxidative stress, disrupting neural function, and impairing reproductive capabilities [8, 9, 10]. Mollusks, as sensitive bioindicators, are particularly vulnerable to metal pollution, and their physiological responses often signal the broader health of aquatic environments [11, 12].

Freshwater mollusks, such as *Lymnaea stagnalis* and *Planorbarius corneus*, exhibit significant physiological and behavioral changes when exposed to heavy metals. Manganese ions, in particular, have been shown to impair respiratory efficiency, reduce growth rates, and hinder reproduction. Sublethal concentrations of Mn^{2+} induce chronic stress and long-term damage to mollusks' physiological systems, affecting their survival and ecological functions [13, 14, 15]. Elevated manganese levels disrupt hemolymph oxygen transport and alter diffusive respiration, leading to decreased population stability in contaminated habitats [2, 16]. *Planorbarius corneus*, as a key species for assessing the ecotoxicological effects of Mn^{2+}, shows significant differences in tolerance between its "western" and "eastern" allospecies. These differences are linked to genetic variability and environmental adaptation [1, 17]. Manganese exposure negatively impacts critical physiological processes such as respiration and reproduction, with "western" populations exhibiting greater resilience due to their adaptation to cooler and less polluted environments [18]. These findings highlight the need for effective conservation strategies and stricter pollution controls to protect aquatic biodiversity from increasing anthropogenic pressures [19, 20, 21].

2. Material and methods

2.1. Materials and places of their collection

Total 250 individuals of *P. corneus* s. l. were studied; all collected by hand: 140 individuals of "western" allospecies (Irsha river, Malyn vicinity, Zhytomyr region, 50°45'55.6"N, 29°13'43.5"E) and 110 individuals of "eastern" allospecies (Psel river, Balakliia vicinity, Poltava region, 49°36'53.9"N, 33°45'50.6"E) (Fig. 1-3). Allospecies were identified by conchiological indexes.

Figure 1. Shells of *P. corneus* s. l.: 1–3 – allospecies "western" (Irsha River, Malyn); 4–6 – allospecies "eastern" (Psel River, Balakliya); 1, 4 – top view; 2, 5 – bottom view; 3, 6 – side view. Scale bars 10 mm.

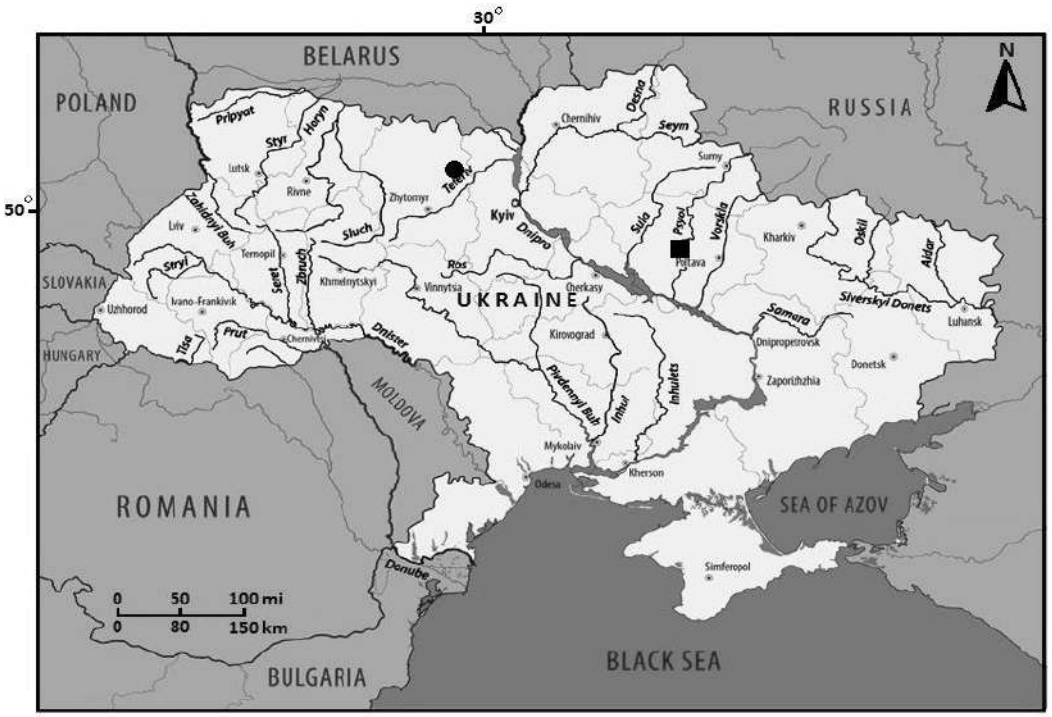
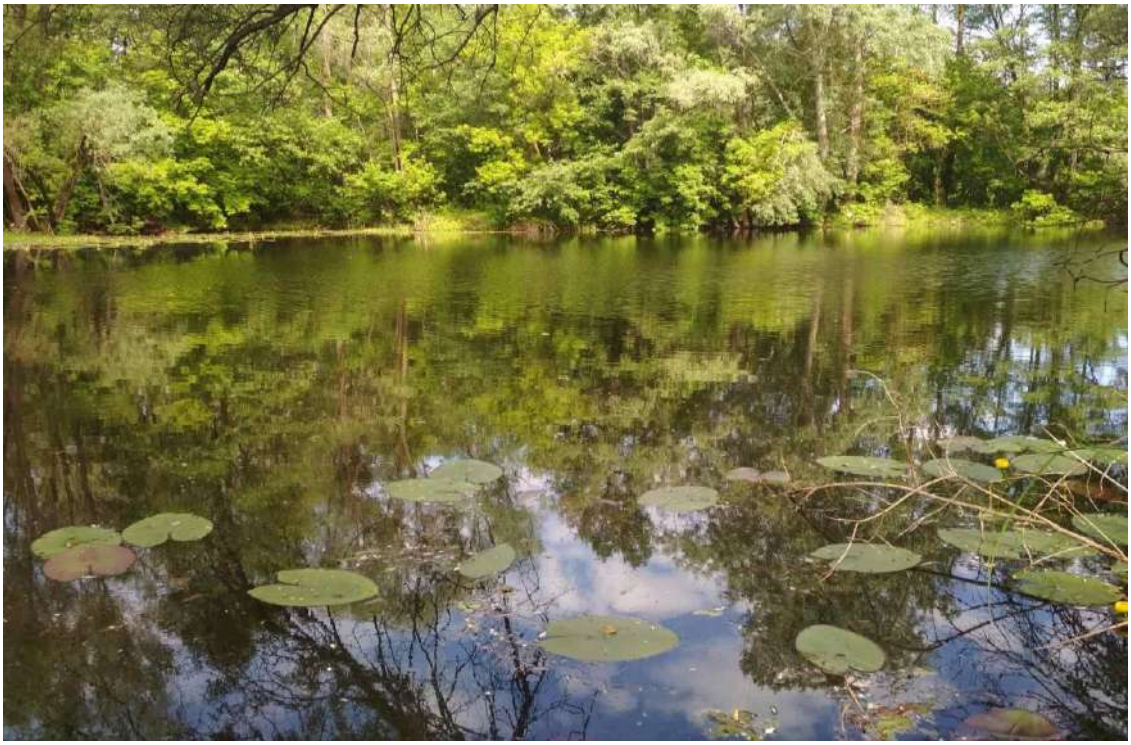


Figure 2. Map showing the type localities of *P. corneus* s. l. allospecies: black circle – "western"; black square – "eastern"

(a)



(b)

Figure 3. Habitats of great ramshorn *P. corneus* s. l.: (a) – Irsha River (Malyn, Zhytomyr region); (b) – Psel River (Balaklia, Poltava region).

2.2. Method acclimation to the laboratory conditions

Collected material having delivered to the laboratory was acclimated to its conditions during 14 days. The conditions were following: 10 L water volume, 4 ind./L density, 20-22 ºC temperature, 7.6–8.4 pH, 8.3–8.9 mg O2/L oxygenation.

2.3. Setting up a toxicological experiment and reagents

The main toxicological experiment was conducted according to [1,2]. Conditions: 100 L aquarium volume, 20-22 ºC temperature, 7.3–7.9 pH, 7.6–8.9 mg O2/L oxygenation, everyday environment renewing. $MnCl_2 \cdot 4H_2O$ (p. a.) in concentrations 0.001–1000 mg/L (calculated per cation) was used as toxicant, exposition – 48 hours. Registration of results – each 10 and 30 minutes, 1, 2, 4, 6, 24 hours. Impact of the toxicant to the experimental individuals was estimated using visual observations of the overall physiological state of mollusks and changes in behavior and physiological reactions caused by intoxicated environment.

2.4. Statistical analysis

Digital results of experiments were analyzed using common methods of basic variation statistics. For each of four studied indexes of pulmonary respiration and for index of surface diffusive (skin) respiration we estimated via these analyzes, in which cases the statistically significant differences ($p \leq 0.05$) and in which cases the highly significant differences ($p \leq 0.001$) took place between compared groups of studied objects.

2.5. Ethical compliance

Throughout the experiments, we strictly adhered to ethical norms and principles governing research involving living organisms, in full compliance with the current laws of Ukraine. As part of our ethical commitment, we emphasize the following:

1 Invertebrate research: the experimental molluscs in our study belong to the category of invertebrates, and we ensured that ethical considerations for their welfare were upheld.

2 Compliance with Ukrainian laws: we affirm that our research is fully compliant with the prevailing laws and regulations of Ukraine, including those governing the ethical treatment of research subjects.

3 Ethical review: this research received approval from the Human or Animal Ethics Committee at Zhytomyr Ivan Franko State University. The committee reviewed and approved the ethical aspects of our study to ensure the humane treatment of the experimental subjects.

3. Results and Discussion

3.1. Characteristics of Mn^{2+}

Comparing to other divalent metals (Cu, Zn, Ni), manganese is weakly toxic microelement to animal hydrobionts. Concerning the freshwater mollusks this point was repeatedly claimed by different authors [5,22]. That is due to the minimal, lethal for 50% of affected individuals, water concentration of Mn^{2+} is 100 mg/L. And its level in Ukrainian water bodies, used for drinking, industrially-household and other purposes, established in accordance with Order of the Ministry of Health of Ukraine (from May 2, 2022, No. 721), is 0.1 mg/L for industrially-household waters and 0.01 mg/L for other water bodies.

Table 1 shows the results of Mn^{2+} analysis of for main toxicological characteristics of *P. corneus* s. lato allospecies under the influence of them by ions during 48 hours. The values of the four groups of compared indicators regarding the survival of "western" and "eastern" allospecies were different, but in all the cases they were higher for the former compared to those

for the latter. The results of the "eastern" allospecies were 1.2–1.5 times lower compared to its counterpart at the Mn^{2+} concentrations.

Table 1. The main toxicological characteristics of *P. corneus* s. lato allospecies under the exposure of Mn^{2+} (48 hours).

Indicator, mg/L	"Western" allospecies	"Eastern" allospecies
Threshold concentration	0.3	0.2
LC_0	85	70
LC_{50}*	450	375
LC_{100}	2750	2550
Toxicity rate	450	350

LC_0 – the highest concentration which caused no mortality of molluscs.
LC_{50} – lethal concentration which caused in accordance 50% mortality of molluscs (* set graphically).
LC_{100} – lethal concentration which caused in accordance 100% mortality of molluscs.

3.2. Latent period (hours)

Table 2 presents information that proves statistically significant differences between allospecies in the length of the latent Mn^{2+} ion intoxication period ($p \leq 0.05$). The "western" allospecies's duration was longer at all concentrations of the toxicant used (Table 2).

Table 2. Latent period (hours) of *P. corneus* s. lato allospecies under the exposure of Mn^{2+}.

Concentration, mg/L	"Western" allospecies	"Eastern" allospecies
	M±SE	M±SE
0.01	25.2±1.09	23.5±1.02*
0.1	4.1±1.15	3.5±1.01*
1.0	2.1±0.06	1.5±0.05*
10	1.2±0.10	0.8±0.11*
100	0.4±0.03	0.3±0.02
1000	0.3±0.09	0.2±0.01

* – statistically significant differences ($p \leq 0.05$) between *P. corneus* s. lato allospecies.

3.3. The mortality (%) of P. corneus s. lato allospecies under the exposure of Mn^{2+}

The data in Table 3 show that within the Mn^{2+} concentration of 10 mg/L, the "western" allospecies and at the same concentration the "eastern" allospecies do not show any physiological changes caused by being subjected to a manganese ion environment. The first individuals of both allospecies, which died at the end of exposure (48 h), were noted for 100 mg/L of Mn^{2+} ion.

Table 3. Mortality (%) of *P. corneus* s. lato allospecies under the exposure of Mn^{2+} (48 hours).

Concentration. mg/L	"Western" allospecies	"Eastern" allospecies
10	0	0
100	30	45
1000	100	100

Table 4. Time-to-death (hours) of *P. corneus* s. lato allospecies under the exposure of Mn^{2+}.

Concentration, mg/L	"Western" allospecies	"Eastern" allospecies
	M±SE	M±SE
1.0	–	1.0±0.03
10	28.6±1.57	26.0±1.12*
100	19.8±0.80	16.1±0.39*
1000	1.5±0.41	1.3±0.23

* – statistically significant differences (p≤0.05) between *P. corneus* s. lato allospecies.

3.4. Rating of various Mn^{2+} concentrations

The species under impact of subthreshold, sublethal, chronic lethal and acutely lethal Mn^{2+} concentrations highly exceeded those of "eastern" allospecies (Table 5).

Table 5. Rating of various Mn^{2+} concentrations (mg/L) according to the effect on *P. corneus* s. lato allospecies.

Subthreshold	Sublethal	Chronic lethal	Acutely lethal
	"Western" allospecies		
0.03–0.003 and lower	10–1.0	80–35	180–90
	"Eastern" allospecies		
0.01 and lower	5–0.1	65–25	160–75

3.5. The lung and direct diffusive respiration

The data in Table 6 proves the more intensive duration of as lung and direct diffusive respiration in the individuals of "western" allospecies comparing to "eastern" allospecies (p≤0.05–0.001).

Table 6. The impact of Mn^{2+} in different concentration on indexes of lung and direct diffusive respiration of *P. corneus* s. lato allospecies.

Toxicant concentration, (mg/L)	n	Indexes of lung respiration				Index of direct diffusive respiration,
		Daily number of inspirations	Interval between inspirations, min	Duration of inspiration, min	Volume of inspiration, number of bubbles	
		M±SE	M±SE	M±SE	M±SE	M±SE
"Western" allospecies (Irsha River, Malyn)						
Control	15	16.98±1.17	50.11±1.39	24.19±1.41	22.48±1.19	48.15±2.48
0.25	21	16.85±1.18	51.20±1.21	25.92±1.10	23.51±1.14	52.21±3.50
2.5	16	18.37±1.26*	49.33±1.73*	24.25±1.07	24.62±1.23*	54.33±4.06*
25	17	20.43±1.41**	16.44±1.25**	11.03±1.01**	11.17±0.92**	18.15±1.44*
250	20	14.51±1.32**	6.09±0.51**	6.43±0.99**	5.59±0.10**	6.51±0.74**
"Eastern" allospecies (Psel River, Balakliya)						
Control	16	15.24±1.11	52.15±1.27	23.17±1.12	20.11±1.13	46.31±2.52
0.25	15	15.11±1.23	50.48±1.39	24.99±1.09	21.12±1.09	47.32±2.49
2.5	20	17.45±1.40*	47.05±1.23*	23.69±1.07	22.34±1.15*	49.10±2.51*
25	21	18.20±1.38**	15.03±1.24**	9.48±1.07**	10.03±0.81**	16.33±0.95*
250	19	13.23±1.24**	5.33±0.43**	4.22±0.94**	4.11±0.75**	4.41±0.83**

n – number of individuals studied; MPC – maximum permissible concentration of ions in the water; M±SE – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

Manganese (Mn^{2+}) is an essential trace element for many organisms, including freshwater mollusks, but its levels must remain within a specific range for physiological processes to function correctly. The results of this study show that the "western" and "eastern" allospecies of *Planorbarius corneus* exhibit distinct responses to Mn^{2+} exposure, which may be related to genetic and environmental adaptations. These findings are consistent with previous studies showing that environmental factors, such as temperature and water chemistry, can influence the resilience of aquatic species to pollutants, including heavy metals like manganese [1, 4, 7].

The observed difference in survival between the two allospecies suggests that the "western" population, adapted to the cooler and wetter conditions of the Polissia zone, is more resistant to Mn^{2+} toxicity compared to the "eastern" population, which is exposed to higher temperatures and drier conditions in the Forest-Steppe zone. This finding aligns with previous research on the role of climate in shaping the ecological tolerance of species to pollutants, such as metals, which are known to become more toxic under warmer and drier conditions [5, 6, 8].

The toxicological results of the study indicate that Mn^{2+} causes a range of detrimental effects in both allospecies, including impaired respiration, behavioral changes, and reproductive disruption. In both populations, high concentrations of manganese led to significant physiological stress, with the "eastern" allospecies showing greater sensitivity. These results are consistent with the findings of other studies on manganese toxicity in freshwater mollusks, where elevated Mn^{2+} concentrations were linked to altered respiratory function and oxidative stress [3, 9, 10].

Despite these toxic effects, manganese also plays a crucial role in the physiological processes of mollusks at low concentrations, such as enzyme activation and regulation of metabolic processes. This highlights the delicate balance between beneficial and harmful effects of manganese, and underscores the need for careful management of Mn^{2+} levels in freshwater ecosystems to maintain ecological health [2, 4, 11].

4. Conclusion

The comparative analysis of two allospecies of *Planorbarius corneus* from Ukrainian rivers demonstrated distinct physiological and toxicological responses to varying concentrations of Mn^{2+} ions. The "western" allospecies exhibited greater resilience and tolerance to manganese exposure, which may be attributed to its adaptation to the harsher climate of the Polissia region, suggesting a heightened ecological stability under environmental stressors. This endurance supports its broader distribution within the forest zones, contrasting with the more limited distribution of the "eastern" allospecies in the warmer and drier Forest-Steppe region.

Furthermore, the impact of manganese exposure highlights the neurotoxic effects that Mn^{2+} exerts on both allospecies, although the effects were more pronounced in the "eastern" population. These findings underscore the necessity for region-specific conservation strategies, as environmental changes and pollution could disproportionately affect certain mollusk populations. The "western" allospecies' tolerance suggests an adaptive advantage in environments with fluctuating manganese levels, whereas the "eastern" allospecies may face greater risks due to its lower resilience and the more demanding environmental conditions

This study also emphasizes the critical role of manganese in aquatic ecosystems, where at low concentrations, it supports vital physiological functions, yet at elevated levels, it poses significant risks to mollusks' neurological health. The findings provide essential insights into how manganese pollution can lead to behavioral and cognitive impairments, contributing to population decline and disrupted ecosystem balance. Recognizing these impacts, regulatory measures should be enhanced to control manganese emissions, particularly in vulnerable areas like the Ukrainian river networks.

Lastly, the observed differences in survival and endurance between the two allospecies of *P. corneus* reflect the broader ecological implications of species-specific responses to metal contamination. This research can inform ecotoxicological models that account for genetic diversity within species and the adaptive mechanisms they deploy against pollutants. Future research should focus on exploring the molecular basis of these responses, as understanding these mechanisms could guide targeted conservation efforts and improve ecological resilience across different climatic regions in Ukraine.

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