Ecologica Montenegrina
ISSN 2336-9744 (online) | ISSN 2337-0173 (print)

Ecologica Montenegrina 52: 57-67 (2022)
This journal is available online at: www.biotaxa.org/em
https://dx.doi.org/10.37828/em.2022.52.9

Article

Influence of some heavy metals to the pulmonary and direct diffusive respiration of the great ramshorn *Planorbarius corneus* allospecies (Mollusca: Gastropoda: Planorbidae) from the Ukrainian river system

OLENA I. UVAYEVA[1*], AGNESSA P. STADNYCHENKO[2], YULIIA V. BABYCH[2], TAMARA V. ANDRIYCHUK[2], YULIIA V. MAKSYMENKO[2], DMYTRO A. VYSKUSHENKO[2], OLGA O. IGNATENKO[2] & TATIANA V. PINKINA[3]

[1]Zhytomyr Polytechnic State University, Chudnovska Str. 103, 10005 Zhytomyr, Ukraine
https://orcid.org/0000-0003-1894-0386
[2]Zhytomyr Ivan Franko State University, Velyka Berdychivska Str. 40, 10002 Zhytomyr, Ukraine
https://orcid.org/0000-0001-7738-4776; e-mail: stadnychenko2016@gmail.com
https://orcid.org/0000-0001-6887-0529; e-mail: b_yulia@i.ua
https://orcid.org/0000-0003-4316-8324; e-mail: andriychuk2012@ukr.net
https://orcid.org/0000-0001-5292-852X; e-mail:tarasova21@gmail.com
https://orcid.org/0000-0002-1233-7650; e-mail: vda@zu.edu.ua
https://orcid.org/0000-0002-8085-9039; e-mail: zts29517@gmail.com
[3]Zhytomyr Polyssia National University, Bouleward Old 7, 10002 Zhytomyr, Ukraine
https://orcid.org/0000-0001-9443-8406; e-mail: pinkinatv61@gmail.com
**Corresponding author. E-mail: bio-2016@ukr.net*

Received 2 February 2022 │ Accepted by *V. Pešić*: 21 March 2022 │ Published online 7 April 2022.

Abstract
The vicaristic genetic allospecies ("western" and "eastern") of superspecies complex *Planorbarius corneus* s. lato from Ukrainian river network have the combined bimodal respiration mode. These molluscs use their true lungs for atmospheric oxygen breathing, and the diffusive (surface) respiration is used for soluble oxygen consumption.

Oxygen transportation to different tissues and organs and carbon dioxide from them to lungs occurs due to respiratory pigment hemoglobin in their liquid internal medium (hemolymph), circulating in vessels, lacunas and sinuses of these animals' open circulatory system.

The aim of present study was to clarify the features and difference levels of lung and surface respiration for "western" and "eastern" allospecies under the impact of heavy metal ions in water environment (MPC: 0.5, 1, 2, 3).

It was established that the toxins could be ranged by their toxicity level for activation of molluscs' respiratory function: Cu^{2+}>Zn^{2+}>Ni^{2+} >Mn^{2+}. The "eastern" allospecies appeared to be more sensitive and less durable for all aforementioned toxins by both lung and direct diffusive respiration, comparing to the "western" allospecies.

Key words: pollutants, respiratory parameters, molluscs, Ukrainian rivers.

Introduction

Long-lasting anthropogenic pressure on the Ukrainian river network have led to the significant increasing of its contamination level. There are many pollutants involved – the components of industrial, agricultural and household dumps and drains. One of the most dangerous type for hydrobionts is the heavy metal ions. However, the microdoses of these elements are not only harmful, but rather vitally essential. Being involved in the cycles of nutrients and energy in water ecosystems and persisting in biogeohemical cycles for a long time, such microelements are quite important in processes of energetic exchange and maintain their components vital activity (Davydova & Tagasov 2002; Kutsenko 2004; Afanasyev & Grodzinsky 2004; Kyrychuk 2006; Kyrychuk & Stadnychenko 2010; Grubinko *et al.* 2012; Linnik 2010; Dudnik & Evtushenko 2018; Babych & Pinkina 2021; Babych *et al.* 2021; Harbar *et al.* 2021). Heavy metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+} etc.) are the obligatory components of many chemical compounds, important for the set of vital biochemical and physiological processes in hydrobionts organisms. The Cu^{2+} is the obligatory component of their respiratory pigments; Zn^{2+} – component of carboanhydrase ferments, catalyzing the lysis of carbonic acid into water and carbonic anhydride and reverse reaction; Ni^{2+} is responsible for particular stages of hematopoiesis, and Mn^{2+} – for growth and development of hydrobionts organisms (Hochachka & Somero 1988; Romanenko 2001; Kyrychuk 2006; Kyrychuk & Stadnychenko 2010; Golovko *et al*. 2015). According to the modernly used scale of toxicity level of heavy metal ions for hydrobionts (Metelev *et al*. 1971), which was applied in our experiment with *P. corneus* s. l. allospecies, the toxicants belong to the following categories: highly toxic (<1 mg/dm^3) – Cu^{2+}, very toxic (1–10 mg/dm^3) – Zn^{2+}, moderate toxic (10–100 mg/dm^3) – Ni^{2+}, low toxic (>100 mg/dm^3) – Mn^{2+}.

The income and accumulation level of heavy metal ions in water animals is driven by both hydro-chemical features of their living environment and physiological and genetic status of these hydrobionts (Oros & Gomoiu 2010; Babych & Pinkina 2021).

It was recently established (Mezhzherin *et al*. 2005; Garbar 2003) and confirmed (Garbar *et al*. 2020; Babych *et al.* 2021), that the only European and West Siberian (till the Ob river basin) Planorbidae representative – *Planorbarius corneus* (Linnaeus, 1758) is not a species as having known before, but superspecies complex *P.* (superspecies) *corneus* s. l. It consists of two vicaristic allospecies "western" and "eastern", whose ranges are separated by narrow (about 100 km) zone of introgressive hybridization, which Ukraine belongs to. The first one occupies the western and central part of Right-Bank Ukraine, and the second one – northern and north-eastern territories of Left-Bank Ukraine, and the extreme south of its Steppe zone (from Siverskyi Donets river to Danube river inclusively). The distribution of ramshorn allospecies coincide with geographical zones of different drought hazard. The "western" allospecies is widespread in the zone of minimal hazard, where the portion of dry days does not exceed 1%. The "eastern" allospecies inhabits mainly the zone with possibility of dry days from 1% to 10%. And in the zone of high drought possibility (more than 10%), the ramshorns inhabit mostly the channels of big rivers: Dnipro, Dnister and Danube (Garbar & Garbar 2006).

Allospecies of *P. corneus* s. l. are well-distinguished by their conchiological features – by absolute size of their shells (the first has the bigger) and 6 metric indexes, by which the speed of shell growth and ratios between their mouths' high to width are estimated. Conchiolin layer coloration of these allospecies is completely different: dark-brown to black in "western" form and brown-yellow or yellow-gray in "eastern". Allospecies are also different by linear parameters of some reproductive system organs (vagina, spermatheca and its ducts) and the indexes related to them (Babych *et al.* 2021).

Freshwater Gastropoda from family Planorbidae possess two respiratory modes: lung and surface (direct diffusive). The lungs of these Pulmonata are the new organs, emerged during their long evolution as the tool for breathing using atmospheric air. During the definitive organogenesis, the lung progenitor forms as protrusion of ectodermal covering of their body long before their mantle cavity forms. Therefore the lungs and mantle cavity innervations operate through the different ganglia of their CNS: lungs are innervated directly by unpaired visceral (abdominal) ganglion and mantle by the pair of parietal ganglia. This was first proved via scrupulous anatomical studies of nervous regulation features in pulmonate Gastropoda (Régondaud 1961).

Indirect nervous regulation of surface diffusive respiration (by body epithelium coverings) is also peculiar in these molluscs. It occurs through the powerful peripheral subcutaneous nervous network controlled by buccal ganglia. This respirational mode is used by adaptive gill of these animals also, which, however, is innervated differently (Minichev & Starobogatov 1979): by parietal ganglion, like right ctenidium in Pectinibranchia molluscs (homologue of adaptive gill).

The lungs of great ramshorn are the clusters of many tightly tangled small branches of the lung vein, which increase the surface of oxygen consumption. It goes through the dorsal wall of lung cavity, embodied with respiratory epithelium. Oxygen comes from atmospheric air into the lungs through the pneumostome and long movable lung syphon, during animal regular emerging under the surface of water (where surface tension acts). This occurs when oxygen level in lungs falls to 2.8 % (Prosser & Braun 1967). Consumed oxygen is distributed among all the tissues in mollusc organism due to transportation by their liquid internal medium – hemolymph. Oxygen distribution from lungs to all the tissues and carbonic acid and water back is performed with hemoglobin (solved in plasma), via the vessels, lacunas and sinuses of their open circulatory system.

The direct diffusive respiration is performed by percutaneously using diffusion of oxygen solved in the water through thin membranes to the cytoplasm of covering epithelium cells no deeper than 1 mm (Krogh 1941). Thus, the lung respiration in molluscs is the daily regularly repetitive process, occurring in the "day-night" mode (Stadnychenko 2013), and the diffusive respiration is the lifelong permanent. The quantitative results of both these processes of oxygen supply (according to both mentioned scientists) are almost identical: 0.025 and 0.03 mg (O_2)/h by 1 g of fresh body mass, respectively. Under the impact of heavy metal ions in water environment, the ramshorn allospecies demonstrate functional impairments of both their respiratory systems, that was not previously studied.

The aim of present study was to compare the physiological response reaction between both of the allospecies using some indices of their lung and direct diffusive respiration under the impact of different ions.

Material and methods

Material was the 1261 samples of *P. corneus* s. l. (Fig. 1): 639 samples of "western" allospecies (among which the 164 and 161 samples were used for studying the lung and direct diffusive respiration, respectively) (Hnyla river, Horodnytsia village, Ternopil region; 49.410655 N, 26.008865 E) and 622 samples of "eastern" allospecies (157 and 154 samples - for studying the lung and direct diffusive respiration, respectively) (Sula river, Romny, Sumy region; 50.738034 N, 33.498816 E) (Figs. 2–4). All samples were collected by hand. Allospecies were identified using conchiological features (Garbar 2003; Mezhzherin *et al*. 2005; Garbar & Garbar 2006). Collected animals were subjected to obligatory (Khlebovich 1981) 15-days acclimatization under the following conditions: water volume 30 l, individuals density 4 ind/l, water temperature 20-23° C, pH 7.7-8.6, oxygenation 8.2-8.8 mg (O_2) /l. Molluscs were daily fed by the mixture of *Miriophyllum spicatum* L., *Alisma plantago-aquatica* L. and *Cladophora* sp.

Animals, assigned for experiment, were weighted (weights WPS 1200/C), and their shells were measured (calipers). Hemolymph was obtained via direct bleeding. Its volume was measured using insulin syringe, its pH – using paper express indicator (produced in PRC), Hb content – using Sali method with Alakrinska modifications (1970).

Toxicological experiment was performed according to Alekseev (1981). The salts $CuCl_2 \cdot 2H_2O$, $ZnCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$ in concentrations 0.5, 1, 2 and 3 MPC were used as toxicants (fisheries MPC – 0.065 mg/dm^3; values were calculated for cation). Indexes of lung respirations were the results of three stages (at 3, 7 and 11 days of experiments) of whole-day animals monitoring according to the Stadnychenko et al. (1996). The intensity of direct surface diffusive respiration was identified via indirect method: we measured, how long the molluscs could maintain their viability without absolute inability to perform lung respiration. Molluscs were kept at the aquariums bottom in the closed water-permeable containers made of fine-mesh kapron with mounted weight.

The obtained results were tested for significant difference presence using methods of basic variation statistics via the Statistica 6.0 soft package.

Results

“Western” allospecies (Hnyla river, Dnister river basin), sample from 06.07.2021. The mean size if its shell (mm): diameter – 26.12±0.54, mouth height – 13.10±0.31 (Fig. 1). “Eastern” allospecies (Sula river, Dnipro river basin), sample from 13.07.2021. Shell diameter (mm) – 24.78±0.24, mouth height – 12.15±0.16 (Fig. 1).

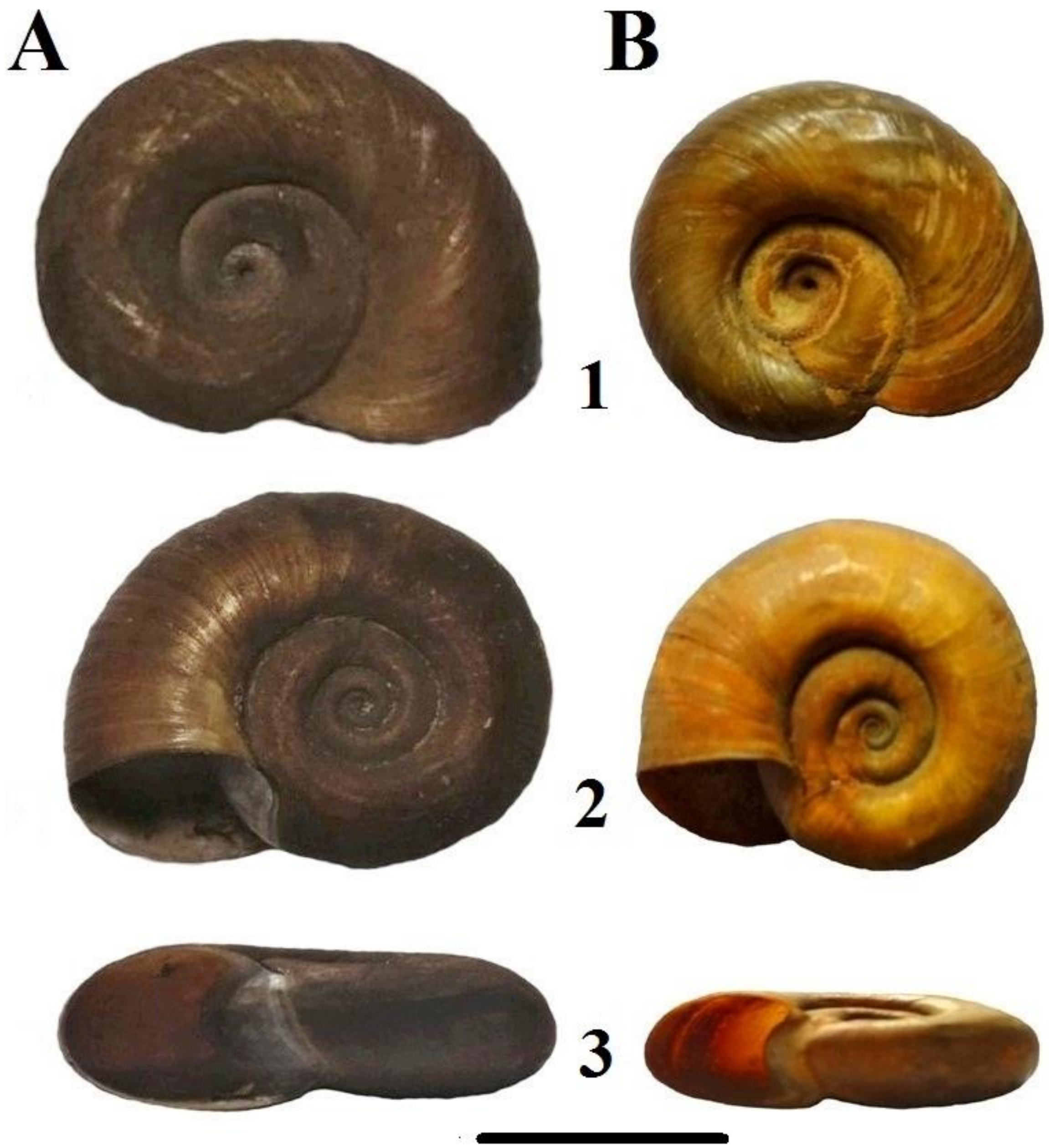

Figure 1. Shells of *Planorbarius corneus* s. lato. (A – allospecies “western”, B – allospecies “eastern”): 1 – top view; 2 – bottom view; 3 – side view. Scale bars: 10 mm. (Photos: Yuliia V. Babych).

The tables 1 and 2 present data on the levels of endurance for objects of toxicological experiments under the impact of heavy metal ions. The endurance coefficients for different Cu^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+} concentrations in water environment appeared lower in “eastern” allospecies comparing to those in “western” allospecies (Table 1). Table 2 shows the difference between two allospecies by the lethal levels of aforementioned toxicants. Under the same concentrations of toxicants used, the lethality of “eastern” allospecies was higher than that of “western” allospecies in all cases.

Figure 2. Map showing the type localities of *Planorbarius corneus* s. lato allospecies: black triangle – «western»; black square – «eastern».

Figure 3. Photo of habitat *Planorbarius corneus* from Hnyla River (Horodnytsia village, Ternopil region) in 2021 (Photos: Yuliia V. Babych).

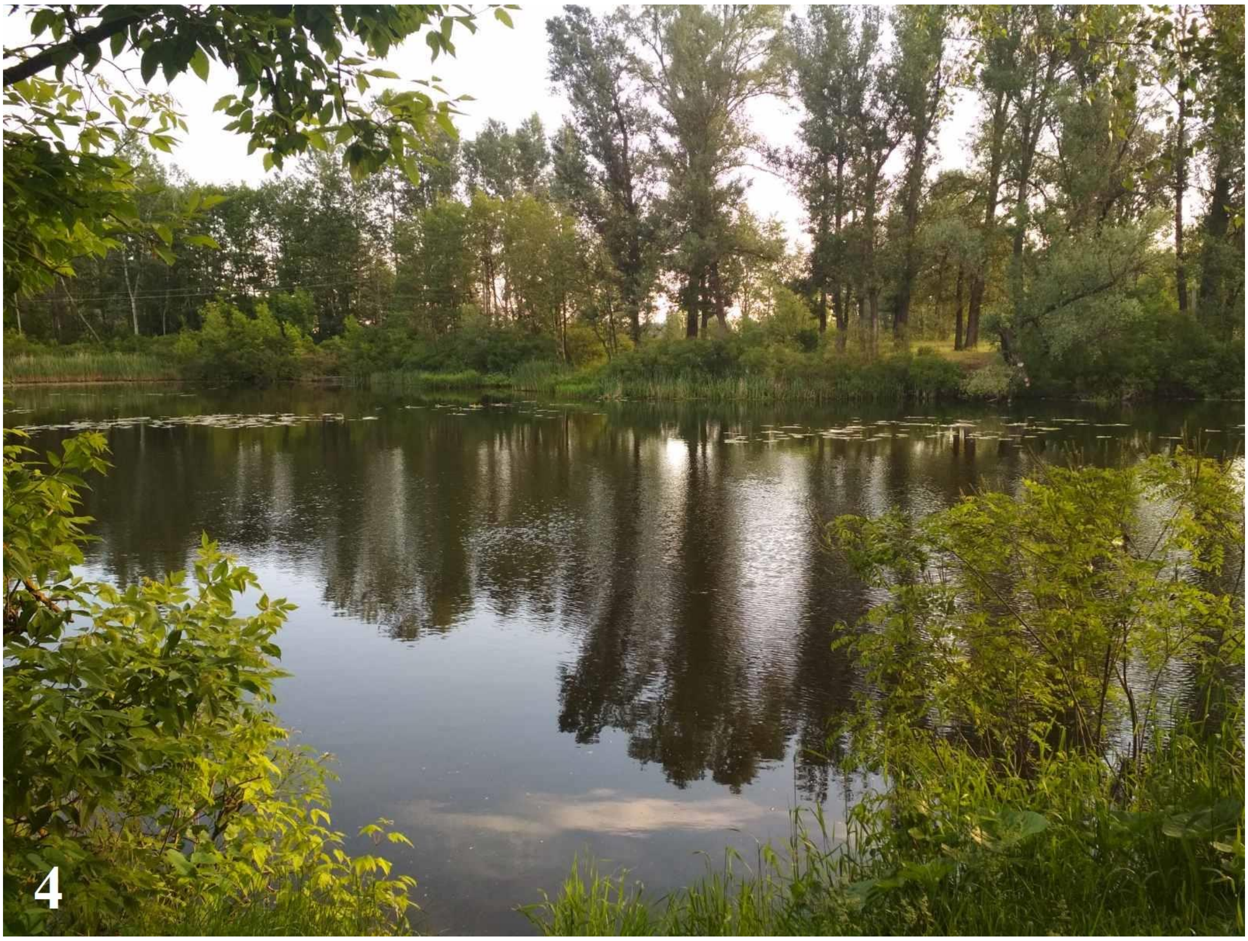

Figure 4. Photo of habitat *Planorbarius corneus* from Sula River (Romny, Sumy region) in 2021 (Photos: Yuliia V. Babych).

Table 1. The mortality (%) of ramshorn allospecies under the exposure of different concentration of heavy metal ions.

Concentration, MPC	"Western" allospecies				"Eastern" allospecies			
	Cu^{2+}	Zn^{2+}	Ni^{2+}	Mn^{2+}	Cu^{2+}	Zn^{2+}	Ni^{2+}	Mn^{2+}
0 MPC	0	0	0	0	0	0	0	0
0.5 MPC	0	0	0	0	2	1	0	0
MPC	10	4	0	0	20	10	0	0
2 MPC	20	10	60	40	40	20	80	50
3 MPC	100	100	100	100	100	100	100	100

Table 2. The coefficient of adaptation (hours) of ramshorn allospecies for the heavy metal ions influence.

Ion	"Western" allospecies	"Eastern" allospecies
Cu^{2+}	4.9	3.3
Zn^{2+}	1.28	1.16
Ni^{2+}	1.13	1.07
Mn^{2+}	1.05	1.01

Table 3. The impact of heavy metal ions in different concentrations on indexes of lung and direct diffusive respiration in *P. corneus* s. l. allospecies.

Ion	Toxicant concentration	n	Indexes of lung respiration				Index of direct diffusive respiration*, h
			Daily number of "inhales"	Interval between "inhales", min	Duration of "inhale", min	Volume of "inhale", number of bubbles	
			Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
"Western" allospecies (Hnyla river, Horodnytsia village)							
	0 MPC	20	17.34±1.18	53.17±1.14	22.24±1.12	20.75±1.23	47.76±2.15
Cu^{2+}	0.5 MPC	19	17.58±1.25	52.83±1.15	22.88±1.30	21.04±1.26	48.22±2.58
	MPC	18	18.34±1.10	48.28±1.26	24.38±1.24	23.23±1.14	50.20±3.72
	2 MPC	18	20.64±1.24	40.25±1.22	27.54±1.16	29.58±1.29	55.67±3.45
	3 MPC	20	14.32±1.18	74.34±1.42	14.92±1.22	12.42±1.09	21.37±2.96
Zn^{2+}	0.5 MPC	19	17.73±1.23	51.86±1.20	23.06±1.24	21.76±1.12	49.04±2.52
	MPC	20	18.84±1.16	47.49±1.17	25.09±1.06	23.85±1.31	51.16±3.16
	2 MPC	19	21.46±1.23	39.66±1.19	28.39±1.23	30.52±1.34	56.78±3.42
	3 MPC	19	14.39±1.14	73.38±1.25	15.16±1.26	12.96±1.26	21.97±2.26
Ni^{2+}	0.5 MPC	19	17.88±1.14	51.24±1.12	23.65±1.08	22.14±1.14	50.08±2.63
	MPC	18	18.89±1.19	47.12±1.13	25.63±1.16	24.54±1.06	52.54±4.11
	2 MPC	20	21.86±1.15	39.13±1.25	28.85±1.12	31.17±1.16	57.28±2.36
	3 MPC	18	14.45±1.11	72.46±1.19	15.52±1.17	13.36±1.17	22.50±3.25
Mn^{2+}	0.5 MPC	20	18.07±1.21	50.91±1.14	24.01±1.14	22.84±1.24	51.15±3.78
	MPC	19	19.49±1.19	46.82±1.19	26.32±1.20	25.04±1.32	53.27±3.45
	2 MPC	19	22.68±1.18	38.25±1.22	29.42±1.06	31.78±1.35	59.38±2.54
	3 MPC	20	14.66±1.24	70.72±1.15	15.88±1.21	13.96±1.24	24.52±3.63
"Eastern" allospecies (Sula river, Romny)							
	0 MPC	19	15.27±1.05	63.61±1.16	19.75±1.14	17.67±1.18	39.62±2.75
Cu^{2+}	0.5 MPC	19	15.64±1.25	62.04±1.20	20.12±1.16	18.09±1.31	40.11±2.54
	MPC	20	16.61±1.16	58.89±1.24	22.28±1.21	20.58±1.24	42.36±2.21
	2 MPC	20	18.48±1.24	50.62±1.32	25.42±1.18	26.46±1.21	46.28±3.54
	3 MPC	18	10.24±1.35	108.56±1.43	12.24±1.23	9.67±1.28	18.84±2.12
Zn^{2+}	0.5 MPC	16	15.87±1.11	61.76±1.18	20.82±1.23	19.03±1.18	41.18±2.21
	MPC	18	16.74±1.20	57.23±1.23	22.91±1.26	21.27±1.24	43.61±2.54
	2 MPC	17	18.82±1.28	49.58±1.29	26.23±1.24	27.24±1.22	48.48±3.05
	3 MPC	19	10.86±1.23	106.12±1.16	12.84±1.27	10.43±1.34	20.69±2.14
Ni^{2+}	0.5 MPC	20	15.98±1.13	60.84±1.16	21.16±1.22	19.14±1.02	43.26±2.58
	MPC	19	17.04±1.20	56.16±1.28	23.04±1.34	21.68 ±1.12	45.15±2.35
	2 MPC	18	19.13±1.23	48.78±1.25	26.88±1.11	27.72±1.36	49.59±2.78
	3 MPC	18	11.06±1.25	105.02±1.12	13.44±1.04	10.57±1.13	22.28±2.32
Mn^{2+}	0.5 MPC	15	16.22±1.28	60.10±1.12	22.02±1.26	19.78±1.20	46.22±2.64
	MPC	16	17.64±1.32	55.89±1.23	24.46±1.28	22.06±1.34	48.12±2.32
	2 MPC	20	19.46±1.23	46.41±1.30	27.36±1.02	28.64±1.18	53.28±3.46
	3 MPC	19	11.47±1.25	104.58±1.17	14.07±1.37	11.09±1.01	23.82±2.19

Note: * the survival of molluscs without lung respiration was identified.

Table 4. The physico-chemical indexes of *P. corneus* s. l. allospecies hemolymph under the exposure of heavy metal ions in different concentrations.

Ion	Toxicant concentra-tion	n	Volume of hemolymph, ml	Mass of hemolymph, g	Hb amount, g%	Hb amount by the body mass, g%/g	Hemolymph pH
			Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
“Western” allospecies (Hnyla river, Horodnytsia village)							
	0 MPC	18	0.62±0.04	0.64±0.05	1.24±0.05	1.68±0.20	7.10±0.16
Cu^{2+}	0.5 MPC	19	0.50±0.07	0.52±0.08	1.08±0.04	1.23±0.16	7.30±0.14
	MPC	17	0.45±0.05	0.46±0.05	0.91±0.03	1.15±0.07	7.50±0.18
	2 MPC	18	0.40±0.04	0.44±0.06	0.82±0.07	1.09±0.16	8.10±0.17
	3 MPC	18	0.36±0.03	0.37±0.03	0.68±0.03	0.94±0.15	8.30±0.15
Zn^{2+}	0.5 MPC	17	0.51±0.05	0.53±0.05	1.12±0.05	1.25±0.16	7.30±0.21
	MPC	19	0.46±0.04	0.48±0.05	0.95±0.06	1.18±0.08	7.60±0.14
	2 MPC	19	0.43±0.04	0.44±0.05	0.81±0.04	1.11±0.11	8.10±0.08
	3 MPC	17	0.38±0.05	0.39±0.05	0.72±0.07	0.99±0.16	8.50±0.09
Ni^{2+}	0.5 MPC	18	0.54±0.08	0.56±0.09	1.10±0.07	1.36±0.12	7.50±0.12
	MPC	19	0.50±0.06	0.54±0.07	0.98±0.06	1.25±0.10	7.70±0.16
	2 MPC	20	0.46±0.04	0.46±0.04	0.86±0.05	1.15±0.14	8.30±0.15
	3 MPC	19	0.42±0.04	0.44±0.05	0.75±0.04	1.04±0.15	8.60±0.13
Mn^{2+}	0.5 MPC	20	0.58±0.06	0.60±0.07	1.14±0.04	1.53±0.14	7.30±0.18
	MPC	19	0.52±0.05	0.54±0.05	1.02±0.05	1.48±0.08	7.80±0.14
	2 MPC	18	0.48±0.06	0.49±0.06	0.90±0.04	1.32±0.11	8.35±0.16
	3 MPC	19	0.44±0.04	0.47±0.06	0.82±0.03	1.12±0.09	8.50±0.12
“Eastern” allospecies (Sula river, Romny)							
	0 MPC	20	0.48±0.02	0.50±0.04	1.14±0.06	1.42±0.24	7.30±0.10
Cu^{2+}	0.5 MPC	19	0.40±0.03	0.40±0.04	0.98±0.05	1.15±0.16	7.50±0.16
	MPC	18	0.35±0.04	0.37±0.05	0.88±0.08	1.06±0.19	7.80±0.17
	2 MPC	18	0.31±0.04	0.33±0.05	0.76±0.09	0.94±0.18	8.20±0.14
	3 MPC	20	0.26±0.04	0.30±0.06	0.62±0.04	0.88±0.21	8.40±0.13
Zn^{2+}	0.5 MPC	17	0.41±0.06	0.42±0.06	1.06±0.04	1.20±0.18	7.20±0.16
	MPC	18	0.37±0.04	0.39±0.05	0.92±0.05	1.14±0.12	7.50±0.12
	2 MPC	17	0.32±0.05	0.32±0.05	0.79±0.06	0.98±0.13	7.80±0.14
	3 MPC	19	0.28±0.05	0.31±0.06	0.64±0.08	0.92±0.17	8.10±0.18
Ni^{2+}	0.5 MPC	18	0.42±0.03	0.43±0.04	1.07±0.08	1.25±0.16	7.40±0.14
	MPC	17	0.39±0.05	0.40±0.08	0.94±0.07	1.19±0.12	7.80±0.18
	2 MPC	17	0.33±0.04	0.33±0.04	0.82±0.04	1.04±0.14	8.00±0.11
	3 MPC	19	0.30±0.06	0.32±0.06	0.70±0.06	0.96±0.17	8.20±0.16
Mn^{2+}	0.5 MPC	19	0.44±0.04	0.44±0.04	1.09±0.06	1.28±0.19	7.50±0.11
	MPC	18	0.40±0.05	0.42±0.06	0.97±0.07	1.21±0.12	7.70±0.18
	2 MPC	19	0.35±0.08	0.37±0.07	0.86±0.06	1.12±0.14	7.80±0.21
	3 MPC	18	0.31±0.06	0.34±0.05	0.76±0.04	1.08±0.05	8.10±0.16

The data presented in Table 3 indicates the significant difference between studied allospecies by the indexes of both their respirational modes (between the control and the experimental groups) depending on the chemical nature of toxicant and its concentration in the experimental environment. The heavy metal ions can be ranged by their damaging impact on molluscs in the following order: $Cu^{2+}>Zn^{2+}>Ni^{2+}>Mn^{2+}$. The increasing of these toxicants' concentrations from 0.5 MPC to 2 MPC was followed by increasing of molluscs' daily number, duration and volume of inspirations along with their decreasing of intervals between them (CV>99.9%). Instead, under the impact of the same toxicants at 3 MPC concentrations, the sharp decreasing of all indexes of lung respiration occurred with increasing of intervals between inspirations (CV>99.9%). The similar shifts in studied mollusks were registered also for direct diffusive respiration. These results correspond well with the data in Table 4 on physico-chemical hematological properties of "western" and "eastern" allospecies in normal conditions as well as under impact of heavy metal ions in concentrations from 0.5 to 3 MPC.

Discussion

In our study, the "western" allospecies of *P. corneus* s. l. outclass the "eastern" allospecies by levels of many absolute indexes of both respiration modes and by set of physico-chemical properties of hemolymph (Tables 3, 4). But there was no statistically significant difference by amount of consumed oxygen (by 1 g of fresh body mass) observed between allospecies.

Keeping the molluscs in the heavy metal ions solutions drove them to the phasic pathological process of intoxication. Under the 0.5 MPC, the first and the longest phase was the latent phase, which was characterized by no changes in features and levels of behavioral (moving, feeding) reaction in molluscs. Under the 1 and 2 MPC toxicants impact, the latent phase was changed to stimulation phase: the increasing of all the life-supplying functions in individuals, including their respiratory capacity increasing. The same toxicants under the 3 MPC concentrations drove the molluscs to the three final phases of irreversible pathological process – longer depressive and much sharper sublethal and lethal phases. The toxicoresistance against all heavy metal ions used in experiment was apparently lower in "eastern" allospecies comparing to "western" one. The first was more sensitive and less endurable for all these toxicants. This was the cause of its less adaptation for all aforementioned toxicants and, therefore, higher mortality comparing to "western" allospecies.

Recently, the increasing year by year global climate warming became the real serious hazard for *P. corneus* s. l. in Ukraine. It causes negative changes in indexes of some abiotic factors, including those supplying respiration needs of *P. corneus* s. l. populations in Ukrainian river network. The analysis of models of as modern ranges of both allospecies, as of hybrid zone (existing and some potential ones by 2050 year) between them indicates the possibility of as contraction their ranges, as impoverishment of overall number and density these populations too (Garbar *et al.* 2020).

Conclusion

The indexes of lung and direct diffusive respiration in the allospecies of *P. corneus* sensu lato under the impact of the different heavy metal ions concentrations in water environment demonstrated that "eastern" allospecies tends to regress under the increased environmental pollution more comparing to "western" allospecies. It can be considered as the result of their spreading in different climatic conditions, which for "eastern" allospecies are not more favorable due to the higher climate drought on the territory of its modern range.

Acknowledgement

The authors express their grateful to corresponding member of NAS of Ukraine Igor A. Akimov (Ukraine, Kiev) for the preliminary discussion the theme and detailed plan of our work and for the critical comments on the first version of our manuscript.

References

Afanasyev, S. A. & Grodzinsky, M. D. (2004) *Methodology for assessing environmental risks arising from the impact of pollution sources on water bodies*. IB, Kiev, 62 pp. (In Russian).

Alakrinska, I. O. (1970) The quantitative characteristics of hemolymph and hemoglobin in great ramshorn *Planorbarius corneus* (Gastropoda, Pulmonata). *Zoological Journal*, 49(3), 349–354. (In Russian).

Alekseev, V. A. (1981) Fundamental principles of comparative toxicological experiments. *Hydrobiological Journal*, 17(3), 92–100. (In Russian).

Babych, Yu. & Pinkina, T. (2021) Influence of heavy metal ions on ecotoxicological indicators of *Planorbarius corneus* (Mollusca, Gastropoda, Pulmonata, Bulinidae). *Visnyk of the Lviv University. Series Biology,* 84, 76–83. https://doi.org/10.30970/vlubs.2021.84.07 (In Ukrainian).

Babych, Yu., Uvayeva, O. & Stadnychenko, A. (2021) Food preferences of the great ramshorn *Planorbarius corneus* (Linnaeus, 1758) allospecies (Mollusca: Gastropoda: Planorbidae) from the Ukrainian river system. *Folia Malacologica*, 29(4), 204–211. https://doi.org/10.12657/folmal.029.023

Davydova, S. L. & Tagasov, V. I. (2002) *Heavy metals as supertoxicants of the XXI century*. RUDN, Moscow, 140 pp. (In Russian).

Dudnik, I. V. & Evtushenko, M. Yu. (2018) *Hydrobiotoxicology: principal theoretical thesises and their application.* Ukrphytosociological centre, Kyiv, 297 pp.

Garbar, A. V., Babych, Yu. V., Stadnychenko, A. P. & Garbar, D. A. (2020) Bioclimatic features of ecological niches and modeling the dynamics of areas of *Planorbarius corneus* allospecies in the conditions of climate change. *Biological research – 2020. Collection of scientific works.* Zhytomyr, 150–153. (In Ukrainian).

Garbar, A. V. & Garbar, D. A. (2006) Genogeographical approach to the taxonomy of molluscs on the example of the allospecies complex of *Planorbarius corneus* s. l. *Ecological-physiological and faunal aspects of malacological research the role of molluscs in bioindication of the state of the environment*, 2, 57–59. (In Russian).

Garbar, D. A. (2003) Genus Planorbarius (Gastropoda, Pulmonata, Bulinidae) in the fauna of Ukraine. *Proceedings of the State Natural History Museum*, 18, 123–126. (In Ukrainian).

Golovko, N., Golovko, T. & Gelikh, A. (2015) Investigation amino-acid structure of proteins bivalve freshwater mussels from the family Anodonta of the northern Ukraine. *Eastern Journal Enterprise Technology*, 77(5/11), 10–16. https://doi.org/10.15587/1729-4061.2015.51072

Grubinko, V. V., Kyrychuk, G. E. & Kurant, V. Z. (2012) The role of amino acids in adaptation to heavy metals at freshwater fishes and mollusc. *Scientific Issue Ternopil Volodymyr Hnatiuk National Pedagogical University. Series: Biology*, 2(51), 71–86. (In Ukrainian).

Harbar, O., Harbar, D., Stadnychenko, A., Babych, Yu. (2021) Ecotoxicological responses of two *Planorbarius corneus s. lato* (Mollusca, Gastropoda) allospecies to exposure of heavy metals. *International Journal of Aquatic Biology*, 9(6), 423–431. https://doi.org/10.22034/ijab.v9i6.1356

Hochachka, P. & Somero, G. (1988) *Biochemical adaptation*. Mir, Moscow, 568 pp. (In Russian).

Khlebovich, V. V. (1981) *Acclimation of animals.* Nauka, Leningrad, 136 pp. (In Russian).

Krogh, A. (1941) *The comparative physiology of respiratory mechanisms.* University of Pennsylvania Press, Philadelphia, 172 pp.

Kutsenko, S. A. (2004) *Fundamentals of toxicology.* Foliant, Saint-Petersburg, 355 pp. (In Russian).

Kyrychuk, G. E. (2006) The features of heavy metal ions accumulation in the organisms of freshwater molluscs. *Hydrobiological Journal*, 42(4), 89–110. (In Russian).

Kyrychuk, G. E. & Stadnychenko, A. P. (2010) Effect of trematoda infestation and zinc ions of the aquatic medium on hemocytes and some hematological characteristics of *Planorbarius purpura* (Mollusca: Gastropoda: Pulmonata: Bulinidae). *Hydrobiological Journal*, 46(5), 111–120. (In Russian).

Linnik, P. N. (2010) Content of metals labille fraction in surface waters as the main element in assessing their potential toxicity. *Hydrobiological Journal*, 46(6), 90–104. (In Russian).

Metelev, V. V., Kanaev, A. I. & Dzasokhova, N. G. (1971) *Aquatic toxicology*. Kolos, Moscow, 247 pp. (In Russian).

Mezhzherin, S. V., Garbar, D. A. & Garbar, A. V. (2005) Systematic structure of the *Planorbarius corneus* s. l. complex (Gastropoda, Pulmonata): analysis of allozyme markers and morphometric traits. *Vestnik zoologii*, 39(6), 11–17. (In Russian).

Minichev, Yu. S. & Starobogatov, Ya. I. (1979) The subclasses of Gastropoda and phylogenetic relations. *Zoologichesky zhurnal*, 58(3), 293–305. (In Russian).

Oros, A. & Gomoiu, M.-T. (2010) Comparative data on the accumulation of five heavy metals (cadmium, chromium, copper, nickel, lead) in some marine species (molluscs, fish) from the Romanian sector of the Black Sea. *Cercetari Marine*, 39, 89–108.

Prosser, L. & Braun, F. (1967) Comparative animal physiology. Mir, Moscow, 766 pp. (In Russian).

Régondaud, I. (1961) Development de la covité pulmonaire et de la covité palleale chez *Lymnaea stagnalis*. *Comptes Rendus de l'Académie des Sciences*, 252, 173–181. (In French).

Romanenko, V. D. (2001) *Basics of hydroecology*. Oberehy, Kyiv, 728 pp. (In Ukrainian).

Stadnychenko, A. P. (2013) The daily cyclicality of lung respiration in freshwater molluscs (Gastropoda, Pulmonata). *Hydrobiological Journal*, 49(3), 44–50. (In Ukrainian).

Stadnychenko, A. P., Slastenko, N. N., Husenko, O. V., Svitelskyi, N. N. & Sychevskyi, A. S. (1996) The influence of trematode invasion and exposure to lead nitrate on the lung and skin respiration in *Lymnaea stagnalis* (Mollusca: Lymnaeidae). *Parazitologiya*, 30(1), 76–80. (In Russian).