

EFFECT OF ZINC IONS ON THE LUNG AND CUTANEOUS DIFFUSIVE RESPIRATION OF THE GREAT RAMSHORN *PLANORBARIUS CORNEUS* ALLOSPECIES (MOLLUSCA: GASTROPODA: PULMONATA: PLANORBIDAE) OF THE UKRAINIAN RIVER NETWORK

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ABSTRACT: Toxicological studies were performed to analyze the effect of Zn²⁺ in different concentrations on the lung and cutaneous diffusive respiration of great ramshorn *Planorbarius corneus* (Linnaeus, 1758) allospecies from the two geographically separate populations. At 0.5 MPC Zn²⁺ in the water containing experimental molluscs, there were no statistically significant changes in the number or duration of inspirations compared to the control. Increasing the concentration of toxicant to 2 MPC was accompanied by a sharp increase in both ($p \leq 0.001$), as also in the volume of inspirations ($p \leq 0.05$). Increasing concentrations to 5 and 10 MPC was followed by drastic and statistically significant impairments of pulmonary respiration indices. Avoidance also collapsed at these higher concentrations, and the accumulation of thick mucus inhibiting respiration resulted in heavy mortality. Trends were the same in both “western” and “eastern” allospecies, although the latter appeared marginally less resistant.

The main reason for changes in respiratory function is the damage inflicted by toxicant on the lung and cutaneous respiratory epithelium. The effects are highly intensified production of mucus which completely blocks oxygen intake by cutaneous epithelium and induces cell death in the respiratory epithelium.

KEY WORDS: allospecies of *Planorbarius corneus* sensu lato; Zn²⁺; lung and cutaneous diffusive respiration

INTRODUCTION

The yearly progressive contamination by heavy metal ions of freshwater as well as salt water bodies in Ukraine is the consequence of increasing anthropogenic pressure on the hydrosphere (VYSHNEVSKY 2000, ROMANENKO 2001, KOTKOVA & SELEZN’OVA 2011, MYSLYVA & KOT 2011, POSTANOVA KABINETU MINISTRIV UKRAYINY 2013, FRID & CASWELL 2017). Despite some positive trends of decreasing levels in the first two decades in XXI century (HIRIY et al.

2011), pollutant concentration in rivers still remains high. One of such pollutants dangerous for animal hydrobionts is Zn²⁺. Its impact on the pulmonary and surface (diffusive) respiration indices in hydrobionts, and on their other physiological functions is important not only in Ukraine, but more generally, as evidenced by the recent appearance of several publications on the matter. This is crucially important not only for European water networks, but for the water

networks on other continents as well (JORGE et al. 2007, KHAYATZADEH & ABBASI 2010, DE LISI et al. 2013, ROSTERN 2017, LI et al. 2019).

It is known (DAVYDOVA & TAGASOV 2002, DUDNYK & YEVTUSHENKO 2013) that Zn^{2+} in microdoses is an essential element, necessary for the vital activities of water animals. It enables normal growth and development of animal hydrobionts. In particular, it prevents hemolymph (liquid internal medium) stability impairments (KYRYCHUK & STADNYCHENKO 2009). The presence of Zn^{2+} as a necessity for all hydrobionts is due to its role as a component of carbonic anhydrase: the ferment catalyzing of carbonic acid lysis into water and carbonic anhydride as well as the reverse reaction of carbonic acid synthesis (HARBAR et al. 2021). Thus, without carbonic anhydrase, the hydrobiont organisms become unable to remove the carbonic acid arising from tissue aerobic respiration.

However, when Zn^{2+} concentration exceeds maximum permissible concentration (MPC) of ions in the water (0.01 mg/L), it becomes harmful for their animal inhabitants (UDRIS & NEJLAND 1981). That is because it belongs to the local action toxicants group by the type of impact and to the very toxic group by the level of toxicity (METELEV et al. 1971).

The heavy metal ions enter the organisms of pulmonate gastropod molluscs usually via percutaneous osmosis and only in lesser amounts ingested as food. In pulmonate molluscs they accumulate mainly in the hepatopancreas; hemolymph circulation provides their distribution to other organs and tissues (KYRYCHUK et al. 2002, KYRYCHUK & STADNYCHENKO 2009).

It is known that some physiological functions in freshwater molluscs worsen under the impact of enhanced Zn^{2+} concentrations: filtration (STADNYCHENKO et al. 2020), hematological (KYRYCHUK &

STADNYCHENKO 2009), growth (KROGH 1941, TSAI et al. 2004), and trophological (PINKINA & PINKIN 2019, STADNYCHENKO et al. 2020). However, the features of respiration in the vicarious genetic allo-species of *Planorbarius corneus* (Linnaeus, 1758) sensu lato have not been well studied. These allospecies of *P. corneus* sensu lato are hydrobionts with two means of respiration; as pulmonates, they obtain their oxygen both through the lungs (atmospheric air oxygen), and through the epidermis from oxygen dissolved in the water.

The species is typically widespread in Ukrainian water networks and are poorly studied concerning the impact of heavy metal ions. The input and level of accumulation of heavy metal ions in these molluscs are driven both by hydrochemical features of their environment and their own physiological and genetic statuses (OROS & GOMOIU 2010, BABYCH & PINKINA 2021). The degree of impairment is related to the toxicant dose received, being linearly dependent on the value of MPC, i.e. on the concentration beyond which effects can be detected. According to our data, the MPC of Zn^{2+} for great ramshorns *P. corneus* sensu lato is 0.01 mg/L (STADNYCHENKO et al. 1996).

The aim of the present study was to perform toxicological experiments and to investigate the level of impact of Zn^{2+} in different concentrations on some indices of lung and cutaneous diffusive respiration, and the behavioural responses involved, in the great ramshorn snail *P. corneus* sensu lato. We also looked for any response differences between “western” and “eastern” allospecies – as well as to estimate the expediency of their usage as allospecies-bioindicators and their respiration index use as test-functions for ecological monitoring (against the background of increasing heavy metal ions contamination of some Ukrainian water bodies, particularly by zinc ions).

MATERIAL AND METHODS

The study involved 207 individuals of the “western” *P. corneus* allospecies (mean diameter of shells 26.32 ± 0.12 mm), collected by hand in the Teteriv river (right Dnipro river tributary; Zarichany village, Zhytomyr region, $50^{\circ}14'11.70''N$, $28^{\circ}38'10.70''E$) in July 2020 (Figs 1–2), and 151 individuals of the “eastern” *P. corneus* allospecies (mean diameter of shells 24.16 ± 0.05 mm), collected by hand in the Psel river (Sumy, Sumy region, $50^{\circ}53'28.10''N$, $34^{\circ}47'49.70''E$) in June 2021 (Figs 1, 3). The topicality of such a study is caused by the detection of increased Zn^{2+} level in the rivers of Polissia and Forest-Steppe Ukraine nature zones during last 1–1.5 decades (mg/L): Teteriv – 0.012–0.026, Kamianka – 0.009–0.025 (LINNIK 1999), Zheriv – 0.04 (KOTKOVA & SELEZN'OVA 2011), – which are 2–4 times more than this ion's MPC.

Allospecies were identified by their conchological features (GARBAR 2003, GARBAR & GARBAR 2006, MEZHHERIN et al. 2005). Before the main toxicological experiment, the obligatory preliminary 15-days animal acclimation for laboratory conditions was performed (following KHLEBOVICH 1981): 10 l of Zhytomyr tap water volume (t° – 20–21 $^{\circ}C$, pH – 7.4–8.3, oxygenation – 8.2–9.4 mg O_2/L), experimental snail density – 4 ind/L, with everyday renewing of environment and feeding (leaves of *Alisma plantago* L.).

The main toxicological experiment was conducted following ALEKSEYEV (1981). The $ZnCl_2 \cdot 2H_2O$ (pure for analysis) was used as a toxicant, in the concentrations corresponding to 0.5, 2, 5 and 10 times the established MPC (all the concentrations in experimental solutions were measured by the Zn^{2+}

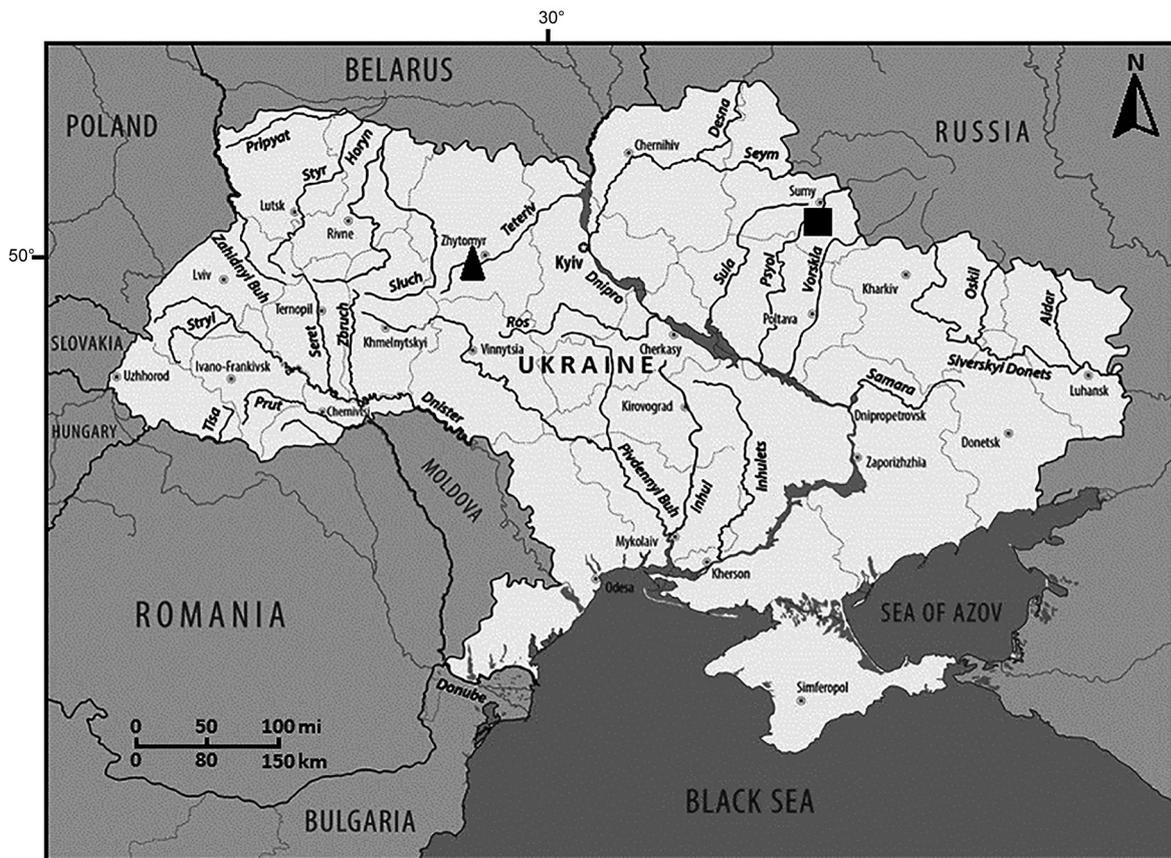


Fig. 1. Map showing the type localities of *Planorbarius corneus* sensu lato allospecies: black triangle – “western”; black square – “eastern”

cation). The salt solutions used in the experiments were prepared using aged Zhytomyr tap water. The snails were exposed to the treatments for seven days, with a change of treated water every two days. Snails used as controls were kept in aged tap water, with the same regime.

The duration of toxicological experiments was 7 days. During the first 5 days of experiment, we calculated and established such indexes of pulmonary respiration as daily number of inspirations and the duration and volume of each for “western” and “eastern” *P. corneus* sensu lato allospecies from a control group and from individuals placed in toxic environments (0.5 MPC, 2 MPC, 5 MPC, 10 MPC), via daily monitoring of the molluscs’ pulmonary respiration. During the last two days (6 and 7) of this experiment, the indirect indexes of the molluscs’ surface diffusive respiration were established for both allospecies remaining alive at the end of the experiment. These indexes were estimated indirectly, by the time surviving without access to pulmonary respiration.

The average number of inspirations was estimated by the number of times snails rose to the surface to extend their respiratory siphons to break the water’s surface tension, an action which is typically followed by an audible “clap”. The duration of “inspiration”

was considered as the time from the “clap” to the moment of individual’s return to the water deep. This was followed by the “inspiration” volume estimation: the snail was sharply pricked by a pin in the foot muscles, and the number of air bubbles, appearing from their lungs through the pneumostome, was counted; this procedure was repeated till this reaction stopped. The level of cutaneous (diffusive) respiration was estimated indirectly by the duration of signs of life when the molluscs were prevented from using the lung. To achieve this, they were confined to the bottom of the aquarium in weighted plastic boxes.

The following behavioural and physiological reactions of the molluscs (218 and 224 specimens of “western” and “eastern” allospecies, respectively) were used as testfunctions for Zn^{2+} impact: movement of experimental animals trying to avoid the toxic environment, levels of mucification of their body surface, and mortality by the end of the seventh experimental day.

Death was recorded when there was no heartbeat, and there was no response to pricking the foot with a sharp pin.

The results of experiments were analyzed using standard methods of basic variation statistics in software Statistica 6.0 (BOROVIKOV 2013).



Figs 2–3. Habitats and shells of great ramshorn *Planorbarius corneus* sensu lato allospecies: 2 – “western” (Teteriv river, Zarichany village); 3 – “eastern” (Psel river, Sumy); A – top view; B – bottom view; C – side view. Scale bars 10 mm



RESULTS

The results of examining lung function are shown in Table 1 along with survival times when only cutaneous respiration was possible. Table 2 shows the behavioural responses and survival at different concentrations. Relative to the controls, 0.5 MPC Zn²⁺ has little effect on functions, behaviour or survival. At 2.0 MPC, however, things change greatly. The number of inspirations increases, as do their duration. Perhaps anomalously, however, volume of inspiration and survival when lung breathing is prevented do not decline. Avoidance increases significantly, as does the mucification of the skin.

At 5.0 and 10 MPC, there is a further change. In effect, lung respiration is inhibited in all aspects, and survival time when prevented from using the lung also decreases. Similarly, avoidance declines, while mucification of the skin is complete. There is significant mortality, approaching 100% at an MPC of 10. During the toxicological experiment, the mortality of “western” allospecies was 14%, and the “eastern” allospecies 24.5%.

It is worth noting the differences, although slight, between the two allospecies. The eastern form appears marginally less tolerant of zinc than the western

Table 1. The impact of different Zn²⁺ concentrations on the lung and cutaneous diffusive respirations in *P. corneus* sensu lato allospecies

Toxicant concentration	n	Lung respiration			Cutaneous respiration (survival under the inability of lung respiration), hours
		Number of “inspirations” per day	Duration of “inspiration”, min	Volume of “inspiration” (number of air bubbles)	
		M±SE	M±SE	M±SE	
“Western” allospecies					
Control	51	18.05±1.29	22.10±1.14	55.01±1.00	56.38±3.10
0.5 MPC	27	19.18±1.17	24.09±0.96	57.13±3.02	58.22±2.54
2 MPC	32	26.09±2.21**	31.11±1.09**	61.91±6.11*	61.17±7.12*
5 MPC	47	12.12±1.13*	16.09±0.85*	31.12±9.18**	31.05±2.57**
10 MPC	50	9.05±0.95**	11.11±0.71**	25.09±5.39**	27.18±5.45**
“Eastern” allospecies					
Control	34	15.36±1.14	19.41±1.22	51.36±1.64	42.64±2.25
0.5 MPC	25	16.12±1.32	21.32±1.05	53.82±2.05	44.52±2.06
2 MPC	27	22.45±1.63**	27.54±1.08**	57.22±3.61*	47.13±3.78*
5 MPC	30	9.67±1.28*	13.10±0.98*	27.36±4.56**	23.34±4.11**
10 MPC	35	7.16±1.16**	9.52±0.84**	22.63±4.87**	19.44±3.47**

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<0.05); ** – highly significant differences (p<0.001).

Table 2. The impact of different Zn²⁺ concentrations on the quick behavioural and physiological reactions in *P. corneus* sensu lato allospecies

Toxicant concentration	n	Avoiding reaction, %	Cutaneous mucification, %	Death by the end of 7 days, %
		M±SE	M±SE	M±SE
“Western” allospecies				
Control	41	0	0	0
0.5 MPC	42	4.51±0.09	9.42±1.02	0
2 MPC	47	28.73±2.11**	74.51±9.27**	3.21±0.12
5 MPC	45	0.55±0.01**	100.00**	9.32±1.03**
10 MPC	43	0	100.00**	78.54±10.12**
“Eastern” allospecies				
Control	44	0	0	0
0.5 MPC	46	6.68±1.03	20.64±2.16	0
2 MPC	45	30.14±3.25**	85.45±11.02**	6.32±0.17
5 MPC	47	1.04±0.04**	100.00**	18.24±1.29**
10 MPC	42	0	100.00**	90.16±9.33**

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<0.05); ** – highly significant differences (p<0.001).

one. In both, there appears to be a two-stage response to increasing toxicity. This second, physiological protective reaction was much noticeable: the powerful mucification of epithelial body coverings, aimed at prevention of the toxicant entrance (PINKINA 2010).

DISCUSSION

As in other pulmonates, vital activity in *P. corneus* depends on the direction and intensity of redox-processes of energy supply, which is dependent on the oxygen concentration in the air, and on the amount dissolved in the water (PINKINA & PINKIN 2019, BABYCH & PINKINA 2021, UVAYEVA et al. 2022b). These molluscs need the atmospheric oxygen for lung respiration, and the dissolved oxygen for the diffusive cutaneous mode. Their lung cavity is situated between the kidney middle line and mantle transverse fold forming the bottom of their supraoccipital cavity (STAROBOGATOV 1967). The roof of lung cavity is rich in blood vessels (branching of lung vein). The lung cavity is connected with the outer environment via the movable respiratory (pneumostome) siphon at the right side of the body. It is formed by a rounded-triangular mantle fold, whose raised up edges are bent to the back side. At the basis of respiratory siphon, there is a massive secretory field, formed by the thick layer of secretory epithelium. Its mucous secretion serves not only for direct hydration of the pneumostome siphon interior side, but also for hydration of the lung cavity walls.

In the second half of the 20th century, it was shown (RÉGONDAUD 1961) that lungs of all Pulmonata are not the transformed part of their mantle cavity possessing dense blood vessels network, but a new organ with high oxygen-adsorbing surface. It allowed snails to colonise terrestrial environments, and enabled a recolonisation of water, even water with suboptimal concentrations of oxygen (UVAYEVA et al. 2022a).

The cutaneous diffusive respiration of *P. corneus* is enabled by the thin walls of the epithelium. It is worth noting that the entire respiratory surface of the body covers in *P. corneus* sensu lato is proportionately much greater than in most pulmonates because of the presence of a large leaf-like thin-walled gill placed slightly backwards from the pneumostome. Crucially, the oxygen entering the epithelium cells is utilized in the very these cells. And it has been shown that the amount of oxygen consumed from the at-

This reaction was the most intensive under the impact of Zn^{2+} in 2 MPC and above (Table 2). This was also observed earlier for great ramshorns from Hnyla river (Horodnytsia village, Ternopil region) and from Sula river (Romny, Poltava region) (UVAYEVA et al. 2022a).

mosphere is almost equal to that consumed from water (KROGH 1941, JONES 1961, STADNYCHENKO 1990, 2013, JORGE et al. 2007).

Under the impact from 0.5 and to 2 Zn^{2+} MPC, both lung and diffusive respiratory indexes of *P. corneus* sensu lato showed the development of defensive processes by enhancing all the indices considered, which increased along with toxicant concentration increasing ($p \leq 0.05-0.001$). However, under the toxicant impact in higher doses (5 and 10 MPC), the level of such a reaction appeared not enough for successful defense against the toxicant. In effect, the reaction changed to one of shutting down to minimize uptake. In the long term, such action can be considered as a depressive phase of pathological processes. Mortality increased, and asphyxia followed on from structural and functional damage of lung and cover epitheliums. This resulted from the development of a thick layer of coagulated mucus over both respiratory surfaces that was associated with initial over-hydration (enlarged cells) and subsequent collapse and death.

The behavioural changes induced at moderate concentrations of zinc are well known from other studies. Beyond such levels, the reactions change, and may be pathological unless exposure is very limited in time. While the snails showed no evident pathology at 0.5 MPC, our study, limited to one week, may underestimate the harmful effects over longer periods. This will be even more true of concentrations of MPC 2 or more. Long term survival in such conditions seems impossible. While there are significant differences in the responses of eastern and western forms ($p \leq 0.05$), they are slight when set against the magnitude of the overall response.

These responses are very clear. The indices of lung function are easily observed, and the species might therefore be used as a bio-indicator of toxicity, the more so as the species is probably capable (via lung breathing) of a greater tolerance than many other aquatic animals.

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