The growth characteristics and survival rates of great pond snail (*Lymnaea stagnalis* L.) juvenile under the heavy metal impact

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Abstract. The study of the long-term effects of toxic substances, in particular, heavy metals, on aquatic organisms is currently a topical issue, due to the increase of anthropogenic pressure on the hydrosphere. The embryonic and juvenile stages of mollusks are more sensitive to toxic effects than adult ones. Consequently, the effects of different concentrations of heavy metal in the aquatic environment on the growth and survival rates of *Lymnaea stagnalis* juveniles were evaluated. In a chronic toxicological experiment, we used chronic lethal, sublethal, and subthreshold concentrations of heavy metals. We conducted 12 toxicological chronic experiments, lasting 60 days, with 1070 specimens of *L. stagnalis* juveniles. The toxic effect of heavy metals exposure in the aquatic environment in young individuals is manifested at much lower concentrations than in adults. The heavy metals ions affect the shell height of *L. stagnalis* juveniles even at the embryonic stage, which is manifested in some cases in the acceleration of their growth, in others – in its slowing down. The chronic experiment indicated that the values of shell height growth of juveniles in most cases correlate with the values of the total body weight growth. Under the influence of sublethal concentrations of heavy metal ions, the survival rates of juveniles are 3–4 times lower than the control. In solutions containing chronic lethal concentrations of Co$^{2+}$ and Mn$^{2+}$, up to 80–90% of juveniles die. The sharper and more rapid response of young snails to toxic effects compared to adult animals can be explained by embryo intoxication.

1. Introduction
In recent decades, anthropogenic factors have accelerated the cycle of geochemical elements, resulting in increasing metal imports into water systems [1,2]. The heavy metals such as Copper (Cu), Cadmium (Cd), Zink (Zn), Cobalt (Co), Manganese (Mn), and Nickel (Ni) are potentially hazardous in combined or elemental forms [3,4]. Most are on the U.S. Environmental Protection Agency’s list of priority metals for monitoring and assessing their harmful effects on aquatic ecosystems [5–8]. Since heavy metals chlorates are highly soluble in water, they are readily absorbed by living organisms [9] and accumulate in the gills, liver, and muscle tissues of various species in a contaminated water ecosystem [10]. Drinking water contaminated with heavy metals becomes one of the main problems for human health [11–13].

Snails are among the invertebrates that could accumulate significant quantities of heavy metals in their tissues, making them suitable test animals for researching the kinetics of metal accumulation and detoxification [7,14–17]. Understanding how metals influence freshwater snails
can help scientists better comprehend the ecotoxicological impact of metal contamination on snails' populations and design water-quality guidelines that sufficiently safeguard protect water organisms \[18–21\]. According to several studies, freshwater snails' early life stages have been found to be among the most vulnerable aquatic organisms to inorganic pollutants, including heavy metals \[22–25\]. Thus, Wang et al. \[26\] found that juvenile freshwater mussels are more sensitive to acute and chronic copper exposure than most other freshwater organisms tested, such as cladocerans \(Daphnia magna\) and \(Ceriodaphnia dubia\), an amphipod \(Hyalella azteca\), fathead minnow \(Pimephales promelas\), and rainbow trout \(Oncorhynchus mykiss\). Therefore, young individuals of freshwater mollusks were used for our study.

A rising number of studies have been published that measure the effects of known lethal concentrations on aquatic organisms \[27–30\]. The study of acute poisoning of aquatic species with the survival rate as the primary criterion is the main focus of researchers \[31–34\]. Growth processes are quite sensitive to the slightest changes in any environmental factor, so the size and mass characteristics of animals placed in a poisoned environment are among the parameters that characterize the body's response to changes in water quality \[35,36\]. The freshwater pulmonates are one of the most commonly used taxonomic groups for toxic experiments because acute effects may be observed within a very short time \[37,38\]. Although chemical spills and other point sources of toxins can cause localized mortality in aquatic animals, freshwater snail populations may be declining as a result of the pervasive impacts of chronic, low-level contamination \(\text{Strayer et al., 2004}\). A number of research have examined the acute toxicity of metals to freshwater mussels, but little is known about the sub-lethal effects of long-term exposure to low-level, environmentally relevant concentrations \[24\]. Recently, it was shown that the great pond snail is either the most sensitive or the second-most sensitive freshwater animal in chronic exposures to Co, Cu, Ni, and Pb, making it particularly important for the creation of future water quality requirements \[39–43\].

2. Research aim and objectives

\textit{Lymnaea stagnalis} was chosen for this study because of its widespread prevalence throughout Ukraine, its sensitivity to heavy metal impact, and its success in laboratory culture \[44,45\]. We studied the features of heavy metals chronic exposure effect on the size-weight characteristics and survival rate of juvenile great pond snails.

3. Material and methods

3.1. Experimental animals

Juvenile great pond snails \(Lymnaea stagnalis\), Linnaeus, 1758) were obtained from the clutches (eggs) of adult snails, which were placed in solutions of heavy metal chlorides and kept for 70 days in laboratory culture. In three years, 12 toxicological chronic experiments lasting 60 days, were performed, in which 1070 young specimens of great pond snail were used.

3.2. Chronic toxicity tests

Toxicological tests were carried out using the Alekseev method, which was developed by Stadnichenko \[46\]. Chlorides of 6 heavy metals (copper, cadmium, nickel, zinc, cobalt, manganese) were used as toxicants. The content of toxic substances was calculated by cation. \textit{L. stagnalis} juveniles placed in clean (non-toxic) water were controls. The water temperature was measured three times a day. The optimum temperature (19–23°C) was maintained due to the periodic ventilation of the room. The illumination close to that the snails are used in natural reservoirs was created, as well as the daily rhythms of activity (respiratory, nutritional), for this purpose the indicators were taken at regular intervals.

The concentration ranges for the toxicological studies were chosen according to a fishery-toxicological approach, which distinguishes four concentrations (table 1). In the chronic
In a chronic toxicological experiment, we used chronic lethal, sublethal and subthreshold concentrations of heavy metals. The range of acute lethal concentrations (as well as other ranges) was determined by us empirically, but it is not discussed in the text of the article due to the short duration of the molluscs’ survival in this range (3-5 days). And in this study, a chronic experiment was set up.

3.3. Determining the dynamics of changes in the size-weight characteristics and survival rates of mollusks
The size and weight measurements (shell height, body weight) of juvenile mollusks were taken every 10 days. The weight of mollusks was determined by direct weighing (weight VLR-200) with an accuracy of 0.0005 g. The height of the shell was determined using a micrometer (accurate to 0.01 mm). The survival rate was determined by counting (every 10 days) the surviving individuals and comparing this number with the total number of individuals taken into the experiment at the beginning.

3.4. Statistical methods
The concentrations measurements were log-transformed. The normality of the sample’s distribution was determined using the Kolmogorov-Smirnov test. The student’s t-test was used to assess the significance of the differences between the two mean values of the samples in case of normal distribution. Wilcoxon Matched Pairs Test was used to test for differences between samples if they are not normally distributed. Equality of variances was tested by ANOVA. The regression analysis was carried out to determine the dependencies between body weight and shell height. The significance level of 5% was accepted in the study. The difference between the means was considered probable if $p < 0.05$. The statistical analysis was performed by Statistica 10 software.

4. Results and discussion
It is known that juvenile gastropods are much more sensitive to heavy metal impact than adults [47, 48], although it would be more appropriate to speak not about sensitivity, but about a much higher dose load that juveniles receive at the same concentration (due to the difference in biomass) compared to adults. That is why the toxic effects of these pollutants are manifested in young individuals at much lower concentrations. The influence of heavy metal ions on the shell height of juvenile great pond snails during hatching suggests that mollusks perceive toxic effects at the embryonic stage, which is manifested in the lower average shell height of mollusks hatched.
in metal solutions compared to the control (table 2). Shell height distribution is not normal for all metals. The differences in shell height compared to the control values are significant for Cobalt ($Z = 4.2, T = 49.5, p < 0.05$), Copper ($Z = 2.11, T = 84, p < 0.05$), Zinc ($Z = 2.3, T = 2515, p < 0.05$), Nickel ($T = 2.0, Z = 3.2, p < 0.05$).

**Table 2.** The shell height of juvenile mollusks (mm) at the moment of hatching.

| Heavy metal ion | N  | Mean ± Stand. Error | Minimum | Maximum | Std.Dev. | Coef.Var |
|-----------------|----|---------------------|---------|---------|----------|----------|
| Cd$^{2+}$       | 86 | 1.47±0.017          | 1.16    | 1.78    | 0.16     | 10.97    |
| Co$^{2+}$       | 34 | 1.38±0.019          | 1.11    | 1.62    | 0.11     | 8.08     |
| Cu$^{2+}$       | 26 | 1.48±0.018          | 1.30    | 1.70    | 0.10     | 6.29     |
| Mn$^{2+}$       | 43 | 1.53±0.017          | 1.28    | 1.75    | 0.12     | 7.70     |
| Zn$^{2+}$       | 88 | 1.50±0.015          | 1.24    | 1.86    | 0.14     | 9.58     |
| Ni$^{2+}$       | 14 | 1.32±0.044          | 1.11    | 1.63    | 0.17     | 12.68    |
| Control         | 41 | 1.56±0.022          | 1.10    | 1.80    | 0.14     | 9.07     |

The largest range of variation and the lowest average shell height have juveniles mollusks that were kept in different concentrations of Nickel ions (CV = 12.7%), which indicates that this metal ions significantly affects the embryonic development of the gastropod pond snail [49]. In addition, the freshwater pulmonate snail, *Lymnaea stagnalis*, is believed to be the most sensitive aquatic organism tested to date on Ni [41]. According to the ANOVA results, the shell height of juvenile great pond snails is significantly dependent on the concentrations of all heavy metals studied ($p < 0.05$). The effect of different concentrations of heavy metal ions on the shell height is manifested in some cases in accelerating their growth, in others - in slowing it down (figure 1).

**Figure 1.** Influence of heavy metal ions on shell height (mm) of juvenile *L. stagnalis* at the moment of hatching. * – differences compared to the control values are significant.
Copper ions at subthreshold concentrations did not affect the shell height of newborn mollusks; however, at sublethal and subthreshold concentrations of copper ions, the average values of shell height were statistically significantly higher than those of the control. The average shell height of juvenile snails at hatching is significantly less than the control ones only when exposed to cadmium ions at chronic lethal concentrations ($p < 0.05$). Under the influence of nickel ions at sublethal concentrations, the size of the embryos is significantly larger ($p < 0.05$) than in the control group. In the remaining studied concentrations of Ni$^{2+}$ the values of the shell height are significantly lower than the control values ($p < 0.05$).

Under the influence of zinc and manganese ions, the shell height of juveniles at the moment of hatching is statistically significantly higher than the control values. And under the influence of cobalt ions, juvenile shell height decreases with increasing concentration of the solution, which indicates an increase in toxic effects with increasing Co$^{2+}$ concentration in the medium (figure 1).

Heavy metals’ impact on snails’ survival is well documented, but their sublethal effects on the growth process are gaining traction [28]. When studying the size-weight characteristics of juvenile mollusks during prolonged (60 days) stay in a medium poisoned with heavy metals, it was found that, as in adult mollusks [50], the shell height growth rate of juvenile mollusks in most cases correlates with the growth of total body weight. Changes in the shell height of juveniles under long-term toxic exposure generally repeat the dynamics of changes in the same parameter in embryos, but there is a clear tendency to a decrease in the growth rate of juvenile great pond snails in the toxic environment (figure 2, 3).

![Figure 2. Increase in shell height (growth) of juvenile L. stagnalis during the 60-day experiment, mm. * – differences compared to the control values are significant.](image)

All the average increases in shell height of juveniles obtained in the experiments are lower than the control values, except for the indicators obtained in solutions of sublethal concentrations of Ni$^{2+}$ and subthreshold and sublethal concentrations of Mn$^{2+}$ (figure 2). Inhibition of shell growth by heavy metals can be explained by the juvenile-snail development suppression was a particularly sensitive end-point, possibly due to Ca homeostasis disruption [51]. Since, *L. stagnalis* need a lot of calcium to keep up with its quick shell creation and rapid growth rates
in the early stages of its life (around 20 percent of body mass per day) [40].

The water balance in the body of juvenile great pond snails at first becomes positive when the concentration of heavy metals in the environment increases (which probably allows at least to some extent to compensate for the toxic effect), but gradually and smoothly shifts to the negative side as the concentration of the toxicant increases [52]. It can be assumed that dehydration of young animals at chronic lethal concentrations of pollutants subsequently leads to their death.

In most of the studied solutions containing heavy metals, under the influence of subthreshold and sublethal concentrations there is no statistically significant difference in weight from control values \( (p < 0.05) \), which indicates a certain resistance of these indicator to toxic effects (Fig 3). However, with increasing concentrations of heavy metals, there is an increase in intoxication, which is reflected in the size-weight values. In chronic lethal concentrations, deviations from the weight of control animals can, in some cases, reach 49%, and in shell height, up to 30%.

![Figure 3. Influence of heavy metal ions on the total body weight (mg) of juvenile *L. stagnalis*.](image)

* \( \) – differences compared to the control values are significant.

The juvenile pond snails are the most sensitive to the influence of Cadmium ions in terms of size-weight parameters. In solutions with \( \text{Cd}^{2+} \), the values of these indicators are the lowest among all those obtained under the influence of the other five studied metals (figure 3).

Individual survival has been discovered to be one of the criteria for the organism’s stability, which is considered in time [53]. When studying the survival of juvenile pond snails exposed to heavy metals, we found that they do not respond to intoxication in the same way that adults do [50]. In adult pond snails there is a stimulation of vital functions under the influence of sublethal concentrations of pollutants, and the values of snails’ survival in solutions of subthreshold concentrations are very close to control values, which suggests that they are immune to toxic effects. Heavy metal ions greatly affect the survival of juveniles. The percentage of juvenile specimens that survived in a toxic environment during the chronic (60 days) experiment is always below the control values, regardless of the concentration of pollutants in the environment. Only when juveniles are exposed to sublethal concentrations of \( \text{Ni}^{2+} \) and subthreshold concentrations of \( \text{Mn}^{2+} \) do survival rates approach 50%, and exposed to other metals, juvenile survival rates are 3–4 times lower than control values in some cases.
The presence of juveniles in solutions of chronic lethal concentrations of heavy metals leads to persistent suppression of their viability, which is manifested in high values of snails’ death. At the same time, a paradoxical situation arises: juveniles react less strongly to those metals that were diagnosed as highly toxic in the acute experiment and more strongly to those that were determined to be slightly toxic [3]. Thus, in solutions of chronic lethal concentrations of Co\(^{2+}\) and Mn\(^{2+}\), up to 80–90% of juveniles die (figure 4).

**Figure 4.** Influence of heavy metal ions on the survival rates (%) of juvenile *L. stagnalis* in the first 30 days of life. * – differences compared to the control values are significant.

A possible explanation for this phenomenon is the following: when a juvenile organism is exposed to highly toxic substances, it immediately experiences severe stress, reparation mechanisms are activated, and this makes it possible to resist the toxic effects to some extent [54–56]. In the case of prolonged exposure to low-toxic substances intoxication of organisms increases slowly, the body initially reacts less rapidly, untimely adaptation mechanisms and slowly increasing negative changes in the functioning of body systems make it unable to resist the toxic effects of heavy metals [57–59]. Under the influence of Zn\(^{2+}\), which has a moderately toxic effect on great pond snails, the survival rates of juveniles in solutions of all its concentrations differ little from each other and range from 20–25% (figure 4).

Thus, the study of the shell height and body weight of juvenile *L. stagnalis* under the influence of heavy metal ions in the aquatic environment showed that the determining factor for changing these parameters is time of exposure. In the first 30 days of the experiment, regardless of the solution concentration and the nature of the metal ion, there were no statistically significant differences in changes in shell height and body weight of great pond snail juveniles compared to control specimens. Only when the animals are kept in a poisoned environment for a longer period of time do the size and weight parameters undergo significant changes in relation to the control values. The dynamics of changes in the shell height and weight of juveniles within a toxic environment indicate that juvenile mollusks are extremely sensitive to heavy metals impact. In general, the size-weight indices decrease with the increasing concentration of pollutants.

Biomonitoring of water bodies is the use of aquatic species, to measure the extent of human influence on aquatic ecological balance. Our research confirmed that the early juvenile stages of *Lymnaea stagnalis* are extremely sensitive bioindicators, allowing even very low levels of
the heavy metals to be detected. The quantitative characteristics of the growth and survival rates of the lake pond juveniles reflect the toxicological situation of the environment and can be recommended as biological criteria of toxicity in biotesting in the system of environmental monitoring of natural water pollution by heavy metal ions.

5. Conclusion
The influence of heavy metal ions on the shell height of juvenile great pond snails at the moment of hatching suggests that mollusks perceive toxic effects at the embryonic stage, which is manifested in the lower average shell height of mollusks hatched in metal solutions compared to the control. The largest range of variation and the lowest average shell height have newborn mollusks that were kept in different concentrations of Nickel ions. Although, in terms of shell height and weight growth, the juvenile pond snails showed the greatest vulnerability to the influence of Cadmium ions during the chronic experiment. In solutions with Cd\(^{2+}\), the values of these indicators are the lowest among all those obtained under the influence of the other five studied metals. Under the influence of sublethal concentrations of heavy metal ions, the survival rates of juveniles are 3-4 times lower than the control. In solutions containing chronic lethal concentrations of Co\(^{2+}\) and Mn\(^{2+}\), up to 80-90% of juveniles die. As a result, it is possible to suggest that poisoning already affects mollusk embryos, as evidenced by the fact that juvenile great pond snails react more sensitively to the impact of the studied pollutants than adult mollusks. The growth and survival rates of L. stagnalis juveniles reflect the environment’s toxicological state and can be considered as biological toxicity criteria in biotesting of natural water pollution by heavy metals.

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