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Chronic effect of low urea concentration on the content of carotenoid pigments in *Lymnaea stagnalis*

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Abstract. Urea is one of the most common nitrogen-containing pollutants in freshwater ecosystems and, if it enters water bodies for a long time, can disrupt the course of metabolic processes in aquatic organisms. Since carotenoids serve as non-enzymatic antioxidants, changes in their content reflect the development of oxidative stress and serve as sensitive biochemical markers of toxic effects. The purpose of the study was to find out the features of the effect of low urea concentration on the content of β-carotene and xanthophylls in the haemolymph, hepatopancreas, mantle, and foot of *Lymnaea stagnalis*. The study was conducted on sexually mature uninfected mollusks that were kept in an environment with urea at a concentration equivalent to twice the maximum permissible concentration for water bodies used for fisheries purposes (2 $MPC_{fisheries}$), for 2, 7, 14, and 21 days. The carotenoid content was determined spectrophotometrically, and the results were processed using two-factor analysis of variance and correlation. It was found that with short-term urea exposure (2 days), the content of β-carotene in the mantle and foot of *L. stagnalis* increased by 1.93-2.88 times ($p < 0.01$-0.05). The content of xanthophylls in these organs significantly decreased by 50.73-56.68%, while no significant differences were found in hepatopancreas. An increase in the duration of exposure to 14-21 days was accompanied by a systemic decrease in the content of β-carotene (by 25.94-69.24%; $p < 0.001$-0.05) and xanthophylls (by 38.89-88.41%) in the hepatopancreas, mantle and foot of *L. stagnalis*, which is probably conditioned by increased pro-oxidant processes and the use of carotenoids in free radical neutralisation reactions. Correlation analysis revealed a statistically significant negative association between urea exposure and β-carotene and xanthophyll content, which indicates depletion of the carotenoid reserve under toxic load. Consequently, chronic exposure to urea in the studied concentration causes tissue-specific changes in the carotenoid profile of *L. stagnalis*, which reflect the transition from compensatory-adaptive reactions to depletion of the non-enzymatic link of the antioxidant system. The results obtained can be used by environmental and fisheries organisations for ecotoxicological assessment of the impact of nitrogen-containing pollutants on freshwater ecosystems, and the content of β-carotene and xanthophylls – as an informative biochemical marker of such effects

Keywords: freshwater shellfish; β-carotene; xanthophylls; oxidative stress; nitrogen-containing compounds

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INTRODUCTION

One of the most pressing environmental issues is anthropogenic pollution of aquatic ecosystems with nitrogen-containing compounds entering reservoirs with industrial and household wastewater, and due to the widespread use of mineral fertilisers in agriculture and fish farming. The accumulation of these substances in the aquatic environment is accompanied by a change in its physico-chemical properties, deterioration of the conditions of existence of aquatic organisms, which leads to the restructuring of physiological and biochemical processes in the body, changes in the limits of their tolerance, and, in general, can negatively affect the functioning of aquatic ecosystems. Under such conditions, the search for informative biochemical parameters that can reflect the early compensatory and adaptive reactions of the body to the action of toxicants even before the appearance of pronounced morphofunctional disorders becomes particularly relevant.

The most common nitrogen-containing fertilisers include urea, which is widely used in agriculture and fisheries both in its pure form and as part of complex mineral fertilisers for fertilising water bodies and stimulating the development of a natural feed base. At natural concentrations, urea plays an important role in the functioning of hydroecosystems, participating in the processes of the nitrogen cycle, and as indicated by N. Arandia-Gorostidi *et al*. (2024), serves as an accessible source of this element for microorganisms and is involved in the processes of its transformation and assimilation. However, excessive intake of this compound in water bodies is accompanied by changes in the physical and chemical properties of the aquatic environment, disruption of biological processes and the development of toxic effects in aquatic organisms of various systematic groups. Due to its high solubility in water and ability to easily penetrate biological membranes, urea quickly enters the tissues of aquatic organisms and is involved in physiological and biochemical processes in the body.

According to A. Ojha *et al*. (2025), urea toxicity may be enhanced by the action of the urease enzyme, which, due to its high affinity for the substrate, catalyses its hydrolysis to form ammonia (NH_3) and carbon dioxide (CO_2) even at low concentrations in the medium. In conditions of excessive urea intake, its hydrolysis is accompanied by increased synthesis of ammonia, which increases the toxic load on aquatic organisms – both due to its accumulation in the aquatic environment and through its synthesis directly in the body. Summarising current data, S. Yun *et al*. (2026) concluded that the toxic effects of ammonia on aquatic organisms are accompanied by impaired energy metabolism, osmoregulation, immune response, and the development of oxidative stress. In this case, the severity of toxic effects is determined by the concentration of the toxicant, the duration of exposure, the parameters of the aquatic environment, and the specific features of aquatic organisms. Experimental studies by Y. Zou *et al*. (2023) showed that the action of NH_3 in fish is accompanied by inhibition of growth rates, impaired energy metabolism, ion homeostasis, and induction of oxidative stress due to excessive development of reactive oxygen species, in particular, superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxyl ($ROO^\bullet$), and hydroxyl ($^\bullet OH$) radicals. The consequence of this is changes in the antioxidant status, activation of inflammatory processes, and violation of physiological homeostasis. Similar patterns have been established for mollusks, in which, according to M. Cong *et al*. (2021) and H. Ge *et al*. (2021), the effect of ammonia is accompanied by a violation of the functioning of the antioxidant system, changes in nitrogen detoxification processes, the development of oxidative stress and structural tissue damage, and the intensity of these changes depends on the level of toxic load.

One of the first compensatory and adaptive responses of the cell to excessive development of reactive oxygen species is the activation of the antioxidant system, which is represented by enzymatic and non-enzymatic links and functions as a single integrated protection system, ensuring the maintenance of pro-oxidant-antioxidant balance, neutralisation of free radicals and protection of the cell from oxidative damage. An important place among the components of the non-enzymatic antioxidant system is occupied by carotenoids, which, as indicated by C. Zhuang *et al*. (2022) and K. Lim *et al*. (2023), effectively neutralise reactive oxygen species, inhibit the processes of lipid peroxidation, maintain the antioxidant status of the body, and promote the adaptation of aquatic organisms to the action of stress factors. Of particular interest are β-carotene and xanthophylls, which, according to X. Hu *et al*. (2025), differ in physical and chemical properties and features of interaction with cellular structures. M. Zbyradowski *et al*. (2022) showed that β-carotene performs a dual function, because it is a precursor of vitamin A and is involved in the antioxidant protection of cells. M. Pasenkiewicz-Gierula *et al*. (2024) noted that xanthophylls, in addition to their antioxidant properties, are characterised by greater polarity and interaction features with biological membranes due to the presence of oxygen-containing functional groups.

Despite a significant number of studies on the enzymatic antioxidant system of hydrobionts, the role of its non-enzymatic link, in particular carotenoids, remains insufficiently elucidated. This is especially true for freshwater mollusks, for which information on tissue-specific changes in the content of β-carotene and xanthophylls with prolonged exposure to nitrogen-containing toxicants is limited. In previous studies, G. Kyrychuk (2022) found that exposure to high concentrations of urea causes tissue-and time-dependent changes in the content of xanthophylls in the body of freshwater mollusks *L. stagnalis*. However, the characteristics of the response of the carotenoid component of the antioxidant system to exposure to urea at a concentration of 2 $MPC_{fisheries}$, corresponding to a moderate level of toxic load, remain insufficiently investigated, which prompted the present study. The purpose of this study was to investigate the features of the effect of urea at a concentration of 2 $MPC_{fisheries}$ (exposure – 2, 7, 14, and 21 days) on the content of β-carotene and xanthophylls in *Lymnaea stagnalis*.

MATERIALS AND METHODS

The object of the study was sexually mature individuals of the common pond snail *Lymnaea stagnalis* (Linnaeus, 1758). Before starting the experiment, the mollusks were acclimated to laboratory conditions for 14 days in aquariums with aerated tap water settled during the day at stable hydrochemical parameters (t = 18-20°C, pH = 7.3-7.7, dissolved oxygen content = 8.5-8.9 mg/dm^3). Urea at a concentration of 2 $MPC_{fisheries}$ (160 mg/dm^3; $MPC_{fisheries}$ = 80 mg/dm^3) was used to model the chronic toxic load. Solutions were replaced daily with freshly prepared ones with the restoration of the initial toxicant concentration, which ensured the constancy of experimental conditions and minimised urea hydrolysis under the action of bacterial microflora. The control group was kept in dechlorinated water without the addition of urea. The duration of exposure to the experiment was 2, 7, 14, and 21 days. The total sample size was 80 individuals of *Lymnaea stagnalis* (10 individuals in the control and experimental groups for each exposure period; n = 10).

For biochemical analysis, haemolymph was selected using a modified targeting method, after which the hepatopancreas, mantle, and foot were prepared. To exclude the influence of trematode infection on the parameters studied, only uninfected individuals were selected for the study. To determine the content of β-carotene and xanthophylls, tissue samples were pre-homogenised, lipid saponification was performed, and then carotenoids were extracted with hexane in a ratio of 1:4. The quantitative content of pigments was determined spectrophotometrically. The content of β-carotene and xanthophylls was expressed in mg/g of raw tissue. A total of 640 detections of β-carotene and xanthophyll were performed. All measurements were conducted in three analytical repetitions, the average value of which was used for further statistical analysis.

Statistical processing of the obtained results was performed using variational statistics methods using Statistica 10.0 software suite. The results are presented as an average (M) with a 95% confidence interval (95% CI). To assess the effect of "toxic effects" and "duration of exposure" factors on the content of β-carotene and xanthophylls in the tissues of *Lymnaea stagnalis*, two-way analysis of variance (Two-way ANOVA) was used to assess the effect of factor interaction. The Tukey HSD test was used for multiple comparison of mean values between groups. Correlation analysis with calculation of the Pearson correlation coefficient (r) was used to assess the strength and direction of the relationship between the toxic effect factor, the duration of exposure, and the content of β-carotene and xanthophylls in the studied tissues (organs). Differences in $p < 0.05$ were considered statistically significant. All studies with experimental animals were performed in accordance with generally accepted international and national bioethical principles for handling aquatic organisms and objects of biological research. The study was conducted in accordance with the ethical standards of Convention on Biological Diversity (1992) and Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

It was found that the effect of urea at a concentration of 2 $MPC_{fisheries}$ caused a restructuring of the carotenoid profile in *L. stagnalis*, which reflects a consistent transition from compensatory and adaptive responses to depletion of the antioxidant defence system. Two-way analysis of variance revealed a statistically significant interaction of the factors "toxic effect" and "duration of exposure" for all the studied tissues, which indicates the complex nature of the mollusk body's response to the toxicant. Presumably, the detected changes in the carotenoid profile are conditioned by the combined action of the studied factors, when with an increase in the duration of exposure, even a moderate concentration of the toxicant gradually leads to a restructuring of metabolism in the body of *L. stagnalis*. Such a response is characteristic of chronic toxic effects, in which the biological effect is determined not only by the concentration of the toxicant, but also by the duration of its action.

In the control group of mollusks, a gradual and stable increase in the content of β-carotene was established during the entire experimental period (2, 7, 14, and 21 days). The most intense accumulation of pigment was recorded in the hepatopancreas and foot (5.8-6.4 times), which may indicate the development of an effective antioxidant reserve under the conditions of the physiological norm. A similar tendency to increase the content was observed for xanthophylls, but the intensity of their accumulation was lower compared to the indicators obtained for β-carotene. The highest values were observed in the hepatopancreas, mantle, and foot of *L. stagnalis*, while in their haemolymph, the values remained relatively low, which is consistent with the transport function of the tissue and the absence of pronounced carotenoid deposition. The highest levels of β-carotene and xanthophylls in hepatopancreas may be associated with the leading role of the organ in metabolism, nutrient deposition, and maintenance of antioxidant homeostasis, while the growth of their content in the foot may be conditioned by the high energy costs of the organ that provides motor activity.

Such accumulation of carotenoids in the control group may be associated not only with the adaptation of shellfish to laboratory conditions, but also with the stabilisation of metabolic processes in the absence of an additional toxic load. As shown by L. Zhang *et al*. (2023) and Y. Liao *et al*. (2025), carotenoids are not synthesised by most animals *de novo*, and come with feed and accumulate mainly in tissues that perform deposition functions or are characterised by high metabolic activity. Given this, a gradual increase in their content in the hepatopancreas and foot of mollusks can be considered as a physiological process of forming an antioxidant reserve, which, according to R. Jia (2025), is an important component of maintaining the antioxidant status of the body and the normal course of redox processes. It was found that for the action of urea in a concentration that was responsible for 2 $MPC_{fisheries}$, the nature of changes in both groups of carotenoids acquired pronounced tissue and temporal specificity. Thus, with the short-term action of urea (2 days), the content of β-carotene increased in the mantle and foot of mollusks by 1.93-2.88 times ($p < 0.01$-0.05), which may indicate early activation of the non-enzymatic antioxidant system in response to pro-oxidant exposure (Fig. 1). The rapid accumulation of β-carotene in the mantle and foot of *L. stagnalis* with short-term exposure (2 days) cannot be explained by its synthesis *de novo* because shellfish lack this metabolic ability. There is a stress-induced redistribution of the endogenous pigment pool. It is likely that the primary toxic stress stimulates the mobilisation of carotenoid reserves from the main storage organ – the hepatopancreas – and their directed transport via the haemolymph to barrier tissues (the mantle), which are in direct contact with the toxic environment, and to metabolically active locomotor tissues (the foot), thereby maintaining their functional integrity under oxidative stress. The probability of such a mechanism is consistent with current ideas about the depository function of mollusk hepatopancreas and the transport role of their haemolymph (Song *et al*., 2025).

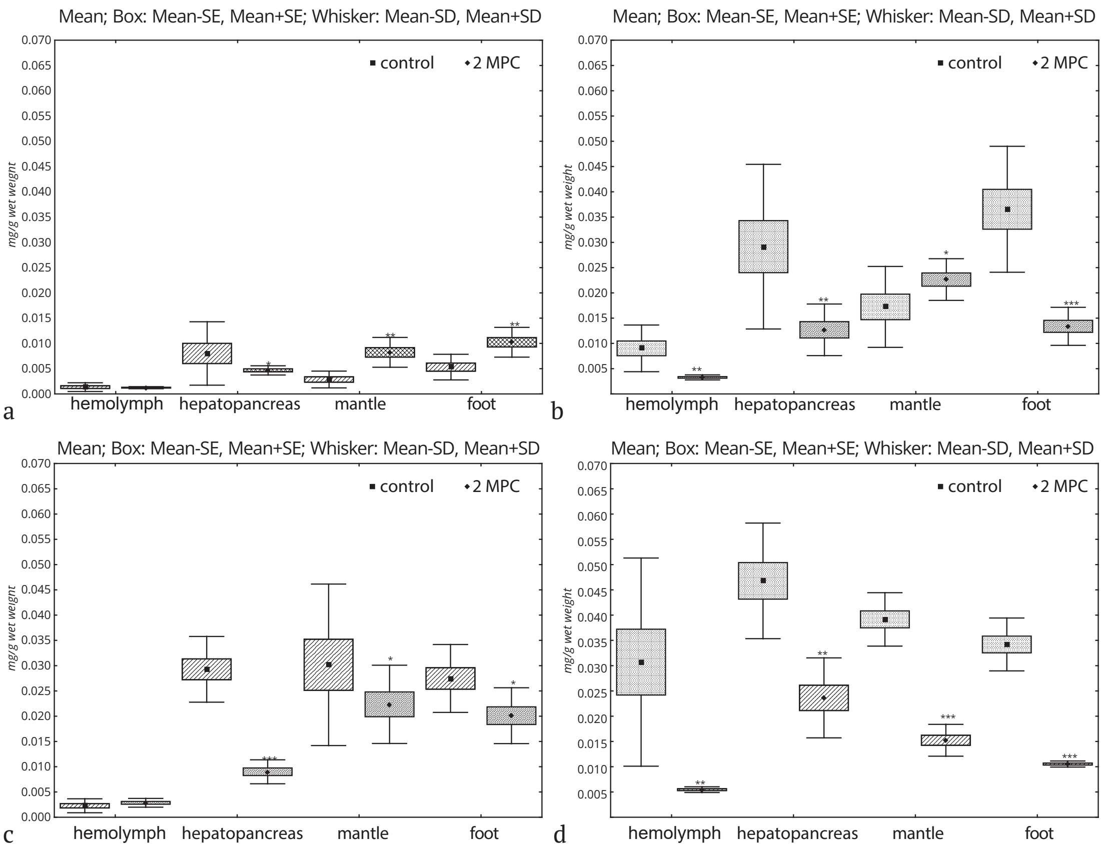

Figure 1. Effect of urea on the content of β-carotene in *L. stagnalis*

Note: A – exposure of 2 days; B – exposure of 7 days; C – exposure of 14 days; D – exposure of 21 days); n = 10 for each experiment variant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Tukey test)
Source: compiled by the authors

Prolonging the action of urea for up to 7 days increased its inhibitory effect on *L. stagnalis*, which was accompanied by a decrease in the content of β-carotene in the haemolymph, hepatopancreas, and foot by 56.45-63.71% compared to the control. The mantle showed an increase in pigment levels by 31.43%, which probably reflects the preservation of the local compensatory response of the tissue directly in contact with the environment. In general, the results obtained indicate a gradual transition in the body of *L. stagnalis* from the compensation stage to the depletion of antioxidant protection, which is probably due to the intensive use of β-carotene to neutralise reactive oxygen species and maintain the redox balance of the cell. This interpretation is consistent with current ideas about the functional role of carotenoids under oxidative stress. In particular, C. Zhuang *et al*. (2022) showed their active involvement in the neutralisation of reactive oxygen species, whereas K. Lim *et al*. (2023) and X. Hu *et al*. (2025) consider these compounds as an important component of the non-enzymatic antioxidant system that ensures the maintenance of cellular redox homeostasis.

With an increase in the duration of exposure to 14 days, there was a decrease in the content of β-carotene in the hepatopancreas, mantle, and foot of mollusks by 25.94-69.24% ($p < 0.001$-0.05) and a simultaneous increase in its level in the haemolymph by 25.69%. This multidirectional dynamics probably reflects the mobilisation of carotenoids from depot tissues and their entry into the haemolymph for further redistribution between organs in order to maintain systemic antioxidant protection under prolonged toxic stress.

Furthermore, a decrease in the content of β-carotene in hepatopancreas may indicate an increased functional load on this organ, which, as noted by T. Zhang *et al*. (2024), plays a leading role in the metabolism, detoxification, and antioxidant protection of shellfish. According to Y. Liao *et al*. (2025), carotenoids accumulated in the body of aquatic animals can be redistributed between tissues according to their metabolic needs and functional state. Therefore, an increase in the content of β-carotene in the haemolymph on the day 14 of exposure can be considered as a manifestation of activation of inter-organ transport of β-carotene, aimed at maintaining the circulating pigment pool at the compensation stage.

Under conditions of prolonged exposure to urea (21 days), a systemic decrease in the content of β-carotene in all studied tissues and organs was established at 49.49-82.19% ($p < 0.001$-0.05). Such dynamics probably indicate the depletion of the carotenoid link of the antioxidant system and a decrease in the compensatory and adaptive capabilities of mollusks, because, as noted by K. Lim *et al*. (2023) and X. Hu *et al*. (2025), with prolonged stress exposure, intense involvement of carotenoids in maintaining homeostasis may be accompanied by a gradual depletion of their tissue reserves. The smallest deviation from the control was recorded in the hepatopancreas, and the largest – in the haemolymph, which may reflect the relative preservation of the depository function of the hepatopancreas and depletion of the circulating pool of β-carotene. According to the severity of changes, the studied tissues were arranged in the following sequence: hepatopancreas → mantle → foot → haemolymph.

As for xanthophylls, with the short-term action of urea (2 days), their content increased by 73.09% ($p < 0.05$) in the haemolymph, while no statistically significant differences were found in hepatopancreas compared to the control (Fig. 2). A significant decrease in the indicator by 50.73-56.68%, respectively ($p < 0.01$-0.05), was recorded in the foot and mantle. This nature of changes indicates that even at an early stage of toxic effects, xanthophylls are intensively used in peripheral tissues that are directly in contact with the environment or are characterised by high functional activity. A similar role of xanthophylls as membrane antioxidants, which are the first to be involved in protecting cells from lipid peroxidation, was noted by K. Lim *et al*. (2023) and X. Hu *et al*. (2025). Prolongation of urea action for up to 7 days was accompanied by a subsequent violation of carotenoid metabolism and a decrease in the content of xanthophylls in most of the studied tissues. In the haemolymph of mollusks, there was a tendency to decrease by 20.06% ($p > 0.05$), while in the hepatopancreas, mantle and foot, the content of xanthophylls statistically significantly decreased by 49.55-57.28% ($p < 0.001$-0.05) compared to the control. An increase in the duration of stay of mollusks in the toxic environment up to 14 days was accompanied by a sharp inhibition of carotenoid metabolism, which was manifested by a decrease in the content of xanthophylls in their hepatopancreas, mantle, and foot by 81.28-88.41% ($p < 0.001$). This nature of changes indicates a deep depletion of the non-enzymatic link of the antioxidant system, when the rate of use of carotenoids probably exceeds the possibility of their accumulation. Similar dynamics under long-term toxic load conditions were described by K. Lim *et al*. (2023), linking reduced tissue carotenoid content to their intensive use to maintain antioxidant protection. The largest deviation from the control was registered in hepatopancreas of *L. stagnalis*, and in their haemolymph, no statistically significant differences were found from the control group ($p > 0.05$).

With the longest exposure (21 days), a pronounced decrease in the content of xanthophylls in all studied tissues and organs was recorded by 38.89-90.49% ($p < 0.001$-0.05). The largest deviation from the control was recorded in the haemolymph, and the smallest – in the hepatopancreas. Presumably, such changes indicate a decrease in the compensatory capabilities of the non-enzymatic antioxidant system with prolonged toxic effects of urea. Under these conditions, a marked decrease in the content of xanthophylls in the haemolymph may indicate depletion of the transport pool of carotenoids, while their deficiency in tissues reflects the intensive use of these pigments in the processes of neutralising reactive oxygen species and maintaining the structural and functional integrity of cells. In addition, differences in the dynamics of changes in the content of β-carotene and xanthophylls established in the tissues of *L. stagnalis* was probably conditioned by the different physical and chemical properties of these compounds and the specifics of their functional role in the cell. As noted by M. Zbyradowski *et al*. (2022), β-carotene is a non-polar carotenoid that is localised mainly in the deep hydrophobic part of biological membranes and, in addition to its direct antioxidant function, acts as a precursor of vitamin A. However, according to W. Gruszecki & K. Strzałka (2005) and M. Pasenkiewicz-Gierula *et al*. (2024), xanthophylls, due to the presence of oxygen-containing functional groups, are characterised by greater polarity, are localised transmembranously, and are oriented perpendicular to the plane of the lipid bilayer. This spatial organisation ensures that they are located at the interface of the "lipids/water" phases, where they more effectively inhibit the processes of lipid peroxidation. It is these features that may explain the faster depletion of the xanthophyll pool compared to β-carotene in conditions of chronic urea intoxication.

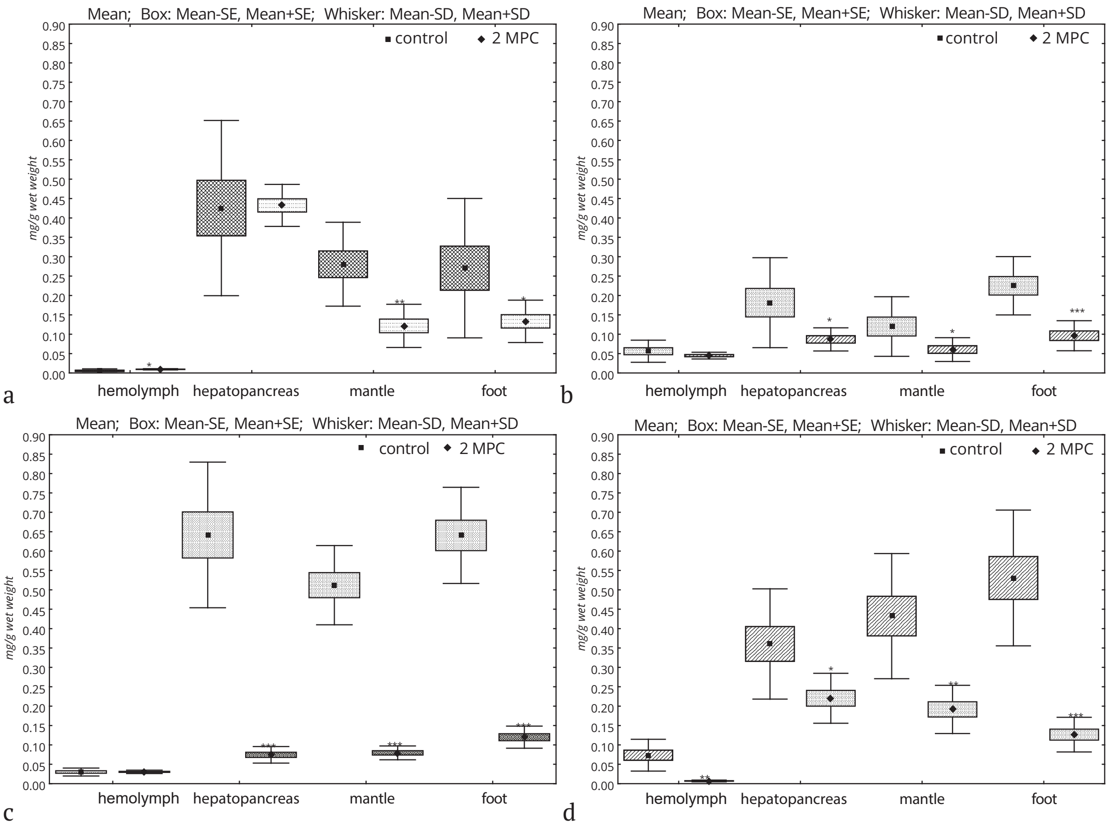

Figure 2. Effect of urea on the content of xanthophylls in *L. stagnalis*

Note: A – exposure of 2 days; B – exposure of 7 days; C – exposure of 14 days; D – exposure of 21 days); n = 10 for each experiment variant; *p < 0.05; **p < 0.01; ***p < 0.001 (Tukey test)

Source: compiled by the authors

An additional factor in a faster decrease in the content of xanthophylls in pond snails due to the action of urea may be a violation of synergistic interactions in the antioxidant protection system. R. Jia (2025) and F. Shah *et al*. (2026) showed that under normal physiological conditions, xanthophylls can regenerate by reducing their radical cations with water-soluble cytoplasmic antioxidants, in particular ascorbic acid. However, the long-term toxic effect of urea is probably accompanied by a violation of nitrogen utilisation processes and the accumulation of ammonium ions in the mollusk's body. As noted by W. Gruszecki & K. Strzałka (2005) and T. Zhang *et al*. (2024), under conditions of ammonia-induced osmotic and oxidative stress, membrane depolarisation, and changes in intracellular pH, xanthophylls are likely involved in maintaining the structural stability of the lipid bilayer of barrier tissues (mantle) and locomotor organ (foot), which may contribute to reducing membrane permeability and maintaining their structural and functional integrity. Thus, unlike β-carotene, xanthophylls were more sensitive to the chronic effects of urea, which was manifested by their earlier and deeper depletion in most of the tissues studied. This suggests that xanthophylls are the first to be involved in neutralising reactive oxygen species and maintaining the structural and functional stability of cell membranes under long-term toxic stress, which is generally consistent with current ideas about the course of oxidative stress in aquatic animals (Ma *et al.*, 2025).

Comparative analysis of the dynamics of β-carotene and xanthophylls showed that tissues (organs) of *L. stagnalis* differ significantly in the nature of the response to the action of urea, and the detected changes have a pronounced tissue-specific and time-dependent character, which may indicate an unequal degree of involvement of different tissues in maintaining homeostasis under toxic conditions. It was found that in the haemolymph of the studied mollusks, the short-term effect of urea (2 days) did not cause statistically significant differences in the content of β-carotene, but an increase in the time of contact of animals with the toxic medium to 7 and 21 days led to a decrease in the level of this carotenoid in the tissue by 63.71 and 82.19%, respectively ($p < 0.01$). Xanthophylls were characterised by a different dynamic, in which their content increased (by 73.09%) after two days of exposure, while at the end of the experiment the indicator decreased by 90.49% ($p < 0.01$) relative to the control. The results obtained may indicate a violation of the transport and intertissue redistribution of carotenoids, accompanied by a gradual depletion of their transport pool.

In the *hepatopancreas* of *L. stagnalis*, the β-carotene content, regardless of the duration of exposure, significantly decreased by 41.77-69.24% compared to the control ($p < 0.05$-0.01). A similar pattern was established for xanthophylls, the content of which decreased by 38.89-88.41% during 7, 14, and 21 day exposure, while no statistically significant differences from the control were found during 48-hour exposure. A special nature of changes was observed in the mantle of pond snail, in which the short-term effect of urea (2-7 days) caused an increase in the content of β-carotene (1.32-2.88 times), and the prolongation of toxicant exposure to 14 and 21 days was accompanied by a decrease in indicators by 25.94-61.09% ($p < 0.001$-0.05) relative to the control. As for xanthophylls, regardless of the duration of exposure, their content significantly decreased by 49.55-84.49% ($p < 0.05$-0.001), which may be due to the barrier function of the mantle and its constant contact with a toxic environment. One of the most sensitive organs to the action of urea was the foot of *L. stagnalis* in which, on the second day of the experiment, the β-carotene content increased by 92.58% ($p < 0.01$), but the prolongation of exposure was accompanied by a decrease in indicators by 26.81-69.20% relative to the control. For xanthophylls, a statistically significant decrease in the content by 50.73-81.28% ($p < 0.05$-0.001) was recorded throughout the experiment. The smallest deviation was registered after two days of exposure, and the largest – on the day 14 of the experiment.

The revealed tissue-specific features of changes in the content of β-carotene and xanthophylls probably reflect the functional specialisation of tissues (organs) of *L. stagnalis* and their unequal contribution to maintaining cellular homeostasis under toxic stress conditions. The intensity of redox processes, the rate of development of reactive oxygen species, and the effectiveness of antioxidant protection differ significantly between individual tissues. In this regard, the different dynamics of the studied carotenoids can be conditioned not only by the intensity of their use in reactions of neutralisation of reactive oxygen species, but also to the specifics of their deposition, transport, and intertissue redistribution. Similar tissue specificity in the distribution and functioning of carotenoids in aquatic animals was described by Y. Liao *et al*. (2025), who noted that the accumulation and redistribution of these pigments depends on the physiological role of individual tissues and their metabolic needs.

Analysis of tissue-specific changes in the content of β-carotene and xanthophylls at the final stage of the experiment (21 days) allowed establishing the same metabolic series according to the degree of deviation from the control: hepatopancreas → mantle → foot → haemolymph. This pattern indicates the preservation of the overall sequence of tissue response regardless of the carotenoid group studied, although xanthophylls were characterised by an earlier and more pronounced deficiency. The results confirm that both groups of carotenoids are involved in a single metabolic response, but differ in the rate of depletion and functional role in adaptive processes. To generalise the identified patterns, a correlation analysis of the relationship between the toxic exposure factor, exposure duration, and the content of β-carotene and xanthophylls in tissues (organs) of *Lymnaea stagnalis* was performed (Table 1).

Table 1. Correlation analysis of the effects of urea and exposure duration on carotenoid content in *Lymnaea stagnalis*

Tissue (organ)	Urea effect		Exposure (2 MPC)	
	β-carotene	xanthophylls	β-carotene	xanthophylls
Haemolymph	-0.33*	-0.32*	0.85*	-0.15
Hepatopancreas	-0.50*	-0.45*	0.71*	-0.48*
Mantle	-0.20	-0.62*	0.31	0.39*
Foot	-0.50*	-0.68*	0.16	0.02

Note: * – statistically significant correlation coefficient ($p < 0.05$)
Source: compiled by the authors

To assess overall urea exposure, correlation analysis was performed for a set of control and investigate values for all exposure times, while the relationship between exposure duration and carotenoid content was evaluated only in the study group. For most tissues, a statistically significant negative correlation was established between the toxic factor and the content of β-carotene and xanthophylls, which indicates a general tendency to reduce the content of carotenoid pigments under the action of urea. For β-carotene, the closest relationship was found in the hepatopancreas and foot ($r = -0.50$; $p < 0.05$), while for xanthophylls it was more pronounced, especially in the foot ($r = -0.68$; $p < 0.05$) and mantle ($r = -0.62$; $p < 0.05$). This suggests that xanthophylls are characterised by a higher sensitivity to the toxic effects of urea compared to β-carotene.

In the experimental group of mollusks, a strong positive correlation was established between the duration of exposure and the content of β-carotene in the haemolymph ($r = 0.85$; $p < 0.05$) and hepatopancreas ($r = 0.71$; $p < 0.05$), and no statistically significant relationship was found in the mantle and foot. For xanthophylls, a significant negative association was established only in hepatopancreas ($r = -0.48$; $p < 0.05$), while a moderate positive association was observed in the mantle, and in the haemolymph and foot, the correlation between exposure duration and xanthophyll content was not statistically significant. The established positive correlation for β-carotene reflects the overall temporal trend of changes in its content in shellfish under the action of urea at a concentration of 2 $MPC_{fisheries}$ and it does not contradict the phase nature of the response established during the analysis of individual exposure times. At the initial stages of the experiment, a compensatory increase in the content of β-carotene was observed in individual tissues, while with an increase in the duration of exposure, a tendency to decrease prevailed.

Thus, the combination of the results obtained allows considering the carotenoid system as a dynamic component of the non-enzymatic antioxidant system, sensitive to changes in environmental factors of the aquatic environment. The revealed features of tissue-specific rearrangement of the content of β-carotene and xanthophylls indicate that the dynamics of individual classes of carotenoids reflects different stages of development of the compensatory and adaptive response of the body, which expands ideas about the mechanisms of antioxidant protection of freshwater pulmonate mollusks and confirms the prospects of using carotenoids as biochemical markers of ecotoxicological monitoring.

CONCLUSIONS

It was found that chronic exposure to urea at a concentration of 2 $MPC_{fisheries}$ causes significant tissue-specific and time-dependent changes in the content of β-carotene and xanthophylls in *Lymnaea stagnalis*. Two-factor analysis of variance confirmed a statistically significant interaction of the factors "toxic effect" and "duration of exposure" on the carotenoid content, which indicates the complex nature of the body's response to toxicant action. At the initial stage of exposure (2 days), multidirectional changes in the carotenoid profile in *L. stagnalis* were established, which were accompanied by an increase in the content of β-carotene and a decrease in xanthophyll counts in the mantle and foot, indicating different sensitivity of individual groups of carotenoids to the toxic effects of urea. An increase in the duration of exposure to 7-21 days led to a progressive decrease in the content of β-carotene and xanthophylls in most of the studied tissues, which may indicate a gradual depletion of the non-enzymatic link of the antioxidant system under conditions of prolonged toxic exposure. Comparative analysis of tissue-specific features of the dynamics of β-carotene and xanthophyll content in *L. stagnalis* showed the greatest deviations from the control values in the foot and mantle, while with the longest exposure (21 days) for both groups of carotenoids, the greatest deviation was recorded in the haemolymph.

Correlation analysis showed a statistically significant negative association between urea exposure and the content of both carotenoid groups. A stronger negative correlation with the toxic factor was established for xanthophylls (up to $r = -0.68$), while a positive correlation was found between the duration of exposure and the content of β-carotene in the experimental group in the haemolymph and hepatopancreas. The obtained results expand the current understanding of the features of the functioning of the non-enzymatic antioxidant system of freshwater lung mollusks under chronic urea exposure and confirm the prospects of using β-carotene and xanthophylls as sensitive biochemical markers of ecotoxicological monitoring of nitrogen contamination of freshwater ecosystems. A promising area of further research is to investigate the relationship between changes in the carotenoid content and the activity of the enzymatic link of the antioxidant system of *Lymnaea stagnalis* by the action of nitrogen-containing toxicants of various nature and concentrations.

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Хронічний вплив низької концентрації сечовини на вміст каротиноїдних пігментів в організмі *Lymnaea stagnalis*

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Анотація. Сечовина є одним із поширених азотовмісних забруднювачів прісноводних екосистем і за тривалого надходження у водойми може порушувати перебіг метаболічних процесів в організмі гідробіонтів. Оскільки каротиноїди виконують функцію неферментативних антиоксидантів, зміни їхнього вмісту відображають розвиток оксидативного стресу та слугують чутливими біохімічними маркерами токсичного впливу. Метою роботи було з'ясувати особливості впливу низької концентрації сечовини на вміст β-каротину та ксантофілів у гемолімфі, гепатопанкреасі, мантії та нозі *Lymnaea stagnalis*. Дослідження проводили на статевозрілих неінвазованих молюсках, яких утримували у середовищі із сечовиною у концентрації, що відповідала двом гранично допустимим концентраціям для водойм рибогосподарського призначення (2 $ГДК_{рибогосп.}$), протягом 2, 7, 14 і 21 доби. Вміст каротиноїдів визначали спектрофотометрично, а результати опрацьовували із застосуванням двофакторного дисперсійного та кореляційного аналізів. Встановлено, що за короткострокової експозиції сечовини (2 доби) вміст β-каротину у мантії та нозі *L. stagnalis* збільшувався у 1,93-2,88 раза ($p < 0{,}01$-$0{,}05$). Водночас вміст ксантофілів у цих органах достовірно знижувався на 50,73-56,68 %, тоді як у гепатопанкреасі значущих відмінностей не встановлено. Збільшення тривалості експозиції до 14-21 доби супроводжувалося системним зниженням вмісту β-каротину (на 25,94-69,24 %; $p < 0{,}001$-$0{,}05$) та ксантофілів (на 38,89-88,41 %) у гепатопанкреасі, мантії та нозі *L. stagnalis*, що, ймовірно, пов'язано з посиленням прооксидантних процесів і використанням каротиноїдів у реакціях нейтралізації вільних радикалів. Кореляційний аналіз виявив статистично значущий негативний зв'язок між впливом сечовини та вмістом β-каротину і ксантофілів, що свідчить про виснаження каротиноїдного резерву за токсичного навантаження. Отже, хронічний вплив сечовини у досліджуваній концентрації спричиняє тканинно-специфічні зміни каротиноїдного профілю *L. stagnalis*, які відображають перехід від компенсаторно-адаптаційних реакцій до виснаження неферментативної ланки антиоксидантної системи. Отримані результати можуть бути використані природоохоронними та рибогосподарськими організаціями для екотоксикологічної оцінки впливу азотовмісних забруднювачів на прісноводні екосистеми, а вміст β-каротину та ксантофілів – як інформативний біохімічний маркер такого впливу

Ключові слова: прісноводні молюски; β-каротин; ксантофіли; оксидативний стрес; азотовмісні сполуки