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Physiological and biochemical adaptations` assessment of the marbled crayfish *Procambarus virginalis* (Lyko, 2017) as an invasive specie of Ukraine

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ABSTACT

Marbled crayfish are the typical invasive hydrobionts spreaded over the lot of world`s waterbodies and create the populations that can reproduce in the presence of only one triploid female, thereby the threat of biodiversity appears and increase the competition with local species. Due to the appearance of the marbled crayfish *Procambarus virginalis* (Lyko, 2017) in the open Ukrainian waterbodies, there is necessity to research it`s adaptation possibilities to the ecological factors to predict it`s possible spread and naturalization. The research aim was to determine the physiological and biochemical features of the adaptations of marbled crayfish as a new species inhabiting the aquatic ecosystems of Ukraine under the conditions of toxicological load on the aquatic ecosystem. The obtained research results indicate significant changes in individual cytological and biochemical indicators of marbled crayfish under the impact of heavy metal ions. A change in these parameters may indicate a rapid cellular response of the crustacean species to the toxic effect of heavy metals. These indicators can be used in the future in studying the adaptation of marbled crayfish in natural waterbodies.

***Keywords*:** heavy metals, marble crayfish, physiology, adaptation

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1. INTRODUCTION

One of the new invasive species that causes a threat to the populations of local crayfish species is the marbled crayfish. *Procambarus* marble crayfish *virginalis* (Lyko , 2017) (until 2017 were known as the form *P. fallax f. virginalis* [8, 9, 17, 19] (Decapoda , Cambaridae) – parthenogenetic crustaceans, which were first found in German reservoirs in 1990 [8, 9, 17]. Initially, they were described as a subspecies of the American species *P. fallax* , whose natural range covers the water bodies of Georgia and Florida (USA) [12]. Recent studies have allowed the separation of marbled crayfish into a separate new species of decapod crayfish *P. virginalis* (Lyko , 2017). These crustaceans came to Europe as aquarium species and were released into the reservoirs of Germany. The appearance of parthenogenetic individuals of this species in natural reservoirs gave rise to its expansion in various countries. They became a threat to local species in a number of countries: the Netherlands, Italy, Slovakia, Sweden, the Czech Republic, Ukraine and Japan [2, 7, 9, 16, 18, 19].

Marbled crayfish are typical invasive hydrobionts that are common in many water bodies and form populations capable of self-reproduction in the presence of only one individual, creating a threat to agriculture and competing with native species. In 2015, the first finds of marbled crayfish were noted in reservoirs of the Dnipropetrovsk and Odesa regions [9], which confirms the expected risks of its introduction and naturalization. In connection with the fact that the water ecosystems of the Dnieper region are characterized by significant pollution by heavy metals [11] , the inhabited crustaceans can either die, unable to withstand the pressure of anthropogenic factors, or adapt to new conditions of existence. High ecological plasticity and ability to parthenogenetic reproduction allows marbled crayfish to quickly increase their numbers in recipient reservoirs and adapt to their conditions. The high adaptability of marble crayfish is associated with epigenetic mechanisms, which are implemented in the DNA molecule and help to read genetic information, activating or deactivating the corresponding genes under the influence of environmental factors. Similar adaptations of crustaceans to living conditions take place at the biochemical and cellular levels, which allows them to live in water bodies with a high level of pollution.

As an invasive species, marbled crayfish cause significant damage to the local ecosystems in which they inhabit. Their food spectrum includes molluscs, worms, insects, small fish and plants – that's why they are direct competitors of aboriginal species of river crayfish. For example, the spread of marbled crayfish in the waters of Madagascar threatens the disappearance of seven endemic species of crustaceans. In the countries of the European Union, they are prohibited by legislation: there is a complete ban on their sale, artificial reproduction and release into natural ecosystems. At that time, in Ukraine, they were openly sold in pet stores, pet markets and through the Internet. In most cases, the cost of one individual does not exceed one euro. In this regard, the risk of their further spread in water bodies of Ukraine is extremely high. In addition to competing with native crayfish for food resources, marbled crayfish can carry dangerous diseases for native crustaceans: WSSV virus, crayfish plague - caused by the fungus *Aphanomyces astaci.*

Today, the study of the possibilities of adaptation of new species of hydrobionts , which appear for the first time in reservoirs with a stable ecological regime, is of particular interest. At the same time, the process of adaptation, which occurs at the biochemical and cellular levels, creates prerequisites for the survival of the population of invasive species. Due to the fact that marbled crafish *Procambarus virginalis* (Lyko , 2017) entered the reservoirs of Ukraine, there was a need to study the possibilities of its adaptation to the ecological conditions of reservoirs in order to further predict its distribution or even acclimatization.

Glial fibrillary acidic protein (GFAP) is a histospecific component of intermediate filaments of the cytoskeleton of astrocytes. GFAP is a specific marker of astrocytes and participates in the formation of glial cells filaments cytoskeleton of cells, takes part in molecular mechanisms of neuron- astrocyte interactions. The use of GFAP as a molecular index of neurotoxicity and a marker of nerve tissue damage is proposed. From the point of view of the marker concept, it is believed that the response of astrocytes to the action of stress factors is non-specific, and the intensity of biosynthesis of GFAP depends on the dose and duration of exposure to the factors, and not on their nature. In some works, the role of GFAP in the mechanism is emphasized formation engram memory [1, 4].

Glial fibrillary acidic protein is a marker of astrocytic cells that provide structural and trophic support for developing neurons [13], and is involved in the processes of neuron growth, migration of neurons, synaptogenesis, etc. [10]. Astroglial protein not only supports the structure of neurons , but is necessary also for the course of neural morphogenesis system [6]. GFAP is important in formation astrocytic processes in response to stimulation cells neurons [3]. Also the expression of GFAP participates in the formation architecture white substances, in processes vascularization , and integrative processes formation hematoencephalic barrier [5]. Therefore, any expression disorders given protein during the period development nervous systems may lead to pathological changes in her development.

In this connection, the issue of comparative characteristics of the influence of adverse factors of various origins, as well as their combined action, on the metabolism of GFAP becomes relevant.

The purpose of the work is to determine the physiological and biochemical features of adaptations of marble crayfish as a new species inhabiting the water ecosystems of Ukraine under the conditions of toxicological load on the water ecosystem.

Task:

- determine the influence of heavy metal ions on the mass and state of tissues and organs of marbled crayfish;
- to investigate histological changes in the antennal glands of marbled crayfish under the influence of increased concentrations of heavy metal ions;
- evaluate changes in adipocytes under the influence of model concentrations of heavy metal ions;
- to assess the state of the hepatopancreas of marbled crayfish under the influence of various concentrations of heavy metal ions;
- to determine the influence of heavy metal ions on enzymatic activity and protein metabolism in marbled crayfish;
- determine the effect of temperature on GFAP of marbled crayfish.

Research object: marbled crayfish *Procambarus virginalis* (Lyko , 2017).

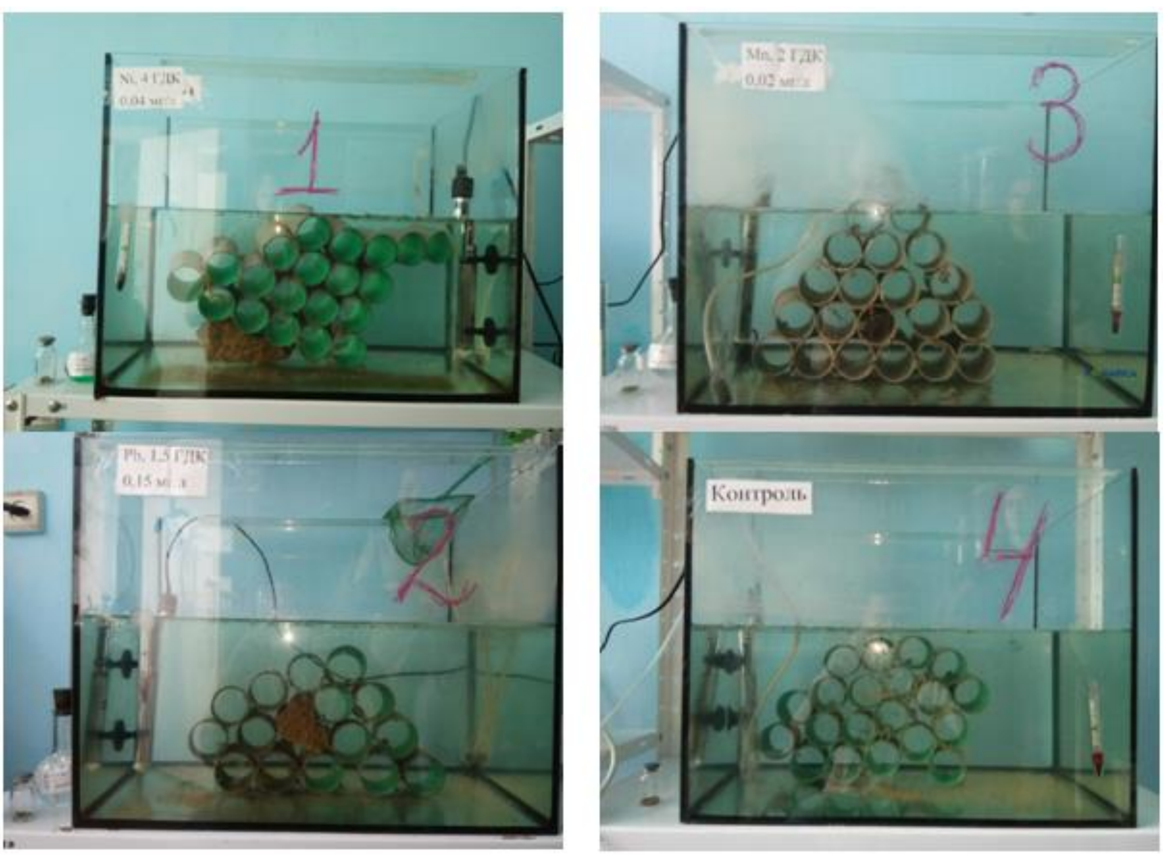
The subjects of research: cytological and histological adaptations, changes in enzymatic and protein metabolism in the tissues and organs of marbled crayfish under conditions of exposure to increased concentrations of heavy metals. Research methods: generally accepted hydrobiological, cytological, histological, biochemical and variational statistical methods.

Scientific novelty of the obtained results. For the first time, the inflow of increased concentrations of heavy metals, which are in the recipient reservoir of the invasion and possible spread of marbled crayfish in Ukraine, was simulated . The nature of the effect of increased concentrations of heavy metals on the physiological and biochemical indicators of marbled crayfish was determined. The adaptive capabilities of the marbled crayfish, which are manifested at all levels of the organization of living matter: biochemical, cellular, population, have been determined.

Practical significance of the obtained results. Given that the physiological, biochemical and cyto -histological parameters of marbled crayfish tissues objectively reflect the state of aquatic organisms in specific conditions of the aquatic environment, the obtained data on changes in cytological, histological, hematological and biochemical parameters can be used to develop express methods for assessing the viability of invasive hydrobionts in conditions of toxicological load of new areas of their existence.

2. MATERIALS AND METHODS

Setting up model experiments. We conducted laboratory model experiments to study the mechanisms of adaptation of the marbled crayfish *Procambarus virginalis* (Lyko, 2017). The influence of different concentrations of heavy metals on the physiological state, histostructure of tissues and organs, and biochemical parameters of marble crayfish was determined. A series of experiments was conducted in 4 aquariums with a working volume of 30 liters. (Fig. 1). A total of 3 series of experiments were conducted.



A

B

Fig. 1(A,B). Experimental aquariums and research crustaceans

The water temperature in the aquariums was maintained at +22 °C. Water aeration was carried out with the help of aerators, the oxygen content in the water was kept at the level of 6–7 mg/l. Twice a week, a complete water change was carried out with the addition of heavy metal salts to the experimental aquariums based on the calculation of the metal ion content: Ni^{2+} – 0,04 mg/l (4 MACs), Pb^{2+} – 0,15 mg/l (1,5 MACs), Mn^{2+} – 0,02 mg/l (2 MACs), Zn^{2+} 0,1 mg/l (10 MACs), Cd^{2+} 0,01 mg/l (2 MACs).

Concentrations of heavy metals were determined from the calculation of their content in the water of the Zaporizhzhya (Dnipro) Reservoir - the main recipient reservoir of this species in the Dnipropetrovsk Region. 15 specimens of marbled crayfish of the same size and age group, obtained parthenogenetically from one female, were planted in each aquarium. Toxicological experiments lasted 21 days. All work with experimental animals was carried out in accordance with the rules of the European Convention on the Humane Treatment of Laboratory Animals, "General Principles of Animal Experiments", adopted by the First National Congress on Bioethics.

Making the hemolymph smears. Hemolymph from marbled crayfish was collected by amputation of 1/3 of the walking leg (pereopod Y) (Fig. 2). Drops of hemolymph were applied to the glass slide, which was stretched by 10–15 mm along the glass. One glass slide is a sample from one crayfish.

Fig. 2. The way of cutting 1/3 part of walking leg

After drying, the smears were painted according to Romanovsky. Smear preparations were fixed according to May-Grunwald for 5–7 s and dried in the wind. Microscopy was performed using immersion objects. The selected hemolymph can be used as a bioindicator of the current state of freshwater crayfish under the influence of environmental factors or the study of their physiological reactions to anthropogenic influence.

Making the histological sections. Marbled crayfish were fixed in a solution of 4% formalin with further processing according to generally accepted histological methods. A Microm HM 325 microtome was used to make sections of marble crayfish. After obtaining the sections, they were placed on glass slides. Next, the sections were deparaffinized and stained with hematoxylin-eosin. After staining, the sections were filled with Canadian balsam and covered with a glass coverslip. Photographs of preparations were taken with a digital nozzle "SciencelabT500 5.17 M", which was connected to a microscope model "MICROmed XS-2610". In each experimental group, at least 50 lobules of the hepatopancreas, 50 lumens of the hepatopancreas, 50 adipocyte cells, 50 green gland cells were analyzed and measured.

Biochemical research methods. Biochemical indicators of marble crayfish were determined in laboratory conditions. Homogenization of tissues was carried out using 0.1 M phosphate buffer (pH 7.4), in a ratio of 1:10. Centrifugation was carried out using a K-70 ultracentrifuge at 20,000 g. The activity of succinate dehydrogenase was determined by the Vexey method on SF-26 at a wavelength of 420 nm. Lactate dehydrogenase activity was determined using standard commercial LDH kits (Filisit-Diagnostika, Ukraine) on a SF-26 spectrophotometer at a wavelength of 340 nm. Alkaline phosphatase activity was assessed using the "Alkaline Phosphatase" reagent kit (Filisit Diagnotyka, Ukraine). Total protein in muscle tissues was determined on a KFK-2M concentration photocolorimeter at a wavelength of 750 nm using the Lowry method.

Statistical data processing. Statistical processing of data was carried out according to generally accepted methods using Statistica 8.0 (StatSoft Inc, USA). All results are presented as mean ± standard deviation (SD). The reliability of the difference between the data samples was established using one-way analysis of variance ANOVA at the significance levels $p < 0.05$, $p < 0.01$, $p < 0.001$.

Determination of the effect of temperature on marble crayfish. The work used 24 marble crayfish, which were divided into 4 groups (n = 6): 1 – control group, +20 °C; 2 – were in an aquarium with a water temperature of +9 °C; 3 – an aquarium with a water temperature of +26 °C; 4 – an aquarium with a water temperature of +23 °C. Obtaining protein fractions from ganglia was carried out by the method of differential centrifugation of the tissue homogenate in a hypotonic buffer (tris - 0.25 mm (pH7.4), ethylenediaminetetraacetate (EDTO) – 1 mm , dithiothreitol – 2 mm , phenylmethylsulfonyl fluoride (FMSF) – 0.2 mm , sodium azide (NaN_3) – 3 mM (the specified reagents were purchased from Sigma, USA) in a ratio of 1:10. The content of total protein in the obtained fractions was measured by Bradford and expressed in mg/ml. Electrophoresis in PAGE was performed in a polyacrylamide gel gradient (7–18%) in the presence of 0.1% sodium dodecyl sulfate . The content of the intact polypeptide of 49 kDa and the composition of the polypeptide fragments of GFAP were determined using immunoblotting. The determination of the relative intensity of the polypeptide zones was carried out by processing the scanned results of immunoblotting with the Image2000 application program (Bio-Techne Corp.) Relative quantitative analysis of polypeptide fragments of GFAP was carried out by comparing the intensity of staining of the corresponding samples. Statistical processing of the results was carried out by one-way analysis of variance ANOVA. Data with $p<0.05$ were considered probable changes.

3. RESEARCH RESULTS

Heavy metals are priority toxic pollutants of fresh water bodies. They pose an extreme danger as pollutants of natural waters, which even in relatively small concentrations can have a negative effect on aquatic organisms.

In connection with the fact that in 2015 the marbled crayfish entered the water bodies of the Dnipropetrovsk region [9], there was a need to study the possibilities of its adaptations to the ecological factors of the aquatic environment in order to further forecast its spread or even acclimatization in the conditions of toxicological pollution of the water bodies of Ukraine.

Effects of Pb, Mn, and Ni ions on the weight and survival of marbled crayfish. Early experiment research marbled crayfish had average weight of individuals 0.68 g and belonged to one-dimensional groups. Compared to the beginning of the experiment marble crayfish in control, experiment with ions Mn and Pb increased their weight by 40.8–65.2%. At the end experiment increase mass in the control aquarium was 0.44 g (60.4%). Biggest increase masses marble crayfish noted in an experiment with ions lead 0.44 g (66.2 %).

At the end experiment statistically reliable difference between by mass crustaceans observed between the control and the experiment with Ni, crayfish during experiment did not grow, and even lost mass – on average, by 3.29%, compared to the beginning of the experiment (Table 1). Ions researched difficult metals in the conducted experiments influenced not only the mass crustaceans, but also on them survival. For 21 days of experiment in an aquarium with lead 26.5% of marbled died crayfish. In the control room the group one individual died due to an accident cannibalism after next molt. In the experiment with Ni, crustacean mortality was the highest and reached 60%.

Table 1. Changes in the weight of marbled crayfish during the experiment (x ± SD)

Biological indexes	Control	Pb	Ni	Mn
Initial weight, g	0.715±0.383 (n = 15)	0.676±0.213 (n = 15)	0.654±0,252 (n = 15)	0.661±0.274 (n = 15)
Final weight, g	1.144±0.425 (n = 14)	1.122±0,681 (n = 11)	0.633±0.154* (n = 6)	0.942±0.382 (n = 13)
Difference, %	60.55*	66.07*	-3.27	40.98*

Note: * - the difference is reliable at $p < 0,05$.

Physiological assessment of the excretory system under the influence of heavy metal ions. The main organs of excretion of marble crayfish are a pair of modified metanephridia - antennal glands, or green glands. These are fairly large rounded glands that are located in the head section and open into ducts near the base of the antennae. Each gland consists of a large coelomic sac, a convoluted tubule, and a urinary bladder.

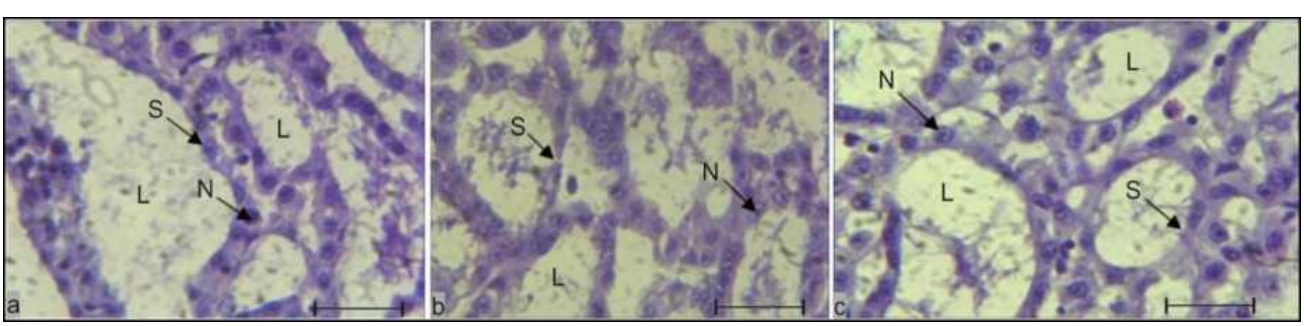


Fig. 3. Histological preparation of the antennal gland *P. virginalis* (Lyko, 2017) a – experiment with Zn, b – experiment with Cd, c – in control S – secretory cells, N – cell nucleus, L – gland lumen; scale – 50 μm

The secretory part of the antennal gland of the marble crayfish had the appearance of a bag divided into numerous chambers lined with a single-layer glandular epithelium that formed a labyrinth (Fig. 3). Rows of glandular cells located on a thin basement membrane are visible on histological specimens. Cubic-shaped cells contained a very large nucleus, in which the nucleolus is clearly visible. The number of nucleoli varied from one to several.

The cells of the antennal gland of marbled crayfish in the control reached the size of 165.4 μm² (Tabl. 2). Glandulocytes had clear edges of cells, a pronounced structure of ducts, a clear basal membrane. Large nuclei of glandular cells in the cross-section reached the size of 51.2 μm². The nuclear membrane had a smooth edge, nucleoli were observed in the nuclei, which were characterized by pronounced basophilia. That is, the structure of the antennal gland of the marble crayfish in the control corresponded to the norm for decapod crustaceans.

Under the influence of zinc, the cells of the green gland of the marble crayfish also had a clear organization, pronounced membranes, complete nuclei and nucleoli. The cross-sectional area of glandulocytes reached 147.7 μm². The cell nucleus occupied approximately 25.5–29.4% and reached the size of 38.4 μm². Compared to the control, no statistically significant difference between cell sizes was observed, however, the size of the nuclei of green gland cells under the influence of zinc ions was 23.5% smaller than the control.

Table 2. Results of histometric analysis of green gland cells P. virginalis (Lyko, 2017) (n = 120, x ± SD)

Research group	The area of glandulocytes, μm²	The area of cell nucleuses, μm²	The value of the nuclear-cytoplasmic ratio
Control	165.4 ± 8.14	51.2 ± 2.88	0.33 ± 0.015
Experiment with Zn, 0,1 mg/l (10 MACs)	147.7 ± 9.98	38.4 ± 1.46**	0.28 ± 0.024
Experiment with Cd, 0,01 mg/l (2 MACs)	141.6 ± 7.61**	42.4 ± 1.74*	0.31 ± 0.021

Note: * – the difference is reliable at $p < 0,05$, ** – the difference is reliable at $p < 0,01$.

The worst histological picture for antennal gland cells was observed in individuals exposed to cadmium ions. The structure of the excretory ducts of the green gland was disturbed in them, the ducts contained a large number of granulocyte cytoplasmic fragments, and the ducts themselves had unclear boundaries. Pyknosis was noted in some cells nuclei, as well as the exit of the nucleolus outside the nucleus – the appearance of micronuclei. A similar phenomenon is explained by the toxicological effect of cadmium.

Glandulocytes compared with control of the antennal gland were 14.5% smaller. The nuclei of glandular cells were also significantly smaller by 17.4% than the control values. In the experiment and the control, the value of the nuclear-cytoplasmic ratio of glandulocytes did not differ statistically and ranged from 0.28 to 0.33 units, which indicates a proportional decrease of both the cytoplasm of green gland cells and their nuclei caused by the influence of heavy metals.

Physiological and histological evaluation of the hepatopancreas under the influence of heavy metal ions. The digestive system of marbled crayfish was represented by a tube, esophagus, stomach, midgut, hindgut and a digestive gland – the hepatopancreas , which consisted of small lobes and opened with its ducts into the stomach. The function of this organ corresponded to the function of the liver and pancreas of vertebrates. The secret of the hepatopancreas of marble crayfish can not only split fats and turn them into an emulsion, but is also able to enzymatically split starch, which is characteristic of the pancreas. In the control , the cross-sectional area of the lobule of the hepatopancreas was equal to 3844.5 ± 44.22 μm ^{2} with a lumen of 1128.8 ± 55.88 μm ^{2} (Fig. 4).

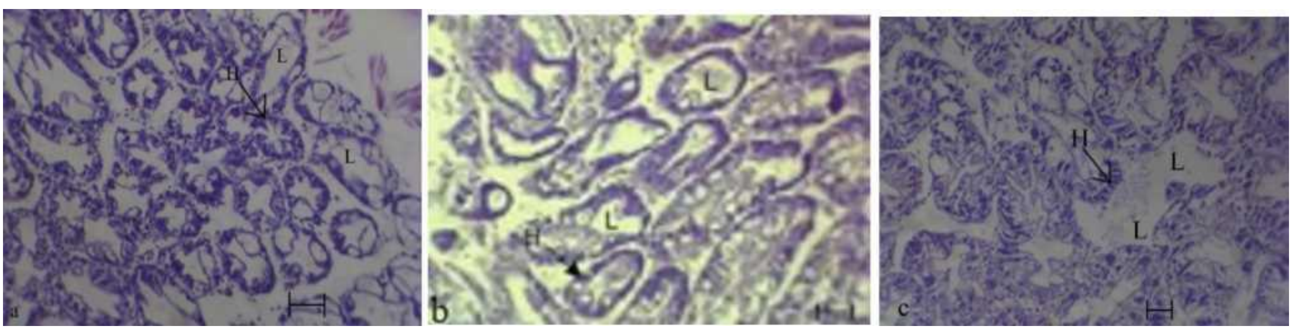


Fig. 4. Histological picture of the hepatopancreas of marbled crayfish *P. virginalis* (Lyko, 2017): a – control; b – experiment with Zn; c – experiment with Cd, H – lobe of the hepatopancreas, L – lumen of the hepatopancreas; Scale: bar – 10 μm

While in the experiment with zinc the structures of the hepatopancreas were increased by 1.2 times, in the experiment with cadmium the largest increase in the structural elements of the tissue was observed – by 1.5 times (Tabl. 3)

Table 3. Results of histometric analysis of hepatopancreas P. virginalis (Lyko, 2017) (n = 100, x ± SD)

Histometric indexes	Control	Experiment with Zn	Experiment with Cd
Lobe of hepatopancreas, μm ^{2}	3844.5 ± 44.22	4339.3 ±15.89***	6278.01 ± 67.46***
Lumen of hepatopancreas, μm^2	1128.8 ± 55.88	1598,3 ±17.01***	1648.65 ±67.46***

Note: *** - the difference is reliable at $p < 0,001$

Even small concentrations of toxic substances caused noticeable changes in the structure of the hepatopancreas of crustaceans, in this regard, the histological structure of the hepatopancreas can be used as a biomarker of the physiological reaction and adaptation of the marble crayfish organism to the toxicity of the environment.

Physiological assessment of the hepatopancreas under the influence of Pb, Ni, Mn. In the control the hepatopancreas lobule was equal to 3844.25 μm ^{2} with a lumen of 1144.63 μm ^{2}.

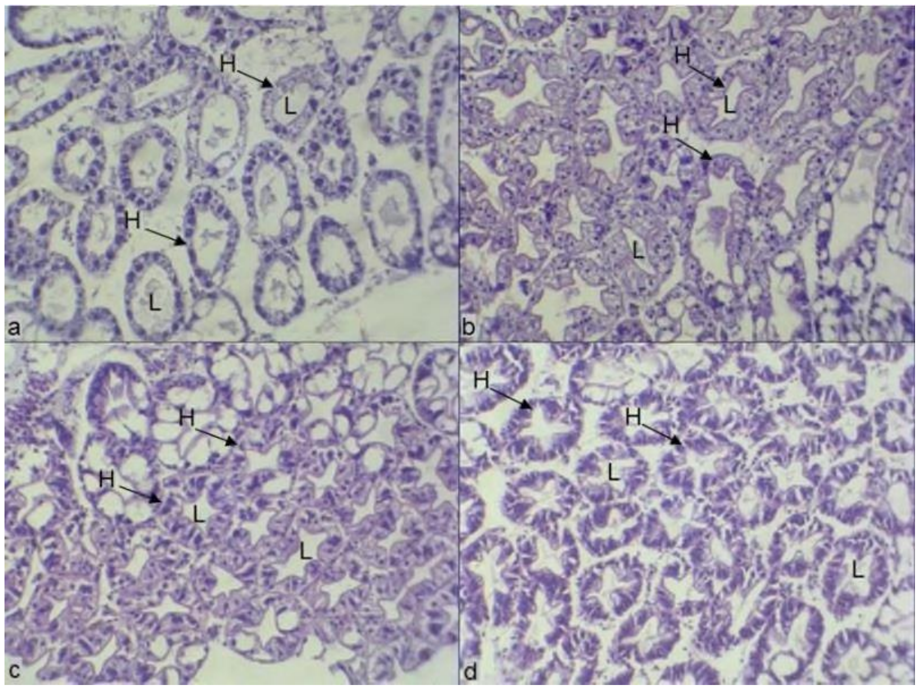


Fig. 5. *P. virginalis* hepatopancreas lobes (Lyko, 2017), tubules in cross section:
a – experiment with Ni, b – experiment with Mn, c – experiment with Pb, d – control,
H – Lobe of hepatopancreas, L – lumen of hepatopancreas; scale bar – 10 μm

While in the experiment with nickel no significant difference was observed. The cross-sectional area of the lobule of the hepatopancreas was 3678.6 μm ^{2} with a lumen of 1036.24 μm ^{2}. In the experiment with manganese and lead, the lobules of the hepatopancreas were smaller by 14.79% and 2.89%, respectively (Table 4). Also, for all crustaceans from the experiment with manganese and lead, a significant decrease in the cross-sectional area of the hepatopancreas lumen was noted by 38.79% and 43.85%, respectively.

Table 4. Results of histometric analysis of hepatopancreas P. virginalis (Lyko, 2017) (n = 50, x ± SD).

Indexes	Control	Group with Ni	Group with Mn	Group with Pb
Lobe of hepatopancreas, μm^2	3844.25±172.14	3678.6±140.48	3276.12±142.7*	3743.23±155.78

Lumen of hepatopancreas, μm^2	1144,63 ±61,14	1036.24 ±97.16	706.56±34.12*	646.2±41.46*

Note: * – the difference is reliable at $p < 0.05$.

Studies of the hepatopancreas of marble crayfish showed changes in the histological structure of the tissue (Fig. 5).

Heavy metals affected the shape of hepatopancreas lobules and the size of the gland lumen. Ni, Mn and Pb ions caused deformation of the glandular parts of the gland and excretory ducts.

Physiological assessment of fat cells under the influence of Pb, Mn and Ni ions. Since adipocytes are highly specialized cells, the main function of which is the accumulation of fat inclusions, their nucleus and mitochondria are shifted to the periphery. The central part of the cells is occupied by a large drop of fat, which is washed out with alcohols during the preparation of the preparations, therefore, on the preparations, the fat cells have the appearance of clear cavities with a displaced nucleus (Fig. 6).

Studies of adipocytes of connective tissue showed a significant difference between the sizes of cells under the influence of heavy metals. The sizes of adipocytes varied in a wide range from 144.13±8.01 μm^2 to 537.11±31.6 μm^2. In the control, the area of adipocytes was equal to 406.96 ±21.64 μm^2.

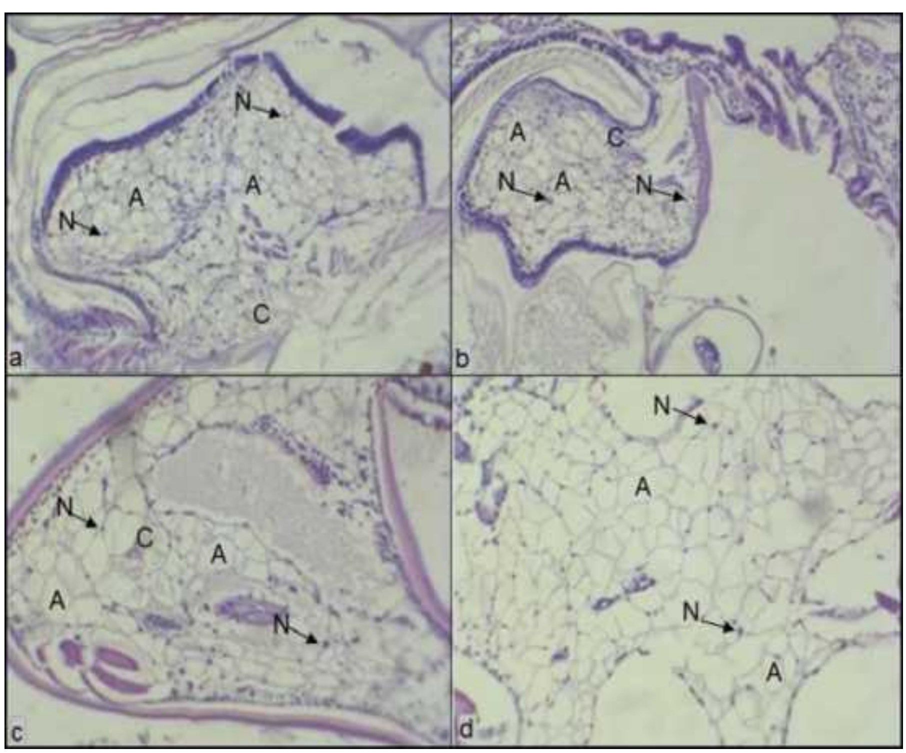


Fig. 6. Fat cells of connective tissue *P. virginalis* (Lyko, 2017): a – Ni; b – Mn, c –Pb, d – control, C – connective tissue, A – adipocytes, N – nucleus; bar – 10 µm

For experimental groups with nickel and manganese, a significant decrease of 2.1 times and 2.8 times was noted, respectively. In the experiment with lead, an increase in the area of adipocytes by 1.31 times was observed.

Physiological assessment of hemolymph under the influence of heavy metals. According to the results of experimental studies, it was established that under the conditions of exposure to lead ions, a significant increase in the area of blasts (by 1.45 times) was noted. In the control group of marble crayfish, the area of blasts was 110.55±27.49 μm^2, after exposure to lead ions, for 21 days, the area of blasts was set at the level of 156.36±47.95 μm^2 (Fig. 7).

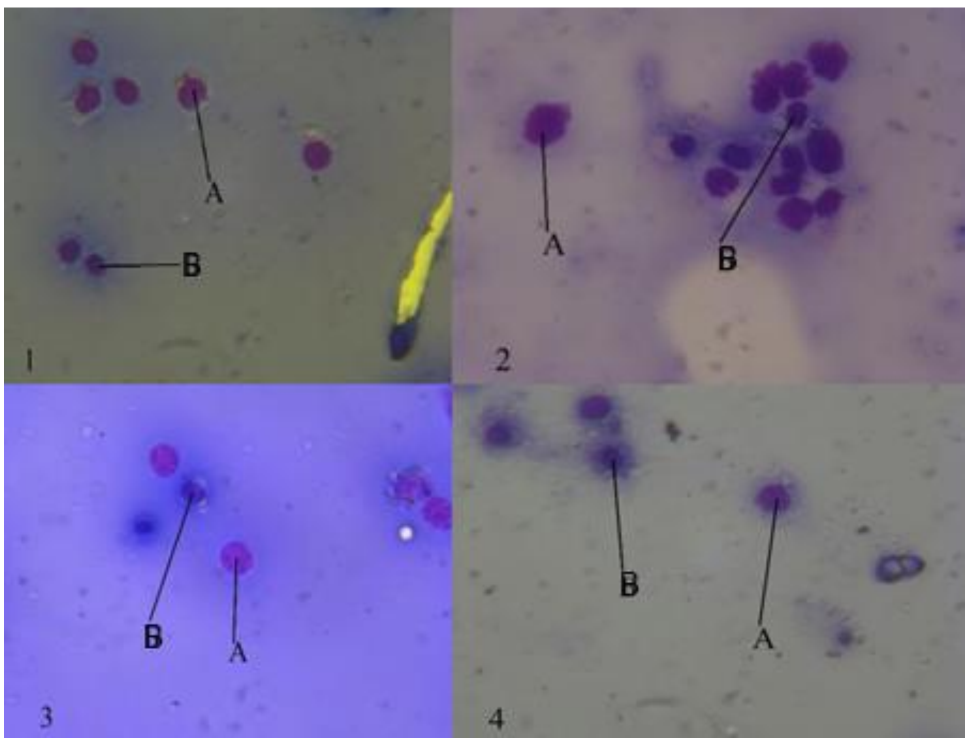


Fig. 7. Hemolymph cells *P. virginalis*: 1– control; 2 – Mn, 3– Pb, 4– Ni, A – blast, B – round hyalinocytes, bar = 10 μm

In studies with the addition of manganese and nickel ions, a slight decrease in cells was observed. The area of blasts under the influence of manganese was 108.26±53.79 μm^2, under the conditions of action of nickel ions - 103.32±38.38 μm^2 (Table 5).

Studies of the size of blasts showed that under the influence of heavy metal ions, their size in the control was 110 μm^2, a significant increase (1.4 times) was noted in the area of blasts that were under the influence of lead ions and amounted to 156 μm^2, and in the experiment with manganese ions and cell reduction was observed with nickel. While the area of round hyalinocytes in the control and in the experiment with manganese did not differ significantly and was 170 μm^2, in the experiment with lead ions the cells decreased by 1.1 times, and in the experiment with nickel ions they decreased by 1.7 times. In contrast to the area of blasts, the obtained data show that the area of round hyalinocytes in the control group and in the group with the addition of manganese did not differ significantly and was 170.63±32.46 μm^2 in the control group, under the influence of manganese ions – 170.82±48.79 μm^2. Under the conditions of action of lead ions, the cells decreased by 1.1 times and amounted to 154.64±71.92 μm^2.

Table 5. Area of hemolymph cells of marbled crayfish, M±SD μm^2

Types of cells	Control	Mn	Pb	Ni
Blast, n = 35	110.52±27.49	108.26±53.76	156.36±47.95**	103.32±38.38
Round hyalinocytes, n =15	170.63±32.46	170.82±48.79	154.64±71.92	96.29±17.46**

Note: ** $p < 0.01$.

Experimental data show that it was the influence of nickel ions that led to the largest decrease in the area of round hyalinocytes (by 1.7 times) and under the conditions of action of this metal, the area of cells was 96.29±17.46 μm^2

The influence of heavy metal ions on the enzymatic activity of marbled crayfish. The use of basic physiological and biochemical parameters makes it possible to assess the peculiarities of the course of metabolic processes in resident species from fragmented habitats and to investigate the process of their adaptation to the new conditions of existence in which they have entered. Because at the molecular level, hydrobionts living in anthropogenically altered conditions often record violations of vital functions. Such changes reflect the influence of factors of the surrounding water environment and allow to assess and diagnose the general state of the water ecosystem. On the basis of determining the enzymatic activity of the physiological and biochemical state of hydrobionts, it is possible to establish the presence of stress reactions under unfavorable habitat conditions.

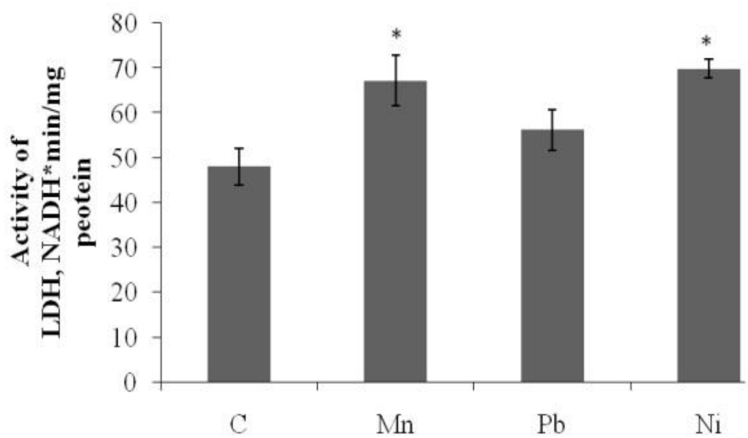
When conducting molecular studies, it is necessary to identify the most informative biochemical markers, which can be used to effectively and clearly assess the physiological state of hydrobionts under the influence of toxicological factors. By assessing the physiological state of species, it is also possible to predict changes in hydrobiocenoses as a whole.

Adaptation of hydrobionts to environmental factors occurs both by morphometric and morphophysiological features, and at the physiological, biochemical, and cytological levels. In this regard, the study of these characteristics on the example of various types of hydrobionts opens up wide opportunities to assess the conditions of a separate aquatic ecosystem.

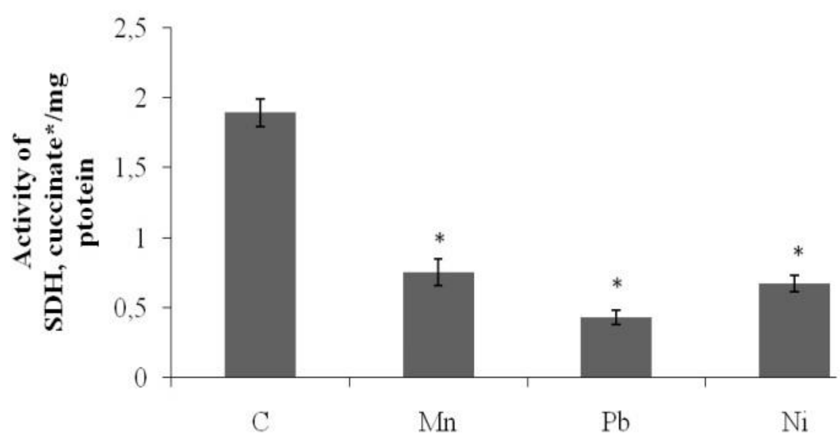
Lactate dehydrogenase is an enzyme that is present in most tissues of various taxa of animals. It is involved in the conversion of lactate into pyruvate and vice versa. A change in the activity of this enzyme is widely used as an indicator of stress. In the body of marble crayfish, the processes of glycolysis were enhanced, which were caused by the effect of the investigated heavy metal ions on the body of the crayfish, in particular, they affected the processes of energy supply. According to the research results, it was established that under the action of manganese and nickel in the muscle tissues of the marble crayfish, the activity of lactate dehydrogenase (LDH) increased by 29.6% and 32.3% compared to the control groups (Fig. 8 A).

In the control group, LDH activity was indicated at the level of 48.04±4.03 NADH/mg. Under the influence of manganese, the activity of lactate dehydrogenase increased to 67.23±5.69 NADH/mg, under the influence of nickel – to 69.84±2.1 NADH/mg. Under the action of lead, non-probable ($P \leq 0.05$) changes in enzyme activity by 15.6% were also noted.

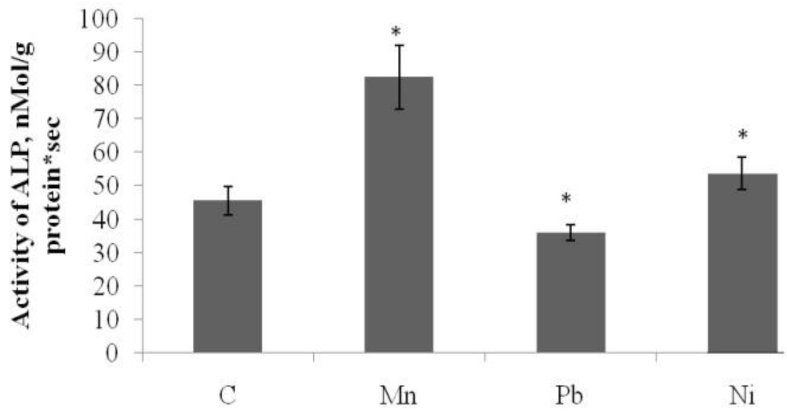
The activity of LDH under the action of lead is indicated at the level of 56.23±4.46 NADH/mg. In contrast, we detected changes in the activity of succinate dehydrogenase (SDH). Oxidizing enzymes, in particular SDH, play an important role in respiratory metabolism. SDH is one of the important oxidizing enzymes in the tricarboxylic acid (TCA) cycle. According to M. Banaae and K. Fahmadi , when acting on river crayfish *Astacus leptotactylus* toxicants of organic origin also revealed a decrease in SDH activity (Fig. 8 B). Suppression of SDH activity in marbled crayfish indicates a deterioration of the oxidative metabolic cycle, and as a result, the involvement of glycolysis in the energy exchange. We know that heavy metals are absorbed in the body, which further contributes to tissue damage and causes dysfunction of specific organs. This is reflected in the change in enzyme activity. First of all, this concerns enzymes involved in the regulation of energy metabolism. These changes could provoke a violation of redox processes in muscle tissues of crayfish.



(A)



(B)



(C)

Fig. 8. Enzyme activity in marble crayfish muscles: A – LDH, B – SDH, C – LF. C – control group, Mn – group with added manganese, Pb – group with added lead, Ni – group with added nickel. n = 5, * – p<0.05 relative to the control.

Determination of the amount of succinate dehydrogenase showed that under the action of manganese, lead, and nickel, the activity of the enzyme decreases by 2.5, 4.3, and 2.8 times, respectively, compared to the control (Fig. 8 B). The content of SDH in the control group was set at the level of 1.89±0.1 nmol of succinate /mg of protein. Under the influence of manganese, the activity of the enzyme decreases to 0.75±0.095 nmol of succinate /mg of protein, under the action of lead – up to 0.43±0.05 nmol of succinate /mg of protein, under the action of nickel – up to 0.67±0.06 nmol of succinate /mg of protein.

Alkaline phosphatase is involved in phosphate hydrolysis and membrane transport, and is also used as an indicator of stress in biological systems. A change in the activity of this enzyme indicates certain disturbances in cell membranes. It is known that alkaline phosphatase is used as a bioindicator of heavy metal exposure in ecotoxicological studies . A change in enzyme activity under the influence of lead may indicate a decrease in the functional activity of a certain organ during intoxication with this heavy metal, as it has an accumulative property. Its action could cause a decrease in the synthesis of molecules of this enzyme. In addition, it could cause a violation of the structure and permeability of the membranes of cell organelles and cause acidosis.

Exposure to nickel and manganese caused the opposite pattern of changes in the activity of this enzyme. This could cause an increase in phosphorylation processes to ensure the normal process of cell growth and proliferation in conditions of their intoxication. Under the influence of the tested concentration of lead in cancer muscles, a decrease in enzyme activity ($p \geq 0.05$) was found by 22.1% compared to the control group. In the control group, the activity of alkaline phosphatase in the muscle tissues of the marble crab was indicated at the level of 45.56±4.21 nmol /g protein* sec, under the action of lead the activity of the enzyme decreased to 35.9±2.3 nmol /g protein* sec. Under the action of nickel ($p \leq 0.05$) and manganese ($p \geq 0.05$) ions, the activity of the studied enzyme increased by 14.1% and 45.8%, respectively. The activity of alkaline phosphatase under the conditions of action of nickel ions is indicated at the level of 53.65±4.76 nmol /g protein* sec , under the action of manganese – 82.4±9.53 nmol /g protein* sec. Changes in enzyme activity also affected the total protein content. The total protein content in the control group was set at the level of 156.08±8.4 mg/g of tissue. Under the conditions of manganese, the total protein content decreased to 106.65±3.81 mg/g of tissue, lead to 132.3±5.92 mg/g of tissue, nickel to 88.9±4.7 mg/g fabrics Thus, under the action of lead, manganese, and nickel ions, a decrease in the total protein content by 16.3%, 32.7%, and 44.1% was established ($p \geq 0.05$), respectively. A significant decrease in the total protein content under the influence of the studied concentration of nickel may be associated with a significant damage to organs by this heavy metal. In general, the action of toxicants could cause catabolism of proteins, which in the process of decomposition into amino acids were used for energy needs in tissue detoxification processes. It should also be noted that the significant use of protein in energy metabolism could cause the hydration of cancer tissues. These are a kind of exhaustion results due to the negative effect of toxic compounds on tissue homeostasis.

Effect of temperature. The results of the study of the total content of proteins show that no significant changes were found under the conditions of temperature stress on marble crayfish *P. virginalis.* The results of immunoblotting indicate an insignificant glial content of fibrillar acidic protein in the control group, and a significant increase in its content in the ganglia of the group of animals that were in an aquarium with a reduced water temperature of +9 ºC (Fig. 9). This may indicate that low water temperature provokes gliosis and induces the expression of GFAP [14, 15]. An increase in water temperature is also accompanied by an increase in glial content fibrillary acidic protein in the ganglia, but not as significantly as observed in the group of cold-water marbled crayfish.

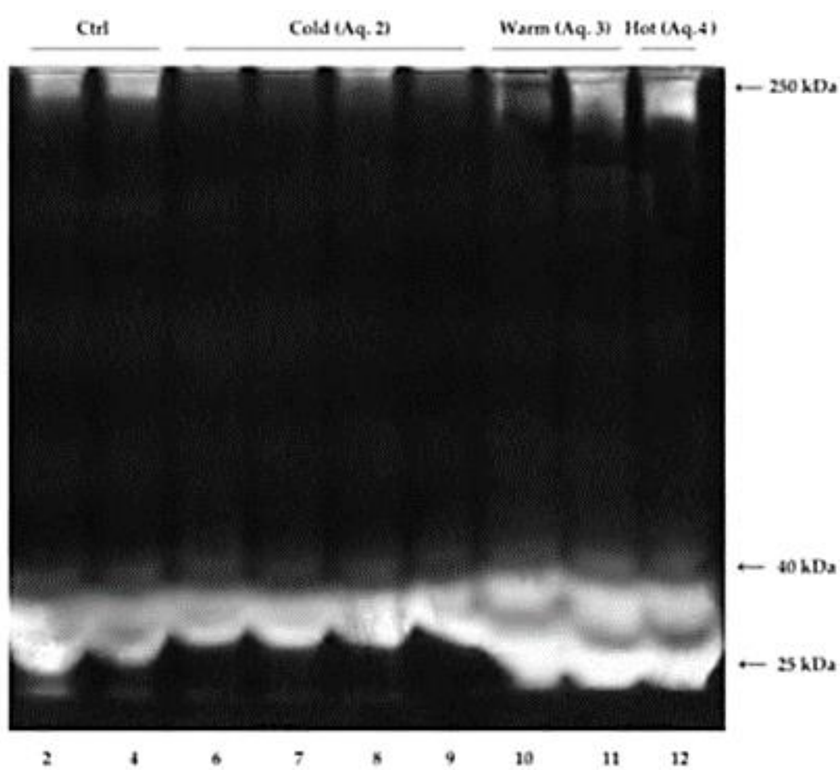


Fig. 9. Results of electrophoresis

In addition, the results of immunoblotting indicate the appearance of low-molecular-weight polypeptide fragments of intact GFAP of 49 kDa under conditions of exposure to low temperature. It is likely that the expression of glial of fibrillar acidic protein may depend on the action of stress factors.

4. CONCLUSIONS

Spread marble crayfish water bodies of Europe caused by wide adaptations opportunities parthenogenetic species, which even in water bodies from tense toxicological condition can increase their numbers . Appearance marble crayfish in water bodies Dnipropetrovsk region may signal about it possible naturalization and further spread of the species in water bodies of Ukraine.

1) Experiments show that compared to the beginning of the experiment marbled crayfish in control, experiment with ions manganese and in an experiment with ions lead increased their weight by 40.8–66.1%. At the end experiment increase mass in the control aquarium was 0.44 g (60.4%). Biggest increase masses marble crayfish observed in an experiment with ions lead 0.44 g (66.2 %). In an experiment with ions nickel , crayfish during experiment did not grow, and even lost mass - on average, by 3.25%, compared to the beginning of the experiment. So, among researched ions difficult metals had the most negative effect on weight Indexes crustaceans ions nickel.
2) The study of the antennal (green) glands of marbled crayfish showed that in the experiment with zinc ions, compared to the control, there was no statistically significant difference between the sizes of the cells, however, the sizes of the nuclei glandulocytes of the green gland under the influence of zinc ions were 23.5% smaller than the control. The worst cyto -histological picture for glandulocytes of the antennal gland was observed in individuals exposed to cadmium ions. Derivative structure ducts antennal their glands were disturbed, the ducts contained a large number of them fragments cytoplasm glandular cells, and the ducts them selves had unclear limits. In some cells noted pyknosis of the nuclei, as well as appearance micronuclei. Similar phenomenon is explained sharp toxicological influence ions cadmium.
3) Studies of the hepatopancreas of experimental cancers showed significant changes in the cytological and histological structure of the tissue. Heavy ions metals influenced the shape and size lobule hepatopancreas and the size of the lumen glands, called deformation glandular departments and outlets ducts hepatopancreas .
4) Studies of connective tissue adipocytes showed that for experimental groups with nickel and manganese ions, a significant decrease of 2.2 times and 2.7 times, respectively, was noted. In the experiment with ions lead observed magnification square adipocytes by 1.32 times.
5) Based on the results of the physiological assessment of hemolymph, it was established that under the conditions of exposure to lead ions, a significant increase in the area of blasts (by 1.4 times)) is noted. Experimental data show that it was the influence of nickel ions that led to the largest decrease in the area of round hyalinocytes by 1.7 times
6) Research results it is established that during the action manganese and nickel in tissues muscles marble cancer activity lactate dehydrogenase (LDH) increased by 29.6% and 32.3% respectively control groups. When acting lead also non- probable ($P \leq 0.05$)

changes were noted for 15.6% enzyme activity . On the contrary this we discovered changes activity succinate dehydrogenase (SDH). Definition quantity succinate dehydrogenase showed that the actions manganese, lead and nickel happens decrease enzyme activity, respectively, by 2.5, 4.3 and 2.8 times compared to the control. When investigated alkaline phosphatases experimental the data showed that actions researched concentration Pb was detected in the muscles of the cancer decrease enzyme activity by 22.1% compared to the control group. For actions ions nickel and manganese is established growth activity of the studied enzyme by 14.1% and 45.8% , respectively. Changes activity enzymes also marked on the general in the city proteins.

7) The results research general content proteins show that reliable no changes were detected under conditions of temperature load on marbled crayfish *P. virginalis*. The results immunoblotting indicate a minor contents glial fibrillar acidic protein in the control group, and essential magnification him contents in ganglia groups of animals that were in an aquarium with a reduced water temperature of +9 ºC. It may testify that low water temperature provokes gliosis and induces expression of the GFKB. Increase water temperature as well is accompanied increase content glial of fibrillar acidic protein in the ganglia, but not so much significantly like this was observed in the group marble crayfish with cold water.

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