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Parameters of the histological adaptation of Marmorkrebs *Procambarus fallax f. virginal* (Decapoda, Cambaridae) to zinc and cadmium ions pollution

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ABSTRACT

Due to the fact that marbled crayfish (Marmorkrebs) got into the reservoirs of the Dnipropetrovsk region in 2015, it was necessary to study the possibilities of its adaptation to environmental factors of reservoirs for further prediction of its distribution or even acclimatization under conditions of toxicological contamination of the ponds of the steppe Prydniprovya. The article presents the results of studies on the influence of heavy metal ions on marbled crayfish. It was determined that zinc and cadmium ions affect weight indicators, survival and reproduction of experimental crayfish. Experimentally simulated concentrations of heavy metals on the example of zinc – 0.1 mg/l (10 MPC) and cadmium – 0.01 mg/l (2 MPC) allowed detecting the reaction of the excretory system of marbled crayfish. It is determined that under the influence of heavy metal ions the area of glandulocytes cells decreases by 10.4–14.8%. Also, there is a decrease in the cellular nucleus, thus, under the influence of zinc, the nucleus of the secretory cells was 23.6% smaller, and under the influence of cadmium by 17.5%. To study the influence of zinc and cadmium on the glandular cells of the green gland of marbled crayfish, the index of nuclear-cytoplasmic ratio (NCR) was used. The indicator of nuclear-cytoplasmic ratio allows estimating the level of metabolism and detecting the manifestation of compensatory reactions of the organism of experimental crayfish. The value of NCR in the experiment and control was not statistically different and ranged from 0.29 to 0.31 units. This indicates an interproportional reduction in both the cytoplasm of the cells of the green gland and their nuclei, caused by heavy metals exposure. The negative influence of heavy metals on the histostructure of hepatopancreas of marbled crayfish was determined; significant changes in the structure of tissue

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were noted, heavy metals influenced the shape of the hepatopancerase lobes and the size of the lumen. The worst histological picture of cells of experimental tissues and organs of marbled crayfish was observed in individuals exposed to cadmium ions due to the toxicological effects of this heavy metal. Investigation of the histological structure of tissues and organs of marbled crayfish will allow the development of measures for the determination of biomarkers for the bioindication of the aquatic environment using freshwater crayfish. The results of such studies have an important fundamental and applied significance for understanding the mechanisms of adaptation of crustaceans to the conditions of toxicological burden on aquatic ecosystems.

Keywords**:** *Procambarus fallax f. virginalis* Martin et al., 2010, marbled crayfish, heavy metals, glandulocytes, hepatopancreas

1. INTRODUCTION

For ecological assessment and determination of the level of influence of heavy metals on aquatic organisms it is necessary to find out their content in hydrobionts, regularities of insertion, accumulation and distribution in organs and tissues. In industrial hydrobionts, the content of heavy metals should be set for the hygienic assessment of production, consumed by human. Biological effects of heavy metals pollution of the environment are manifested primarily in direct toxic effects on hydrobionts, leading to damage of their physiological systems and mass death of organisms. In addition, there is a violation of primary products and trophic links, as well as the balance between auto- and heterotrophic organisms, which leads to a disruption of the biotic cycle and the destabilization of aquatic ecosystems. Besides direct toxic effects on the body heavy metals cause dangerous remote biological effects, mutagenic, embryotoxic, gonadotoxic effects, etc.

All these circumstances make it possible to determine the problem of contamination of water ecosystems with heavy metals, as one of the first of its ecological significance.

Currently, the anthropogenic factor has a significant impact on the formation of the elemental composition of the water of the Zaporizhzhya (Dnipro) reservoir. The average annual concentrations of dissolved forms of almost all heavy metals in the water of the Samara Bay (right tributary of the Zaporizhzhya (Dnipro) reservoir), except for lead and mercury, exceed the MAC for fishery ponds. There is also a permanent water pollution of the reservoir with cadmium and zinc.

Cadmium is one of the most harmful pollutants in the aquatic environment. Even its residual concentrations can cause irreversible functional disturbances, deformations, even the death of hydrobionts. For toxicity for freshwater hydrobionts, cadmium belongs to the first place. In many countries, the content of cadmium in water is strictly regulated for various types of water use. In accordance with the recommendations of FAO/WHO experts (Foodand Agricutural Organization / World Health Organization), the content of cadmium in drinking water is regulated at 1 mg/l. In recent years, the concentration of cadmium in the water of the lower part of the Zaporizhzhya (Dnipro) reservoir is an average of 0.001 mg/l, which is an order higher than the clark values, but does not exceed the levels of boundary concentrations. The average content of cadmium in the Samara bay is 6 times higher than its amount in the lower section of the reservoir, and 60 times higher than the Clark values. Also, in this area of the reservoirs there is an increasein the levels of fish-farming MACs by 20%.

Zinc gets to the natural waters as a result of natural processes of destruction and dissolution of rocks and minerals. A considerable amount of zinc gets into water bodies with technogenic pollution such as waste water from ore dressing factories and galvanic workshops, parchment paper production, mineral paints, etc. In water, it exists mainly in the ionic form or in the form of its mineral and organic complexes. It occasionally occurs in insoluble forms, in the form of hydroxides, carbonates, sulfides etc.

The most important external source of zinc in the Zaporizhzhya (Dnipro) reservoir is the inclined runoff, which changes the distribution of zinc in the littoral, and in the high-water years even in the profundal of reservoir. The role of atmospheric precipitation in the formation of zinc regime is small, because the reservoir is in the zone of weak rains.

The average concentration of zinc in water in the course of several decades in the water area of the reservoir varied from 0.0059 to 0.18 mg/l. Since 2004, there is a significant difference between the average annual concentration of zinc in the waters of the Samara Bay and the lower part of the reservoir (Viyskove Village), which is 73%. The average content of zinc (0.008 mg/l) in the area of Viyskove Village does not go beyond the boundary norms and clark values. The average concentration of this element in the waters of the Samara Bay exceeds 3 times the fishing-MAC and clark values and is 0.03 mg/l.

As the Zaporizhzhya (Dnipro) reservoir is located on the territory of the industrial region, the content of zinc in water is 1.3–2.3 times higher than in other reservoirs of the Dnieper (Kyiv, Kaniv and Kremenchug).

Thus, zinc and cadmium are part of a group of heavy metals, the content of which in the water of the Zaporizhzhya (Dnipro) reservoir exceeds the fishery's MAC and forms a toxicological background. In this regard, the study of adaptation of invasive hydrobionts to the action of these metals becomes particularly relevant.

Priority toxic pollutants of fresh water are heavy metals; they pose a huge danger as pollutants of natural waters, which, even at relatively low concentrations, can negatively affect aquatic organisms [12]. The biological effects of heavy metal contamination of the aquatic environment are manifested primarily in direct toxic effects on hydrobionts, which leads to damage to their physiological systems.

The study of adaptation possibilities of new species of hydrobionts, which are first introduced to reservoirs with a sustainable ecological regime and the formed toxicological background, is of particular interest. In this case, new species can either perish without withstanding the pressure of anthropogenic factors, or vice versa, adapt to new conditions. At the same time, the adaptation process, which takes place at the biochemical and cellular levels, creates the preconditions for the survival of the population of the invasive species.

Due to the fact that marbled crayfish got into the reservoirs of the Dnipropetrovsk region in 2015, it was necessary to study the possibilities of its adaptation to environmental factors of reservoirs for further prediction of its distribution or even acclimatization under conditions of toxicological contamination of the ponds of the steppe Prydniprovya [8]. Marbled crayfish (or marmorkrebs) *Procambarus fallax f. virginalis* Martin et al., 2010 (Crustacea, Decapoda, Cambaridae) – parthenogenetic crayfish that were discovered in the German waters in 1990 [10]. This species is a subspecies of its American congener *Procambarus fallax* (Hagen, 1870), which covers the area of natural water reservoirs of southern Georgia and Florida (USA). Crayfish got in Europe as an aquarium species and most likely have been infused in Germany reservoirs [4]. As an invasive species marbled crayfish hit the waters of the Netherlands [7], Italy, Slovakia [9], Sweden [3], Czech Republic and

Ukraine. Marmorkrebs are also an invasive species. They have been introducted in many places, and have established populations in at least three countries, damaging agriculture and threatening native species.

The main feature of the marbled crayfish is that its population consists entirely of triploid females and reproduction occurs by parthenogenesis [11]. All individuals are female, and the offspring are genetically identical to the parent [1,5,11]. Thus, marmorkrebs conveniently used as model objects in biological studies [10].

Therefore, our research is aimed at determining the influence of heavy metal ions on the physiological state and histostructure of tissues and organs of marbled crayfish.

2. MATERIALS AND METHODS

We conducted a laboratory model experiments to study the mechanisms of adaptation of marbled crayfish (Marmorkrebs) *Procambarus fallax f. virginalis* Martin et al., 2010. The effect of different concentrations of heavy metals on physiological state and histostructure of the excretory system of marbled crayfish was determined.

Figure 1. Staging an experiment: A – aquarium with Zn, 0.1 mg/l (10 MPC); B – aquarium with Cd, 0.01 mg/l (2 MPC); C – control aquarium, D – Marmorkrebs *Procambarus fallax f. virginalis* Martin et al., 2010.

The experiment was conducted in 3 aquariums with working capacity of 30 liters (Fig. 1). The water temperature was maintained by thermostat and was +24 °C in all aquariums.

Oxygen regime was maintained by the compressor, the oxygen content in the water of aquariums was 8 mg/l. Twice a week it was carried out a complete replacement of water in aquariums and toxicants were added at the rate of concentrations of heavy metals Zn – 0.1 mg/l (10 MPC), Cd – 0.01 mg/l (2 MPC). Concentrations of heavy metals were determined by their content in water of Zaporizhzhya (Dnipro) reservoir – the main reservoir recipient of this species. Feeding crayfish occurred every day with the same quantity of food. In each aquarium there were put 11 specimens of the marbled crayfish with the same size-age group and derived from one parthenogenetic female. Feeding of crayfish was carried out once a day by the universal feed of the brand "Tetra", the series "Crustaceans", with a daily dose of 5% of the mass of individuals. Weighing of individuals was carried out every 7 days. On the last day of the experiment, it was conducted determination of the fertility of marbled crayfish by direct counting the number of eggs. The experiment lasted 21 days.

To investigate the impact of heavy metals on histological structure of tissues and organs of marbled crayfish histological research methods were used. Individuals of control and experimental groups at the end of the experiment were fixed in 4% formalin solution, followed by processing by conventional histological methods. To produce slices microtome MC-2 was used. Histological sections were stained using haematoxylin-eosin. Photographs of preparations were made by a digital microscope attachment «Sciencelab T500 5.17 M», which was connected to a microscope Jenaval. Histological preparations were described using histology atlases of crustaceans [14]. The value of nuclear-cytoplasmic ratio (NCR) was calculated as the ratio of the core area to the area of the cell.

The statistical analysis of the results was conducted in Statistica 8.0 (StatSoft Inc., USA). The differences between the selections were found using one-way ANOVA and considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$. All results are presented as the means ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Table 1. Indicators of crayfish growth in experimental and control aquariums (x ± SD)

Control			Experiment with Zn			Experiment with Cd		
Weight in the beginning, g	Weight in the end, g	%	Weight in the beginning, g	Weight in the end, g	%	Weight in the beginning, g	Weight in the end, g	%
2.34	3.01	28.63	2.56	3.12	21.88	2.32	2.98	28.45
0.79	0.91	15.19	0.93	1.09	17.20	1.02	1.08	5.88
1.03	1.13	9.71	1.79	2.56	43.02	1.04	2.03	95.19

0.71	1.74	145.07	0.94	1.19	26.60	0.76	0.98	28.95
0.45	0.45	0.00	0.91	1.77	96.67	0.48	0.68	41.67
0.70	0.89	27.14	0.49	0.61	22.45	1.89	2.55	34.92
1.69	2.23	31.95	1.86	2.68	44.09	0.96	1.16	20.83
0.94	1.34	42.55	0.96	1.72	79.17	0.41	0.53	32.50
1.00	1.38	38.00	0.89	0.83	–6.74	1.61	–	–
1.17	1.44	23.08	0.77	–	–	0.86	–	–
1.31	1.48	12.98	0.49	–	–	0.74	–	–
1.31±0.21	1.6±0.24	24.43	1.3±0.23	1.7±0.29	31.06	1.34±0.25	1.5±0.25	11.19

Note: «–» – the individuals died.

Growth of crayfish in a control and experimental aquariums.

At the beginning of the experiment, all marbled crayfish were in one dimensional and weight group, the average weight of individuals was in the range from 1.31 to 1.34 g (Table 1). On the 21st day of the experiment, the mass gain in the control aquarium was 0.32 g.

The largest increase in the mass of marbled crayfish was observed in the experiment with zinc 0.41, but we did not notice statistically significant difference between the weight indices of marbled crayfish. Despite this, the smallest mass gain in crustaceans was noted in the experiment with cadmium and was only 0.15 g. Excessive concentrations of heavy metals can cause the loss of hydrobionts. During the experiment, no crayfish was lost in the control.

At that time, on the 21st day of the experiment in the aquarium with zinc 18.19% of marble crayfish died. The highest mortality rate was observed in the experiment with cadmium and reached 27.28% (Table 2).

Table 2. Crayfish survival in experimental conditions

Control	Experiment with Zn	Experiment with Cd
100%	81.81%	72.72%

At the end of the experiment, some marbled crayfish put out eggs. In control, the percentage of females with caviar was the largest and amounted to 29.1%. Somewhat fewer females laid eggs in experiment with zinc – 11.1%. In the experiment with cadmium, egg deposits were not observed (Table 3).

Table 3. Research of reproductive indices of marbled crayfish (x ± SD)

Experiment conditions	Control (n = 11)	Experiment with Zn (n = 9)	Experiment with Cd (n = 8)
Percentage of females with eggs	29.1%	11.1%	0%
IAF indicator, units	84.5±2.44	78.0±1.63	–
Weight of sex products, mg	30.0±1.4	25.0±1.4	–
Weight of one egg (n = 50), mg	0.36±0.02	0.32±0.02	–
Diameter of one egg (n = 50), mg	1.5±0.05	1.7±0.05*	–

Note: In an experiment with cadmium crustaceans did not have eggs. * – difference significant at $p < 0.05$.

The study of the influence of heavy metals on the reproductive performance of hydrobionts is important because they negatively affect the reproduction of aquatic biological resources and cause genetic mutations.

Histological picture of tissues and organs of marbled crayfish under the influence of heavy metals. Heavy metals affect the physiological state of hydrobionts, which is reflected on their tissues and organs, therefore histological studies of tissues and organs can be used as biological markers for the determination of the negative effects of toxicants.

Physiological evaluation of antennal glands under the influence of heavy metals.

The main organs of secretion in marbled crayfish are a pair of modified metanephridium, antenatal glands or green glands. These are quite large rounded glands located in the head part and open through the ducts in the main segments of the antennas. Each gland consists of a large coelomic sac, a convoluted tubule and a bladder.

The secretory part of the antennal gland of marbled crayfish has the form of a sac, divided into multiple chambers, lined with single-layer glandular epithelium (Fig. 2).

On histological preparations there are marked rows of glandular cells located on a thin basal membrane. Cubic-shaped cells contain a very large nucleus in which a well-visible nucleolus is presented. The number of nucleoli can vary from one to several.

The secretion of the gland is accumulated in the apical part of the cell, while the protoplasm is diluted, and is partially lost for creation of secretion. On the outer part of the cell there are processes that turn into large vesicles containing the secret and liquid protoplasm. Then these bubbles are separated from the cell and for some time lie in the bore of the gland in the form of droplets or bubbles.

The cells of the antennal gland of marbled crayfish in the control had a size of 166.08±10.13 μm^2 (Table 4). Glandulocytes were characterized by the presence of clear edges of the cells, pronounced structure of the ducts, and a sensitive basal membrane. The cells had large nuclei with a cross-sectional area of 51.31±3.92 μm^2. Nuclear membrane had a clear edge, there were observed nucleoli, characterized by basophily. That is, the structure of the antennal gland of marbled crayfish in control corresponded to the norm for decapods.

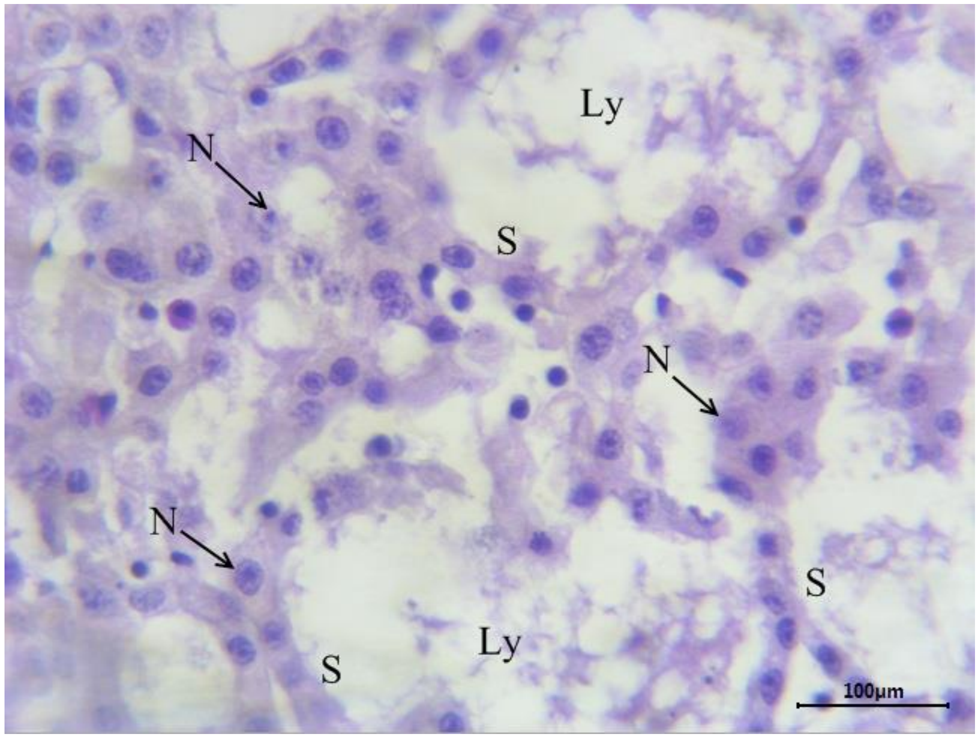


Figure 2. Green gland of crayfish, control: S – secretory cells, cells of an antennal gland, granulocyte, N – nucleus, Ly – labyrinth, luminal of gland.

Table 4. Results of histometric analysis of cells of an antennal gland of marbled crayfish ($n = 120$, $x \pm SD$)

Researched group	Cell area, μm²	Nuclear area, μm²	Value of NCR
Control	166.08±10.13	51.31±3.92	0.31±0.01
Zinc, 0.1 mg/l (10 MPC)	148.77±10.11	39.19±1.44**	0.29±0.02
Cadmium, 0.01 mg/l (2 MPC)	141.44±7.60**	42.32±1.74*	0.31±0.02

Note: * – difference significant at $p < 0.05$, ** – difference significant at $p < 0.01$

Under the influence of zinc, the cells of the green gland of marbled crayfish were also characterized by a clear organization, pronounced membranes, integral nuclei and nucleoli (Fig. 3). The cross-sectional area of the glandulocytes reached 148.77±10.11 μm². The cell nucleus occupied approximately 26–29% and reached 39.19±1.44 μm². Compared with control, there was no statistically significant difference between cell sizes, but it was noted that the sizes of the nuclei of the green gland under the influence of zinc ions were 23.6% less than in control.

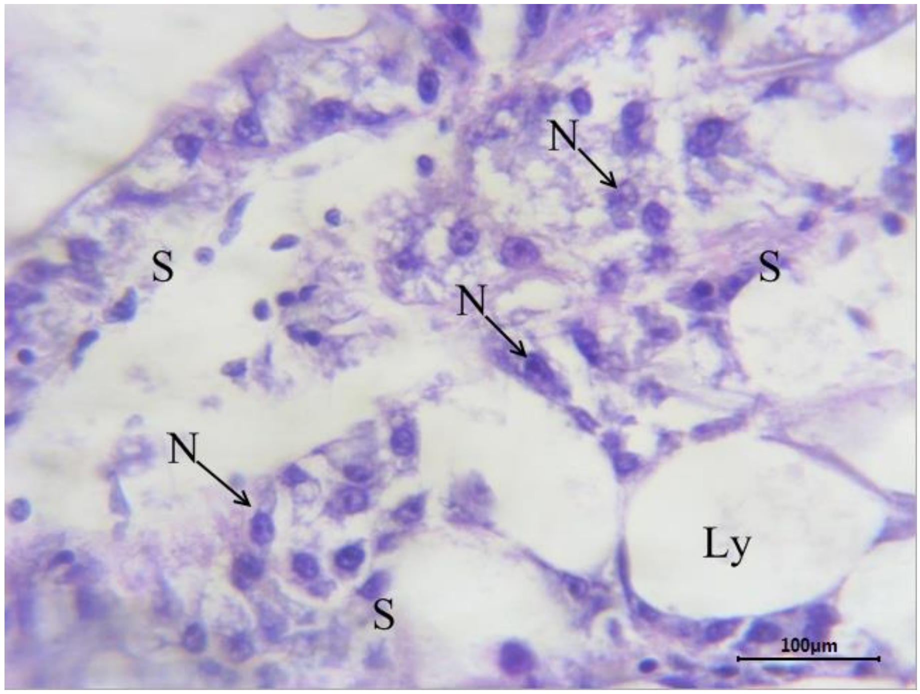


Figure 3. Antennal gland of marbled crayfish influenced by zinc: S – secretory cells, cells of an antennal gland, granulocyte, N – nucleus, Ly – labyrinth, luminal of gland.

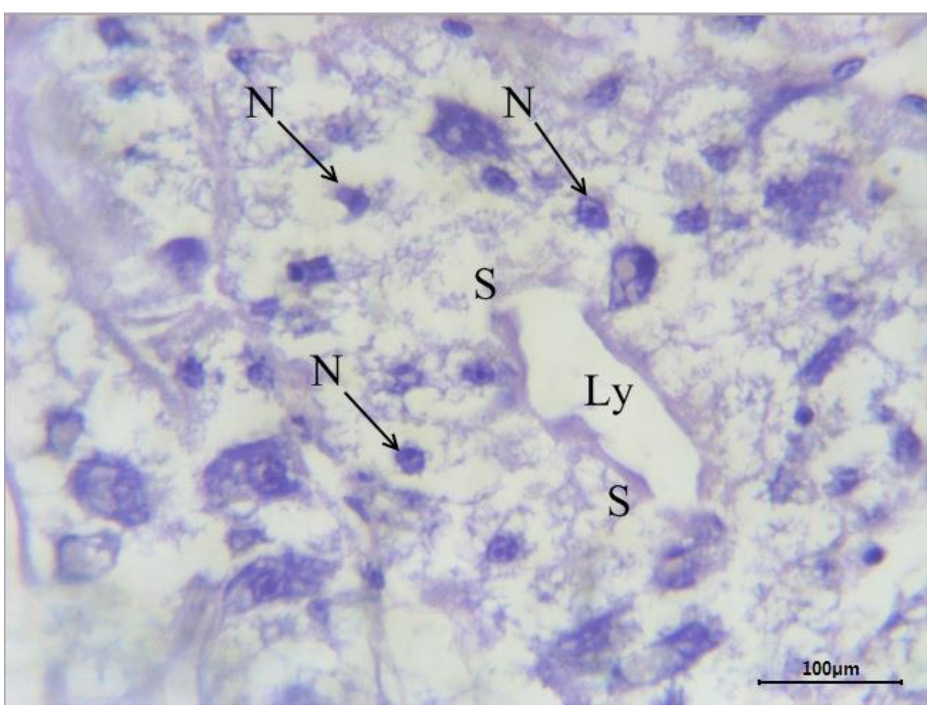


Figure 4. Antennal gland of marbled crayfish influenced by cadmium: S – secretory cells, cells of an antennal gland, granulocyte, N – nucleus, Ly – labyrinth, luminal of gland.

The worst histological picture for antenatal gland cells was observed in individuals exposed to cadmium ions. The structure of the excretory ducts of the green gland was broken, they had fuzzy boundaries, contained a large number of fragments of the granulocyte cytoplasm. In some cells there was a pyknosis of nuclei, as well as the output of a nucleolus beyond the nucleus, the appearance of the micronucleus. This phenomenon is explained by the toxicological effects of cadmium (Fig. 4).

Compared to control, the glandulocytes of the antennal gland were 14.8% smaller, their cross-sectional area reached 141.44±7.60 μm². The nuclei of the glandular cells also were significantly lower by 17.5% compared to the control parameters, and their area reached 42.32±1.74 μm².

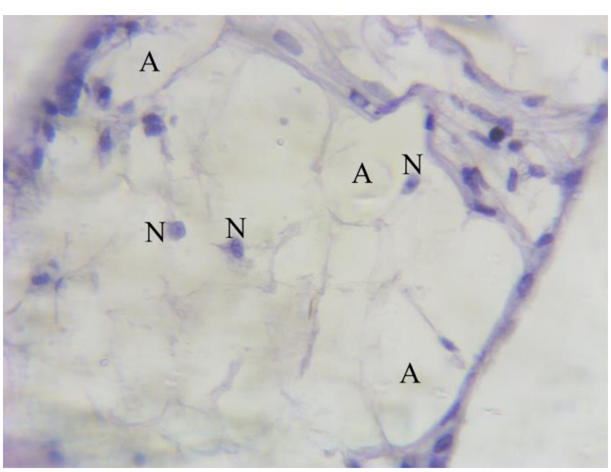
Physiological evaluation of fat cells under the influence of heavy metals.

The study of adipocytes of connective tissue showed that significant difference between cell sizes under the influence of heavy metals was not observed. Dimensions of adipocytes fluctuated in a wide range from 215 μm^2 to 2178 μm^2. And on average they reached the sizes 872–994 мкм^2. Thus, such concentration of heavy metals at the expiration of the experiment at 21 days did not affect the lipid metabolism in the organism of marbled crayfish (Table 5).

Table 5. Fatty cells of connective tissue of marbled crayfish (n = 100, x ± SD)

Indicator	Control	Experiment with Zn	Experiment with Cd
Adipocyte area, μm^2	872.64±87.22	985.31±66.88	994.50±101.86

The adipose tissue is a kind of connective tissue. Almost all of the fat cell, the specific function of which is the accumulation and exchange of fat, is filled with a fat drop that surrounds the cytoplasm with displacement of the nucleus at the periphery. The main physiological significance of adipose tissue is that it carries the function of the energy depot of the body and protects it from heat loss.



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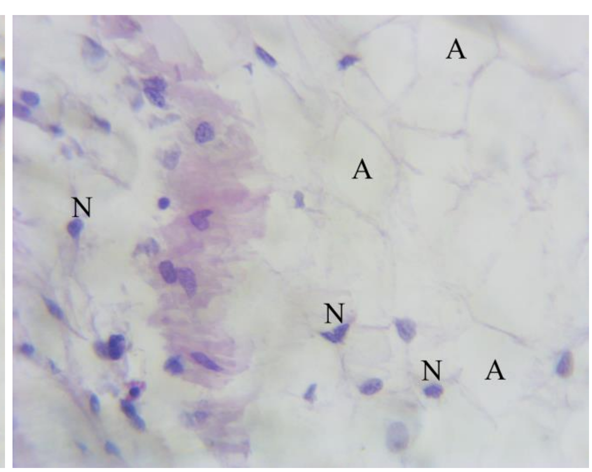

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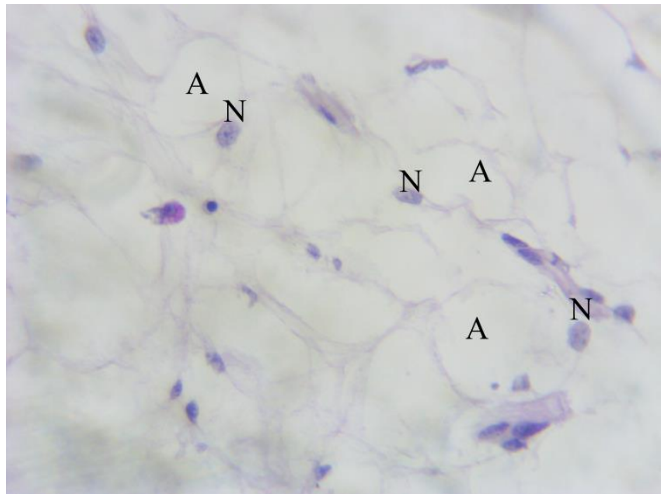

C

Figure 5. Fat cells of connective tissue of marbled crayfish: A – experiment with zinc; B – experiment with cadmium, C – control; A – Adipocyte cell, N – nucleus (ob. lens×40, oc. lens×10).

The fibrous connective tissue consists of fibers of collagen and elastin. It is characterized by considerable strength, which is conditioned by the good development of collagen bundles interwoven with each other, as well as elastic fibers. Fat cells in the experimental group are considerably larger than in the control group, this is due to the accumulation of heavy metals in the connective tissue (Fig. 5).

The adipose tissue can be used for evaluation of the physiological state of crustaceans.

Physiological evaluation of hepatopancreas under the influence of heavy metals.

The digestive system of marbled crayfish is represented by a tube, consisting of the esophagus, the stomach, the middle gut, the rectum and the digestive gland, hepatopancreas, which consists of small lobes and opens with its ducts in the stomach.

The function of this organ corresponds to the function of the liver and pancreas of the vertebrates, where the name "hepatopancreas" came from. The secretion of hepatopancreas of marbled crayfish not only can split fats and transform them into an emulsion state, but also can transform starch into sugar, which is typical for the pancreas.

In control, the cross-sectional area of the hepatopancreas was 164.52 μm^2 with a lumen of 39.03 μm^2 (Fig. 6).

While in the experiment with zinc, the structures of hepatopancreas were increased by 1.2 times, and in the cadmium test, the largest increase in structural elements of tissue was observed in 1.5 times (Table 6).

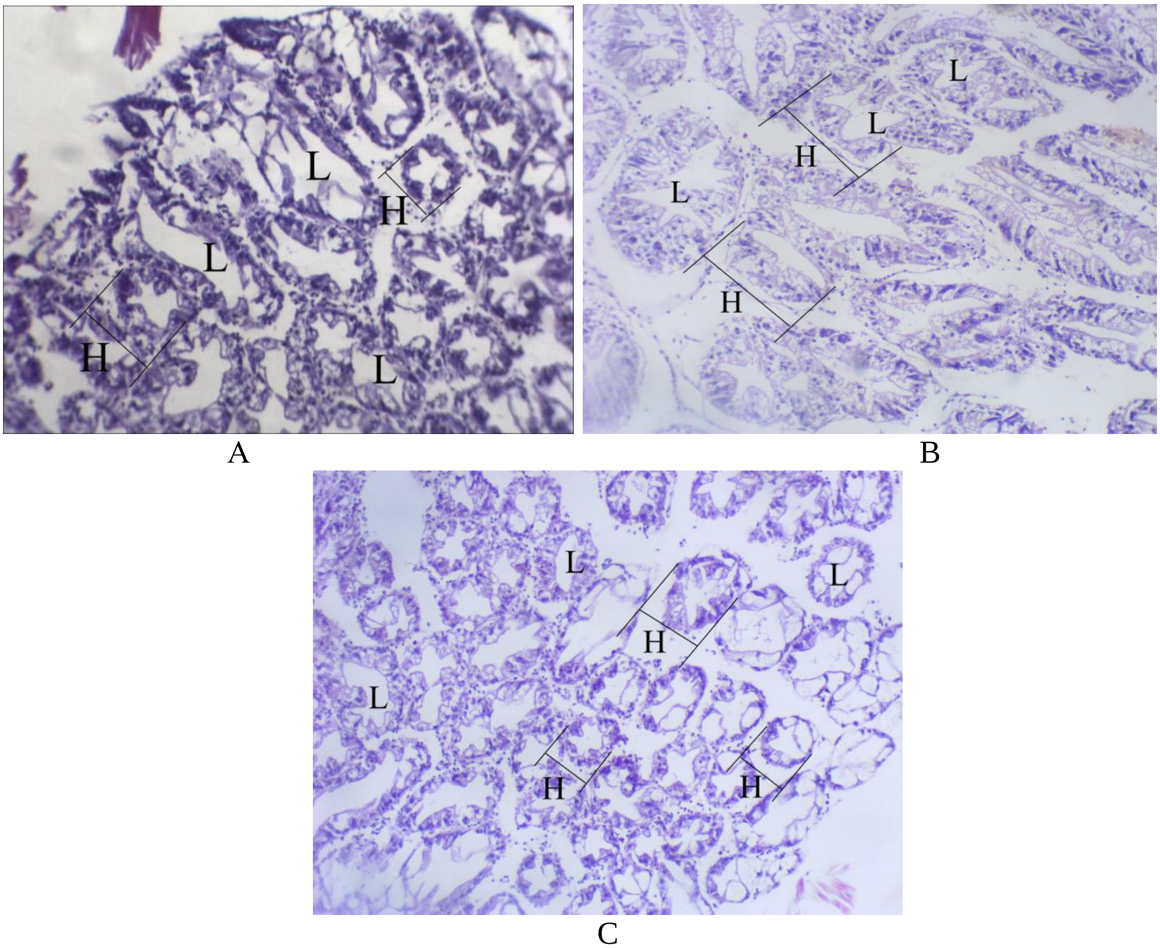

Figure 6. Hepatopancreas of marbled crayfish, tubules in cross-section: A – experiment with zinc; B– experiment with cadmium; C–control; H – Lobes of hepatopancreas, L – Hepatopancreas lumen (ob. lens×8, oc. lens×10).

Table 6. Histometric parameters of hepatopancreas of marbled crayfish (n = 100, x ± SD)

Parameters	Control	Experiment with Zn	Experiment with Cd
Lobes of hepatopancreas, μm^2	164.52±1.87	185.47±0.68 ***	268.96±2.91 ***
Hepatopancreas lumen, μm^2	39.03±0.52	54.07±0.39 ***	56.37±1.08 ***

Note: *** – difference significant at $p < 0.001$.

Research of hepatopancreas of marbled crayfish has shown significant changes in tissue structure. Heavy metals influenced the shape of the hepatopancerose lobes and the size of the lumen.

Investigation of the histological structure of tissues and organs of marbled crayfish will allow the development of measures for the determination of biomarkers for the bioindication of the aquatic environment using freshwater crayfish. The results of such studies have an important fundamental and applied significance for understanding the mechanisms of adaptation of crustaceans to the conditions of toxicological burden on aquatic ecosystems.

4. CONCLUSIONS

1. The appearance of marbled crayfish in the reservoirs of the Pridniprov'e may signal its possible acclimatization and further spread of the species through the territory of Ukraine. The rapid spread of the species in water bodies of Europe is caused by the wide possibilities of parthenogenetic form of marbled crayfish to adaptations, even in reservoirs with a tense toxicological state.

2. Experimentally simulated concentrations of heavy metals on the example of zinc – 0.1 mg/l (10 MPC) and cadmium – 0.01 mg/l (2 MPC) allowed to detect the reaction of the excretory system of marbled crayfish. It is determined that under the influence of heavy metal ions the area of cells of glandulocytes decreases by 10.4–14.8%. Also, there is a decrease in the nucleus cell apparatus, so, under the influence of zinc, the nucleus of secretory cells was 23.6% lower, and under the influence of cadmium lower by 17.5%.

3. The value of the nuclear-cytoplasmic ratio allows estimating the level of metabolism and detecting the manifestation of compensatory reactions of the organism of marbled crayfish. Thus, in the experiment and control, the value of NCR was not statistically different and ranged from 0.29 to 0.31 units, indicating a decrease in both the cytoplasm of the cells of the green gland and their nuclei caused by the influence of heavy metals.

4. The greatest negative effect was shown by the influence of cadmium ions: the structure of the excretory ducts of the green gland was broken, they had fuzzy boundaries, contained a large number of fragments of the cytoplasm of glandulocytes, the nucleus pyknosis was observed, as well as the appearance of the micronucleus.

5. At the end of the experiment, some marbled crayfish laid eggs. In control, the percentage of females with eggs was the largest and amounted to 29.1%. Somewhat fewer females laid eggs in experiments with zinc – 11.1%. In an experiment with cadmium, egg deposition was not observed.

6. Excessive concentrations of heavy metals can cause the loss of hydrobionts. During the experiment, no crayfish was lost in the control. At that time, on the 21st day of the experiment in the aquarium with zinc 18.19% of marbled crayfish died. The highest mortality rate was observed in the experiment with cadmium and reached 27.28%

7. The study of adipocytes of connective tissue showed that there was no significant difference between the size of cells under the influence of heavy metals. Dimensions of adipocytes fluctuated in a wide range from 215 μm^2 to 2178 μm^2. And on average they reached sizes 872–994 μm^2. Thus, such concentration of heavy metals at the 21-day exposure did not affect the lipid metabolism in the marbled crayfish.

8. Investigation of hepatopancreas of marbled crayfish showed significant changes in tissue structure. Heavy metals influenced the shape of the hepatopancerose lobes and the size of the lumen. In control, the cross-sectional area was 164.5 μm^2 with a lumen of 39 μm^2. While in the experiment with zinc, the structures of hepatopancreas was increased by 1.2 times, and in the cadmium experiment, the largest increase in structural elements of tissue was 1.5 times.

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