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PARAMETERS OF HISTOLOGICAL ADAPTATION OF MARBLED CRAYFISH *PROCAMBARUS FALLAX F. VIRGINALIS* (DECAPODA) TO THE POLLUTION WITH ZINC IONS

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The article presents results of the research on the influence of zinc ions on the histological structure of antennal gland cells of marbled crayfish *Procambarus fallax f. virginalis* Martin et al., 2010 (Decapoda). It is found that glandulocytes and their nucleuses gradually diminish in size under the influence of heavy metals, and the nuclear-cytoplasmic ratio remains the same, which probably is an adaptation of excretory system to the impact of zinc ions.

Marbled crayfish, Procambarus fallax, zinc, heavy metals, histology

В статті наведено результати досліджень впливу іонів цинку на гістологічну структуру клітин антенальної залози мармурового рака *Procambarus fallax f. virginalis* Martin et al., 2010 (Decapoda). Встановлено, що під впливом важких металів закономірно зменшуються розміри гландулоцитів та їх ядер, при цьому стало зберігається ядерно-цитоплазматичне відношення, що певно є адаптаційною реакцією видільної системи на вплив іонів цинку.

Мармурові раки, Procambarus fallax, цинк, важкі метали, гістологія

The foreground water pollutants are toxic heavy metals; they are extremely dangerous natural water pollutants as even in relatively low concentrations they may adversely affect aquatic organisms. The main biological effects of heavy metal pollution of the water environment are the direct toxic effects on aquatic organisms, which lead to the damage of their physiological systems [1].

Multi-year research has shown that in the water of Zaporizke (Dnipro) reservoir and its tributaries there are constant violations of regulatory requirements of SanPiN-88 for containing Cd, Mn, Cu and in some areas for the content of Zn, Ni and Fe. These heavy metals can reduce the number, inhibit growth and cause death of aquatic organisms, sensitive to toxins.

The study of adaptive capacity of new species of aquatic organisms, which first move to water with sustainable environmental regime and formed toxicological background, is of particular interest. In this case, new species may die because of impact of anthropogenic factors, or vice versa, to adapt to new conditions. The process of adaptation that occurs at the biochemical and cellular level is a prerequisite of survival of invasive species populations [2].

Marbled crayfish *Procambarus fallax f. virginalis* Martin et al., 2010 (Decapoda) is a North American typical alien species, which was brought to Europe as an aquarium species. Because of considerable popularity in aquaristics it has come to Europe, Asia and Africa, where, most likely as a result of negligence of aquarians was brought to natural waters [3, 4]. Thus, since 1990 parthenogenetic forms of marbled crayfish appeared in ponds of Germany and the Netherlands [5]. In 2007-2008 it appeared in Italy [6], in 2015 in the Czech Republic [7] and in Ukraine [8].

Due to the fact that marbled crayfish has appeared in waters of Ukraine, it became necessary to study the possibility of its adaptation to the environmental conditions of water bodies to predict its spread, or even acclimatization under conditions of toxicological water pollution of steppe Prydniprovya. The purpose of our study was to determine the effect of heavy metals on physiological state and histostructure of excretory system of marbled crayfish.

Materials and methods

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

For this experiment we used 3 aquariums with working capacity of 30 liters. The water temperature was maintained by thermostat at a level of + 24 °C in all aquariums. Oxygen regime was maintained by the compressor, the oxygen content in the water of aquariums was 8 mg/l. Water in aquariums was completely changed twice a week and toxicants were added at the rate of concentrations of Zn ions – 0,1 mg/l (10 MAC). Concentrations of heavy metals were determined by their content in water of Zaporizke (Dnipro) reservoir, the main recipient reservoir for this species. Crayfish were fed every day with the same quantity of food. In each aquarium there were 11 specimens of the marbled crayfish of the same size and age group from one parthenogenetic female. The experiment lasted 21 days.

The impact of heavy metals on histostructure of antennal gland of marbled crayfish was studied by histological research methods. Individuals of control and experimental groups at the end of the experiment were fixed in 4 % formalin solution and treated by conventional histological methods [9]. Sections were made by MC-2 microtome. Histological sections were stained using hematoxylin-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 [10]. The value of nuclear-cytoplasmic ratio was calculated as the ratio of the nucleus area to the area of the cell.

Statistical data processing was carried out by conventional methods using software packages for personal computers Microsoft Excel 2007 and STATISTICA 6.0.

Results and discussion

The main excretory organ of marbled crayfish is a pair of modified metanephridium, called antennal or green glands. This is a rather large rounded gland, located in the head part, which opens by channels in the basic segments of antennas. Each gland consists of a small coelomic sac, tubules and bladder.

Secretory part of antennal gland of marbled crayfish looks like a bag divided into numerous chambers, covered with a single layer of glandular epithelium (Fig. 1). In histological preparations there are notable lines of glandular cells, which are on thin basal membrane. Cells have cubic form; they have the very large nucleus with clearly visible nucleolus. Number of nucleoli may vary from one to several.

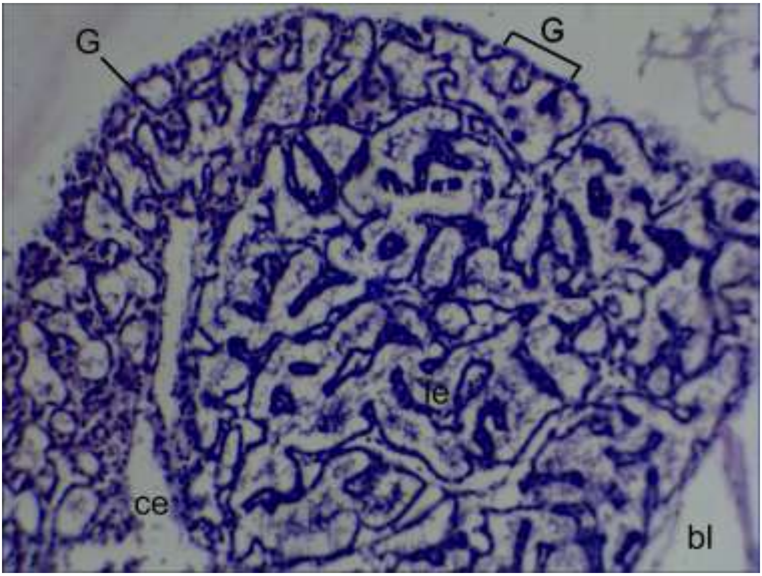

Figure 1 – Green gland of crayfish, control: G – glandulocytes, le – labyrinth of the gland, ce – coelomic sac. bl – bladder (8x lens)

Secretion of gland is accumulated in the apical part of the cell; protoplasm is diluted and partially spent on creating secret. On the outside of the cells outgrowth appear turning into large bubbles containing secret and liquid protoplasm. Then the bubbles break away from the cells and lie in the lumen of the gland in the form of drops or bubbles.

After separation of apical part, cell becomes low enough and slot appears on its free surface (Fig. 2). Gradually gland cells recover and grow to normal size, repeating secretory cycle.

Cells of antennal gland of marbled crayfish in the control group had size of 166,08 ± 10,13 um^2. Glandulocytes had a clear edge of cell, sharp structure of ducts, distinct basal membrane. The cells had large nucleuses with cross-sectional area of 51,31 ± 3,92 um^2. Nuclear membrane had a clear edge and nucleoli with basophilia were observed in the nucleuses. Thus, the structure of antennal gland of marbled crayfish of control group was normal for decapod crustaceans.

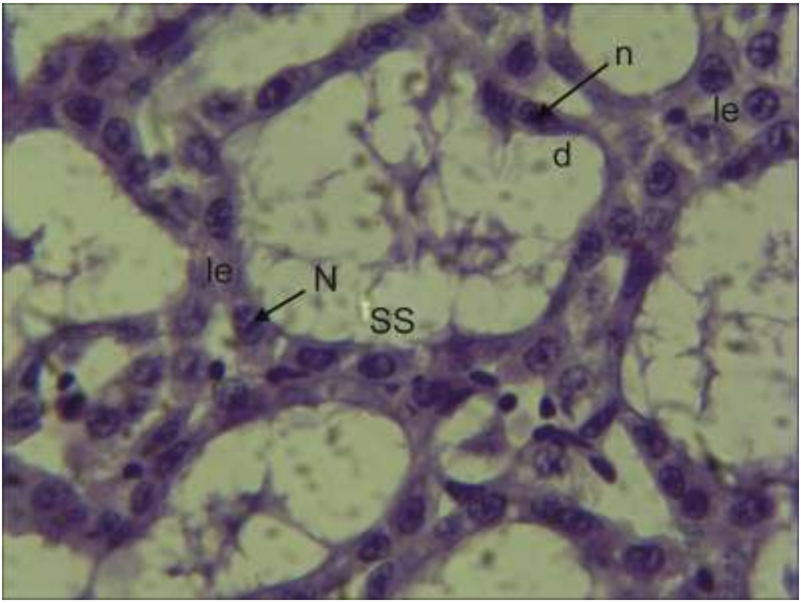

Figure 2 – Histology of antennal gland of marbled crayfish, control: le – labyrinth of the green gland, SS – secretory section, d – duct, N – nucleuses of glandulocytes, n – nucleoli (40x lens)

The value of NC ratio in the experimental and control groups did not vary statistically and ranged from 0.29 to 0.31 units. This indicates an inter-proportional reduction of cytoplasm and nucleuses of green gland cell due to the influence of heavy metals.

Under the influence of zinc cells of green gland of marbled crayfish also had a clear organization, sharp membrane, whole nucleuses and nucleoli (Fig. 3). Cross-sectional area of glandulocytes was 148,77 ± 10,11 um^2. Nucleus occupies about 26–29 % of cell and reached dimensions of 39,19 ± 1,44 um^2.

Compared to control group there was no statistically significant difference between the size of the cells, but it was noted that the size of nucleuses of green gland under the influence of zinc ions were 23.6 % lower than in the control group.

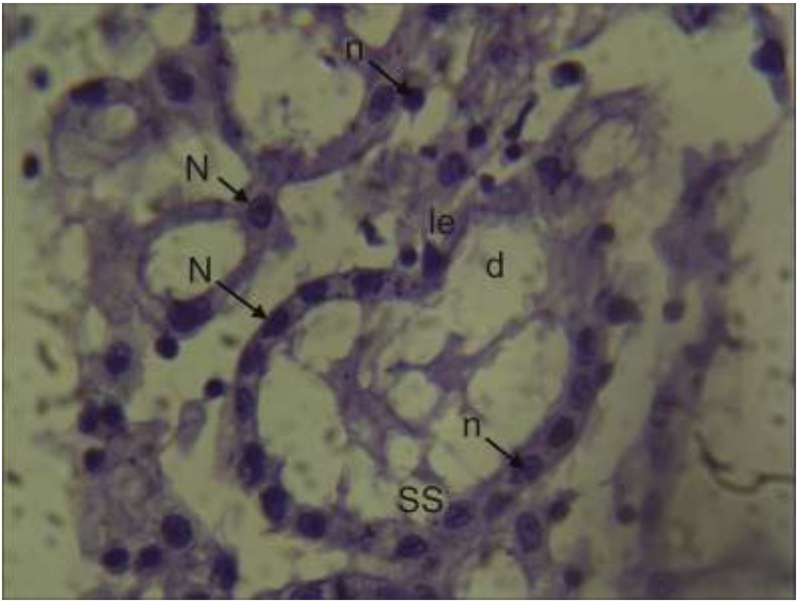

Figure 3 – Antennal gland of marbled crayfish under influence of zink: le – labyrinth of the green gland, SS – secretory section, d – duct, N – nucleuses of glandulocytes, n – nucleoli (lens 40x)

Thus, under the influence of heavy metals the structure of antennal glands of marbled crayfish changes. The size of cells and nucleuses of glandulocytes is gradually reduced, however nuclear-cytoplasmic ratio was kept at the same level, which is probably the excretory system adaptive response to the impact of heavy metals.

Conclusions

1. Experimentally simulated concentration of heavy metals on the example of zinc 0,1 mg/l (10 MAC) revealed reaction of excretory system of marbled crayfish. It was determined that under impact of heavy metals cell area of glandulocytes decreases by 23.6 %.

2. The value of NC ratio in the experimental and the control groups did not vary statistically and ranged from 0.29 to 0.31 units, indicating an inter-proportional reduction of cytoplasm nucleuses of green gland cells due to the influence of zinc ions.

3. The value of nuclear-cytoplasmic ratio allows evaluating the metabolic rate and detecting expression of compensatory reactions of marbled crayfish. Thus, the value of NC ratio in the experimental and the control groups did not vary statistically and ranged from 0.29 to 0.31 units, indicating an inter-proportional reduction of cytoplasm nucleuses of green gland cells due to the influence of zinc ions.

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