Acta Biol. Univ. Daugavp. 17 (2) 2017
ISSN 1407 - 8953

EFFECT OF ZINC AND CADMIUM IONS ON HISTOSTRUCTURE OF ANTENNAL GLANDS OF MARBLED CRAYFISH *PROCAMBARUS FALLAX* (HAGEN, 1870) *F. VIRGINALIS* (DECAPODA)

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Marenkov O., Holoborodko K., Voronkova Y., Gorban V. 2017. Effect of zinc and cadmium ions on histostructure of antennal glands of marbled crayfish *Procambarus fallax* (Hagen, 1870) *F. virginalis* (Decapoda). *Acta Biol. Univ. Daugavp., 17 (2): 211 – 216.*

Research results about the effects of cadmium and zinc ions on the histological structure of cells of antennal glands of marbled crayfish *Procambarus fallax* (Hagen, 1870) *f. virginalis* (Decapoda) are presented in the article. It is determined that size of glandulocytes and their nuclei affected by to heavy metals naturally reduces however nuclear-cytoplasmic ratio is stably preserved, which is probably the excretory system adaptive response to the impact of heavy metals ions.

Key words: cadmium, zinc, marbled crayfish, glandulocytes, *Procambarus fallax* (Hagen, 1870) *f. virginalis*.

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INTRODUCTION

Marbled crayfish *Procambarus fallax* (Hagen, 1870) *f. virginalis* (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 aquarians was released to natural waters (Martin et al. 2010a). Thus, since 1990 it was marked the appearance of parthenogenetic forms of marbled crayfish in ponds of Germany and the Netherlands (Chucholl and Pfeiffer 2010; Martin et al. 2010b). Marbled crayfish have been found in the wild in the Netherlands (Holdich D. M. and Pöckl M. 2007), Sweden (Bohman et al. 2013) Hungary (Lőkkös et al. 2016), Slovakia (Lipták et al. 2016), Madagascar (Jones et al. 2009) and Japan (Kawai T. and Takahata M. 2010) probably through release or escape from aquaria. Marmorkrebs are one of the most widely distributed species of crayfish in the international pet trade (Faulkes 2015). In 2007–2008 it was recorded its appearance in Italy (Nonnis Marzano et al. 2009), in 2015 in the Czech Republic (Patoka et al. 2015) and in Ukraine (Novitsky R.A. and Son M.O. 2016).

Such rapid expansion is explained by that the population of marbled crayfish is presented by only triploid parthenogenetic females which lay unfertilized eggs, from which “cloned” genetically homogeneous individuals of this species are born (Martin et al. 2010a). Males (parthenogenetic forms) of this species were not found either in vivo or in vitro studies.

Due to the fact that marbled crayfish has entered waters of Ukraine, the necessity to study the possibility of its adaptation to environmental conditions of water bodies with an aim to predict its spread, or even acclimatization under conditions of toxicological water pollution of steppe Prydniprovya (Holoborodko et al. 2016). The purpose of our study was to determine the effect of heavy metals on physiological condition and histostructure of excretory system of marbled crayfish.

The priority water pollutants are toxic heavy metals; they are an extreme danger as natural water pollutants that even in relatively low concentrations may adversely affect aquatic organisms. Biological effects of heavy metal pollution of the water environment are primarily in the direct toxic effects on aquatic organisms, leading to the defeat of their physiological systems.

Years of research scientists have shown that in the water of Zaporozhye (Dnipro) reservoir and its tributaries it is constantly observed violations of regulatory requirements SanPiN-88 for containing Cd, Mn, Cu and in some areas the content of Zn, Ni and Fe. These heavy metals can reduce the size, inhibit growth and induce 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 be lost or could not stand the pressure 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 populations of invasive species.

MATERIAL AND METHODS

We conducted a laboratory model experiments to study the mechanisms of adaptation of marbled crayfish *Procambarus fallax* (Hagen, 1870) *f. virginalis* (Decapoda). The effect of different concentrations of heavy metals on physiological state and histostructure of the excretory system of marbled crayfish was determined..

The experiment was conducted in 3 aquariums with working capacity of 30 liters. The water temperature was maintained by thermostat and was + 24^0 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 MAC). 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. The experiment lasted 21 days.

To investigate the impact of heavy metals on histostructure of antennal gland 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 (Mumford et al. 2007). 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 (Shields and Boyd 2014). The value of nuclear-cytoplasmic ratio was calculated as the ratio of the core 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 eliminative organ of marbled crayfish is a pair of modified metanephridium, antennal or green glands. This is a rather large rounded gland, located in the head part and opens by channels in the basic segments of antennas (Shields and Boyd 2014). Each gland consists of a small coelomic sac, tubules and bladder.

Secretory part of antennal gland of marbled crayfish looks like bag divided into numerous chambers that are covered with a single layer of glandular epithelium (Fig. 1). In histological preparations there are notable lines of glandular cells, which are on thin basement membrane. Cells have cubic form, contain the very large core with clearly visible nucleolus. Number of nucleoli may vary from one to several. Secretion of gland accumulates in the apical part of the cell; thus protoplasm is diluted and partially spent on creating secret (Shields and Boyd 2014). 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 the 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 had size of 166.08±10.13 um^2 (Table 1). Glandulocytes were characterized by a clear edge of cell, frank structure of ducts, distinct basal membrane. The cells had large cores with cross-sectional area of 51.31±3.92 um^2. Nuclear membrane had a clear edge karyosomes with marked basophilia were observed in the nuclei.

Table 1.Results of histometric analysis of antennal gland cells of marbled crayfish

Research group	**Area of cells, μm^2 (n=120)**	**Area of nuclei, μm^2 (n=120)**	**Value of NCR (n=120)**
Control	166.08±10.13	51.31±3.92	0.31±0.01
Zink, 10 MAC (0.1 mg/l)	148.77±10.11	39.19±1.44*	0.29±0.02
Cadmium, 2 MAC (0.01 mg/l)	141.44±7.60*	42.32±1.74*	0.31±0.02

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

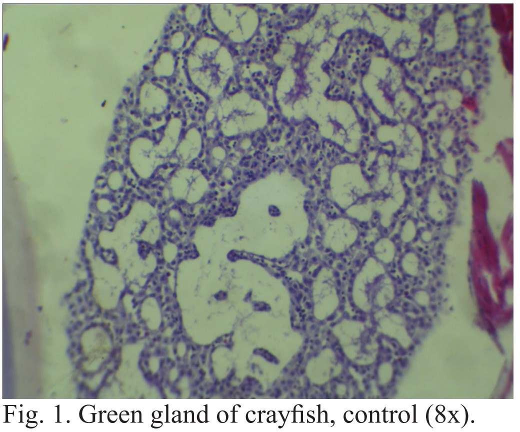

Fig. 1. Green gland of crayfish, control (8x).

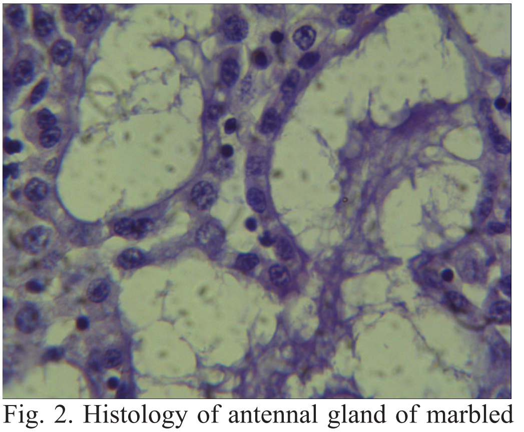

Fig. 2. Histology of antennal gland of marbled crayfish control (40x).

Thus structure of antennal gland of marbled crayfish of control met norms for decapod crustaceans (Shields and Boyd 2014).

Under the influence of zinc cells of green gland of marbled crayfish were also characterized by a clear organization, frank membrane, whole nuclei and nucleoli (Fig. 3). Cross-sectional area of glandulocytes was 148.77±10.11 um^2. Nucleus occupies about 26–29% and reached dimensions of 39.19±1.44 um^2. Compared with controls statistically significant difference between the size of the cells were not observed, but noted that the size of nuclei of green gland under the influence of zinc ions were 23.6% lower than in the control group.

Worst cell histology of antennal gland was observed in individuals, which were under the influence of cadmium ions. The structure of the ductless of green glands was broken; they had fuzzy boundaries and contained a large number of fragments of glandulocytes` cytoplasm. In some cells there was a pyknosis of nuclei and nucleoli outlet beyond the core – the appearance of micronuclei. This phenomenon is explained by the toxicological impact of cadmium.

Compared with the control glandulocytes of antennal gland were 14.8% lower; area of their cross-sectional reached 141.44±7.60 um^2. The nuclei of glandular cells were significantly lower at 17.5% than the control, but their area has reached 42.32±1.74 um^2.

To determine the effect of heavy metals on glandular cells of green glands of marbled crayfish index of nuclear-cytoplasmic ratio (NCR) was used. The ratio between the areas of cytoplasm and nuclei of cells of the excretory system is an important histomorphological characteristic which allows evaluating the metabolic rate and detecting expression of compensatory reactions. Resize of nuclear and nuclear-cytoplasmic ratio may serve as an indicator of inflammation and certain forms of pathologic changes in secretory cells. The value of NCR in the experiment and control statistically varied and ranged from 0.29 to 0.31 units. This indicates an inter-proportional reduction of green gland cell cytoplasm, and their nuclei due to the influence of heavy metals.

Thus, under the influence of heavy metals changes in the structure of antennal glands of marbled crayfish were marked. The size of cells and nuclei of glandulocytes is naturally reduced, thus nuclear-cytoplasmic ratio was kept, which is probably the excretory system adaptive response to the impact of heavy metals. The largest negative effect was indicated under the influence of cadmium ions because it is one the most toxic heavy metals.

CONCLUSIONS

1. The appearance of marbled crayfish in reservoirs of steppe Prydnyprovya may signal a

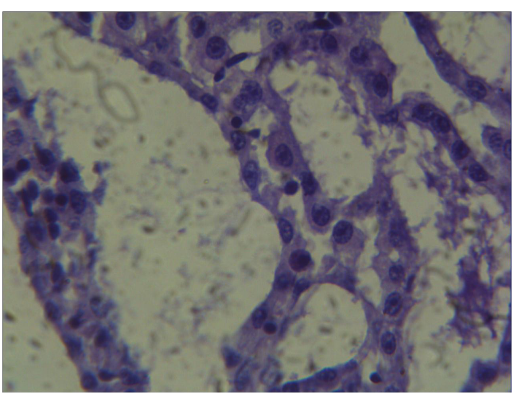

Fig. 3. Antennal gland of marbled crayfish under influence of zink (40x).

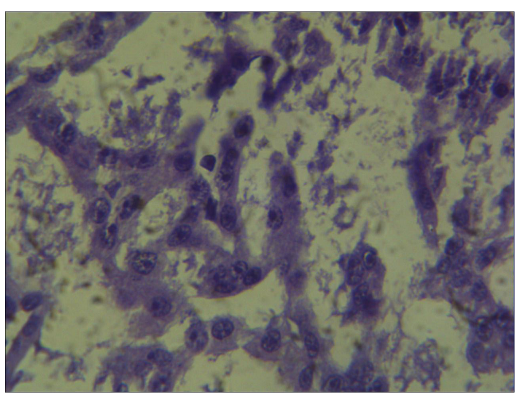

Fig. 4. Antennal gland of marbled crayfish under influence of cadmium (lens 40x).

possible acclimatization and its further spread of this species through Ukraine. The rapid spread of species in the European waters is caused by many features of parthenogenetic forms of marbled crayfish to adaptations even in waters with hard toxicological conditions.

2. Experimentally simulated concentrations of heavy metals for example zinc – 0.1 mg/l (10 MAC) and cadmium – 0.01 mg/l (2 MAC) revealed reaction of excretory system of marbled crayfish. It was determined that under impact of heavy metals cell area of glandulocytes decreases to 10.4–14.8%. Also, there is a reduction of nuclear system of cells; thus under the influence of zinc nuclei of secretory cells were lower by 23.6%, while under the influence of cadmium by 17.5%.

3. The value of nuclear-cytoplasmic ratio allows evaluating the metabolic rate and detecting expression of compensatory reactions of marbled crayfish. Thus, in the experiment and the control value of NCR has not varied statistically and ranged from 0.29 to 0.31 units, indicating an inter-proportional reduction of green gland cell cytoplasm, and their nuclei due to the influence of heavy metals.

4. The largest negative effect was indicated under the influence of cadmium ions: the structure of ductless of green glands was broken, they had fuzzy boundaries contained a large number of fragments of cytoplasm of glandulocytes, the pyknosis of nuclei and the appearance of micronuclei was observed.

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Received: 26.08.2017.
Accepted: 14.10.2017