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# Impact of Low-Molecule Acidic Peptides on Growth and Histological Structure of Inner Organs of Marbled Crayfish *Procambarus fallax* (Hagen, 1870) *F. virginalis*

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**Abstract.** The results of studies on the effects of low molecular weight acidic solution peptides on the growth and development of the marbled crayfish artificial cultivation.An increasing weights of juvenile freshwater crayfish under the influence of dietary supplement "Albuvir" drug. With the use of histological methods of research, found the impact of 0.01 % solution of the drug on the state of the marbled crayfish lobules of hepatopancreas and fat cells. Developed a method for growing juvenile freshwater crayfish with "Albuvir", which allows to increase the weight gain of crustaceans on 24.3–27.2 % and reduce the level of cannibalism at 20 %.

## 1. Introduction

Marbled crayfish (or marmorkrebs) *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Crustacea, Decapoda, Cambaridae) – parthenogenetic crayfish that were discovered in the German waters in 1990 [1; 2]. 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) [3; 4]. Crayfish got in Europe as an aquarium species and most likely have been infused in Germany reservoirs [5; 6]. As an invasive species marbled crayfish hit the waters of the Netherlands, Italy, Slovakia, Sweden, Croatia and Ukraine [7–14]. The main feature of the marbled crayfish is that its population consists entirely of triploid females and reproduction occurs by parthenogenesis [15]. All the offspring is genetically uniform. Thus, marmorkrebs conveniently used as model objects in biological studies [16].

The goal of this work was to study the effects on growth and development of freshwater crayfish latest immunostimulatory additive, low molecular weight solution of acidic peptides as drug "Albuvir". A feature of the drug is the almost complete lack of a toxicity (peptides are broken down into amino acids) and the impossibility of adaptation on the part of the virus and the body of the animal - amino acids arrange themselves in the body of the animal. The drug is widely used in animal husbandry and veterinary medicine as preventive and immunopotentiating substance, but it can also be used in fish farming and aquaculture [17].

The main objective of research was to determine the effect of the new anti-virus biologically active drug "Albuvir" on a linearly-weighted indices and condition of the tissues and organs of juvenile marbled crayfish.

## 2. Materials and methods

As experimental objects used the same age generation of parthenogenetic marbled crayfish *P. fallax* f. *virginalis*, obtained from one of the female (Fig. 1). Thus, in the experiment used genetically homogenous material.

Fig. 1. Marbled crayfish in the beginning of the experiment.

At the beginning of the experiment specimens were one size-weight group, two weeks of age. Research was conducted in aquarium conditions on the basis of Department of General Biology and Aquatic biological resources Oles Honchar Dnipropetrovsk National University. At each tank the same number of individuals have been planted – 43 specimen. The aquarium water was changed every week, and the drug "Albuvir" was added to the experimental aquaria. The concentration of the drug in the water was 0.01 %. Feeding of crayfish was carried out once a day, a universal bottom feed brand Nature "Somiki", the daily dose – 5 % by weight of aquatic organisms (in the control and experiment fed the same amount of food). The water temperature in the experimental and control aquaria was $21^0$C. Constant temperature is maintained thanks to the thermostat. For optimal oxygen conditions used by the compressor.

Features of marmorkrebs were recorded by morphometric analysis using a digital caliper. Measuring the length was done up to 1 mm. All animals were weighed with accuracy up to 0.01 g. Before weighing each individual was dried with filter paper until wet spots cease to appear on it.

During the experiment, the quality of water was determined in the following hydrochemical parameters: pH, chloride content ($Cl_2$, mg/l), nitrates ($NO_2$, mg/l), nitrites ($NO_3$, mg/l), total hardness (GH, ◦d), carbonate hardness (kH,◦d). Water analysis was performed using an express-test "Tetra Test 6 in 1".

Histological methods of research were used to study the effects of the drug on tissues and organs of crayfish.Tissues and organs were collected out of animals of the experimental and control groups, then collected samples were fixed in 4 % formalin with subsequent processing according to conventional histological techniques [18]. For sectioning usев sledge microtome MC-2. Sections were colored using hematoxylin and eosin. Photos were made using a digital camera "Sciencelab T500 5.17 M", which was connected to the microscope of the company "Biolam 70". Description of histological structures were made using histology atlases of crustaceans [19].

Statistical analysis of the material was carried out using Microsoft Excel and STATISTICA 6.0 software packages for personal computers.

## 3. Results and discussion

The experimental and control aquarium average weight of crayfish at the beginning of the experiment both experimental and control groups was 0.06±0,001 g. Fluctuations between the minimum and maximum mass index does not exceed 10 % (Fig. 2).


A B

Fig. 2. Young marbled crayfish: A – experiment, B – control.

According to the research found that by hydrochemical indicators, water quality in the tanks did not differ significantly (Table 1).

Table 1. Water quality indicators

| Indicators | Control | Experimental |
|---|---|---|
| pH | 6.8–7.2 | 7.2–7.6 |
| $Cl_2$, mg/l | 0.8–1.5 | 0.8–1.0 |
| $NO_2$, mg/l | 0–0.7 | 0–0.5 |
| $NO_3$, mg/l | 0–0.9 | 0–1.0 |
| GH,◦d | 16.0–17.5 | 16.0–16.9 |
| kH, ◦d | 5.5–6.2 | 5.8–6.1 |
| Temperature,◦0C | 21.0 | 21.0 |

The pH in the control aquarium ranges from 6.8 to 7.2 units, and the experimental – from 7.2 to 7.6 units, i.e. in a test tank under the influence of the drug, water was more alkaline. The total hardness of the water fluctuated in the control aquarium within 16.0–17.5 d, at a time when in the experimental aquarium stiffness was slightly less – 16.0–16.9◦d. The carbonate hardness of the water were within 5.5–6.1◦d, oscillation index did not exceed 0.3 units.

Chloride content does not deviate from the norm (1–1.5 mg/l) and reached: in the control aquarium 0.8–1.5 mg/l in the experimental – 0.8–1.0 mg/l. The concentration of nitrates in the water of the control tank was in the range 0–0.7 mg/l, experimental – 0–0.5 mg/l. The content of nitrite in the control tank ranged from 0 to 0.9 mg/l in the experimental – 0–1.0 mg/l. Thus, the hydrochemical conditions of the marbled crayfish in aquariums were identical.

When evaluating crayfish's weight growth for 10 weeks, it was found to be increasing by almost 4.6 times in the experimental aquarium, and in the control aquarium – by 3.6 times (Table 2).

Table 2. Crayfish weight indicators

| Indicators | Experiment, M±m | Control, M±m |
|---|---|---|
| Mass at the beginning of experiment, g | 0.06±0.001 | 0.06±0.001 |
| Mass at the end of experiment, g | 0.28±0.003 | 0.22±0.003 |

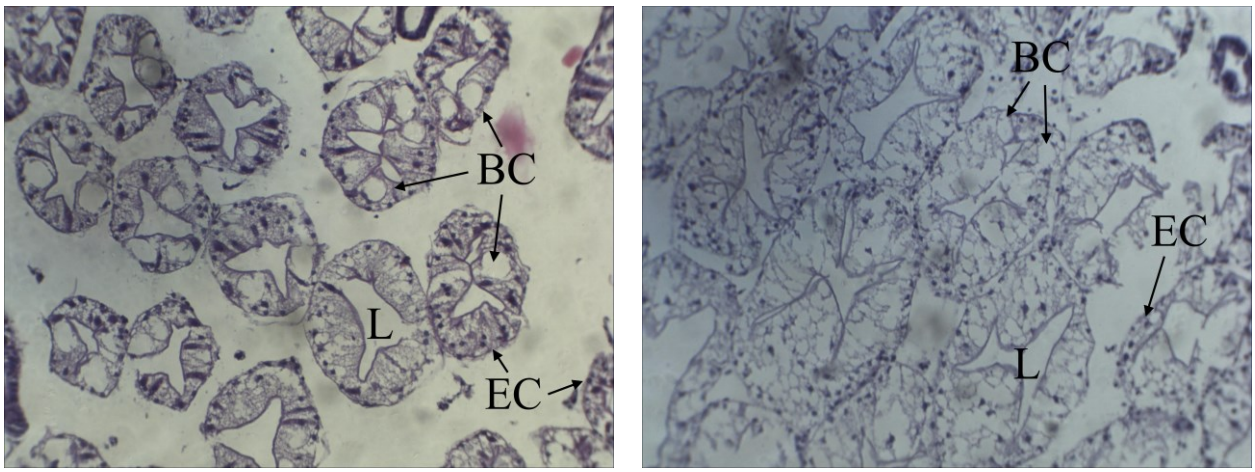
At the end of the experiment the difference between the mass of animals in the control and the experiment aquarium was 27.2 % ($p<0.05$). It was noted that cases of cannibalism in the experimental group of crayfish occurred 20 % less than in the control group.

Note that when adding the drug solution to the water tank during the first day, water was turbid, its transparency then returned to the original state. At the same time the introduction of "Albuvir" did not cause crayfish any state of discomfort, they were active, responded well to food. A single dose of food they ate for 15–25 minutes, while in the control aquarium it took 30–50 minutes.

Epithelium of hepatopancreatic tube contains 4 different types of cells. The tubes are collected in a pair of common ducts draining into the midgut.

The blind end of each tube is represented by embryonic cells (E-cells) which are precursors of other types of cells [19]. Proximally to E-storage cells R-cells, vacuolated B-cells and F-fibrillar cells are located (Fig. 3).

During the research of hepatopancreas of marbed crayfish has been found that the structure and size of the lobules of hepatopancreas in individuals of control and experimental groups differed significantly. In the control, area of the cross-section slices of lobules of hepatopancreas reached 31332.11±1189.72 $\mu m^2$, while in the experiment segments were 42.3 % ($p <0.05$) bigger – 42147.38±4475.77 $\mu m^2$ (Fig. 3). The size of the tubule rift did not differ significantly – in the control area rift of the tubular reached 6301.07±409.37 $\mu m^2$, in the experience – 6868.51±652.33 $\mu m^2$. Thus, increasing the size of lobules of hepatopancreas was due to tissue proliferation and accumulation of fat inclusions in them.

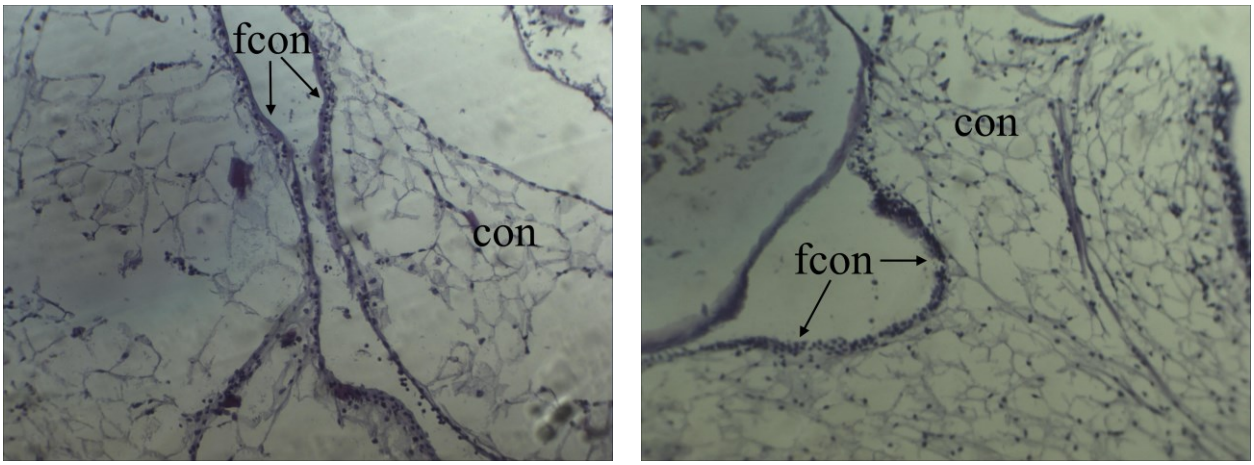



A B

Fig. 3. Marbled crayfish hepatopancreas, canals in transverse section in control (A) and experiment (B): BC – B-cells, EC – E-cells, L – rift of the tubules.

The fibrous connective tissue of marbled crayfish consists of collagen and elastin fibers. It is characterized by considerable strength, due to good development of collagen bundles, intertwined, and elastic fibers. In this structural tissue fibroblasts and histiocytes cells were found. Adipose tissue is a type of connective. Almost entirety of the fat cell, the specific function of which is the accumulation and metabolism of fat, is filled with the oil drop, which is surrounded with the cytoplasm and the nucleus being pushed out to the periphery. The main physiological value of adipose tissue of marbled crayfish is that it accomplishes the energy depot function of the body – during starving the amount of fat in the cells decreases, while during nourishing diet increases.

Analysis of loose connective tissue of marbled crayfish showed that connective tissue cells in individuals exposed to the drug "Albuvir" were characterized as fairly large in size compared with the control. Size of cells in the control were 948.09±69.22 $\mu m^2$, in the experience – 1403.76±80.37 $\mu m^2$ (Fig. 4). Thus, in the experiment, connective tissue cells were almost 48.1 % more than in the controls ($p <0.05$).




A B

Fig. 4. Fibrous cells of connective tissue of marbled crayfish:
A – experiment, B – control: con – connective tissue, fcon – fibrous connective tissue.

Size increase the fat cells in the experimental group of individuals is associated with the accumulation of nutrients in the connective tissues of crustaceans, which may be caused by the influence of "Albuvir" solution.

Thus, developed a method of using the low molecular weight acidic peptides for growing juvenile freshwater crayfish, the implementation of which allows to increase the rate of growth of aquatic organisms and reduce the level of cannibalism.

The method is as follows. Capacity for growing crayfish before adding the drug is carefully cleaned from food residues and excrement. Constant aeration and water filtration is provided through the use of a compressor and a mechanical filter. Create a 0.01 % concentration of low molecular weight acidic peptide which is added with graduated pipette or a graduated cylinder and stirred in a container to distribute the drug. Crayfish feeding is exercised daily. The frequency of application of the drug – 1 times per week for 60 days.

The results are an important basis for optimizing the biotechnology cultivation of freshwater aquatic organisms. The results of researches are introduced into the practice of «Optim-Vet» veterinary center.

## 4. Conclusions

1. It was found that, the mass of juvenile crayfish increased by almost 4.6 times in the control aquarium, and in 3.6 times in the experimental aquarium in 10 weeks of the experiment. The difference between the mass of the control and experimental aquariums individuals was 27.2 % ($p < 0.05$). It was noted that in the experimental group, cases of cannibalism were recorded at 20 % less likely than individuals in the control group.

2. It was found that the structure and size of the lobules of hepatopancreas in individuals of control and experimental groups differed significantly. In control, the cross sectional area segments of hepatopancreas reached 31332.11±1189.72 $\mu m^2$, while the slices in the experiment were more than 42.3 % – 4475.77±42147.38 $\mu m^2$. The value of the tubule rift did not differ significantly – in the control area of the rift of the tubular reached 6301.07±409.37 $\mu m^2$, in the experiment – 6868.51±652.33 $\mu m^2$. This means that the increase in the size of the lobules of hepatopancreas occurred due to tissue proliferation and accumulation of fat inclusions in these tissues.

3. Histological examination of loose connective tissue of marbled crayfish have shown that fat cells in individuals exposed to the drug "Albuvir" was significantly large in size compared with the control. The size of cells in the control reached to 948.09±69.22 $\mu m^2$, in the experiment – 1403.76±80.37 $\mu m^2$. Thus, in the experiment fat cells was almost 48.1 % higher than the control. Increasing the size of fat cells in the experimental group of individuals is associated with the accumulation of nutrients in the connective tissues of crustaceans, which may be caused by the influence of "Albuvir" solution.

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