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SHORT COMMUNICATION

Histogenesis and morphological characteristics of hyaline cartilage of the pumpkinseed juveniles *Lepomis gibbo*sus (Linnaeus, 1758) (Actinopterygii, Centrarchidae)

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ABSTRACT

The article presents the structure feature of the hyaline cartilage of two-month-old fry of the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758). It is found that 4 cm long fry have cartilaginous skeleton, in which the bony tissue is replacing cartilage one. It is noted that the chondroblasts have a flattened or oval shape and reach a size of 77.10 ± 25.55 μm ^{2}. Eventually, the growth of the cytoplasm of cells and the differentiation of chondroblasts into chondrocytes leads to the decrease in nuclear-cytoplasmic ratio from 0.159 to 0.086 units. The definitive chondrocytes reach a size of 142.82±14.78 μm^2, which is almost twice as large as chondroblasts. However, the dimensions of the nuclei of cells of the cartilaginous tissue are not statistically different and range from 11.4 to 11.8 μm^2. As the fish grows, the bony tissue gradually replaces cartilage tissue of the skeleton, involving chondroclasts. The study was carried out within the research scientific work "Assessment of the physiological, biochemical and cytological status of native and alien aquatic organisms under conditions of anthropogenic transformation of aquatic ecosystems" (state registration number 0117U006751).

***Keywords*:** histology, fish, pumpkinseed, hyaline cartilage, chondroblasts, chondrocytes, *Lepomis gibbo*sus

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1. INTRODUCTION

Currently, the sectoral research institutes of Ukraine almost do not research histology of hydrobionts due to the outdated material and technical base and the lack of specialists in this field. Despite this, the need for studying the structural components of the tissues of hydrobionts is quite large in the educational and scientific process [7]. The scientist`s interest in the histology of aquatic organisms is very high, because the state of cells of tissues and organs of aquatic animals can indicate their adaptive capabilities and structural and functional responses to environmental factors [2]. The study of histological structure of tissues and organs of bony fishes is necessary for a better understanding of their physiology and development processes. The study of the morphology of the cartilaginous tissue of bony fishes at different development and growth stages is a necessary link in the study of the ontogenetic diversity of fish and can be useful for further applied research and developments in the field of aquatic biological resources and aquaculture. Studies of the tissues and organs of the alien species, which have expanded their habitat and got into new ecological conditions, are informative [5]. One of these invasive species is the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), a representative of the North American ichthyofauna, which has already totally acclimatized in the waters of Ukraine and is still increasing its number [1, 9]. Information regarding the developmental processes and physiology of this species in Ukrainian water bodies is limited and fragmentary [6]. In this regard, the **purpose of** our studies is to investigate the structure and functional features of the cartilaginous tissue of juveniles of bony fishes using the pumpkinseed`s juveniles as an example.

2. MATERIALS AND METHODS

The basis of the study is study our own research materials about pumpkinseed fry in the water area of the Zaporizke reservoir in summer of 2016. The ichthyological material was selected at two monitoring stations located in the Samara Bay of the Zaporizke Reservoir (Odinkovka village 48º50′60″N, 35º18′87″E, Novoselovka village 48º57′35″N, 35º23′50″E). The permits for the special use of aquatic biological resources allowed scientific research catches of juveniles. Juvenile fish were caught in third decade of August in shallow waters of Samara bay using a 10-meter long and 1 m high minnow seine, made of bolting cloth № 7. Pumpkinseed juveniles were measured within the accuracy of 1 mm and weighed within the accuracy of 0.01 g [14]. We analyzed 30 fingerlings of pumpkinseed by the following biological parameters: L = 3.96 ±0.06 cm, l = 3.16 ±0.06 cm, m = 0.83 ±0.05 g, age: 2 months.

Histological methods of research were used to study the histological structure and morphology of the cartilaginous tissue of fingerlings of bony fishes. Fish fry were fixed in a 4% formalin solution with further treatment after conventional histological methods [11]. Sections were made using MS-2 microtome. Histological sections were stained with haemotoxylin-eosin. Photographs of the preparations were made using a digital camera for the microscope “Sciencelab T 500 5.17 M”, connected to the Jenaval. 100 microscope chondroblasts and chondrocytes were analyzed and measured. Histological sections were described using fish histology atlases [7, 13]. The nuclear-cytoplasmic ratio (NC ratio) was calculated as the ratio of the area of the cell nucleus to the area of the cytoplasm [3]. The data was statistically processed according to standard methods using the software package Statistica

8 .0 (StatSoft Inc., USA). All results are presented as the mean ± standard deviation (SD). The significance of differences between the data samples is determined byone-way ANOVA test with a significance level of p <0, 05.

Bioethical norms were not violated during the research [10].

3. RESULTS AND DISCUSSION

Cartilaginous tissue belongs to a highly specialized group of connective tissues with obvious mechanical functions [4]; it also performs supporting, protective functions and participates in water-salt metabolism of fish. It is widely represented in the body of all vertebrate animals without exception. The tissue contains highly developed intercellular substance, and the number of cells is relatively small. Cartilage tissues are part of the respiratory system of fish, ventral parts of the ribs, articular surfaces and intervertebral discs. They consist of cells and intercellular substance with a high optical density [7].

Hyaline cartilage forms most of the skeleton of fish embryos; cartilaginous tissue is the original substance for the development of the bones of the fish skeleton in the process of embryonic histogenesis [14]. Hyaline cartilaginous tissue forms hyaline cartilage and is the most common type of cartilaginous tissue. In embryos and larvae of the pumpkinseed, almost the whole skeleton consists of this tissue, in adults the hyaline cartilage remainson the articular surfaces of bones and at the edges of the ribs.

In a living organism, the hyaline cartilage is translucent with a blue-white tone. Its color depends on the location in the body and the age of the fish. Fibrillarity manifests itself only with special methods of treatment of preparations and in polarized light [8]. The intercellular substance of hyaline cartilage, like all types of cartilaginous tissue, mainly consists of a collagen-chondrin protein. The intercellular substance of hyaline cartilage of fish is highly hydrophilic, which determines its density, turgor and promotes diffusion of nutrients, water and salts. However, large protein molecules that tend to be antigens do not pass [15].

The cartilaginous tissue of the pumpkinseed, like most bony fishes, develops from the sclerotome mesenchyme. In the period of embryonic histogenesis, mesenchymal areas appear in the areas of prechondral tissue, with more compact arrangement of cells than in surrounding mesenchyme. In such areas, the mesenchymal cells differentiate into chondroblasts, which actively produce an intercellular substance. Later, in amorphous intercellular substance, thin collagen fibers begin to appear so the areas of the primary cartilaginous tissue is coloured with acidic dyes. The amount of amorphous substance significantly increases, which allows chondroblasts to move away from each other. The intercellular substance changes: chondroitinsulfuric acid appears in it, which, combining with proteins, forms a chondromucoid, giving the tissue basophilic properties.

The mesenchyme, which surrounds the young cartilaginous tissue forms a perichondrium. The perichondrium contains collagen fibers and chondroblasts. In the area of the young cartilage the chondrocytes are round or elongated, small, parallel to the perichondrium. In the zone of the mature cartilagecells are larger, oval, located in isogenic groups of 4–6 cells (Fig. 1). Collagen fibers in the intercellular substance form a mesh invisible at standard color.

The cells of the perichondrium differentiate into chondroblasts, which provide interstitial cartilage growth [7]. The growth of the cartilage from the side of the perichondrium is quite intense, as it is rich in blood vessels and the metabolism is much more intensive, compared to

the central part of the cartilage. In adult individuals of pumpkinseed, further growth of the cartilaginous tissue is mainly caused by an increase in the proportion of intercellular substance. At the same time, an increase in the mass of tissue disrupts the trophism of the chondrocytes, which leads to a gradual degeneration.

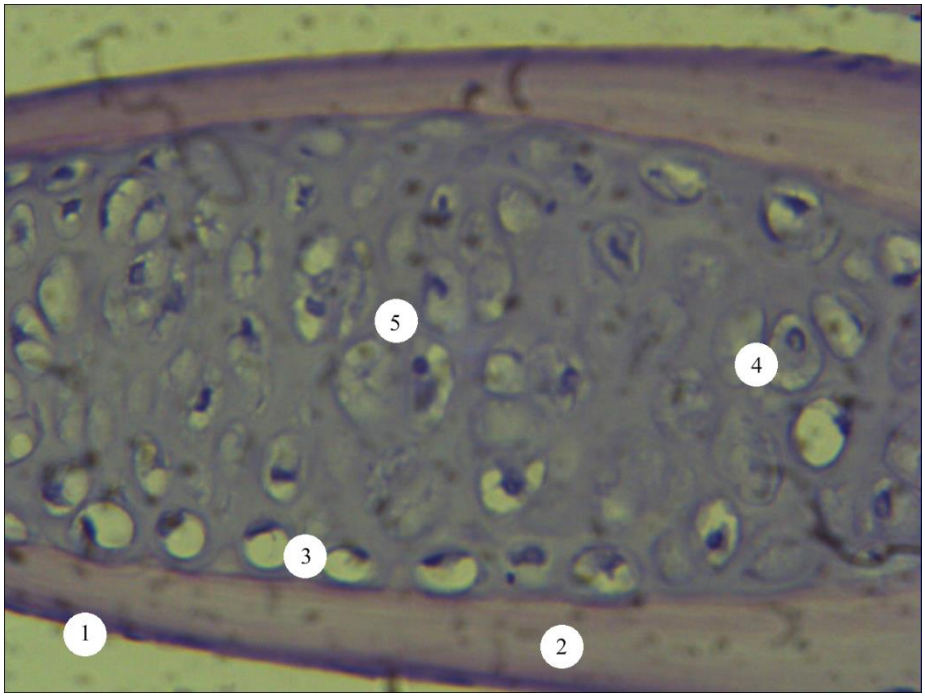

Figure 1. Hyaline cartilaginous tissue of the rib of the pumpkinseed *L. gibbosus* (Linnaeus, 1758).The preparation of O. N. Marenkov. 1 – top layer of the perichondrium, 2 – deep layer of perichondrium, 3 – chondroblasts of the young cartilage zone, 4 –chondrocytes of mature cartilage, 5 – isogenic groups of cells. Hematoxylin-eosin. X400.

Deep zones of cartilage become oxyphilic due to the disappearance of chondroitinsulfuric acid and the transformation of chondromucoid into an albumoid. Further growth of the highly differentiated cartilage leads to the calcium saline deposit in the intercellular substance around the cartilaginous cells appearance of shiny fibers that are optically different from the collagen ones. These zones of cartilage lose elasticity, become soft, resorbed, and eventually they emerge into voids in which blood vessels grow from the perichondrium. Destroying the calcified cartilage, pericyte of blood vessels differentiate into bone cells, the osteoblasts, subsequently giving rise to bone tissue in place of cartilage. During the development of the cartilaginous tissue of fish programed differentiation forms: stem cells – semi-stem cells – chondroblasts – chondrocytes. Chondroblasts are young flattened or oval cells capable of proliferation and synthesis of intercellular substance of cartilage. They are descendants of stem cells. The cytoplasm of chondroblasts has a well-developed granular and agranular endoplasmic reticulum and Golgi complex [7]. Peripheral, apposition growth of the cartilage happens with the participation of chondroblasts. Chondroblasts evolve into chondrocytes during the development of cartilage. The chondroblasts of pumpkinseed are on average 77.10±25.55 μm^2 in size, they

are darker than chondrocytes, because they contain a large number of organelles and organic compounds. The dimensions of the pumpkin seed`s chondroblasts nucleus range from 8.2 to 15.9 μm^2 (Table 1).

Table 1. Histometric analysis of cells of hyaline cartilage of fry fish *L. gibbosus* (Linnaeus, 1758) (n = 100, x ± SD).

Index	n = 100	The magnitude of the NC ratio
Area of chondroblasts, μm^2	77.10 ± 25.55*	0.159*
Area of chondroblast nuclei, μm ^{2}	11.39 ± 2.04	
Area of chondrocytes, micron^2	142.82 ± 14.78*	0.086*
Area of chondrocyte nuclei, μm ^{2}	11.81 ± 2.41	

Note: * – the difference is significant for p<0.05.

Chondrocytes are the main kind of cartilaginous cells. They have an oval, rounded shape, which depends on the degree of differentiation. They are located in special cavities in the intercellular substance alone or in groups by two-four, and in the center of the cartilage by six or eight cells. It happens because in deep layers of cartilage the density of the intercellular substance increases and the cartilage cells after division do not have the ability to move away from each other. Groups of cells lying in the same cavity are called isogenic groups. They are formed by dividing one cell. Chondrocytes of pumpkinseed reach an average of 142.82±14.78 mm^{2,} which is almost twice more than chondroblasts (p<0.05). Chondrocyte nuclei have the same dimensions as the nuclei of chondroblasts and reach 11.81±2.41 μm^2. The NC ratio of chondrocytes is 0.086 units, which is almost one half as much the NC ratio of the chondroblasts.

There are three types of chondrocytes in isogenic groups. The first type of chondrocyte is characterized by a high nuclear-cytoplasmic ratio, the development of vacuolar elements, the presence of mitochondria and ribosomes. The second type of chondrocytes is characterized by a decreased nuclear-cytoplasmic ratio, intensive development of the granular endoplasmic reticulum and the Golgi complex. The third type of chondrocyte has a low nuclear-cytoplasmic ratio, characterized by a high development and an ordered arrangement of the granular endoplasmic reticulum.

Chondrohistogenesis. The cartilaginous tissue of fish develops both in the embryonic and postembryonic periods during regeneration. The source of development of cartilaginous tissues is the mesenchyme. In those places of the embryo's body, where the cartilage is being formed, the mesenchyme first strengthens; the cells lose their processes, multiply intensively and adhere to each other, creating a certain pressure, called the turgor. Such areas are called chondrogenicis lands. The mesenchymal cells of these islands differentiate into chondroblasts, the cells that form a cartilaginous tissue. In the cytoplasm of such cells, the number of free ribosomes increases, and parts of the granular endoplasmic reticulum appear. In the next stage, the primary cartilaginous tissue, the cells of the central region (primordial chondrocytes)

increase in size and become rounded. In their cytoplasm a granular endoplasmic reticulum develops, where the synthesis and secretion of fibrillar proteins (collagen) occurs. Thus, a hydroxyphilic intercellular substance is formed. Later, the stage of differentiation of the cartilaginous tissue begins – the chondrocytes acquire the ability to synthesize both fibrillar proteins and proteoglycans associated with non-collagen proteins. On the periphery of the cartilaginous lining on the border with the mesenchyme, there is a perichondrium – a shell covering the cartilage from the outside. It consists of the outer fibrous and internal chondrogenic layers. In the chondrogenic zone, the cells, dividing intensively, differentiate into chondroblasts, which retain the ability to synthesize DNA, multiply, and to synthesize the components of the intercellular substance.

Cartilage cells located in the center of the young cartilage, for some time, retain the ability to mitotic division. By increasing the number of these cells, an internal mass of the cartilage increases, which is called interstitial cartilage growth. It is observed in embryogenesis, as well as in the regeneration of cartilaginous tissue. As the cartilage grows and develops, the central areas move away from the nutrient vessels and begin to experience a deficit in the trophic (Fig. 2). As a result, chondrocytes lose their ability to reproduce.

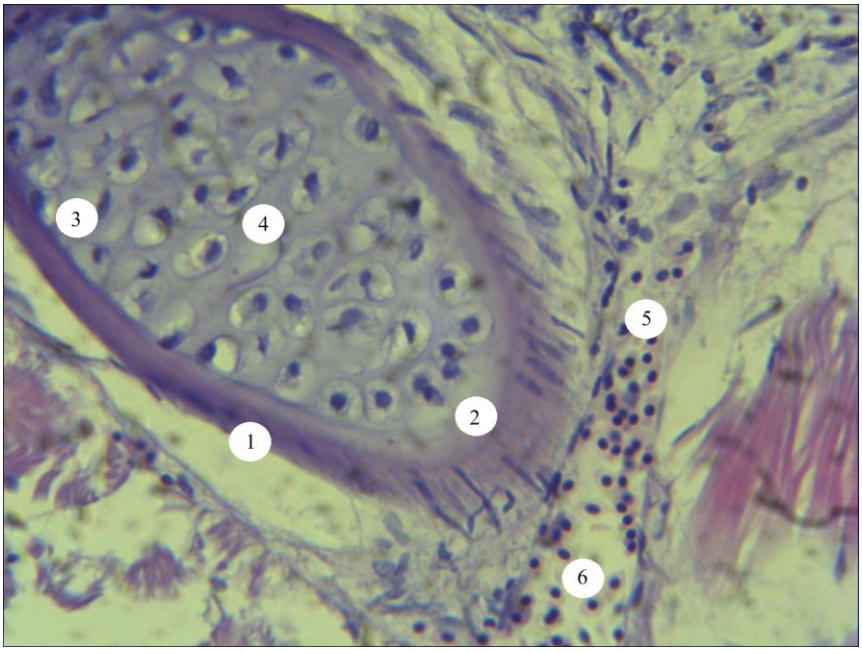

Figure 2. Hyaline cartilage of the rib of the pumpkinseed *L. gibbosus* (Linnaeus, 1758). The preparation of O.N. Marenkov. 1 – top layer of the perichondrium, 2 – deep layer of perichondrium, 3 – chondroblasts of the young cartilage zone, 4 – chondrocytes of mature cartilage, 5 – blood vessel, 6 – erythrocytes. Hematoxylin-eosin. X400.

The last stage of development of differentiated cartilage is accompanied by a deepening of the contradictions between tissue growth and nutrition; conditions begin to be unsatisfactory for the functioning of cells and intercellular substance. Calcium salts (liming) are deposited near the cells, and asbestos dystrophy is observed in the center of the cartilage (Fig. 3).

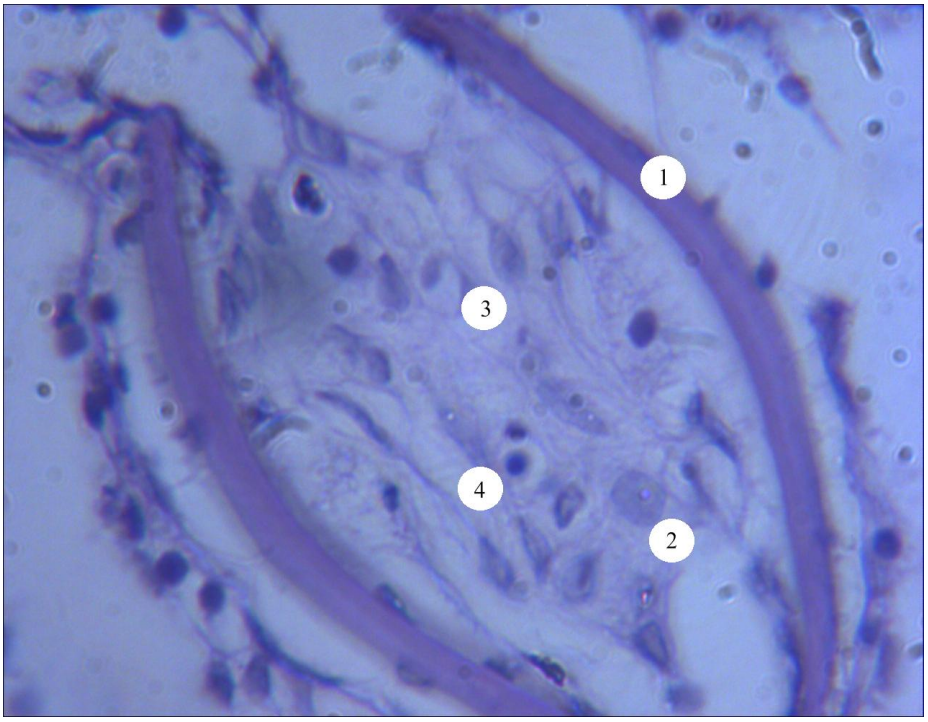

Figure 3. Development of bone in the place of cartilage: 1– perichondrium, 2– chondroclus, 3 – bone cells, 4 – erythrocyte. Hematoxylin-eosin. X400.

During the further growth of the fry of the pumpkinseed, the ossification of the cartilaginous models of the internal skeleton happens due to the replacement of the cartilaginous tissue with the bony one, which is characteristic of most bony fishes [8, 12].

Further studies may be pointedto biochemical studies on age-related changes in the skeleton of bony fishes. A promising area of research is the use of radiography methods to identify pathologies and abnormalities in the structure of the skeleton of fish caused by the impact of anthropogenic factors.

4. CONCLUSIONS

Thus, the two-month-old fry fish of the pumpkinseed with a length of 4 cm mostlyhave a cartilaginous skeleton, which is represented by a hyaline cartilaginous tissue. The surface of the hyaline cartilage is covered with perichondrium, underneath which there is a layer of chondroblasts, which cells have average dimensions of 77.10±25.55 μm^2. Later, chondroblasts increase in size due to the growth of the cytoplasm and differentiate into chondrocytes that perform a structural function. Cells of chondrocytes are twice larger than cells of chondroblasts. The dimensions of the nuclei of cells of the cartilaginous tissue are not statistically different and range from 11.4 to 11.8 μm^2. During the growth of fish, the bony tissue gradually replaces cartilage tissue of the skeleton, involving chondroclasts.

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