

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Morphological Indicators of Skeletal Muscles in Carp in Case of the Use of Quickened Growth Technology.

Natalya A Slesarenko<sup>1\*</sup>, Elena I Shilo<sup>2</sup>, and Pavel N Abramov<sup>1</sup>.

<sup>1</sup>Moscow state Academy of Veterinary Medicine and Biotechnology - MVA by K.I. Skryabin, Academic Skryabin Street 23, Moscow, 109472, Russia

<sup>2</sup>Belgorod State Agricultural University named after V. Gorin, Vavilov Street 1, Belgorod, 308503, Russia

### ABSTRACT

The article analyzes the impact of technological conditions of fish farming on the growth of skeletal muscles as a physiological function of the body. Myogenesis patterns in carp with specific nutritional adaptation are determined. The influence on morphometric parameters of muscle as an organ of anthropogenically simulated conditions of fish farming is presented. The results obtained serve as the background for forecasting the productive qualities of fish.

**Keywords:** carp, muscle, ontogenesis, meat.

*\*Corresponding author*

## INTRODUCTION

Scientific substantiation of the effectiveness of the use of new technologies for growing fish is one of the urgent problems of modern fish farming [1, 2].

When growing carp, two- and three-year technologies are used. According to the basic technology [3], a modified three-year turnover with fish productivity of 1700-3100 kg/ha is for the production of commercial fish in polyculture. It includes growing carp under yearlings weighing 25-30g, two-year-olds – 200-300g and three-year-olds – 1200-2500g with high fish-holding density. The technology of growing large fish seed and commercial fish at a two-year (intensive) turnover at a productivity level of 4600-7000 kg/ha is developed for large under yearlings (80-100 g), commercial two-year-olds weighing up to 1200 g.

Artificial selection of breed fish is aimed at selection of fast-growing and similar in size fish. In recent decades, new information about ecological and biological characteristics of fish has been obtained. It is shown that both in the early stages of development and in adult fish, abiotic (temperature, day length, water flow characteristics, hypoxia) and biotic factors of the environment (food availability, parasitic infections, joint breeding of several fish species) influence the signaling pathways that regulate the behaviour of fish and their metabolism.

The growth of fish in early ontogenesis is connected with the overall energy potential of development and the high degree of variability of its morphological features. According to some authors, the density of fish in water is one of the key environmental mechanisms that limit individual growth. When deteriorating nutritional conditions, the rate of linear-mass growth decreases and variability increases which is manifested in the increase in the proportion of small and large fish that allows the body to use the food resources of the reservoir more fully, in general, and freshwater ecosystems, in particular [4]. It is known that initially fish gains weight when food supply is abundant. Since the growth of fish fry is connected with their food activity, intensive feed on zooplanktons in early ontogenesis is apparently accompanied by increased muscular growth, intensive division and growth of muscle fibers.

The issues of histogenesis of muscle tissue are highlighted in a number of foreign and domestic works. According to A. Rowleson, A. Veggetti, the formation of muscle fibers occurs in three phases [5]. The first phase is the formation of the myotome from the paraxial mesoderm of the embryo. The second phase is the proliferation of muscle fibers due to the migration of cells from growth zones (stratified growth). The third phase of myogenesis is the "mosaic growth" associated with the replenishment of the muscle component directly inside the myotome by the formation of new fibers between the already existing ones that give the muscle tissue a mosaic pattern. In most species, mosaic growth begins after fish enter the larval period of development. Axial musculature in carp of standard length (up to 5 cm) contains subpopulations of myosatellite cells of different differentiation stages [6]. Postembryonic increase in the muscle mass of bony fish occurs with two different mechanisms, including hypertrophy and hyperplasia [7]. The increase in the diameter of muscle fibers (hypertrophy) is achieved by absorbing the nuclei of additional myoblasts to maintain a relatively constant ratio in the cytoplasm [8]. The formation of new fibers is connected with hyperplasia which was studied in carp. Since most muscle fibers rarely reach diameters of more than 50 and 240 microns, a large fish "creates" (recruits) new fast muscle fibers at some time to reach it. The increase or slowdown of hyperplasia depends on biological characteristics of fish. It has been noted that muscles in young herring show a greater number of fine fibers than in salmon on a similar area of muscle fibers when they rise up to 15°C [9]. Hypertrophy of muscle fibers in Atlantic salmon embryos occurs at high temperature, and hyperplasia occurs at low temperature, which is expressed in large number of muscle fiber nuclei as compared with incubation in warm water. It has been found that carp larvae incubated at 18°C and grown at 28°C had a larger cross-sectional area than those grown in water at a temperature of 18°C [10].

The purpose of the study is to establish patterns and peculiarities of the structural formation of carp muscle tissue at various stages of postnatal ontogenesis in case of the use of two- and three-year growth technologies and present the scientific justification of more effective one.

## MATERIALS AND METHODS

The research was carried out at the Department of Morphology and Physiology of Animals in Belgorod State Agricultural University named after V. Gorin and at the Department of Anatomy and Histology of Animals named after Professor A.F.Klimovin Moscow State Academy of Veterinary Medicine and Biotechnology –MVA named after K.I. Scryabin, as well as on Novooskolsk zonal fish farm in Novooskolsk District and Borisov fish farm in Borisov District of the Belgorod Oblast that use different approaches to growing carp. The objects of the study were three fish species: carp (*Cyprinus carpio* L., 1758), grass carp (*Ctenopharyngodonidella* Val., 1844), silver carp (*Hypophthalmichthys molitrix* Val., 1844) that belong to the Osteichthyes class (bony fish), the Cypriniformes group (carp-like fish), the Cyprinidae family (carps). 72 fish of each species were selected according to the principle of analogues: fingerlings and under yearlings aged 15 to 135 days, two-year-olds at the age of 500 days, three-year-olds at the age of 860 days grown under the conditions of two- and three-year-growth technologies. A set of techniques included morphometry, anatomical preparation, light microscopy of histological sections, scanning electron microscopy (SEM), statistical processing of the data. Macroscopic morphometry with subsequent determination of absolute and average daily growth was carried out according to the method of I.F. Pravdin (1966). The histological study was carried out according to E.V. Mikodina. The study of the structure of the muscle tissue was carried out with a light microscope Axiostar PLUS by Carl Zeiss equipped with a digital camera and AxioVision software (version 4.8.2.) by Carl Zeiss; refinement of the data was carried out with a scanning electron microscope Quanta 200 3D in the Center for Collective Use of Scientific Equipment of BelGU "Diagnostics, structure and properties of nanomaterials".

## RESULTS OF THE RESEARCH

The leading factor determining the rate of growth of fish larvae (500-600 mg), as is known, is the initial (first 30-40 days) fish-holding density. When a three-year technology is used, it is on average 150 thousand carps, and when a two-year technology is used – 40 thousand carps per hectare of water surface. The increased density of fish leads to rapid elimination of the natural food supply (zooplankton for carp) and, as a result, to a slowing growth rate. Thus, the average weight of under yearlings after three-year growing was  $15.2 \pm 0.5$  g, and after two-year growing –  $123.4 \pm 4.0$  g.

High adaptive potential of fish growth confirms the fact that technological conditions regulate the growth rate of carp: in all the studied species, the under yearlings are increased in weight by 50 to 411 times, in length by 6 to 14 times; two-year-olds – in weight by 10 to 17 times, in length by 2 to 3.3 times; the weight of three-year-olds in comparison with two-year-olds increases only by 3-5 times, length – by 1.5-1.8 times. The initial growth rate of skeletal muscles of carp determines the growth of fish at subsequent stages of ontogenesis which is confirmed by the dynamics of carp growth processes: slow growth in response to unfavorable food conditions at the age of 30-135 days when a three-year growth technology was used. In a polyculture with carp, silver carp gains weight by 190 to 301 times; herbivorous grass carp – by 128 to 302 times, respectively.

We have established a direct proportion between body weight and body length (0.985), body length and body height (0.982); body length and body width (0.990); body length and cross-sectional area m. lateralis dorsalis (0.989); body width and cross-sectional area m. lateralis dorsalis (0.997); cross-sectional area m. lateralis dorsalis and body weight (0.990) of fish.

A comparative analysis of the micrometric parameters of the dorsal lateral muscle (m. Lateralis dorsalis) of carp indicates a high variability in the diameter of its fibers (1 to 60  $\mu$ m). Taking it into account, fibers were divided into three classes: Class I (1-20  $\mu$ m), Class II (21-40  $\mu$ m) and Class III (41-60  $\mu$ m). Fibers of Class I were considered as hyperplastic, fibers of Class III were referred to as hypertrophic.

**Table 1: Distribution of muscle fibers of Type I in carp skeletal muscles under the three-year-growth conditions, %**

Age, days	Weight, g	Classes of muscle fibers,%		
		I (1–20 μm)	II (21-40μm)	III (41-60 μm)
35±3	2±0.04	-	90±3.2	10±0.4
60±3	10±0.3	90±2.7	10±0.4	-
70±3	12±3	94±2.7	6±0.1	-
135±3	17±0.4	10±0.5	90±2.6	-
390±3	57±2.5	50±1.5	50±1.5	-
420±3	82±2.7	10±0.5	80±2.5	10±0.4
500±3	247±5	10±0.5	60±1.2	30±0.9
860±3	1300±40	30±0.9	35±1.1	35±1.3

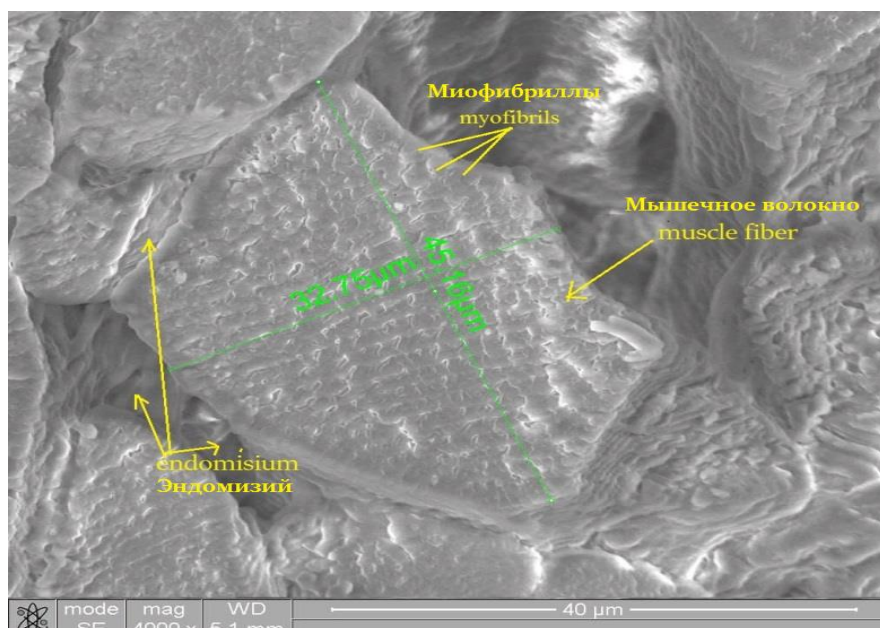
Differences between the compared values are reliable (P≤0.05)

**Table 2: Distribution of muscle fibers of Type I in carp under the two-year-growth condition, %**

Age, days	Weight, g	Classes of muscle fibers,%		
		I (1–20 μm)	II (21-40μm)	III (41-60 μm)
35±3	17±0.5	13±0.4	87±1.3	-
60±3	42±1.2	75±2.8	25±0.7	-
70±3	52±1.2	71±2.3	29±1.0	-
135±3	80±3.2	60±3.3	40±1.6	-
390±3	94±3.0	12±0.6	58±0.9	30±1.0
420±3	183±4.5	10±0.4	90±2.4	-
500±3	700±28	25±0.7	75±1.4	-
860±3	1200±35	-	70±1.4	30±1.0

Differences between the compared values are reliable (P≤0.05)

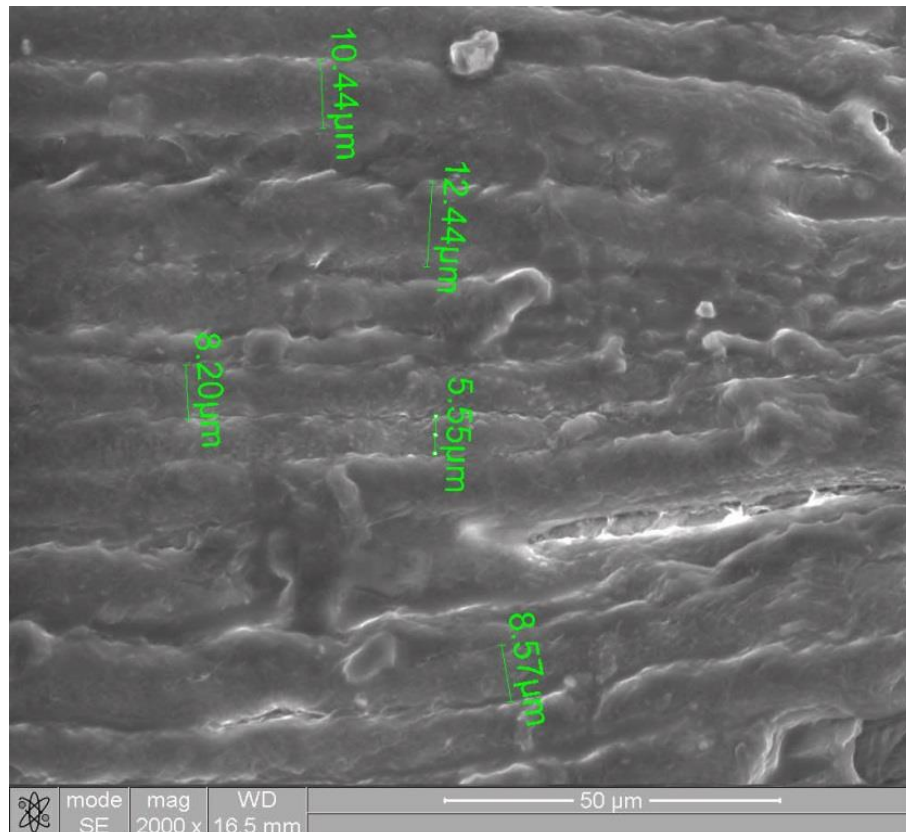
Regardless of the growth technology, fibers of Class II (20-40 μm) predominated in 35-day-old under yearlings that indicates intensive growth of muscle tissue. The increased cell division was observed in 60-day-old under yearlings that resulted in the increase in fibers of Class I (10-20 μm). The newly formed fibers continued to grow up to the age of 135 days, their hypertrophy recapitulated at the subsequent stage of ontogenesis which is confirmed by the ratio of “small” forms to “medium” ones (Fig. 1).



**Fig 1: Structural organization of muscular tissue in carp (m = 300 g) at the age of 500 days in case of the use of a three-year-growth technology. SEM image × 4000**

At the age of 500 days, intensively grown two-year-olds were on average 6 times larger than those grown using a three-year-growth technology. Fibers of all the three types were observed in them with predominance of Class II (medium), large fibers (40-70  $\mu\text{m}$ ) were found in 30% of cases when a three-year growth technology was used.

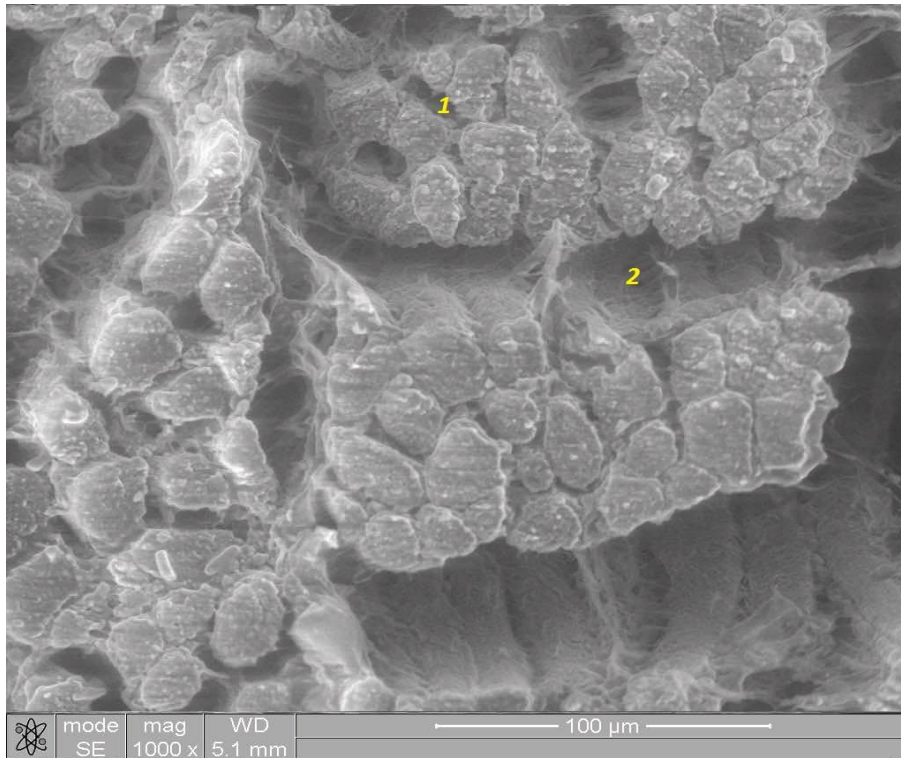
A comparative analysis of the structural organization of the muscular tissue of commercial carp ( $m = 1200 \text{ g}$ ) has shown that in three-year-old fish (860 days, weight of  $1300 \pm 40 \text{ g}$ ), a third of the fibers had a diameter of 1 to 20  $\mu\text{m}$ . A similar pattern was also found in silver carp that confirms the statement of Panov V.P. that muscular tissue growth in carp is not only due to hypertrophy, but also hyperplasia. This concept is consistent with the opinion of Weatherley A. who noted that the complete process of increasing the number of muscle fibers occurs faster in small adult fish than in large ones.



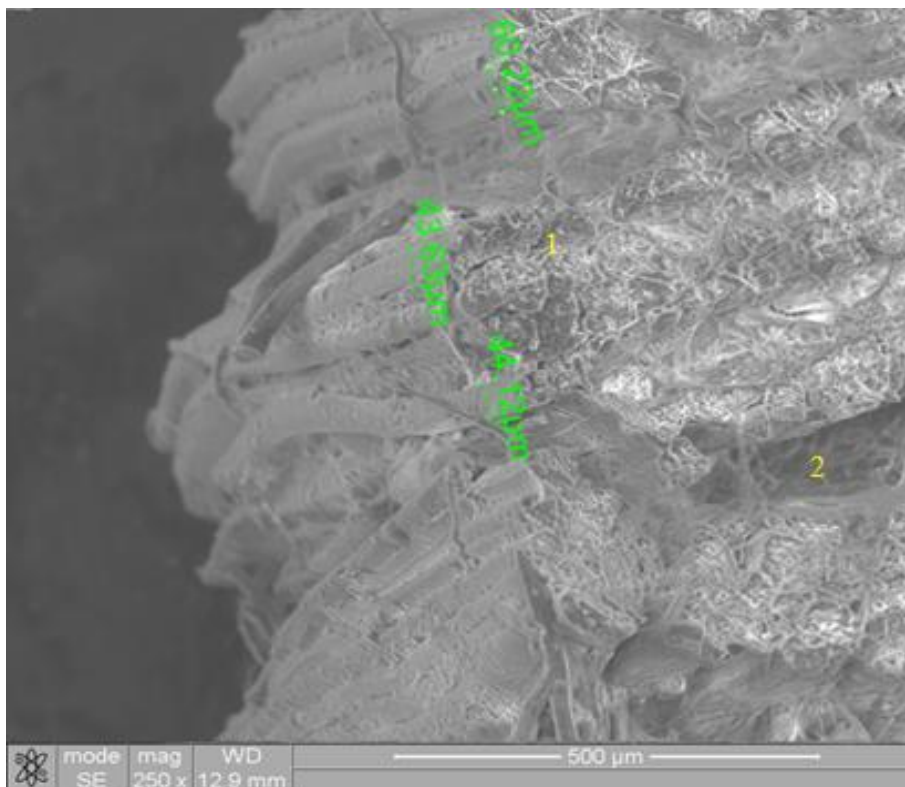
**Fig 2: Micro morphology of muscular tissue of silver carp (weight of 2362 g) at the age of 860 days in case of the use of a three-year-growth technology. SEM Image  $\times 2000$**

The following pattern in the distribution of fiber classes was revealed: fibers reached the maximum diameter both in the under yearlings and in the two-year-olds at the end of the autumn season—the period when fish reach their maximum body weight. It can be assumed that the seasonal dynamics of the morphometric parameters of muscle fibers is determined by the intensity of metabolic processes occurring in the fish body that is consistent with the literature data (Johnston, I.A., 2000, p.318). Good nutrition of fish in August-September and the absence of stress factors cause hypertrophy of muscle fibers.

Commercial three-year-old carps and fast-growing two-year-olds had almost the same average diameter of fibers (36-37  $\mu\text{m}$ ), but the endomysium value in fast-growing two-year-old carps was 30% higher than that in three-year-old fish that can affect body weight and commercial quality of fish (Fig.3,4).



**Fig 3: Micro morphology of muscular tissue in three-year-old carp weighing  $1200 \pm 40$  g. There are muscularfascicles and a connective tissue stroma: 1 – endomysium, 2 – perimysium. SEM-image  $\times 1000$**



**Fig 4: Muscular fascicles in two-year-old carp in case of the use of a two-year-growth technology ( $m = 1200 \pm 36g$ ): 1 – endomysium, 2 – perimysium. SEM-image  $\times 250$**

## CONCLUSION

Patterns and specific features of postnatal growth and development of skeletal muscles in carp species: carp (*Cyprinus carpio* L.), grass carp (*Ctenopharyngodonidella* Val.), silver carp (*Hypophthalmichthys molitrix* Val.) caused by the influence of nutritional adaptation, dynamic stereotype and growth technology have been established.

According to the research findings, it can be concluded that the growth of muscle fibers in carp is the result of a permanent alternation of hypertrophy and hyperplasia throughout the life of fish. It is due to the peculiarities of metabolic processes in the body of fish as water poikilothermic animals. In aquaculture, the morphometric parameters of the muscular tissue of fish are significantly influenced by anthropogenically simulated growing conditions. The choice of one or another technology for growing fish, that corresponds to the conditions for life (primarily the availability of a natural food supply), can directly influence the growth, development, structure of muscular tissue, change the consumer properties of fish meat.

## REFERENCES

- [1] Bagrov A.M., Bondarenko L.G., Gamygin E.A. Technology of pond pisciculture 2014:358.
- [2] Burlachenko I.V., Yakhontova I.V. Proceedings of VNIRO 2015; 153: 137-153.
- [3] Alami-Durante H., Fauconneau B., Rouel M., Escaffre, A.M. J. Fish. Biol. 1997; 11: 1285-1302.
- [4] Ennion S., Gauvry L., Butterworth P., Goldspink, G.J. Exp. Biol. 1995; 198: 1603-1611.
- [5] Fauconneau B., Alami-Durante H., Lacoche M., Marcel J., Vallot D. Aquaculture 1995; 129: 265-297.
- [6] Johnston I.A. San Diego: Academic Press 2000: 318.
- [7] Semenov E.I., Semenov E.I., Tremasov M.Y., Matrosova L.E., Tarasova E.Y., Kryuchkova M.A., Smolentsev S.Y., Korosteleva V.P. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(1): 1860-1868.
- [8] Rowlerson A., Vegetti A. Muscle development and growth. Academic Press 2001: 103-140.
- [9] Vieira V.L., Norris A.A., Johnston I.A.J. Aquaculture 2007; 272: 100-109.
- [10] Weatherley A.H., Gill H.S., Lobo A.F.J. Fish Biol. 1998; 33: 851-859