

Morphohistochemical profile of red and pink muscles in freshwater fish

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Summary

The morphohistochemical profile of red and pink muscles from the caudal portion of the trunk at the height of the horizontal septum in the *Carassius auratus gibelio* and *Perca fluviatilis* 20–25 cm long from the Garešnica (Croatia) fish farm have been studied. Muscle samples, taken on arrival from live fish, were frozen in liquid nitrogen, cut on the cryo cut into 10 µm thick slices stained with hemalum and eosin and with the procedure for the detection of succinate dehydrogenase (SDH). The diameter of muscle fibers from the obtained preparations was measured using a micrometer scale with 10 µm divisions on a Nikon microscope. The red muscle of the *Carassius auratus gibelio* and *Perca fluviatilis* is known to have a triangular cut. It is positioned laterally beneath the skin and runs craniocaudally along the horizontal septum. Underneath the surface red muscle there is a pink muscle, shorter than the red one. In the *Carassius auratus gibelio* it is wide and divided into the surface (pink I) and deep (pink II) layer, while in *Perca fluviatilis* it is narrow, short and unchanged, but comprises a narrow transitional zone towards the red muscle. The red muscle is composed of red muscle fibers of small diameter with potent SDH activity. The pink muscle is composed of two types of fibers – intermediary small-diameter muscles of pronounced SDH activity and large-diameter muscles of less pronounced SDH activity as well as white muscles of large diameter with poor SDH activity. Intermediary fibers in the *Carassius auratus gibelio* accounted for 77.80% of the fibers in the pink I layer and 44.90% of the fibers in the pink II layer. White fibers in the same fish accounted for 22.20% of the fibers in the pink I layer and 55.10% of the fibers in the pink II layer. Intermediary fibers in the pink muscle of the *Perca fluviatilis* accounted for 58.80% fibers, whereas white fibers accounted for 41.20% of all fibers.

Keywords: red muscle, pink muscle, *Carassius auratus gibelio*, *Perca fluviatilis*, morphohistochemical profile, SDH activity

The lateral muscle of the fish trunk and tail is markedly segmented, clearly showing the myotomes that are mutually separated by thin connective tissue septa to which muscle fibers are attached. Myotomes have a structure consisting of three types of muscle fibers: red, intermediary and white, placed so as to form longitudinally positioned muscles, differentiated by their appearance, position and function (1, 2, 12).

White muscle fibers are dominant in number and largest in diameter. They are placed in a deeper layer of the myotome and comprise a majority portion of the lateral muscle, the so called „white muscle”. Due to the high incidence of the areal white large-diameter fibers, the white muscle is capable of bearing a heavier load (17). Red muscle fibers are the smallest in diameter. They are positioned at the surface part of the myotome, underneath the skin and run along the horizontal septum in the craniocaudal direction forming the so called „red muscle”. The red muscle runs as

a band along the lateral surface portion of the trunk along the horizontal septum. Intermediary (or pink) fibers are larger in diameter than the red ones, but shorter than the white ones, and are positioned between the red and white muscle composing the so called „pink muscle”. This muscle does not appear in all fish species (11). In some fish it has a double layer (sheat-fish), whereas in others it forms a narrow surface transient zone (*Lebistes reticulatus* and *Gobius paganel-lus*) in which some fibers have the properties of red fibers, and others have the properties of intermediary fibers (12). In addition to the red, white and intermediary fibers, red and pink muscles have been found to contain the so called „tonic fibers”, differentiated from the aforementioned by their histochemical properties and ultrastructure (9).

The red and pink muscles are very interesting from the morphological and functional points of view, and the study was aimed at determining the relationship

between them in two fresh water fish species (*Carassius auratus gibelio* and *Perca fluviatilis*) that appear in nature or are cultivated and are differentiated in appearance and swimming mode.

Material and methods

In the study of the red and pink muscles we used samples taken from the trunk of twenty *Carassius auratus gibelio* and twenty *Perca fluviatilis* of both sexes, 20-25 cm long, obtained from the Garešnica (Croatia) fish farm. Muscle samples of approximately 1 cm³, taken from the caudal part of the trunk at the height of the horizontal septum, were immediately frozen in liquid nitrogen, cut on the cryo cut into 10 µm slices and stained with hemalun and eosin (15) to show muscular structure, the activity of succinate dehydrogenase (13) and metabolic activities of muscle fibers. These histological and histochemical preparations were studied for their muscle structure and fiber diameters using a micrometer scale with 10 µm divisions on a Nikon microscope. The diameter of the fibers was measured at the widest and lowest points so as to obtain a realistic diameter of every measured fiber. The obtained data were statistically analyzed using Student's t test and are shown in the tables for every layer of the red and pink muscle and fiber type, i.e. the tables show areal incidence of each type of fibers in the studied red and pink muscles.

Results and discussion

The trunk muscle of both studied fish species is known to be segmented, with clearly distinguished myotomes. Each myotome is composed of a surface and a deep layer, both visible by the unaided eye along the whole trunk. A deep layer lighter in color, corresponds to white muscles, and constitutes a major portion of muscle tissues in both fish. The surface layer is dark-colored and corresponds to the red muscle. It runs as a band beneath the skin in the craniocaudal direction along the horizontal septum and has a triangular shape in the transversal cut. A red layer can be clearly divided into a surface dark-colored part (red muscle) and a thicker lighter part (pink muscle). A pink muscle is medially connected with the white one, and laterally with the red one. In both fish it is shorter than the red muscle.

Both muscles, red and pink, are constituted of differently sized diameter muscle fibers (tab. 1). The diameter of all fibers in the *Carassius auratus gibelio* range from 20-100 µm, and in the *Perca fluviatilis* from 20-150 µm. Generally, the diameter of the peripheral fibers is smaller and increases towards deeper parts.

SDH activities show the existence of three different layers in the dark-colored muscle tissues of the *Carassius auratus gibelio* trunk. The red muscle layer closest to the surface comprises muscle fibers of a small diameter that exhibit marked SDH activity and get very intensely stained by the applied procedure. These fibers can be considered red fibers. This layer resembles a well-developed surface band situated beneath the skin (fig. 1). In the *Carassius auratus gibelio*, a deeper layer, the pink muscle, can be divided into two sub-layers: pink sub-layer I (pink I) and pink sub-layer II (pink II). The pink muscle runs along few adjacent myotomes. This layer has a structure with a varying number of muscle fibers of small and large diameter. Small-diameter pink fibers are relatively intensely stained during the presence of SDH activity, although less intensely than the fibers of the surface red muscle. These fibers can be considered as intermediary fibers. Large-diameter muscle fibers of this layer show poorer SDH activity, i.e. a lower level of staining, and can be considered as white fibers (fig. 2). Intermediary fibers in the pink sub-layer pink I (pink I) are stained more intensely than the intermediary muscle fibers in the pink sub-layer II (pink II). Pink sub-layer I contains many more intermediary fibers (77.80%) than white fibers (22.20%), and pink layer II (pink II) contains some more white (55.10%) than intermediary muscle fibers (44.90%) (tab. 1).

In the *Perca fluviatilis*, the surface dark-colored muscle is less developed and can be divided into a wider red surface muscle and a deeper, very narrow pink muscle (fig. 3). The surface red muscle is structured by small-diameter muscle fibers exhibiting more intense SDH activity than the corresponding muscle fibers in the *Carassius auratus gibelio*. The zone of the red muscle fibers of different diameter and with marked SDH activity forms a narrow borderline between the red and pink areas. The pink muscle is very short and narrow, comprising small-diameter intermediary fibers with relatively marked SDH activity and large-diameter white fibers with poor SDH activity (fig. 3). Actually, the *Perca fluviatilis* red muscle has a structure with only the red muscle fibers, whereas the pink muscle comprises intermediary and white muscle fibers in the proportion of 58.80% : 41.20% (tab. 1).

As is the case with other teleosts, trunk and tail muscles in the *Carassius auratus gibelio* and *Perca fluviatilis* are markedly segmented and the myotomes are clearly distinguished. Moreover, a dark-colored

Tab. 1. Diameter and percentage of some type of muscle fibers in red and pink muscle layer

Fish	Red muscle layer		Pink I muscle layer				Pink II muscle layer			
	µm ± SE	%	intermed. fibres µm ± SE	%	white fibres µm ± SE	%	intermed. fibres µm ± SE	%	white fibres µm ± SE	%
<i>Carassius auratus gibelio</i>	36.09 ± 10.55	100	31.36 ± 9.67	77.80	77.95 ± 10.11	22.20	22.90 ± 4.53	44.90	89.47 ± 7.59	55.10
<i>Perca fluviatilis</i>	30.28 ± 8.10	100	35.00 ± 10.48	58.80	122.50 ± 19.20	41.20	-	-	-	-

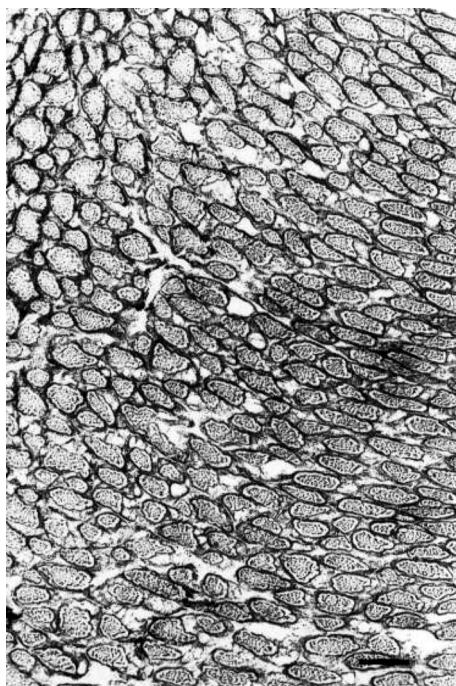


Fig. 1. *Carassius auratus gibelio*, activity of SDH in red layer (scale bar = 100 μ m)

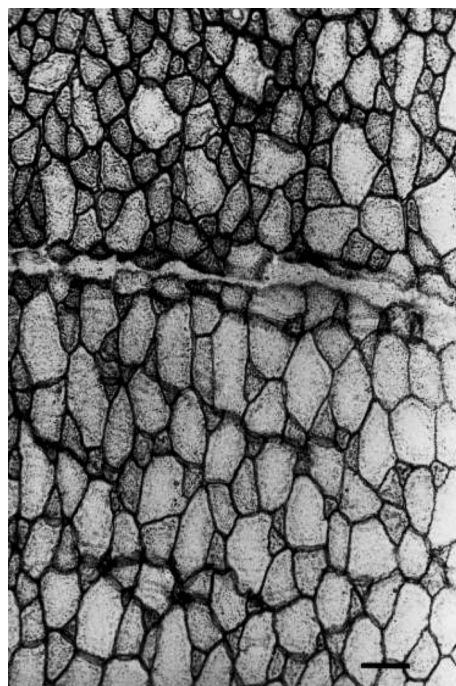


Fig. 2. *Carassius auratus gibelio*, activity of SDH in pink layer, pink I is upper and pink II is et lower part of figure (scale bar = 100 μ m)

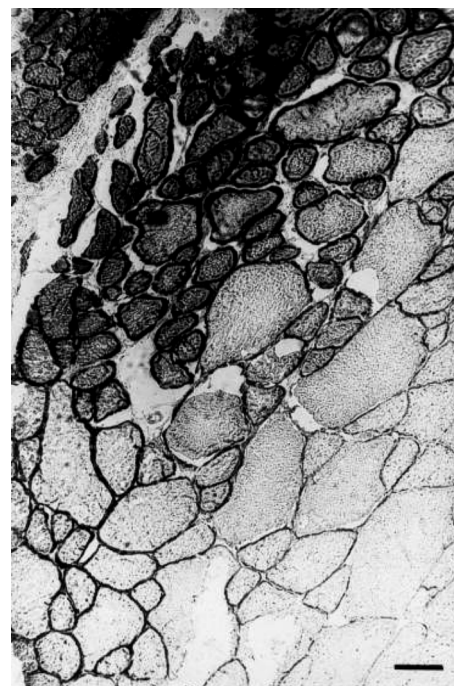


Fig. 3. *Perca fluviatilis*, activity of SDH in red layer (upper left), in pink layer (middle) and white muscles (lower) (scale bar = 100 μ m)

surface muscular portion positioned at the surface along the horizontal septum is clearly distinguished. A dark-colored muscle in both studied fish species is composed of the surface red and deeper pink part, which is in accordance with the findings of Mascarello et al. (12). Both the red and the pink muscles are structured by differently sized diameters of fibers which is also in line with the findings of Devicenti et al. (7), and of different SDH activity, which corresponds to the findings in other studied fish species. Red muscles comprise small-diameter fibers with marked SDH activity, whereas pink muscles comprise the fibers of small, medium and large diameter. Small and large diameter fibers exhibit somewhat poorer SDH activity than the red muscle fibers, whereas large-diameter fibers exhibit markedly poor SDH activity. Based on the SDH activity and diameter it was possible for us to classify the red muscle fibers of small diameter with potent SDH activity into red oxidative fibers, as established by Carpené et al. (3), Sängner et al. (16) and Ramirez-Zarzosa et al. (14). Large-diameter fibers in the pink muscle with moderate SDH activity were classified as intermediary, oxidatively glycolytic, according to Martinez et al. (11), and very large-diameter fibers in the pink muscle with poor SDH activity were classified as white glycolytic fibers, all in line with the findings of many authors who studied other fish species (3, 4, 9). Regarding the aforesaid it can be concluded that red muscle fibers utilize aerobic metabolism, they contract slowly and tire more slowly and are, hence, capable of the prolonged, steady and slow swimming, as in the search for food. On the other hand,

white muscle fibers (of the pink muscle) utilize anaerobic metabolism, they contract rapidly and tire faster, and because of their large diameter they are very strong (17) and, thus, capable of fast short and explosive swimming, as in chasing prey or escaping predators. There is a theory that the pink muscle is not engaged in swimming, but is rather a transition to the red and white fibers (3, 6, 7). Intermediary fibers of the pink muscle have the properties of the red and white fibers, meaning that they have a combined aerobic-anaerobic metabolism. Therefore, they are capable of faster but prolonged swimming and have greater endurance which enables somewhat faster and more prolonged swimming (5).

Findings have shown that the studied fish exhibit great similarities as well as significant dissimilarities as to the organization and structure of both muscles. In both species the red muscle is structured by red small-diameter fibers with potent SDH activity. The pink muscle has a structure of intermediary small- and large-diameter fibers with poor to moderate SDH activity and of white large-diameter fibers with poor SDH activity. However, the pink muscle in the *Carassius auratus gibelio* is larger than in the *Perca fluviatilis*, although both fish species are similarly sized and have structures of two layers: surface and deep, both clearly distinguishable, according to Mascarello et al. (12), as in the catfish. Both layers possess structures of different proportions of intermediary and white fibers. The surface layer is dominated by intermediary and the deeper layer by white fibers. The pink muscle of the *Perca fluviatilis* is short and narrow with an

almost similar proportion of white and intermediary fibers. In addition, the *Perca fluviatilis* has a narrow transitional zone of red fibers in the red fibers between the red and pink muscle, as is the case with *Lebistes recitulus* and *Gobius paganellus*, described by Mascarello et al. (12). Tonic fibers described by Kilariski and Kozłowska (9) have not been recorded in the studied muscles of the *Carassius auratus gibelio* and *Perca fluviatilis* trunk.

The increased number of white muscle fibers in the deep pink sub-layer of the *Carassius auratus gibelio* is in line with the increased number of white fibers in the pink parts closer to the white muscle tissues, as can also be seen in the unsegmented pink layer of the *Perca fluviatilis*. The size difference between the red and pink muscle in the studied species is probably due to their different lifestyle and swimming mode. While looking for food the *Carassius auratus gibelio* is in permanent motion with frequent acceleration, which requires well-developed red and pink muscles. The *Perca fluviatilis* lies in ambush waiting for prey, so it swims less, at a steady rate and placidly which may explain the poorer development of its pink muscle. On the other hand, high areal incidence of the white fibers in the pink muscle of the *Perca fluviatilis* shows the ability for a short, rapid burst of swimming during the chase for prey or retreat from enemies.

Conclusions

1. Both fish species have well-developed red and pink muscle, the latter being less pronounced than the former one. In the *Carassius auratus gibelio* it is larger and divided into the surface and deep layer, while in the *Perca fluviatilis* it is smaller and undivided.

2. The red muscle has a structure of small-diameter red muscle fibers with potent SDH activity. The pink muscle has a structure of intermediary small- and large-diameter fibers with moderate SDH activity and of white large-diameter fibers with poor SDH activity.

3. The percentage of the intermediary fibers in the surface layer of the *Carassius auratus gibelio* pink muscle exceeds the percentage of the white fibers, and in the deeper layer it is lower than the percentage of the white fibers. In the *Perca fluviatilis* the percentage of the intermediary fibers in the pink muscle is somewhat higher than the percentage of the white fibers.

4. Areal incidence of the red fibers in the red and pink muscle in both fish species is the lowest. In the *Carassius auratus gibelio* the intermediary fibers cover more than half the surface, whereas white fibers cover one third of the surface of both muscles together. In the *Perca fluviatilis* the white fibers cover 4/5 of both muscles surface, and the intermediary cover only 1/10 of the surface.

5. The detected structural differences and variations of organization between the red and pink muscles arise from their manner of living and swimming mode of the studied species.

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