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DIFFERENCES BETWEEN WHITE AND RED MUSCLE FIBERS DIAMETER IN THREE SALMON FISH SPECIES

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Abstract: Because of skeletal muscle is the main contributor to body weight in most fish, it is probable that the species of the fish is limited by the growth of this tissue. Several aspects of both somatic size and skeletal muscle growth was investigated in this research work included a total of 20 brown trout (*Salmo truta m. fario Lineus*), 20 brook trout *(Salvelinus alpinus*) and 20 rainbow trout (*Oncorynchus mykkis Walbaum*), the average weight of 200 grams. Gathered data showed that rainbow trout has a faster increasing white muscles then other two fish species at same body weight. Main peak of diameter white muscles was 31-40 µm (30.55%) and 41-50 µm (22.15%) for rainbow trout. In mean time in the other two fish groups (brown trout and brook trout) was 21-30 µm (40.1% or 39.27%) and 31-40 µm (39.27% or 33.85%) of measured cross sectional areas. Distribution measured cross sectional areas of red muscles laniary goes down from the <20 µm to >71 µm.

Key words: muscle fibers, diameter, rainbow trout, brown trout and brook trout**.**

Introduction

The main edible part of the fish myotome is composed of white muscle fibers. The number of muscle fibers recruited to reach a particular girth varies between families and strains and is influenced by environmental factors including diet, exercise, light and temperature regimes *(Johnston, 2000)*. In most fish, two types of muscle fibers are found; red fibers form a thin lateral superficial sheet just under the skin, whereas white fibers make up the underlying mass of the myotome *(Rabah 2005)*.

The majority of studies on muscle growth have measured the average cross-sectional areas or diameters of fibers at various stages of the life cycle.

However, average fiber size is a relatively insensitive and unreliable indicator of hypertrophic growth because of the recruitment of new muscle fibers. For example, the average fiber diameter in the white myotomal muscle of the rainbow trout remained within the range 90–95 mm between 34 and 52 cm body length due to the addition of new fibers, but increased to 135–140 mm at 62 cm body length once recruitment had ceased *(Stickland, 1983)*.

Muscle growth can therefore be studied as the contribution of hyperplasia (increase in fiber number) and hypertrophy (increase in fiber size) to muscle growth by various forms of histological methods combined with morphometric analysis *(Rowlerson and Vegetti, 2001)*. Diameter of red and white muscles fibers have a sigmoid characters, red muscles fibers becomes constant diameter 30-40 µm at body length 25-35 cm, in same time diameter of white muscles fibers have diameter 80-120 µm for same length *(Greer 1970)*.

In rainbow trout, ratio of cross-sectional area of red and white muscle was 25:1 *(Stickland, 1983)*. White muscle fibers in the size class 10–20 µm represented 10% between 2.2 and 10 cm body length, falling to 1% at 52 cm and were absent in 63 cm fish due to the cessation of new fiber recruitment.

Several studies have demonstrated that recently recruited fibers in fish are relatively small in size and that these fibers increase in cross-sectional area through hypertrophic growth. Because hyperplasia is associated with small fibers and hypertrophy is correlated with fibers of greater dimensions, the size of individual fibers can therefore be used to assess muscle growth at different life stages.

Hyperplastic and hypertrophic growth patterns have been described for several species of fish representing different taxonomic families which display a broad range of maximum reported sizes *(Weatherley and Gill, 1980, 1981, 1988; Stickland, 1983)*.

Texture is one of the criteria of flesh quality. It is a sensory characteristic for the consumer and an important attribute for the mechanical processing of fillets. Very soft texture is frequently reported and the industry is requesting methods able to measure fish texture, and is also seeking answers to what causes fillet softness *(Kiesling et al., 2006)*.

Materials and Methods

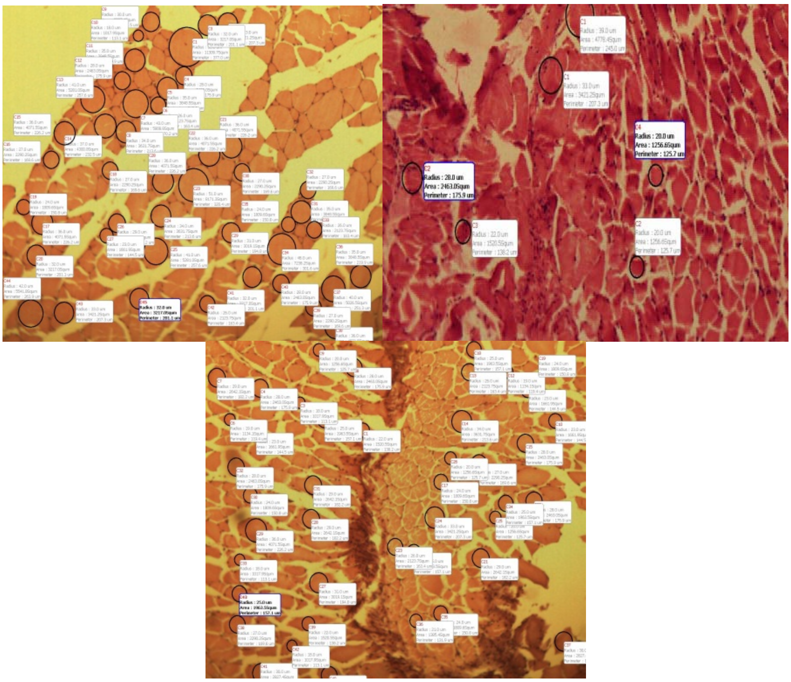
Sampling

Sixty fish in total were selected at random from a population from cages: *rainbow trout, brown trout and brook trout* ranging in length from 20-25 cm and average weight 200 g. None of the fish selected were maturing and all had been fed a commercial trout diet. They were anesthetized by solution of *p-aminobenzo etil ester, 50 mg^{-1}* before slaughtering. Measured of body weight by *„Electronic Scale“BIRE K3052-P High Precision*.

Histological techniques and measurement of fiber size

Samples of white and red muscles were taken from epaxial myotome from left and right side (2x2x1 cm). Tissue blocks were orientated so fibers were cut at a right angle to their main axis during sectioning. After that the samples left in 10% formalin until histological investigation. Muscle sections were stained with haemotoxylin and eosin and mounted on microscope slides. Diameter of white and red fibers was measured by electro-microscope „MOTIC BA 200"enlarged 250x. All photos adjusted by software *„Motic images plus 2.0 ML"*.

The cross-sectional areas of individual fibers within each section were determined using an image analysis system consisting of a Leica RM 2145 computer with corresponding image analysis software and a video camera attached to a microscope. This setup enabled a field of muscle fibers viewed under the microscope to be projected onto the computer monitor screen.



Picture 1. ***White fibers Brown trout, Rainbow trout and Brook trout, Hematoxilin–eozin, 250X***

Results and Discussion

Relationships between the cross sectional areas of white muscle taken from three fish species showed significant differences between rainbow trout and the other two fish species. Main peak of diameter white muscles was 31-40 µm (30.55%) and 41-50 µm (22.15%) for rainbow trout. In mean time in the other two fish groups (brown trout and brook trout) was 21-30 µm (40.1% or 39.27%) and 31-40 µm (39.27% or 33.85%). Gathering data showed that rainbow trout has a faster increasing white muscles then other two fish species at same body weight (Table 1 and Figure 1).

Table. 1. Cross sectional area of white muscles three fish species %

Cross sectional area of white muscles three fish species %							
	< 20µm	od 21-30µm	od 31-40µm	od 41-50µm	od 51-60µm	od 61-70µm	> 70µm
Brook trout	14.97	39.27	33.85	9.04	1.80	0.51	0.51
Brown trout	17.25	40.11	26.16	11.43	3.49	0.58	0.97
Rainbow trout	11.30	19.65	30.55	22.15	6.65	7.25	2.45

As it easy to sea main peaks for brown trout and brook trout was between 21-30 µm, for Rainbow trout was between 31-40 µm and 41-50 µm. In this study, samples of rainbow trout were grown at higher rates than other two groups of fish.

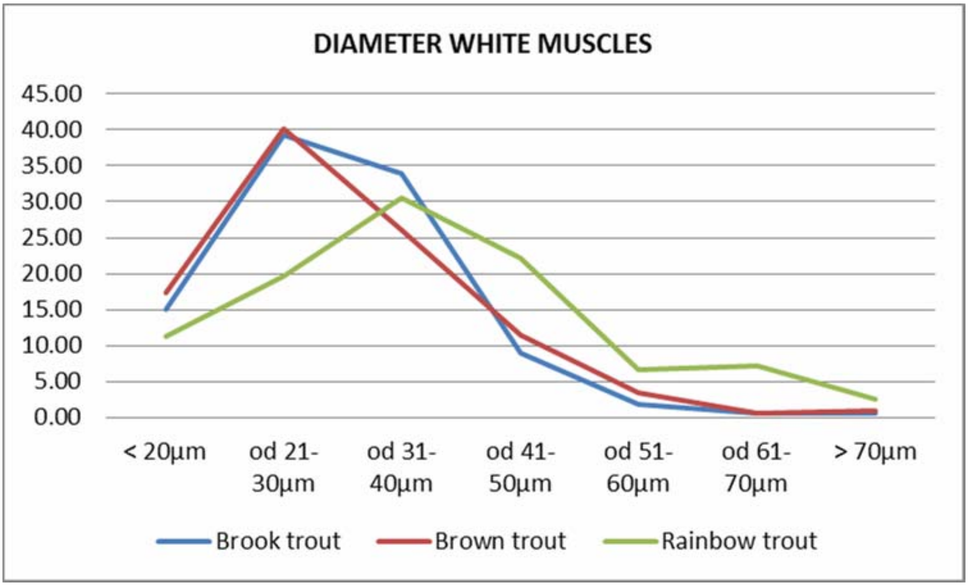


Figure 1. Cross sectional area of white muscles three fish species

Results in table 2 showed that there is a linear relationship between red muscle fiber cross sectional area and fish species in all three groups. Percentage of cross sectional areas were the highest for the lowest cross sections less than 20 µm, in all fish, between 49.05-59.40% than goes down to as diameter was bigger.

Table 2. Cross sectional area of red muscles three fish species %

Cross sectional area of red muscles three fish species %							
	< 20µm	od 21-30µm	od 31-40µm	od 41-50µm	od 51-60µm	od 61-70µm	> 70µm
Brook trout	49.05	27.83	16.03	3.77	1.88	1.41	0.00
Brown trout	59.40	33.66	5.94	0.99	0.00	0.00	0.00
Rainbow trout	51.43	29.67	14.76	2.09	1.48	0.57	0.00

Distribution measured cross sectional areas of red muscles laniary goes down from the <20 µm to >71 µm (Figure 1). Between 76.88% in brook trout to 78.10% in rainbow trout was measured cross sectional area of red muscles means that main distribution of red muscles were between these two measurements, and red muscles are actually not with bigger cross sections.

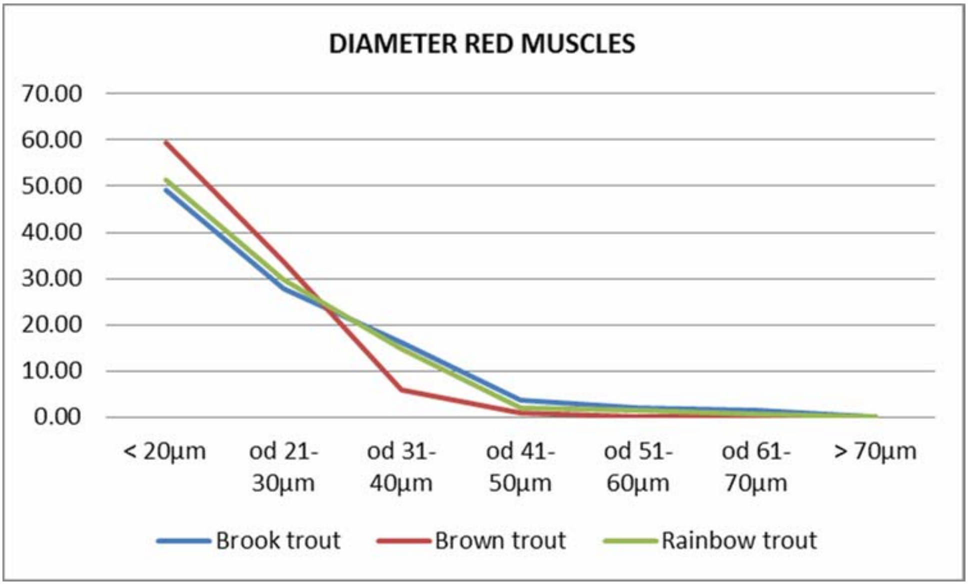


Figure 2. Cross sectional area of red muscles three fish species

The fiber cross section areas for white muscle in the present study varied deepens on fish species as it shown in analyzes from 31-40 µm (30.55%) and 41-50 µm (22.15%); this is in accordance with *Rabah (2005) and Johnston et al. (2004)*. The swimming muscle of salmon is divide into concentric layers of red muscle, they characterized by small fiber diameters, and white muscle that have larger fiber diameters (Figure 2). The red muscle fibers are relatively small and surrounded by a rich store of lipid droplets while the white fibers are relatively large and without lipid droplets.

Weatherley et al. (1988) and *Zimmerman et al. (1999)* proposed that beyond a critical fiber size the continuation of hypertrophic growth would result in impairing growth changes. According to their hypothesis the ultimate size to which a fish could grow would correlate with the attainment of this final critical size by all comprising fibers. They suggest that the critical fiber diameter at which surface area becomes limiting ranges from 120 to 270 in fish. In this study there were few diameters bigger than 60 µm it means that results show simiillar meassuring like in *Rabah (2005).*

The swimming muscle of salmon is divide into concentric layers of red muscle, they characterized by small fiber diameters, and white muscle that have larger fiber diameters. The red muscle fibers are relatively small and surrounded by a rich store of lipid droplets while the white fibers are relatively large and without lipid droplets.

The large range of fibre diameters seen in the white muscle of rainbow trout give it a characteristic mosaic appearance which was once assumed to be a mixture of small and larger fibres *Johnston et al. (2000)* and *Gjedrem (1997)*.

Conclusion

The growth of red and white muscle was investigated in three salmon fish species; brown trout, brook trout and rainbow trout, using fish from 20 to 25 cm in length. In the white muscle, fibre hyperplasia, initially, accounted for all groups of fish muscle growth to 50 µm but relative contribution was decreased in brown and brook trout. The results for the red muscle are more variable and hence more difficult to assess so it can say that cross sectional areas were less than 40 µm in all fish species.

Razlike u dijametru belih i crvenih mišićnih vlakana kod tri vrste salmonidnih riba

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Rezime

Zato što su skeletni mišići osnovni nosioci mase većine riba to je verovatno povezano sa vrstom i rastom ovog tkiva. Nekoliko aspekata veličine i mišićnog rasta je istraživano u ovom radu. Istraživanjima je ukupno obuhvaćeno 60 uzoraka ribe, po 20 uzoraka potočne pastrmke *(Salmo truta m. fario Lineus.),* potočne zlatovčice *(Salvelinus alpinus)* i kalifornijske pastrmke *(Oncorynchus mykkis Walbaum),* prosečne mase 200 grama.

Rezultati istraživanja su pokazali da kalifornijska pastrmka ima brži razvoj (hiperplaziju) belih mišićnih vlakana u odnosu na druge dvije grupe riba u odnosu na telesnu masu. Najveći broj izmerenih dijametara belih mišićnih vlakana kod kalifornijske pastrmke se nalazio od 31-40 µm (30,55%) i 41-50 µm (22,15%), dok su se u isto vrijeme izmereni dijametri belih mišićnih vlakana kod druge dve grupe nalazili u delu od 21-30 µm (40,1% or 39,27%) i 31-40 µm (39,27% ili 33,85%). Distribucija izmerenih dijametara crvenih mišićnih vlakana se linijarno kretala od <20 µm do >71 µm.

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