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LIGHT MICROSCOPE STUDY OF *ONCORHYNCHUS KISUTCH* MUSCLE DEVELOPMENT

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Key words: Oncorhynchus kisutch, white fibers, Red fibres.

ABSTRACT

Study of microscopic structural changes of red and white muscle of coho salmon, *Oncorhynchus kisutch*, through different developmental stages and through transition from parr to smolt revealed developmental variation in fibre size where white fibres were larger than red ones. The increase in fibre area, characterizing all stages of development, was associated with reduction in both fibre and capillary densities. In large fish the reduction in red muscle capillary density was 10 fold lesser than that of white muscle.

INTRODUCTION

In most fish, two types of muscle fibres are found; red fibres form a thin lateral superficial sheet just under the skin (the Seitenlinie or superficial lateral muscle), whereas white fibres make up the underlying mass of the myotome. In some teleosts, such as salmonids (Greene, 1913) the red and white fibres are intermingled throughout the myotome, in yet others (scombroids), in addition to the superficial lateral strip of red muscle, there is also a deeper-lying red portion of the myotome (Kafuku, 1950; Braekkan, 1959). The two types of muscle fibres are more clearly distinct from one another than the types of muscle fibres in higher vertebrates.

MATERIALS AND METHODS

(i) The data examined in this study were obtained from 0.5-1.0µm thick resin sections (T.S.) of muscles fixed at resting length by immersion in the buffered of glutaraldehyde. The reason for using this technique is partly because such data made the analysis of fine structure much easier allows reference to earlier data on fish muscle, and avoids ambiguity with the lack of specific markers for fish blood vessels (Egginton, 1990). Semithin sections (0.5 µm) were collected onto a glass slide and stained with Toluidine blue 2 or 3 seconds after the sections have been dried onto the slide.

(ii) Sections of muscle were examined using Olympus microscope with a drawing arm attachment. Most workers (Greer-Walker, 1970; Johnston *et al*., 1975; Weatherley *et al*., 1979, 1980a; Stickland, 1983), have used fibre area or have assumed that the fibres are circular and calculated the equivalent diameter.

(iii) Muscle morphometrics: Fibre density and capillary density against fibre size. The use of a counting frame having left and lower exclusion edges (Figures 1&2) is an established method for counting the capillaries and muscle fibres. This unbiased sampling helps to eliminate the influence of capillary size and morphology (Egginton, 1990). The number of each, was counted at a magnification of 40 X , number of both white fibres and capillaries (Figure 1) was drawn using a light microscope in which the sample white fibre area was 0.02 mm. The number of white fibres in this sample area was 25 fibres (WF 25) and twelve capillaries (C 12). The sample was taken from a seawater-adapted coho salmon.

(iv) At a magnification of 40 X, the sample area was 0.022 mm^2. However not all this area was filled with muscle fibres but there were some empty spaces. Therefore, these spaces had to be taken into account and corrected, when calculating fibre area. This is achieved by marking the boundary of each fibre on a grid (Figure1).

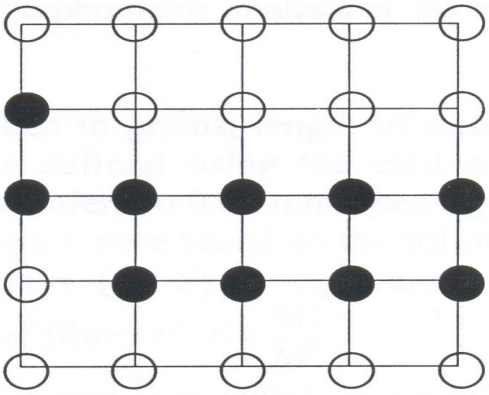

Figure 1. Method of counting fibers in small body mass.

Figure 1 showing:
Ps = 15 (area without fibres) (∘)
Pf = 10 (area with fibres) (•)
The fraction of sample occupied by space= Ps/ Pf+PS

$$\frac{15}{(10+15)} = \frac{15}{25} = 0.6$$

=60% of sample area =60%*total sample area=60%*0.022=0.013 mm^2
To calculate the occupied area with fibres:

$$\frac{1-Ps}{Pf+Ps} = 1 - 0.6 = 0.4$$

The occupied area with fibres = 0.022* 0.4 = 0.009 mm^2 or
= total sample area- unoccupied area
= 0.022-0.013=0.009 mm^2
Weight of sample was taken in grams, length of muscles in centimetres and the sample area was defined using the calibration of the optical microscope and it was equivalent to 0.02 mm^2 (Figure 2).
The calculation of all samples were based on the following equations
The capillary to fibre ratio (C: F) is equivalent to the number of capillaries to the number of fibres $C:F = \frac{NC}{NF}$
The capillary density (CD; mm^{-2}) is equal to the number of capillaries divided by the sample area in mm^{-2}

$$CD = \frac{NC}{sample\ area}$$

And the fibre density (FD) is equal to number of fibres divided by sample area mm^{-2}.

$$FD = \frac{NF}{sample\ area}$$

The fibre area (μm^2) is equal to $\frac{1}{FD} * 1000000$

It should be borne in mind that for morphometric analysis of structure, the anatomical capillary supply, can only be related to the functional capacity of a system, e.g. potential maximum rates for delivery of oxygen and removal of metabolites. The limitations of such analysis and relative value of commonly used indices are discussed in Egginton (1990).

RESULTS

The swimming muscle of salmon is divisible into concentric layers of red muscle (characterised by small fibre diameters, high capillary density and a profusion of lipid droplets), white muscle (having larger fibre diameters, few capillaries and no lipid droplets) and pink muscle (that lies between the two major types and has intermediate characteristics). All of these properties are visible in Figure 2, which shows a transverse section of muscle from a 14g coho salmon. The red muscle fibres (RM) are relatively small and surrounded by a rich store of lipid droplets (LD) while the white fibres (WF) are relatively large and without lipid droplets. The pink fibres (PF) are intermediate in size and vascularisation. Figure 3 shows white and red muscle fibres and demonstrates the difference in size between white fibres and red fibres. Between the two fibre groups there is a range of fibre sizes (mosaic muscle).

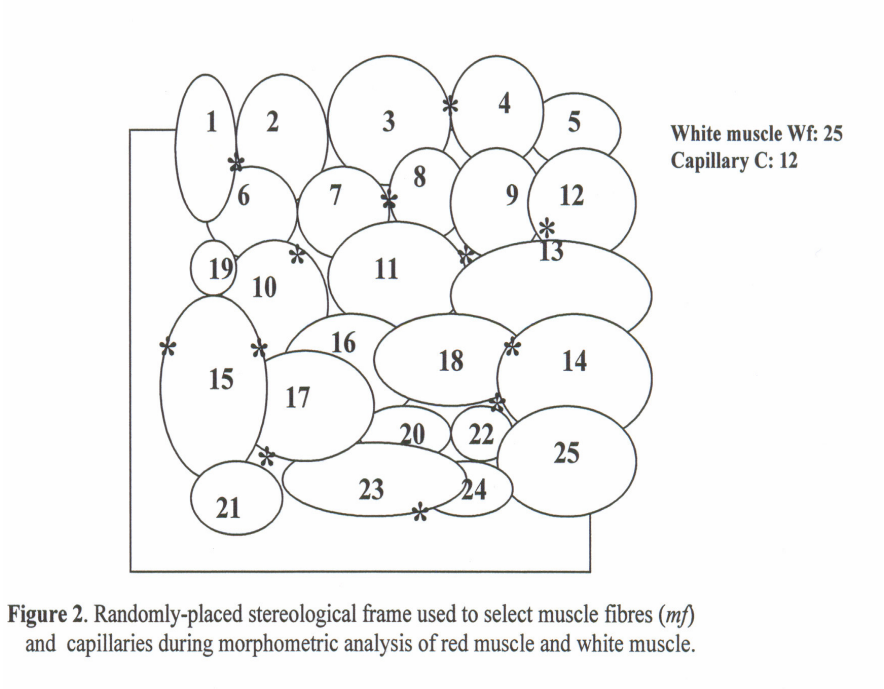

Figure 2. Randomly-placed stereological frame used to select muscle fibres (*mf*) and capillaries during morphometric analysis of red muscle and white muscle.

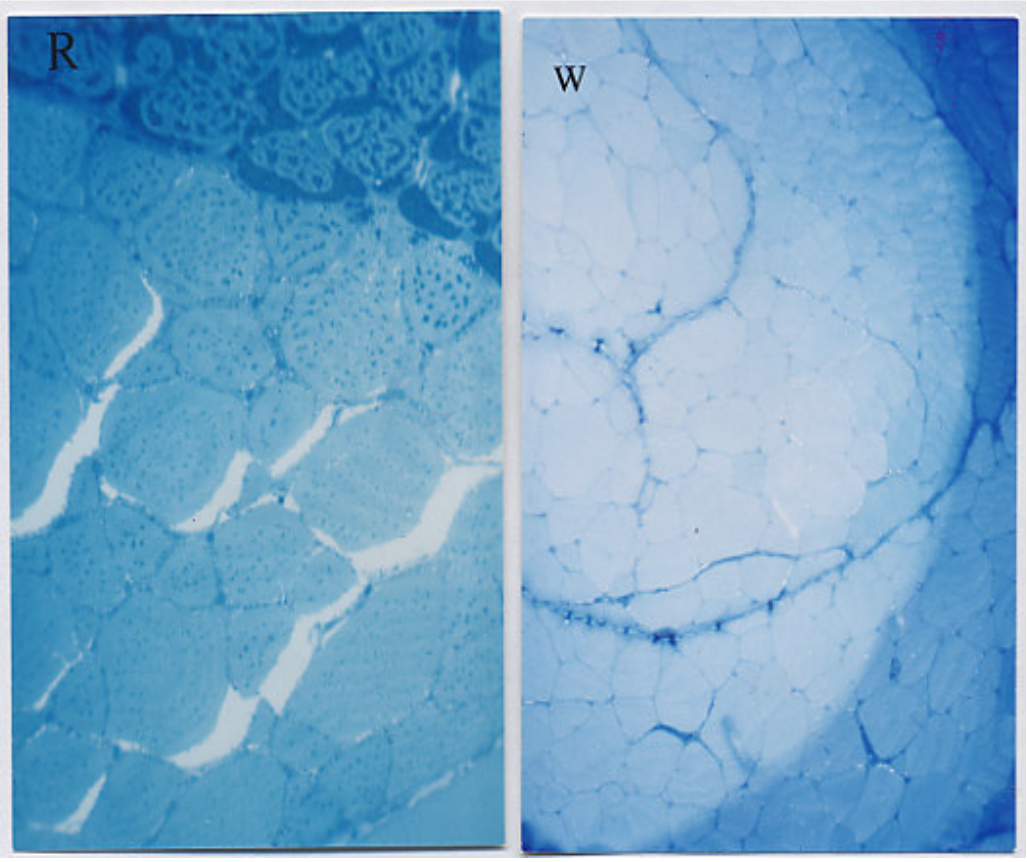

Figure 3: Photomicrograph of semithin section of the swimming muscle of coho salmon C2 (mass 14.9g, length 8.35cm) to show white (W) and red (R) muscle fibres. The average white fibre area is larger than red fibres. Between the two fibre groups there is a range of fibre sizes. X40.

A

B

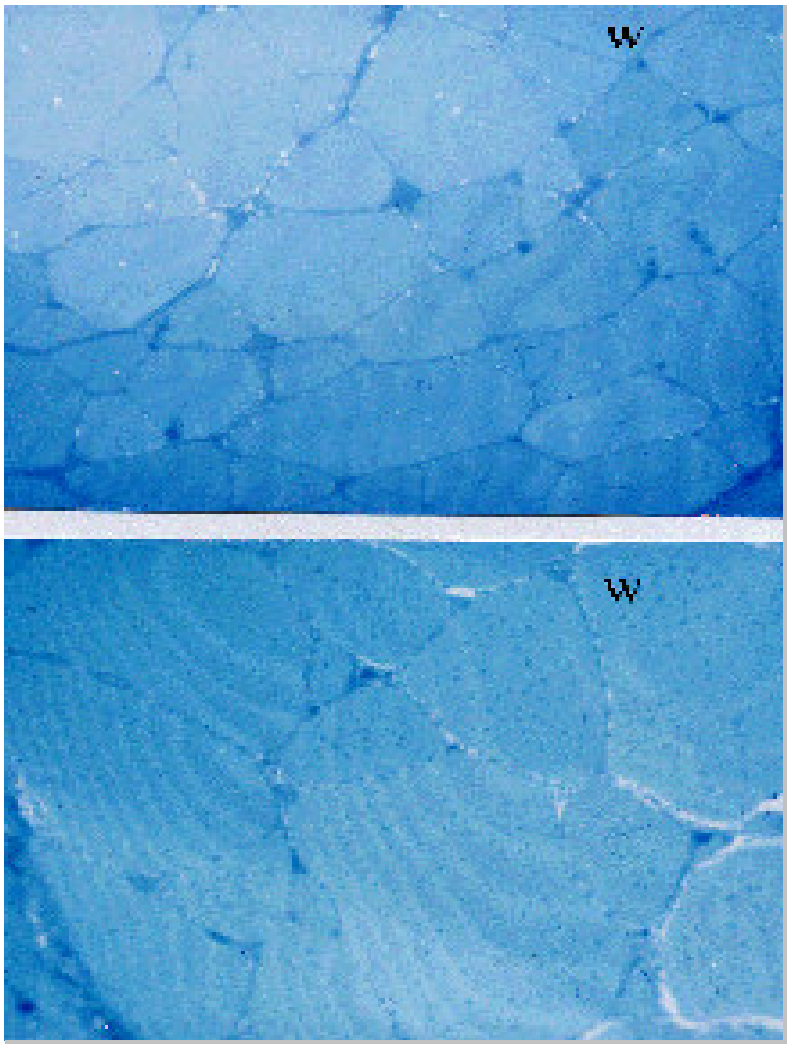

Figure 4: Photomicrograph of semithin section of the white muscle fibres from coho salmon (mass 3.99 g length 5.63 cm). It is clearly shown that the size of white fibres is relatively large. A small number of mitochondria are visible as small dots in the white muscles. A (X 25) &B (X 40).

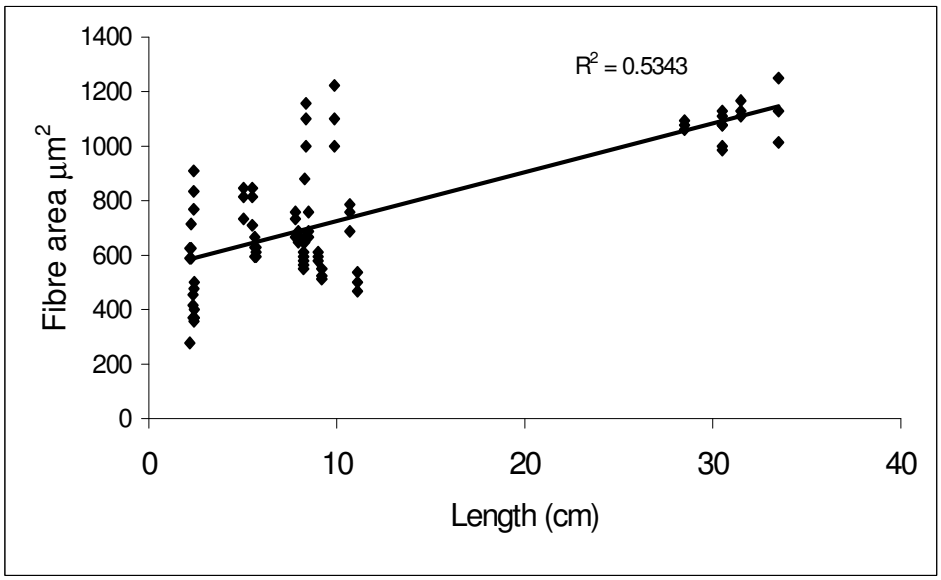

Figure 5: The cross-sectional area of red muscle fibers plotted against red muscle length for coho salmon. Area does not change markedly with length of musculature.

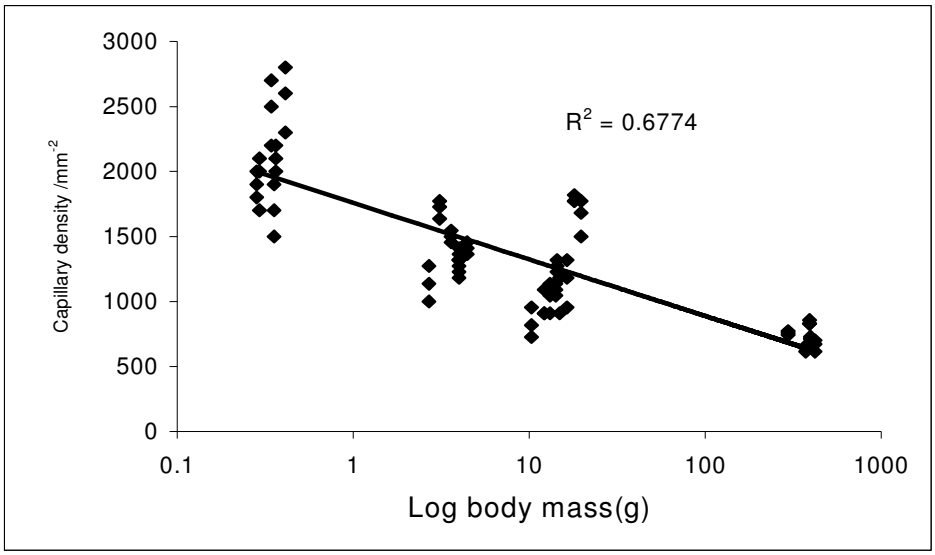

Figure 6: Capillary density (CD) in red muscle from developing coho salmon plotted against the log of body mass. CD decreases progressively with body mass down to a value of around 700 / mm^2 in 400g fish.

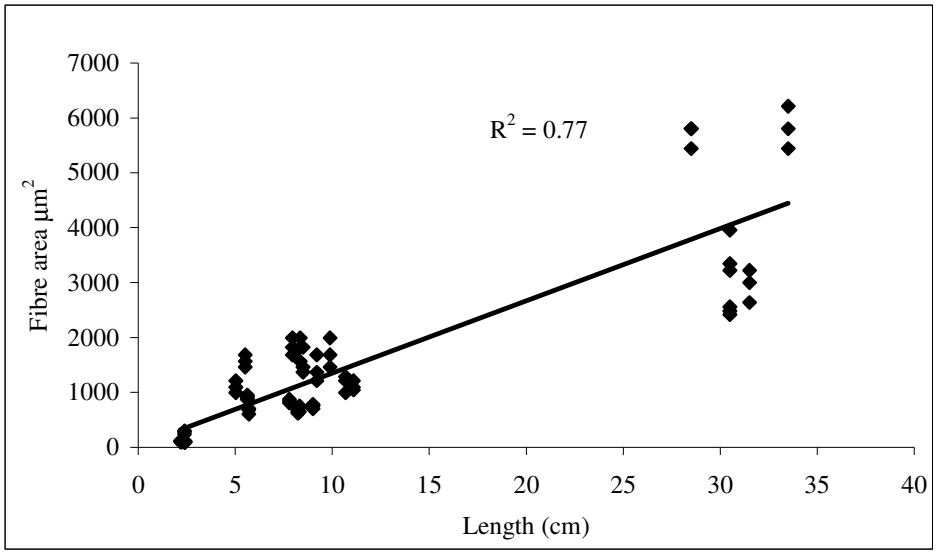

Figure 7: Cross-sectional area of white muscle fibres plotted against fish length for coho salmon. Area increases proportionately with length.

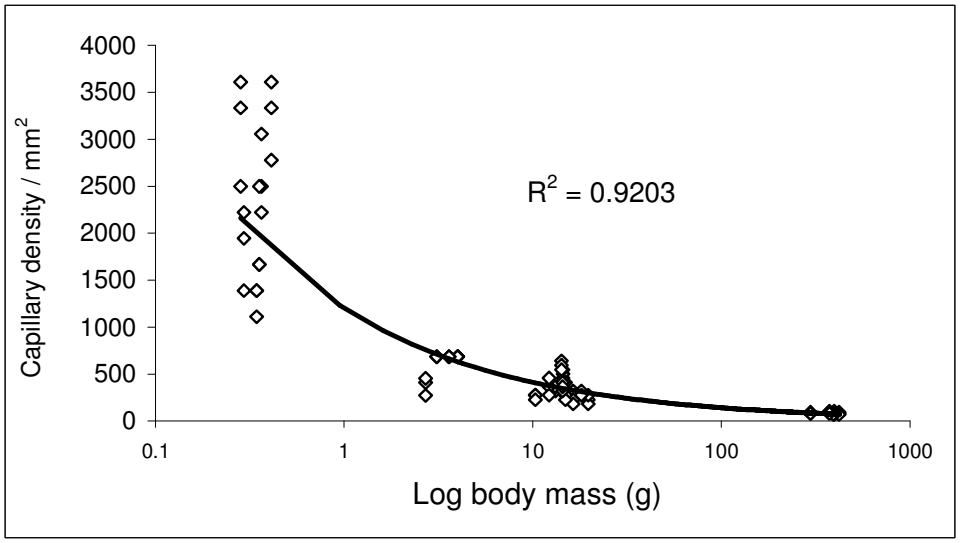

Figure 8: Capillary densities (CD) in white muscle plotted as a function of a logarithmic plot of body mass in developing coho salmon. CD decreases as body mass and consequently fibre area increases, down to a minimum of about 80 / mm^2 in 400g fish.

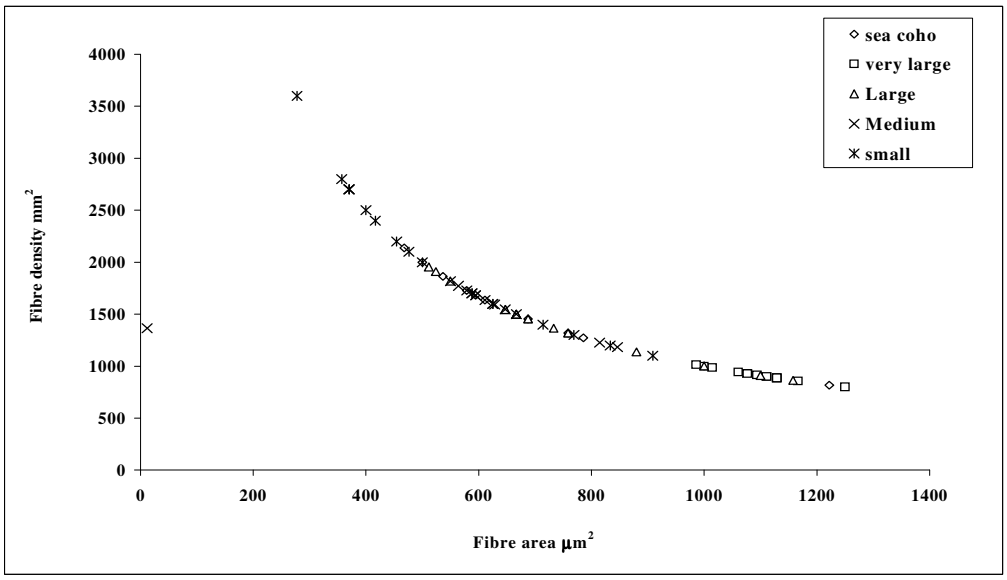

Figure 9: Muscle fibre density plotted against fibre area for red muscle from coho salmon, ranging in mass from 0.3g to 420g. As the fibre area increased the fibre density decreased exponentially down to 1,000 mm^2.

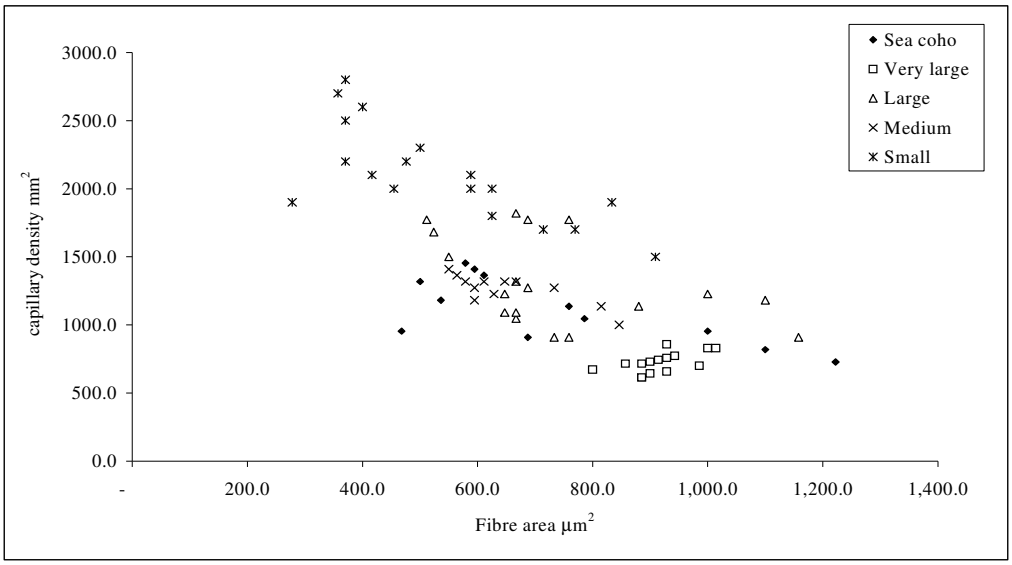

Figure 10: Capillary density plotted against fibre area for red muscle from coho salmon. As the fibre area increased the capillary density decreased to about 700/mm^2

DISCUSSION

The fibre areas for red and white muscle in the present study varied over the same range (around 1, 000 μm^2 but up to 4.000μm^2 for red muscle and around 2.000μm^2 and up to 8.000μm^2 for white muscle). This is in accordance with Egginton and Johnston (1983) in their work on conger eel. Red and white muscle fibre area increased while fibre density decreased as the coho salmon grew from 0.3g to 350g in mean mass. The results of the present study agree in general with data collected by (Johnston *et al*., 2000), who found that the density of muscle fibres in Atlantic salmon (*Salmo salar*) decreased with increasing body weight. However, the changes in fibre area and density of red muscle were only evident in the present study when comparing the small fish with large ones. Over the range of sizes between 0.3g and 15g (i.e. a 50 fold increase in mass accompanying a 4 fold increase in length) the red muscle fibre area changed by only about 1.4 fold, while fibre density was reduced by about 70%.

Hyperplastic growth occurs in a second, postembryonic stage in fish that grow to a large size (e.g. those used for aquaculture such as salmon, sea bass and carp) resulting in a large increase in the total number of fibres. The relative timing of the main hyperplastic growth processes in relation to the life cycle varies between species (Koumans and Akster, 1995; Rowlerson and Veggetti, 2002). As was described in the introduction, early growth up to 35 cm was predominanted by hypertrophy followed by a phase (35-75cm) hyperplasia predominated again. Similar growth phases have been recorded for rainbow trout (*Oncorhynchus mykiss*) (Weatherley *et al*, 1980b), where hyperplasia is again the main growth process up to 20 cm, and hypertrophy assumes increasing importance until at around 60 cm it becomes the sole growth process. Willems (1976) found a good correlation between muscle fibre growth and overall growth in glass eels (*Anguilla anguilla*) of 5.5-7 cm, but the relationship disappeared after migration into fresh water (33-40cm). Although Johnston *et al*. (2000) found that the density of muscle fibres decreased with increasing body weight in Atlantic salmon, average values were relatively constant for fish of 3.5-6.5kg. They concluded that variation in muscle fibre density within a family, often 65 to 140 fibres mm^{-2} muscle, can be significantly greater than that between families.

The white fibres form the bulk of the musculature and are heterogenic in size; being larger than the red fibres they have a glycolytic metabolism and high myosin ATPase activity (Carpene *et al*., 1982). The slow (red) and fast (white) fibres have a different pattern of development. In larval and juvenile coho salmon red muscle grows and develops chiefly by hyperplasia, while white muscle grows by hypertrophy. In mammals muscle growth during normal postnatal development is due almost exclusively to muscle fibre hypertrophy with very little contribution from fibre hyperplasia (Rows & Goldspink, 1969) except perhaps in the very early postnatal period in some mammals (Rayne & Grawford, 1975). Within the mass of large fibres there were small diameter fibres, which give the muscle a mosaic appearance, observations comparable with Boddeke *et al.* (1959) and also Johnston *et al.* (1975). These small fibre may be an early stage in the development of the larger white fibres, thus the range of fibre size reflects the different cohorts of fibre recruitment.

In agreement with Egginton (1987) as fish increased in size capillary density decreased but there was no change in capillary to fibre ratio (C: F). This occurred in both white and red muscles, (as previously mentioned in the introduction) there is a strong relationship between most vertebrate muscle fibre size and capillary supply. Throughout the fishes' development, it was found that as fibre area increased capillary density (CD) decreased. In white muscle it

reaches about 100 mm^{-2} in larger fish (300g); whereas in the red muscle capillary density was much higher at all stages and reached up to 1000 mm^{-2} in larger fish. The decrease in CD and increase in capillary to fibre ratio (C: F) with mean fibre area, did not change from around 1000 μm^2 suggesting that thereafter capillarization appears to be independent of fibre size. Other criteria may become important, possibly involving reorganisation of pathways for intracellular diffusion. Muscle as mitochondrial spacing or fibre lipid content. The present data are in accordance with are also comparable to earlier observation (Johnston *et al.,* 1972, 1974, 1975; Carpene *et al*., 1982)

In the coho salmon in the parr smolting differentiated white muscle fibres from interstitial cells producing collagen containing confluent myofibrils. Numerous mitochondria and an extensive sarcotubular system was observed with fibres being separated by a sarcolemma. Within the white fibres the myofibrils were expanded by sarcoplasm as a result of development (growth).

The use of light microscope revealed a rich store of lipid droplets within the red muscle. Lipid droplets are usually more abundant in red than white fibres constituting 11% of the fibre volume in brook trout (*Salvelinus fontinalis* Mitchill) and plaice (Pleuronectes platessa) (Johnston & Moon, 1981, Johnston, 1982). In fatty fish such as anchovy a layer of fat cells occurs between the skin and red muscle layer, while in other species, for example mackerel (Bone, 1978) and eel, adipocytes are widely distributed among the white muscle fibres.

Although the present results revealed no differences between the capillary density in the red muscle of fish before and after smolting (seawater fish coho salmon), this does not rule out a role for the Root effect in oxygen distribution to active muscle in juvenile fish (parr). In a further study we shall use electronic microscope to measure mitochondrial volume density and examine the lipid droplets in more detail. This may reveal a more subtle role for the early appearance and subsequent loss of the Root effect in coho salmon.

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