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Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*)

N. C. STICKLAND

Department of Anatomy, Royal (Dick) School of Veterinary Studies,
Edinburgh EH9 1QH

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INTRODUCTION

It is now generally accepted that, in mammals, muscle hypertrophy during normal postnatal growth is due almost exclusively to muscle fibre hypertrophy, with very little contribution from fibre hyperplasia (Rowe & Goldspink, 1969) except, perhaps, in the very early postnatal period in some animals (Rayne & Crawford, 1975).

The far fewer studies that have been carried out on muscle growth in fish suggest, however, that hyperplasia may be an important factor in muscle hypertrophy during postlarval growth (Greer-Walker, 1970; Weatherley, Gill & Rogers, 1979) although not all studies are in agreement with this (Kryvi & Eide, 1977). Some of the discrepancies may be due to the fact that the fish are not usually studied throughout their whole period of growth, and it is quite likely that the relative contributions of fibre hypertrophy and hyperplasia to muscle growth change during the lifespan of the fish, as is the case in prenatal muscle development in mammals (Stickland, 1981).

As in most teleost fish the lateralis muscle of the rainbow trout can be divided into a superficial layer, near the lateral line, of red fibres and, beneath this, a far larger mass of white fibres. Within the large mass of white muscle there are small diameter fibres which give the muscle a mosaic appearance (Boddeke, Slijper & Van der Stelt, 1959), but these small fibres were shown by Johnston, Ward & Goldspink (1975) to have the same histochemical properties as the large white fibres. Weatherley, Gill & Rogers (1980) suggest that these small fibres are a stage in the development of the larger white fibres. The lateralis muscle of the rainbow trout does therefore provide a means of studying the growth of red and white muscle fibres separately.

The present investigation was carried out in order to estimate the contribution of fibre hyperplasia and hypertrophy to the growth of red and white muscle in the rainbow trout during the whole period of postlarval growth.

MATERIALS AND METHODS

Seventeen rainbow trout (*Salmo gairdneri*) ranging in length from 2·2 cm (immediately postlarval) to 71 cm (approximately the normal maximum size of rainbow trout) were obtained from Hopewood Trout Farm, Peebles. Immediately after killing the fish by a sharp blow to the head, a complete transverse slice of lateralis muscle was removed from one side of the fish at a level two thirds of the length from the head end. This slice was frozen in dichlorodifluoromethane (Arcton 12, I.C.I.), which was cooled to its melting point (−158 °C) with liquid nitrogen. The slices were then cut at −20 °C, using a cryostat, in order to obtain 20 μm transverse sections of the complete lateralis muscle (red and white components) from one side. The sections were stained with haematoxylin and eosin. Low power photomicrographs of whole sections were made, as well as high power photomicrographs of red muscle and three regions of white muscle dorsal to the lateral line, namely, superficial, middle and deep.

A Videoplan image analyser (Reichert-Jung) was used to estimate the cross sectional area of the red and white muscle portions in the complete sections. The analyser was also used to measure 100 fibres from each of the high power photomicrographs of red muscle and superficial, middle and deep white muscle. The measurements obtained were fibre cross sectional areas (fibre areas) and diameters of circles with equivalent areas (fibre diameters). For the superficial white muscle measurements, the two or three most superficial layers of fibres were not included in the measurements because these are known to have slightly different properties and to be generally smaller than the remainder of the white muscle, being known as pink fibres (Johnston *et al.* 1975).

The fibres in the high power photomicrographs were counted so that estimates of the number of fibres per unit area were obtained for both red and white muscle. These estimates, together with the red and white muscle cross sectional area measurements, were used to calculate the total number of fibres in the complete sections. Enough photomicrographs were used to ensure that approximately 5 % of the total number of fibres were counted.

The effects of growth on fibre size (both cross sectional area and diameter) and fibre number were examined by plotting these parameters against fish length. Fibre diameter measurements were used so that a comparison could be made with the results of several other workers who have used this parameter. Relationships were defined by regression equations, using simple transformations (e.g. log, power, exponential) as necessary to find the best-fit regression line.

RESULTS

The relationships between the cross sectional areas of red and white muscle from one side of the fish and fish length are shown in Figure 1. The regression coefficients of the two power curves (Table 1) are not significantly different, which indicates that red and white muscle increase in cross sectional area at the same rate throughout growth (as measured by fish length increase). The ratio of cross sectional area of white muscle to that of red muscle remained at about 25 : 1 throughout growth.

The increase in the total number of red muscle fibres and white muscle fibres (from one side of the fish) with increasing fish length is shown in Figure 2. Table 1 shows that for the white muscle the best fit curve is a polynomial, indicating (Fig. 2) that fibre number increases rapidly at first and then slowly, reaching a plateau, at about 65 cm, of 150000 muscle fibres. The relationship between red fibre number and length is much more variable and none of the regressions attempted (logarithmic, power, exponential, various degree polynomials) was significantly better than the linear regression (Table 1) shown. Owing to the large amount of variability, however, (indicated by the high S_b value) it is not possible to state whether the red fibre number rate of increase changes during growth, although there is clearly an overall increase.

The increases in mean fibre cross sectional area for red and white muscle with length (Fig. 3) are exponential in form (Table 1). This means that for every 20 cm

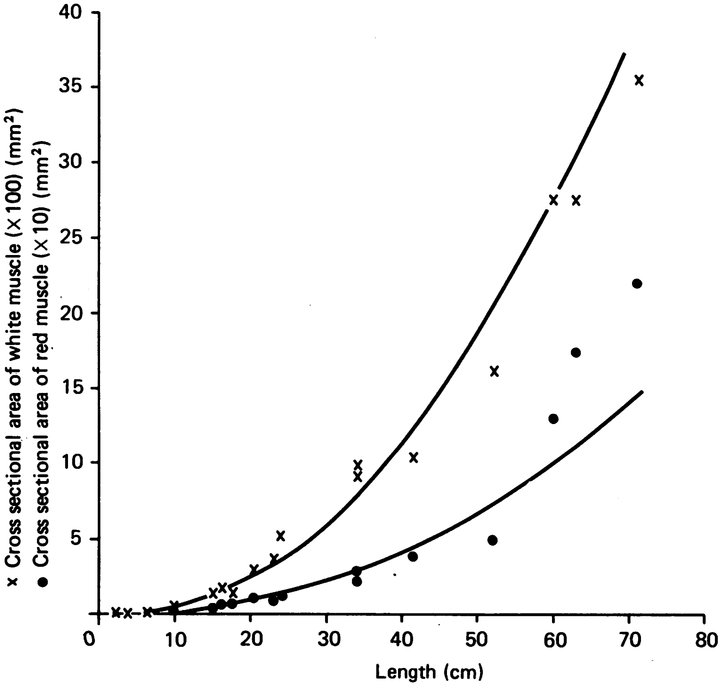

Fig. 1. Total cross sectional area of white muscle and red muscle versus fish length.

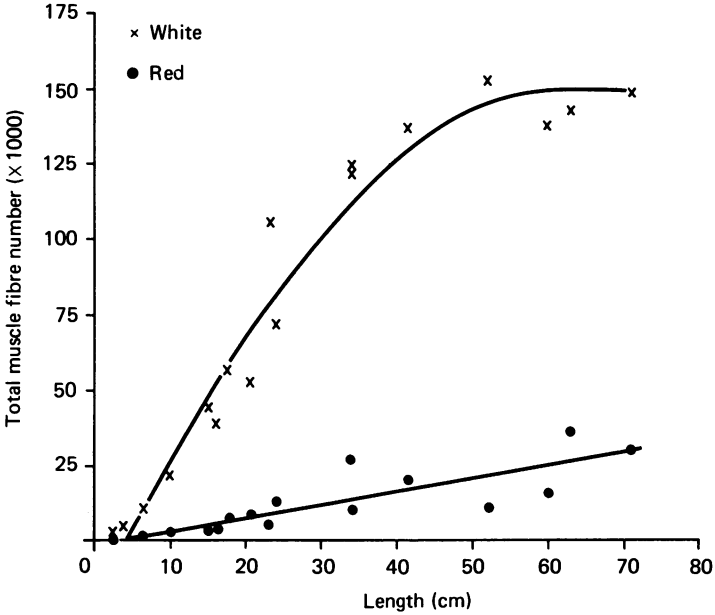

Fig. 2. Total muscle fibre number in complete cross sections for white muscle and red muscle versus fish length.

Table. 1 *Equations of computed regression lines*

Figure	Parameters X	Y	Line	S_b
1	Length	White muscle CSA	$Y = 0{\cdot}372.X^{2{\cdot}18}$	0·04
	Length	Red muscle CSA	$Y = 0{\cdot}0168.X^{2{\cdot}13}$	0·07
2	Length	Total white fibre no.	$Y = 5505.X-44.X^2 -22215$	$S_{b1} = 570$, $S_{b2} = 8{\cdot}0$
	Length	Total red fibre no.	$Y = 434.X-1055$	74
3	Length	Mean white fibre CSA	$Y = 1383.1{\cdot}041^X$	0·004
	Length	Mean red fibre CSA	$Y = 502.1{\cdot}034^X$	0·004
4	Length	Mean white fibre diameter	$Y = 1{\cdot}64.X+29{\cdot}5$	0·12
	Length	Mean red fibre diameter	$Y = 24{\cdot}9.1{\cdot}017^X$	0·002
—	White muscle CSA	Total white fibre no.	$Y = 54033.\log X-49724$	5929
—	White muscle CSA	Mean white fibre CSA	$Y = 5{\cdot}17.X+1786$	0·22
—	Red muscle CSA	Total red fibre no.	$Y = 10252.\log X+831$	7489
—	Red muscle CSA	Mean red fibre CSA	$Y = 24{\cdot}6.X+823$	3·0

CSA, Cross sectional area. S_b, Standard error of regression coefficient. Units: Length, cm; Muscle CSA, mm^2; Fibre CSA, μm^2; Fibre diameter, μm.

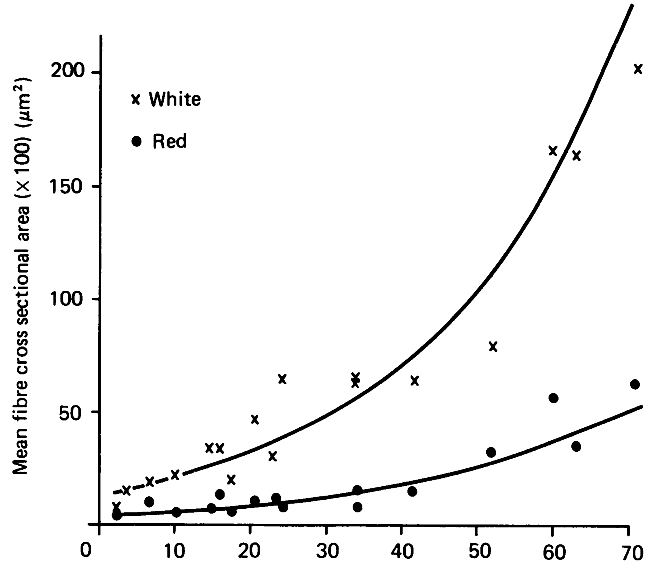

Fig. 3. Mean muscle fibre cross sectional area for white muscle and red muscle versus fish length.

increase in fish length the mean red fibre cross sectional area doubles; the mean white fibre cross sectional area doubles in a slightly shorter time (about every 17 cm). In other words, mean fibre cross sectional area increases at an increasing rate with growth.

Increase in mean white fibre diameter (Fig. 4) shows a linear relationship (Table 1) with fish length, whereas the increase in mean red fibre diameter exhibits an exponential relationship (Table 1) with fish length. From an examination of the 'red' points in Figure 4, however, it would appear that, although the relationship may be

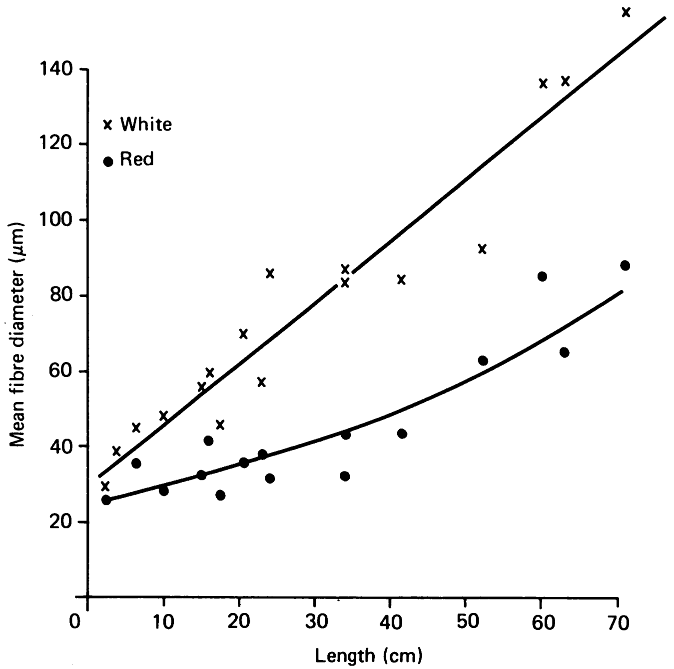

Fig. 4. Mean muscle fibre diameter for white muscle and red muscle versus fish length.

exponential overall (this gave the best-fit regression when using all the points), there is little change in fibre diameter until about 40 cm, which is possibly also shown in Figure 3 for mean red fibre cross sectional area.

Table 1 shows that there is a linear relationship between muscle fibre cross sectional area and total muscle cross sectional area for both red and white muscle, whereas muscle fibre number exhibits a logarithmic relationship with total muscle cross sectional area, also for both white and red muscle. The latter relationship demonstrates that muscle fibre number increase slows down as muscle cross sectional area increases.

Histograms were constructed for muscle fibre diameter distributions for each fish. Figures 5 and 6 show these distributions for white and red muscle, respectively, in representative fish taken at approximately 10 cm intervals. Each white fibre diameter histogram (Fig. 5) is the average for the three white sampled areas (superficial, middle and deep) in each of which 100 fibres were measured. It should be pointed out that within each fish the histograms for the three areas did not show any apparent significant differences and, in particular, did not show evidence of one area having a larger proportion of smaller fibres than another. Figure 5 shows that the increase in mean white fibre diameter during growth is due to an increase in width of the histogram so that, even at 52 cm, there are still some fibres in the smallest size class (10–20 µm) although they represent less than 1 % of the total population at this stage compared to over 10 % at the 2·2 and 10·0 cm stage. In contrast, Figure 6 shows that the increase in mean red fibre diameter is due to the distributions shifting to the right so that, even at 34·0 cm, there are no fibres less than 20 µm in diameter. The distributions are much narrower for the red muscle, especially when it is appreciated that the classes are 5 µm wide in the red (Fig. 6) compared to 10 µm in the white

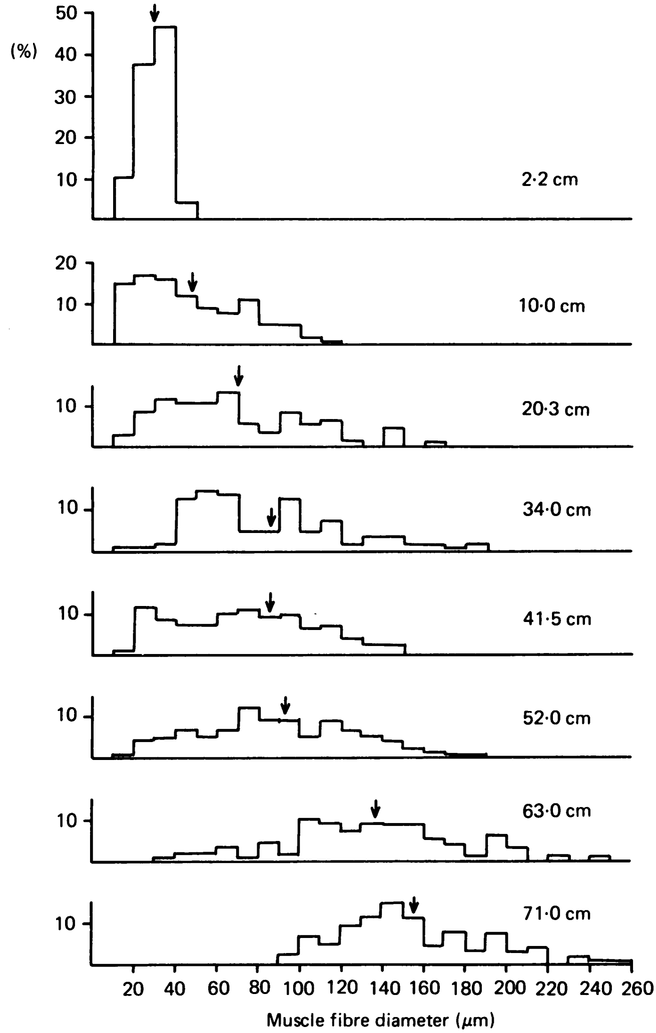

Fig. 5. Muscle fibre diameter percentage frequency histograms for white muscle from fish of lengths indicated alongside each histogram. The arrows mark the mean fibre diameter for each histogram.

(Fig. 5). In fact, for all the histograms shown in Figures 5 and 6, except for the 2·2 cm stage, the variances (based on standard deviations quoted as percentages of the means) are significantly greater ($P < 0{\cdot}05$) for the white muscle than the red.

DISCUSSION

It has been shown that the cross sectional area of both red and white muscle is proportional to approximately the square of fish length (Fig. 1, Table 1), which is perhaps not surprising when plotting an area against a length. Kryvi & Eide (1977), however, found linear relationships between red and white muscle cross sectional areas and fish length in the shark, although this possibly could be explained by the fact that only a relatively narrow range of lengths, namely about 5–25 cm, was used. They also concluded that white muscle cross sectional area increases more than that

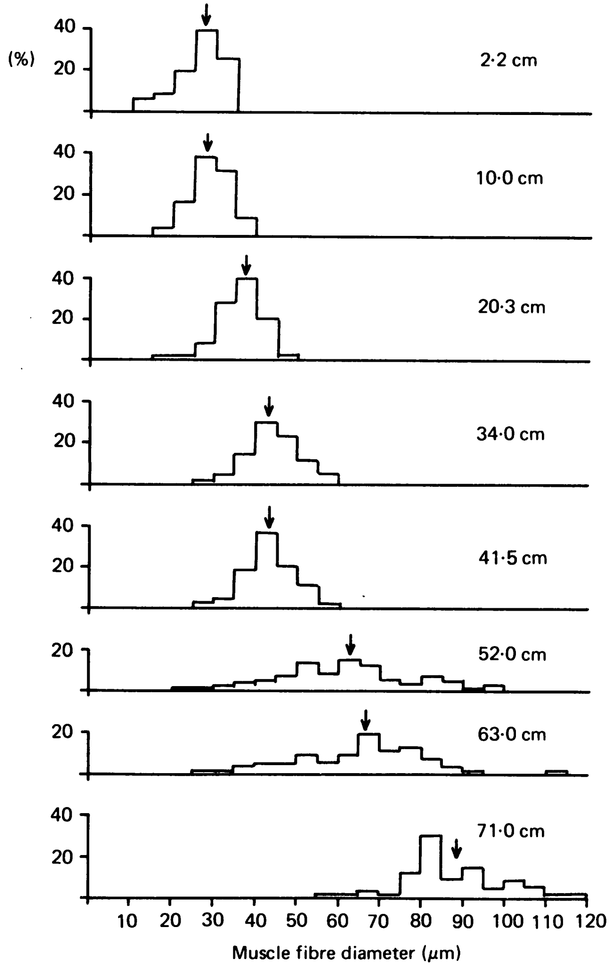

Fig. 6. Muscle fibre diameter percentage frequency histograms for red muscle from fish of lengths indicated alongside each histogram. The arrows mark the mean fibre diameter for each histogram.

of red muscle over the period of study whereas, although the equations in Table 1 show a slightly larger rate of increase in white cross sectional area than that in red, the differences are not significant in this present study.

The rate of increase in the total number of white muscle fibres was found to decrease with increasing fish length (Fig. 2). As far as the red muscle fibres are concerned, there is a definite increase over the length range studied but, owing to the variability found, it is not possible to state with certainty how the rate of increase varies during growth. In contrast to these results, Kryvi & Eide (1977) found no increase in white muscle fibre number in the shark and only a slight increase in that of red. Greer-Walker (1970), however, did find an increase in the number of red and white muscle fibres (added together) throughout the growth of cod from about 5 to 120 cm, but with no rate decrease at the upper end of the range.

It is possible that any change in muscle fibre number with growth may be due to a change in the arrangement of muscle fibres within the muscle. An apparent increase in fibre number may be due to the growth of intrafascicularly-terminating fibres entering the plane of section. However, the lateralis muscle of fish is segmented into myomeres which are separated by sheets of connective tissue, the myocommata. Within these myomeres the muscle fibres are only a few millimetres long and run, roughly parallel to the long axis of the body, from one myocomma to the next. No fibre terminations have been observed between the myocommata when muscle samples have been teased under a microscope (personal observation). It is also known that, although individual fibres extend only from one myocomma to the next, successive fibres lying on opposite sides of myocommata can be teased out as long threads over many myomeres; these threads are known as muscle fibre trajectories (Alexander, 1969). These observations, together with the fact that the arrangement and number of myomeres do not change with growth, indicate that any increase observed in the number of muscle fibres per cross section must be due to the formation of new muscle fibres.

As far as muscle fibre diameter is concerned, there is a continuing increase when plotted against length (Fig. 4) which appears exponential overall for the red muscle although linear for the white. In his study on the cod, however, Greer-Walker (1970) found that red and white muscle fibre diameters increased in a sigmoid manner which, in the white muscle, was followed by a decrease from 83 cm onwards.

The relative contributions of hyperplasia and hypertrophy to increase in muscle cross sectional area were calculated for both white and red muscle and the results are shown in Figure 7. This figure was calculated by estimating the increase in fibre number per unit length (every 5 cm) from Figure 2, and the increase in fibre cross sectional area per unit length from Figure 3, and expressing one increase as a percentage of the two combined. This figure shows that, for the white muscle (Fig. 7*a*), hyperplasia is at first the major contribution to the increase in muscle cross sectional area but throughout growth its relative importance decreases so that at the end of the trout's growth (65–70 cm) increase in muscle cross sectional area is due entirely to fibre hypertrophy. Based on biochemical analyses of rainbow trout muscle (DNA content and ratio of fresh weight to DNA), Luquet & Durand (1970) concluded that hyperplasia accounted for 70 % of the increase in muscle weight up to 400 g body weight (about 33 cm) and thereafter continued to account for 90 %, cells having reached 75 % of their final size by 400 g. It must be appreciated, however, that the hyperplasia to which the authors referred was increase in the number of nuclei, not fibre hyperplasia, and their conclusions on cell size indicate only that the cytoplasmic to nuclear ratio shows little increase after 400 g.

It is interesting to note from Figure 5 that the first histogram in which there are no fibres in the smallest size class (10–20 μm) is that for the 63 cm fish. It may be reasonable to assume that a lack of these very small fibres indicates that no new fibres are being formed, i.e. that hyperplasia has ceased. If this is true then the histogram results are in agreement with Figure 7*a* which shows that hyperplasia does, in fact, cease by this 63 cm stage. Weatherley *et al.* (1979) produced histograms for fibre diameters in the white muscle of rainbow trout up to about 33 cm in length. They did not count the number of fibres but deduced, in agreement with this present investigation, that, as the number of fibres in the smallest size class was reducing, so hypertrophy became relatively more important than hyperplasia in muscle growth.

The large range of fibre diameters seen in the white muscle of trout give it a characteristic mosaic appearance (Boddeke *et al.* 1959) which was once assumed to be a mixture of small red and larger white fibres. Johnston *et al.* (1975), however, showed that both the small and large fibres of this mosaic muscle had the same

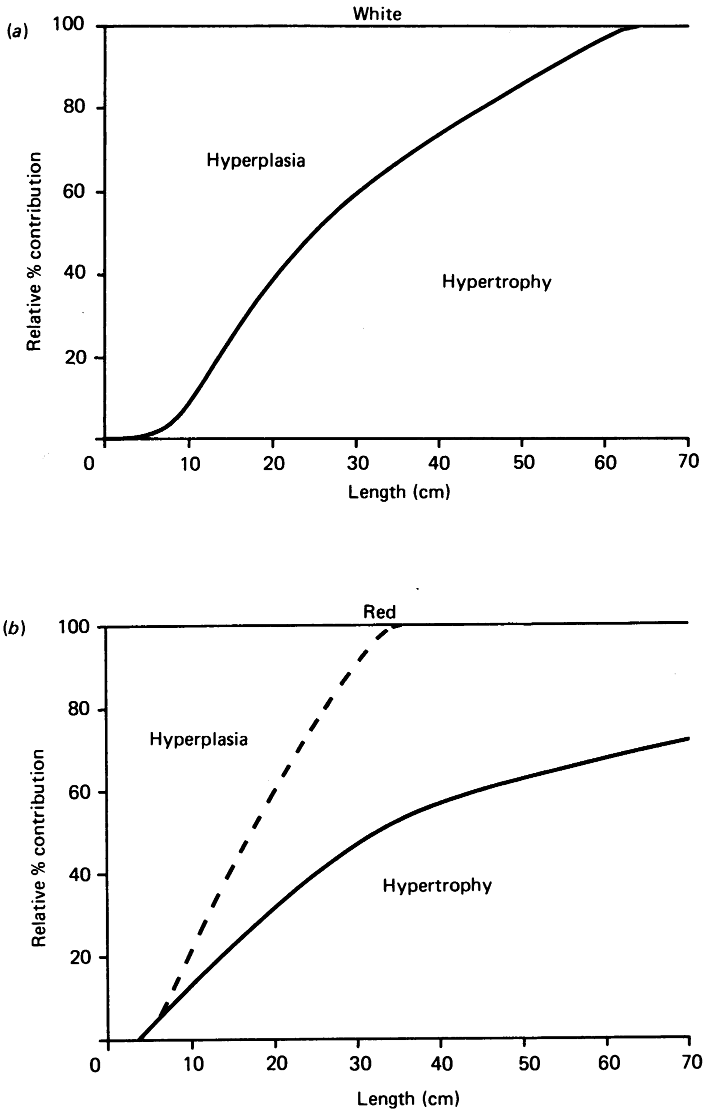

Fig. 7 (*a–b*). Relative percentage contributions of hyperplasia and hypertrophy to total muscle cross sectional area growth versus fish length for (*a*) white muscle and (*b*) red muscle. The broken line in (*b*) is explained in the text.

histochemical properties. Carpenè & Veggetti (1981) working on Mugilidae fish species found small fibres in the white muscle with different histochemical properties from the rest of the white muscle. They concluded that small fibres were produced at certain times of the year and enlarged to evolve mature white fibre properties. It seems reasonable to assume here, therefore, that the smallest fibres are the newly formed fibres which gradually enlarge, hence the widening histograms in Figure 5. It is possible that the seasonal variation observed by Carpenè & Veggetti (1981) may be present in the rainbow trout. For example, in Figure 4, the fish at 24 cm has, in relation to its very small length difference, much bigger white muscle fibres than the fish at 23 cm. The former was obtained in November whereas the latter was obtained in June. The data from this investigation are insufficient, however, to indicate whether this seasonal variation is significant in the trout; more work needs to be carried out in order to elucidate this point. On the other hand, a difference in fibre diameters between fish at the same length may be due to these fish having different growth rates with the fastest growing fish having smaller fibres (Weatherley *et al.* 1979).

As mentioned earlier the red muscle situation is difficult to assess. The continuous line in Figure 7*b* is based on the 'red' lines in Figures 2 and 3. If a disappearance of fibres in the smallest size class is an indication of the cessation of hyperplasia, then, from Figure 6, this occurs at 34 cm (the first stage when there are no fibres less than 20 μm). This would then support the validity of the broken line shown in Figure 7*b*, so that from 34 cm onwards all increase in red muscle cross sectional area is due to fibre hypertrophy. However, although it is perhaps most likely that hyperplasia does cease by about 34 cm, the variability in the results (particularly those shown in Figure 2 'red') means that the possibility that hyperplasia continues throughout growth cannot be ruled out, althouth its relative contribution must decrease.

In conclusion, it has been shown that postlarval muscle growth in the trout is due, firstly and mainly, to hyperplasia, which is then gradually replaced by hypertrophy as the major factor until hyperplasia ceases completely. Although this is somewhat analogous to the prenatal growth of mammalian muscle (Stickland, 1981), there is little hyperplasia postnatally in mammalian muscle (Goldspink, 1962). The newly formed fibres found in growing fish muscle are probably produced from satellite cells or persistent myoblasts rather than by fibre splitting. No evidence of fibre splitting has been observed in the samples used in this investigation. The exact mechanism of fibre production is, however, being presently examined in this laboratory. Another interesting question posed by the results described here is how the new muscle fibres became innervated. Easter (1979) found that there was no increase in axon number in the trochlear nerve supplying the superior oblique eye muscle in the goldfish during growth, although the number of neuromuscular synapses increased greatly to accommodate the more numerous muscle fibres. The innervation of the newly formed fibres found in the lateralis muscle is presently being investigated in this laboratory.

SUMMARY

The growth of red and white muscle was investigated in the rainbow trout, using fish from 2·2 cm to 71 cm in length. In the white muscle, fibre hyperplasia, initially, accounted for all muscle growth but its relative contribution decreased as the contribution from fibre hypertrophy increased. At and above about 65 cm there was no hyperplasia, and this corresponded approximately to the stage when there were no more fibres in the smallest size class (less than 20 μm). The results for the red muscle are more variable and hence more difficult to assess. Although red fibre hyperplasia may continue throughout growth, at 34 cm there are no fibres in the smallest size class, which possibly indicates no new fibre formation beyond this stage.

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