

Patterns of muscle growth in early and late maturing populations of Atlantic salmon (*Salmo salar* L.)

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Received 26 April 1999; received in revised form 10 March 2000; accepted 15 March 2000

Abstract

Muscle growth was investigated in two populations of Atlantic salmon (*Salmo salar* L.) derived from an early maturing stock of West Coast Scottish origin (strain X) and a late maturing stock (strain Y) of Norwegian origin. Fish from six families per population were PIT-tagged and reared together in a 5 × 5 × 5 m sea cage between April 1997 and September 1998. The distribution of muscle fibre diameters in different age classes was investigated using non-parametric smoothing and bootstrapping techniques. Body mass and the total cross-sectional area of white muscle at the level of the first dorsal fin ray increased at a significantly faster rate in strain X than in strain Y after the first 6 months in seawater (April–October). The relative contributions of fibre recruitment and hypertrophy to muscle growth were very different for the two populations. The

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number of white muscle fibres per trunk cross-section was around 150,000 in June and 250,000 in July for both populations. The majority of fish sampled in June, July and August 1997 showed a unimodal distribution of fibre diameter. Between July and August fish from strain Y recruited two times more fibres for each square millimeter increase in white muscle cross-sectional area than fish from strain X. Fibre number in strain X had caught up with strain Y by January with 545,000 fibres per cross-section. There was no significant increase in the number of white fibres in strain Y between January and September 1998, such that growth was entirely by fibre hypertrophy. In contrast, fibre number continued to increase in strain X until the final sample in June, reaching 718,000 per trunk cross-section. All fish showed a bimodal distribution of fibre diameter in the October and subsequent samples with significant differences between strains. The right-hand peak of the distribution was shifted towards higher diameters in strain X than in strain Y, indicating superior hypertrophic growth performance in the early maturing population. For example, in the May 1998 sample, the 95th percentile of fibre diameter was at 215 μm in strain X and 171 μm in strain Y. In March and subsequent samples, the left-hand peak of the distribution was shifted to higher fibre diameters in strain Y relative to strain X, reflecting the cessation of new fibre recruitment in strain Y. Immature fish within strain X had a lower density of small diameter fibres and the right-tail of the distribution was shifted to higher diameters relative to fish that had begun to sexually mature. Thus greater muscle fibre hypertrophy was observed in immature than maturing fish of the same strain. It was concluded that the superior growth performance of the early maturing population was associated with a longer period of fibre recruitment and greater fibre hypertrophy than in the late maturing population, although these differences were not directly related to sexual maturation. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Atlantic salmon; Muscle fibres; Growth; Sexual maturation

1. Introduction

Muscle fibre formation occurs at the foetal stage in mammals and subsequent growth involves the elongation and hypertrophy of the fibres already present at birth (Rowe and Goldspink, 1969). In fish, with the exception of species that only have a maximum length of a few centimeters, the recruitment of muscle fibres occurs throughout the growing period (Weatherley et al., 1988). Different muscle fibre types can be recognised, arranged in discrete layers within the myotomes (Johnston et al., 1974; 1975; Carpenè et al., 1982; Rowlerson et al., 1995; Ramírez-Zarzosa et al., 1998). Slow twitch muscle fibres, supplied primarily by aerobic metabolic pathways (Johnston et al., 1977; Bone, 1978), are located in a thin superficial strip that forms a thicker layer at the major horizontal septum. The red muscle layer, which increases in thickness towards the tail, is responsible for powering slow speed swimming and accounts for a relatively small proportion of the total muscle volume in most species (Johnston et al., 1977; Bone, 1978). White muscle comprises the major edible part of the myotome and is composed of fast twitch fibres (Hudson, 1969; Johnston, 1980). The white muscle is active during high speed swimming and is primarily dependent on anaerobic metabolic pathways for its energy supply (Driedzic and Hochachka, 1976).

Muscle is a post-mitotic tissue and the nuclei required for post-embryonic fibre recruitment and hypertrophy are derived from satellite cells (see Koumans and Akster,

1995; Johnston, 1999 for reviews). The satellite cells represent a muscle stem cell population and their division products committed to differentiation (Yablonka-Reuveni and Rivera, 1994; Schultz, 1996). These division products are either absorbed into muscle fibres as they hypertrophy or else they fuse together to form new myotubes on the surface of existing fibres that mature into small diameter muscle fibres (3–5 μm diameter). In Atlantic salmon (*Salmo salar*), a cross-section of the anterior part of the trunk contains around 5,000 white muscle fibres at hatch, increasing to 10,000 by first feeding (Johnston and McLay, 1997) and 150,000 to 200,000 following smoltification (Higgins and Thorpe, 1990; Johnston et al., 1999). Muscle fibre recruitment continues during seawater growth reaching more than 1 million in two-sea-winter salmon reared under commercial conditions (Johnston, 1999). Individual white muscle fibres increase in size (hypertrophic growth) until they reach their maximum diameter of around 240 μm (Johnston et al., 1999). Thus growth in body girth relies on new fibre recruitment but is largely driven by fibre hypertrophy. The number and size distribution of muscle fibres present in the myotomes of any particular age class reflects both genetic factors and the growth history of the fish (Weatherley and Gill, 1985; Johnston, 1999).

The tendency of wild Atlantic salmon to home to their natal streams to spawn promotes reproductive isolation and genetic differentiation between populations (Verspoor, 1997). Wild salmon show significant phenotypic diversity between and within river systems with respect to body size and age at maturity (Schaffer and Elson, 1975; Thorpe and Mitchell, 1981), morphology (Riddell and Leggett, 1981) and physiological characteristics including digestion and growth rates (Taylor, 1991; Nicieza et al., 1994). In some cases, such population differences have been shown to be heritable in breeding experiments (Riddell and Leggett, 1981; Nicieza et al., 1994; Thorpe et al., 1983).

There is some evidence for genetic influences on muscle growth patterns in salmonids, particularly the number and size distributions of muscle fibres. Valente et al. (1998) determined the concentrations of RNA, DNA and protein in the skeletal muscle of a fast (Cornec) and slow (Mirwart) growing strain of rainbow trout (*Oncorhynchus mykiss*). The higher DNA:protein ratio observed in the Cornec strain was interpreted in terms of a smaller average cell size, but a larger number of cells per unit weight compared to the Mirwart strain. In a subsequent paper, the percentage of fibres less than 25 μm diameter was shown to be greater in the fish from the fast than slow growing strain (Valente et al., 1999). Muscle growth patterns have also been shown to vary between triploid and diploid individuals from the same families in both rainbow trout (Suresh and Sheehan, 1998) and Atlantic salmon (Johnston et al., 1999). Triploid Atlantic salmon recruited around one-third fewer fibres to reach a given muscle cross-sectional area than diploids, but showed a compensatory increase in hypertrophic growth (Johnston et al., 1999). The reduced number of muscle fibres in triploids was associated with a lower density of muscle satellite cells (Johnston et al., 1999).

In salmon farming, sexual maturation marks the end of the useful period of cultivation since associated deteriorative changes reduce flesh quality and lower market value. The proportion of fish maturing as grilse after one sea-winter has a strong genetic as well as an environmental component (Hansen et al., 1992; Taranger et al., 1998). Strains with a high proportion of early maturing fish generally have faster growth rates

than strains with a high proportion of late maturing fish but must be harvested early in the year when market prices may be lower. The main aim of the present study was to investigate the influence of genetic stock and sexual maturation on muscle fibre recruitment and hypertrophy during the seawater stages of Atlantic salmon reared under semi-commercial conditions. The relationship between the different densities of muscle fibres found between the populations and the texture and colour of the flesh was investigated in an accompanying paper (Johnston et al., 2000a).

2. Materials and methods

2.1. Fish stocking and culture

Two populations of Atlantic salmon (*S. salar* L.) were studied corresponding to a high grilising stock of West Coast Scottish origin (strain X derived from the “Lochy” stock), and a low grilising stock of Norwegian origin (strain Y derived from the “Namsen” stock). Each population had been maintained for at least five generations as part of Marine Harvest McConnell’s genetic improvement programme. Individually PIT tagged (passive integrated transponder, supplied by Fish Eagle Co., Gloucestershire, England) and freeze-branded S1 smolts were transferred from Loch Arkaig (freshwater) to Loch Duich (seawater) on the 14th April 1997. Fish were stocked into a steel-construction cage (5 × 5 × 5 m) with a 12 mm mesh cube net. The cage was stocked with 585 smolts with an average mass of 55 g, representing six families of strain X and six families of strain Y. Fish were fed by hand to satiation between one and three times per day depending on season. The diet used was a standard commercial ration of the Ecolife[®] series from BioMar Ltd. Composition ranged from 23/49 (oil/protein) in the smaller pellet size to 28/46 in the larger. The temperature was recorded throughout the growing cycle (Fig. 1A).

2.2. Fish health

The sea cages were stocked with Goldsinney wrasse (*Ctenolabrus rupestris*) (1 per 50 salmon) in an attempt to control sea lice infestations. Additional treatments were carried out with dichlorvos on the 23rd May 1997 and with hydrogen peroxide at 2- to 4-week intervals as necessary between December 1997 and August 1998.

2.3. Sampling

The mass of each fish was measured at 5- to 6-week intervals. Fish were batch-netted into a sample weigh bin, anaesthetised with benzocaine in acetone, identified by PIT tag, and mass, fork length and maturity status recorded. At each weighing fish were removed for muscle analysis to ensure a good representation of the different families in the

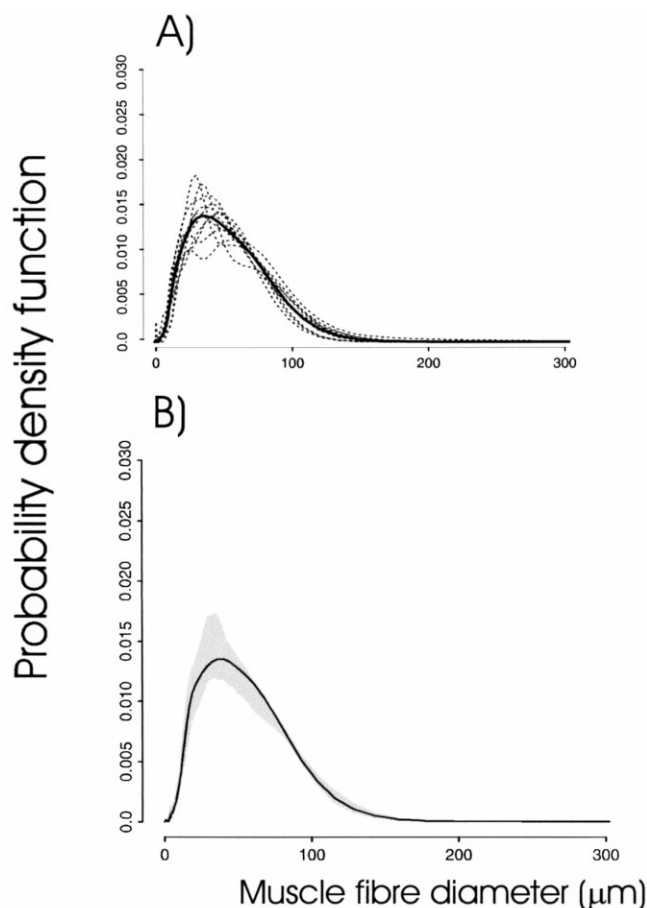


Fig. 1. (A) PDF of muscle fibre diameter for the June 1997 sample of strain X. The dotted lines represent individual fish and the solid line the average PDF. (B) Variability band around the average PDF (solid line) for the fish illustrated in (A) constructed using 100 bootstrap estimates (shaded area).

populations. On each date between June 1997 and October 1997, five fish each were sampled from families designated Xrn, Xrh, Yr3, and Yr + , and a further three fish each from families designated Xlh, Yldu and Yrh. For the January and March 1998 samples, six fish each were analysed from families Xrn and Xrh, three fish from family Xlh, and four fish each from families Yr3 and Yr + . May 1998, eight fish were sampled from family Xrn and six fish from families Xrh and Xlh. The June sample comprised three fish from family Xlh, four fish from family Xli, and three fish from family Xl + . The final sample in September 1998 comprised six fish each from families Yr3, and Yr + , and three fish each from families Yrh and Yldu. All fish were identified by their brand and cross-referenced against PIT number. Fish were starved for 7 days before being killed by percussion stunning.

2.4. Carcass processing

Sampled fish were gill tagged and placed on ice. Following evisceration, gonads and liver were weighed, and fish gutted mass recorded. Salmon sampled between June 1997 and March 1998 were sectioned at a point immediately behind the dorsal fin. The anterior half of the carcass was transported to St Andrews University on ice for the analysis of muscle cellularity. Fish used for taste panel assessment (strain X: May 1998, June 1998; strain Y: Sept. 1998) were sent on ice to Pinneys of Scotland (Annan, Scotland). At Pinneys one fillet was removed for smoking and organoleptic investigation. The second fillet was left on the skeleton and the anterior portion, including the dorsal fin, was sent to St Andrews on ice for the analysis of muscle cellularity.

2.5. Quantification of muscle growth

A range of families was sampled in each age class as described above and the data combined for the analyses of muscle growth patterns in the two populations. To avoid problems associated with shrinkage the number and diameter distributions of muscle fibres was determined using frozen sections. A 5 mm thick transverse steak was prepared at the level of the first dorsal fin ray and either photographed with a digital camera or traced on an acetate sheet. The total cross-sectional area of the white muscle was digitised using an Image Analysis System (Tema2, ScanBeam, Denmark). The steak from one half of the body was divided up into a number of labelled blocks. The number of blocks made from each cross-section of the trunk varied from 5 in the smallest fish to 12 in the largest fish. Blocks were mounted on cork strips and frozen in 2-methyl butane cooled to near its freezing point (-159°C) in liquid nitrogen. Samples were wrapped in tinfoil to avoid desiccation and stored in a liquid nitrogen refrigerator until they could be processed. Frozen sections, 8 μm thick, were cut, air dried, and stained with Mayer's haematoxylin. The outlines of 150 to 200 muscle fibres were digitised from each block using the Image Analysis System and fibre diameters were computed. Small diameter fibres less than 10 μm diameter were identified by the presence of myofibrils. The number of fibres sampled in successive blocks was plotted against the accumulative estimate of the total number (cross-sectional area of white muscle per steak, mm^2 / Σ muscle cross-section areas sampled, mm^2) multiplied by the Σ number of fibres counted until a stable estimate was obtained ($\pm 3\%$) (Johnston et al., 1999). In most cases, around 1000 muscle fibres were digitised per fish, distributed approximately equally between the different blocks (see above).

2.6. Statistics

The effects of population origin on muscle cellularity parameters was tested using ANCOVA with either body mass, fork length or muscle cross-sectional area as a co-variate (SPSS statistical software, SPSS Inc., USA). In cases where the data failed tests for non-uniform variance values were transformed to the natural logarithm prior to

ANCOVA. Body mass and length, and muscle parameters at particular sample dates were compared using a Mann–Whitney Rank Sum test, or in those cases where tests for normality and equal variance in the data were passed, by a Student's *t*-test.

Non parametric statistical techniques were used to fit smoothed probability density functions (PDFs) to the measured fibre diameters using the kernel approach. The statistical methods used are described in Silverman (1986) and Bowman and Azzalini (1997) and their application to the study of muscle fibre size distribution in Johnston et al., (1999). The construction of a smooth PDF of muscle fibre diameters has advantages over the use of histograms as used in most previous studies of muscle growth. These advantages include the preservation of the value of the realisation and the ease with which graphical and statistical comparisons can be made between groups (Johnston et al., 1999). The construction of a variability band around the mean PDF using bootstrap techniques allows inference about the existence of unimodal, bimodal or trimodal distributions (Bowman and Azzalini, 1997). In contrast, the shape of the distribution obtained using histograms is very dependent on the bin interval chosen.

The average smooth PDFs for strain X (f^x) and strain Y (f^y) were estimated using the diameters pooled over each group and the mean optimal smoothing parameter was calculated for each fish in an age class (Bowman and Azzalini, 1997). Values for the smoothing parameter varied between 0.15 and 0.28 and showed no systematic differences between populations. In order to restrict diameters to positive values, density functions were estimated for the natural logarithm of diameter and then transformed back to the original scale. A two-stage sampling procedure was used to account for the hierarchical nature of the data. First, an equal number n of fish was selected at random from the two populations at each sample period and then 500 muscle fibres were selected at random per fish. To estimate f^x and f^y , the kernel estimator was applied to the fibre diameters pooled over the group. In order to obtain consistency when comparing tail percentiles the maximal diameter within an age class was fixed at 110% of the maximum diameter sampled. Bootstrap techniques were used to distinguish underlying structure in the distributions from random variation (Bowman and Azzalini, 1997; Johnston et al., 1999). For each bootstrap sample the average density was estimated and the area between this estimate and f^x or f^y was shaded. The final shaded area, which is referred to as the variability band, represents the maximal polygon created by the 100 bootstrap estimates of density. An example of the construction of a variability band for the smoothed distribution of fibre diameter in the June 1997 sample is shown in Fig. 1A,B. The Kolmogorov–Smirnov two-sample test statistic was used to test the null hypothesis that $f^x = f^y$ over all diameters. To approximate the *P*-value of this statistic, 100 bootstrap samples were drawn from the distribution that satisfied the relevant null hypothesis. This was accomplished by re-sampling in three stages: (1) within each group, n fish were sampled with replacement, (2) ignoring original groupings, group labels were randomly assigned such that there were n fish in each group and (3) a smooth bootstrap sample of 500 fibres for each fish was generated (see Davison and Hinkley, 1997). To supplement this test, the two density curves were compared graphically by constructing a variability band around the density estimate \hat{f}^{x+y} estimated by pooling fish over the age-class and using the mean smoothing parameter. Any region where \hat{f}^x and \hat{f}^y lay outside of this “reference” band provides

evidenced for a major difference between the densities. Finally, the 5th, 10th, 50th, 95th, and 99th percentiles of muscle fibre diameter were calculated. In cases where the Kolmogorov–Smirnov test statistic was significant a Rank Sum two-sample non-para-

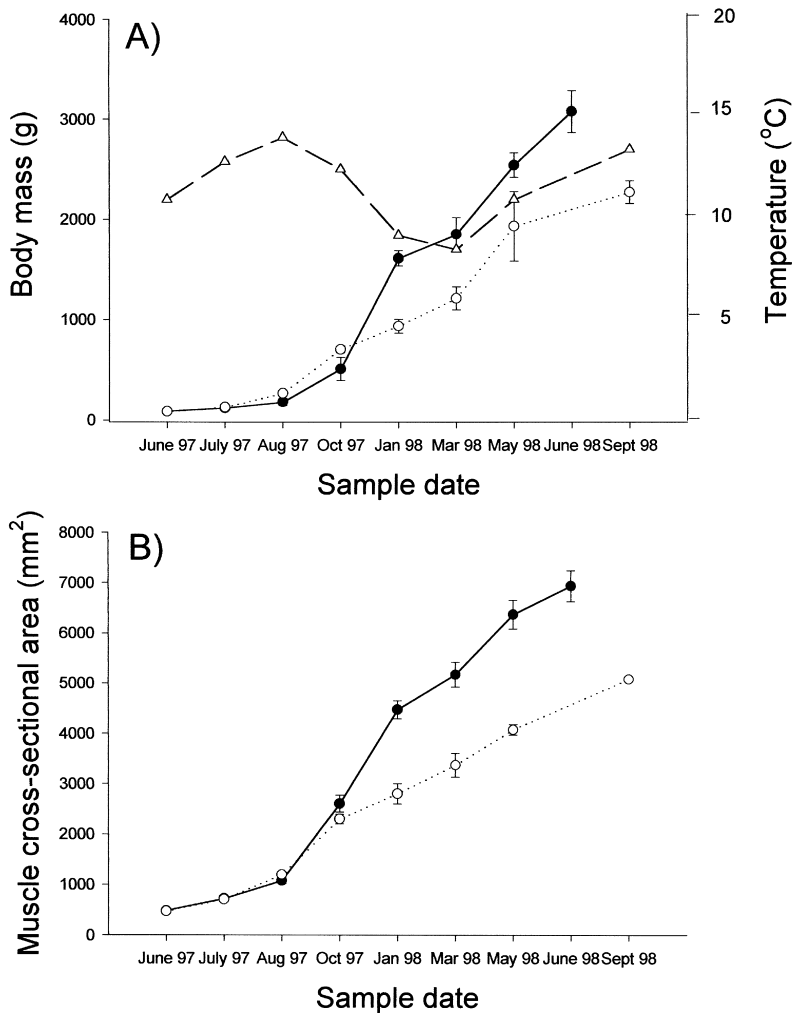


Fig. 2. (A). The body mass of Atlantic salmon (*S. salar*) from strain X (solid symbols/line) and strain Y (open symbols/dotted line) during the course of the growth trial. The triangles (dashed line) represent the average sea temperature for the month in which the samples were taken. (B) The cross-sectional area of white muscle (mm²) at the level of the first dorsal fin ray in fish from strain X (solid symbols/line) and strain Y (open symbols/dotted line) during the course of the growth trial. The fish were sampled on the 5th June, 8th July, 13th August, 21st October 1997 and the 20th January, 2nd March, 6th May, 22nd June and the 8th September 1998. Values represent mean \pm SE. The number of fish per sample date was as follows: strain X: June–October 1997, 13; January and March 1998, 15; May 1998, 20; and June 1998, 10 ($n = 112$); strain Y: June–October 1997, 16; January–May 1998, 8; and September 1998, 18 ($n = 98$).

metric test was used to test the hypothesis that the median value of the specified percentile was equivalent between strains.

3. Results

3.1. Body mass

There was no significant difference in the initial body mass of fish (~ 55 g) from the two populations. At the first sample point 6 weeks following seawater transfer the mean body mass of the fish was 89.8 ± 5.4 g for strain X ($n = 11$) and 86.6 ± 3.1 g for strain Y ($n = 16$) (mean \pm SE). The growth in body mass (Mb) of the two salmon populations during the whole course of the trial is shown in Fig. 2A. The average body mass of the August sample was significantly higher in strain Y than strain X (t -test; $P < 0.05$) (Fig. 2A). However, from this point on the growth trajectories of the two populations began to cross over with no significant differences between strains at the October sample (Fig. 2A). From January 1998 onwards, the average body mass of fish from strain X was significantly higher than for strain Y. For example, in January the average body mass of the early maturing population (strain X) sampled was 72% higher than for the late maturing population (strain Y) (t -test; $P < 0.001$). ANCOVA with the whole data set with the time from the first sample as a co-variate revealed a highly significant difference between populations ($P = 0.0021$).

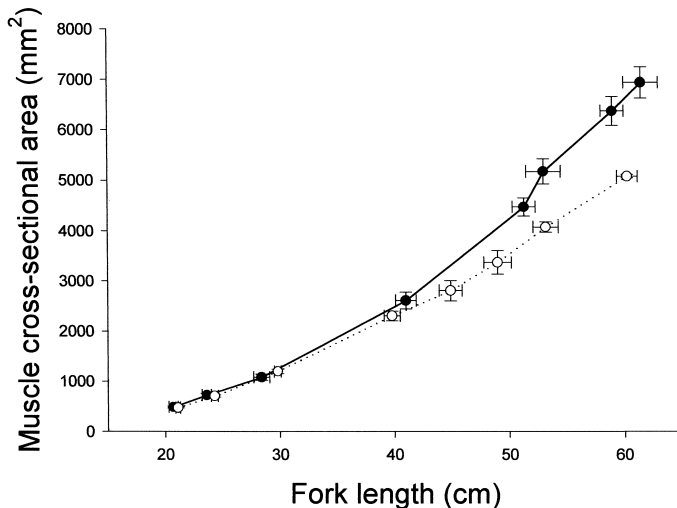


Fig. 3. The relationship between the cross-sectional area of white muscle at the level of the first dorsal fin ray and fork length for Atlantic salmon (*S. salar*) from strain X (solid circles/solid line) and strain Y (open circles/dotted line). Values represent mean \pm SE. The number of fish sampled is shown in the legend to Fig. 2.

3.2. Muscle growth

There was no significant difference in the cross-sectional area of white muscle at the level of the first dorsal fin for the June, July, August and October 1997 samples (Fig. 2B). From the January sample onwards the cross-sectional area of white muscle was a

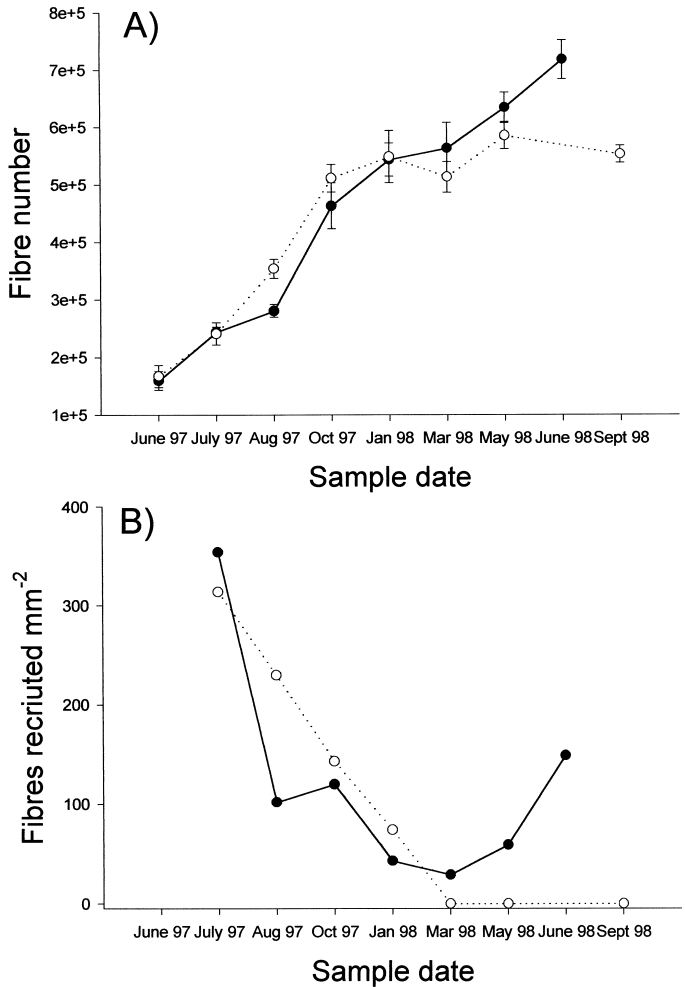


Fig. 4. (A). The number of white muscle fibres per cross-section of the trunk at the level of the first dorsal fin ray in fish from strain X (solid symbols/line) and strain Y (open symbols/dotted line) during the course of the growth trial. See the legend to Fig. 2 for other details of the numbers and dates of the samples. (B). The number of white muscle fibres recruited for each 1 mm² increment of growth in white muscle cross-sectional area since the previous sample period for salmon from strain X (closed circles) and strain Y (open circles). For example, the July 1997 point represents the increase in average white muscle cross-sectional area between June and July divided by the average difference in the number of fibres.

significantly greater in strain X than strain Y (Fig. 2B). For example, for the May 1998 sample the muscle cross-sectional area was 47.5% greater for strain X than strain Y (Mann–Whitney Rank Sum Test; $P < 0.001$). This difference in the cross-sectional area of white muscle between populations was not simply a reflection of the difference in body mass shown in Fig. 2A. For fish greater than about 40 cm fork length the muscle cross-sectional area was significantly greater in the early than late maturing population (Fig. 3), indicating differences in fish shape. ANCOVA of white muscle cross-sectional area for the whole data set with either fork length or body mass as a co-variate revealed highly significant differences between populations ($P < 0.005$). During the trial, the cross-sectional area of white muscle increased 14.3-fold in strain X and 10.7-fold in strain Y (Fig. 2B).

3.3. Fibre recruitment

The relationship between fibre number and body mass for each population during the trial is illustrated in Fig. 4A. The number of white muscle fibres per trunk cross-section was around 150,000 and 250,000 in June and July respectively, with no significant differences between populations. However, major differences in muscle fibre recruitment patterns became apparent between populations in subsequent samples (Figs. 4A and 5). In the August sample there were 27% more white muscle fibres per cross-section in strain Y ($334,000 \pm 16,200$, $n = 16$) than in strain X ($278,500 \pm 9,900$, $n = 13$) (mean \pm SE) ($P = 0.001$; Mann–Whitney Rank Sum Test) (Figs. 4A and 5). The number of additional white fibres recruited for each square millimeter increase in muscle is shown in Fig. 4B. On average between July and August 230 fibres were recruited for

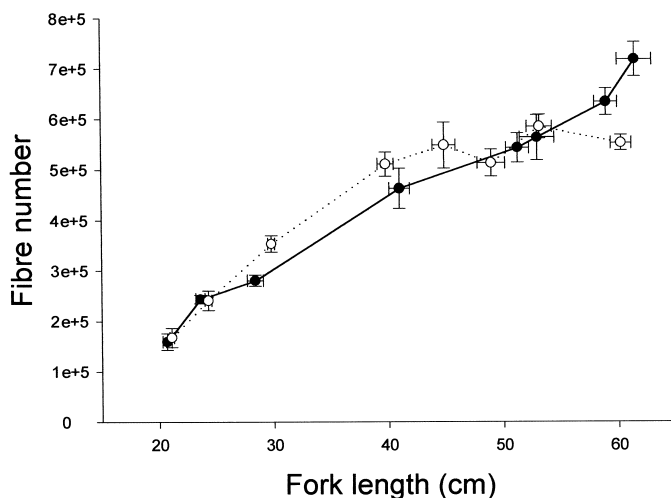
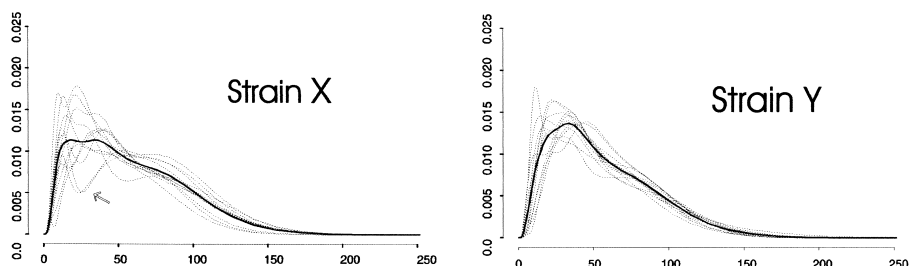
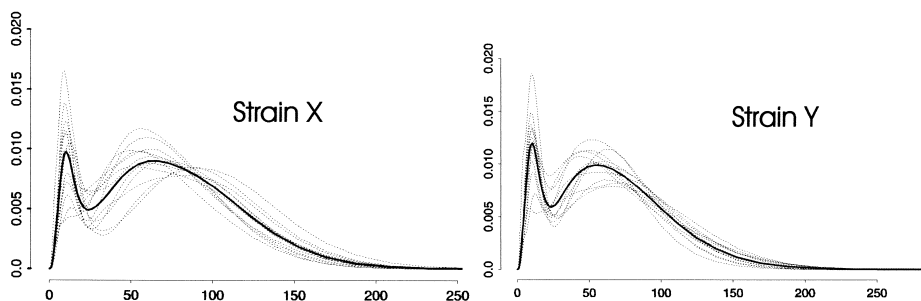


Fig. 5. The number of white muscle fibres muscle at the level of the first dorsal fin ray and its relation to fork length for Atlantic salmon (*S. salar* L.) from strain X (closed circles/solid line) and strain Y (open circles/dotted line). Values represent mean \pm SE. The number of fish sampled is shown in the legend to Fig. 2.

A) August 1997



B) October 1997



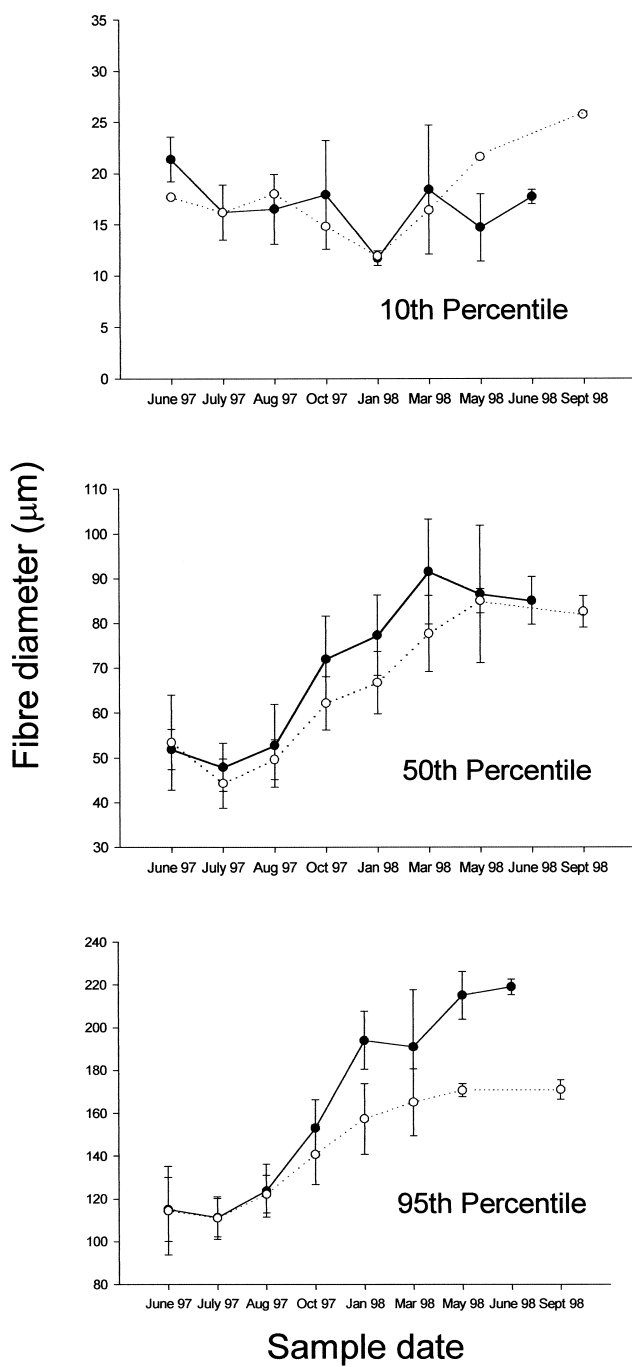
Muscle fibre diameter (μm)

Fig. 6. PDFs of muscle fibre diameter for Atlantic salmon (*S. salar* L.) sampled in (A) August and (B) October 1997. The dotted lines represent individual fish and the solid line the average density estimate for the group. The arrow in (A) illustrates two fish that showed a bimodal distribution of muscle fibre diameters.

each 1 mm^2 growth in muscle cross-sectional area in strain Y compared with 102 fibres in strain X (Fig. 4B). The average number of fibres remained 13% higher in strain Y than in strain X for the October sample, although the difference between populations was no longer significant. Thus for salmon of 30 to 45 cm average fork length there were more white muscle fibres per cross-section of the trunk in the late (strain Y) than in the early maturing (strain X) population (Fig. 5).

Strain X had caught up with strain Y in terms of fibre number by the January sample with about 545,000 fibres per trunk cross-section in each population (Fig. 4A). There was no significant increase in muscle fibre number after January 1998 in strain Y (Fig.

Fig. 7. The 5th, 50th and 95th percentiles of white muscle fibre diameter calculated from the smoothed PDFs for strain X (solid circles) and strain Y (open circles) plotted against sample data. The values represent mean \pm SE. A total of 10 fish from each population were analysed in each of the June, July, August and October 1997 samples, 8 in January 1998, 8 in March and May 1998, 10 of strain X in June and 18 of strain Y in September.



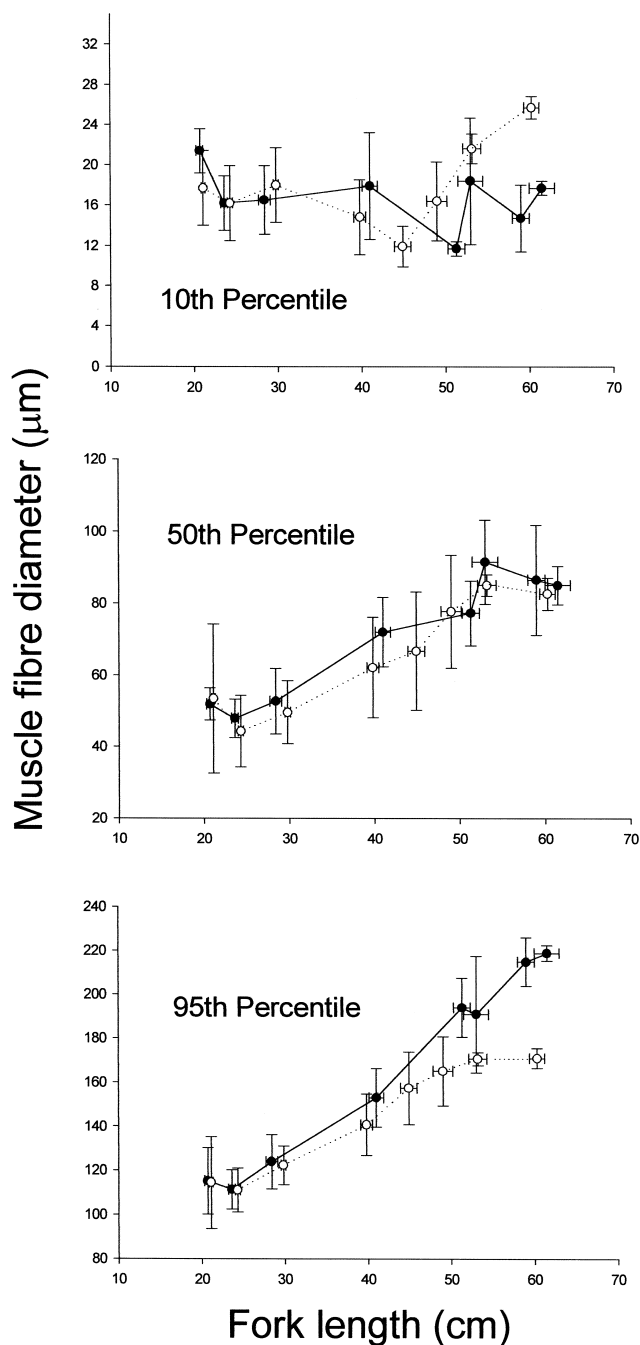
4A) such that growth in the cross-sectional area of white muscle (Fig. 2B) was entirely by fibre hypertrophy (Fig. 4B). Thus the number of white muscle fibres per trunk cross-section in strain Y was relatively constant for fish of 40 to 60 cm fork length (Fig. 5). In contrast, fibre number continued to increase between January and September 1998 in fish from strain X (Fig. 4A). By the June sample fibre number had increased to 718, $100 \pm 33,800$ in fish of 61.5 ± 1.5 cm fork length (mean \pm SE, 10 fish) (Fig. 5). The number of fibres recruited for each 1 mm^2 increase in white muscle cross-sectional area was in the range 30–60 during the late autumn, winter and early spring periods but increased to 150 between May and June (Fig. 4B).

The period between October 1997 and June 1998 when fish from strain Y had largely stopped recruiting new fibres corresponded to the point at which body mass (Fig. 1A) and white muscle cross-sectional area (Fig. 1B) started to increase at a much slower rate than in strain X. Thus part of the variation in growth rate between the two populations after the first 5 months of the trial (April–October) was related to very different patterns of muscle fibre recruitment. For the entire data set here was a significant main effects of population on the number of muscle fibres per myotome with both body mass ($P < 0.01$) and muscle cross-sectional area as a co-variate ($P < 0.001$).

3.4. Muscle fibre hypertrophy

Unimodal distributions of muscle fibre diameter were observed in June, July and August 1997, using the evidence of the variability bands. Fig. 1 illustrates (A) the individual smoothed distributions and (B) the variability band constructed for the June sample from strain X. Two of the individual fish from the early maturing population had a bimodal distribution of fibre diameters in August (arrow in Fig. 6A). However, by the October 1997 sample there was strong evidence of a bimodal distribution of fibre diameters both for individual fish (Fig. 6B) and with respect to the variability bands constructed for each group (not illustrated). The reference bands and Kolmogorov–Smirnov tests indicated no statistically significant differences in the fibre distributions in the first three samples (not illustrated). Fig. 7 shows the 10th, 50th and 95th percentile of fibre diameter calculated from the smoothed distributions. Between June and August the 5th, 50th, and 95th percentile of fibre diameters were in the region of 15, 50 and 120 μm , respectively, and were similar for the two populations (Fig. 7). In October, the left-peak in muscle fibre diameter was around 15 μm in both strains with right-peaks in the distribution at 70 and 60 μm in strains X and Y, respectively (Fig. 6B). The 50th and 95th percentiles of fibre diameter were 72 and 153 μm , respectively for strain X and 62 and 141 μm , respectively for strain Y (Fig. 7) ($P < 0.01$; Mann–Whitney Rank Sum Test). There were no significant differences in the 10th, 50th and 95th percentile of fibre diameter with respect to fork length in the June to October 1997 samples (Fig. 8).

Fig. 8. The 5th, 50th and 95th percentiles of white muscle fibre diameter calculated from the smoothed PDFs for strain X (solid circles) and strain Y (open circles) plotted against fork length. The values represent mean \pm SE. A total of 10 fish from each population were analysed in the June, July, August and October 1997 samples, 8 in January 1998, 8 in March and May 1998, 10 of strain X in June and 18 of strain Y in September.



The left-peak of fibre density was around 20 μm for both strains in January with right-peaks at 100 and 80 μm for strain X and Y, respectively (Fig. 9A). Evidence from

the reference band and Kolmogorov–Smirnov test indicated a significant difference in overall distribution of fibre diameters between strains in January ($P < 0.05$) (Fig. 9A). In January, the 50th and 95th percentiles of fibre diameter were both significantly higher in strain X (77 and 191 μm , respectively) compared to strain Y (66 and 154 μm , respectively) ($P < 0.001$) (Mann–Whitney Rank Sum Tests) (Fig. 7), although this was

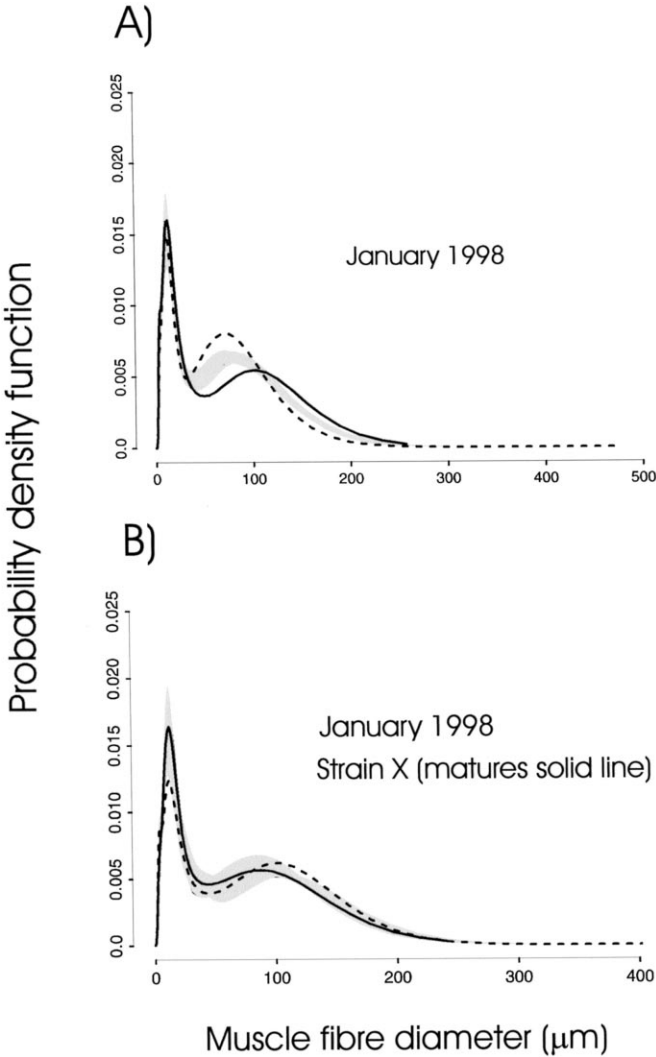


Fig. 9. (A) Average PDFs of fish from strain X (solid line) and strain Y (dotted line) sampled in January 1998. The shaded area encloses 100 bootstrap estimates of the total population, strain X + strain Y. (B) Average PDFs of immature (dotted line) and maturing (solid line) fish from strain X sampled in January 1998. Areas where the solid and dotted lines lie outside the shaded area provide evidence for differences in the density distributions between populations.

simply a function of differences in fork length (Fig. 8). The maximum muscle fibre diameter in both strains was about 200 μm .

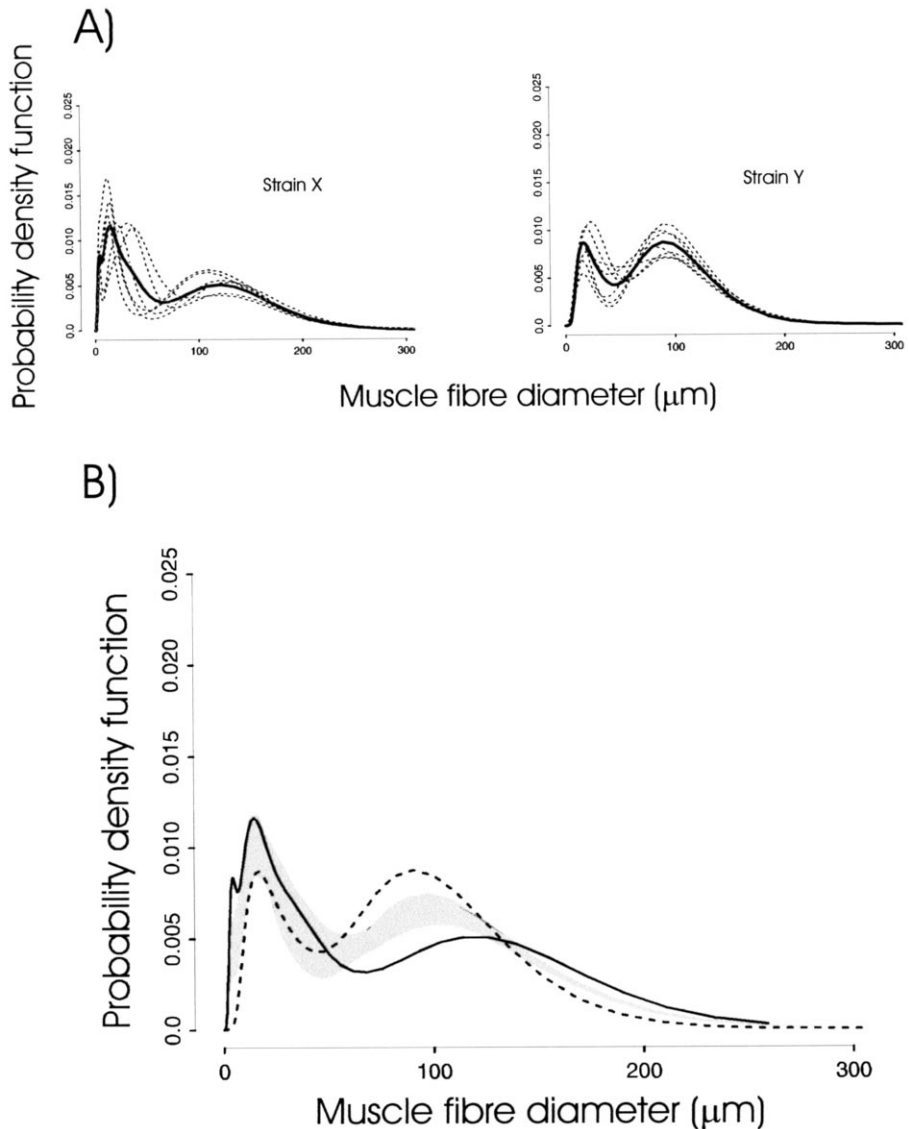


Fig. 10. Distribution of muscle fibre diameter in Atlantic salmon from two populations sampled in May 1998. (A). The PDF of muscle fibre diameter of individual fish (dotted lines) and the average for each population (solid lines). (B). The average PDF for strain X (solid line) and strain Y (dotted line). The shaded area encloses 100 bootstrap estimates of the total population, strain X + strain Y. Areas where the solid and dotted lines lie outside the shaded area provide evidence for differences in the density distributions between populations.

Fig. 9B shows the average PDF of immature and maturing fish from strain X. There was a higher peak density at 20 μm in the maturing fish although this fell within the reference band for the combined populations, indicating it was not significant (Fig. 9B). In contrast, there was a right-shift in the secondary peak of density to higher fibre diameters in the immature fish which fell outside the reference band consistent with a better hypertrophic growth performance ($P < 0.05$; Fig. 9B). Thus greater hypertrophic growth was observed for white muscle fibres in immature than maturing fish within strain X. In contrast, the right peak in the distribution (Fig. 9A) and the 95th percentile of fibre diameter (Fig. 7) were at higher values in strain X than in strain Y. This suggests that the greater hypertrophic growth performance of fibres in strain X was not

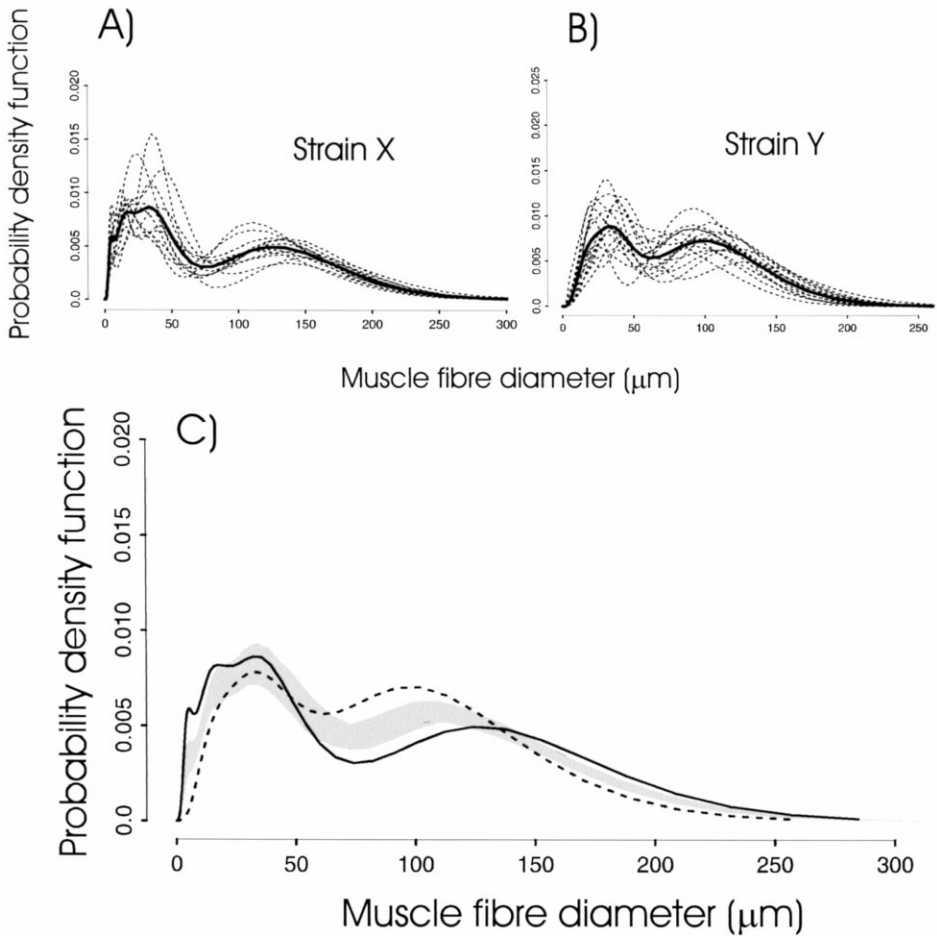


Fig. 11. Distribution of muscle fibre diameter in Atlantic salmon from two populations sampled in (A) June 1998 (strain X) and (B) September (strain Y) at the end of the growth trial. (C) The average PDF for strain X (solid line) and strain Y (dotted line). The shaded area encloses 100 bootstrap estimates of the total population, strain X+strain Y. Areas where the solid and dotted lines lie outside the shaded area provide evidence for differences in the density distributions between populations. Ten fish were analysed from each population.

directly related to sexual maturation but rather to some other genetic difference between the populations.

In all subsequent samples, the right-hand peak of maturing fibres was shifted to higher diameters in strain X than in strain Y, and was 100 and 75 μm , respectively, in March (not illustrated) and 123 and 90 μm , respectively, in May 1998 (Fig. 10A). The smallest diameter size classes were absent from fish of the late maturing population in the May sample (Fig. 10B), reflecting the virtual cessation of new fibre recruitment in strain Y (Fig. 4A). Consequently, the 5th percentile of fibre diameter was also at a significantly higher value in strain Y than in strain X (Fig. 7) ($P < 0.01$; Mann–Whitney Rank Sum Test). Fish from strain Y only reached a similar fork length (~ 59 cm) to the May sample of strain X in September. At this fork length the 95th percentile of fibre diameter was greater in strain X than strain Y, whereas the 10th percentile was greater in strain Y than strain X (Fig. 8), reflecting the cessation of fibre recruitment in the late maturing population. It should be noted that because of heavy sea lice infestation and the need for frequent medication the growth performance of fish in this trial was relatively poor. A final sample of the remaining fish was taken in June for strain X (100% maturing) and in September from strain Y (100% immature) (Fig. 11A,B). The condition factor of the strain X fish (1.23 ± 0.02) was significantly higher than for strain Y fish (1.03 ± 0.02) at the time of harvest (mean \pm SE, $n = 15$ per group) (ANCOVA with fork length as co-variate; $P < 0.001$). The reference band (Fig. 11B) and Kolmogorov–Smirnov test indicated a significant difference in overall distribution of fibre diameters in the final samples ($P < 0.05$). Individual variation in the distribution of fibre diameters was much greater for strain Y than strain X (Fig. 11A). The variability bands constructed around the average density estimates from each population indicated that the

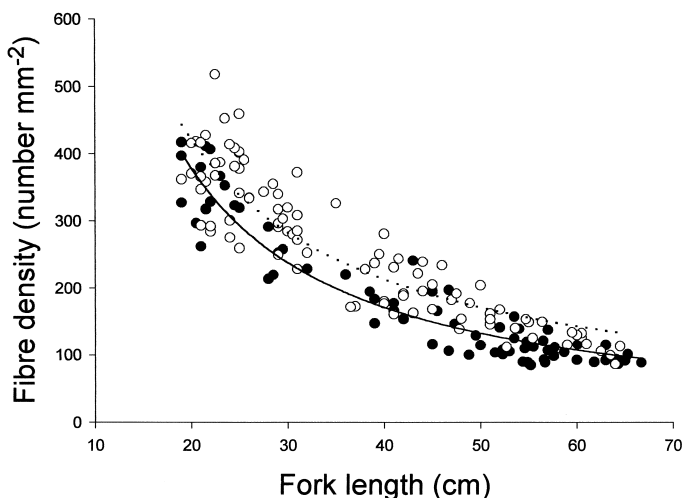


Fig. 12. The relationship between fork length (cm) and the density of white muscle fibres at the level of the first dorsal fin ray in Atlantic salmon (*S. salar* L.) from two populations, strain X (solid circles) and strain Y (open circles). The fitted lines represent a power function of the form $Y = aX^b$. The equations for the lines were as follows: strain X: $a = 11,600 \pm 1800$, $b = -1.14 \pm 0.04$, $r^2 \text{ adj} = 0.89$, $P < 0.001$; strain Y: $a = 8000 \pm 1500$, $b = -0.98 \pm 0.060$, $r^2 \text{ adj} = 0.80$, $P < 0.001$.

fibre diameter distributions were bimodal (not illustrated), although several individual fish from strain X had a trimodal distribution (Fig. 11A). The maximum of the right-hand peak of the average density was at 130- μm diameter in strain X and 100- μm diameter in strain Y (Fig. 11B). The 95th percentile of fibre diameter was at 220 μm in strain X and 171 μm in strain Y ($P < 0.001$; Mann–Whitney Rank Sum Test) (Fig. 7). Thus at the end of the trial there was evidence for a superior hypertrophic growth performance of white fibres in strain X compared to strain Y. The maximum diameter of white fibres was around 240 μm . The density of muscle fibres is plotted against fork length in Fig. 12. Muscle fibre density was significantly higher in strain Y than in strain X (ANCOVA with fork length as co-variate; $P < 0.01$). For fish less than 40 cm this was largely due to the initially greater rate of fibre recruitment in strain Y than strain X (Fig. 5). Following the January sample, in fish greater than 40 cm fork length, the higher numbers of white muscle fibres per myotomal cross-section in strain X than strain Y (Figs. 4A and 5) was offset by the greater rate of fibre hypertrophy (Fig. 8), resulting in a lower fibre density (Fig. 12).

4. Discussion

Muscle growth in fish is a complex process involving the recruitment and subsequent hypertrophy of muscle fibres. The process has been described over the complete life cycle for just a few fish species including Atlantic cod (*Gadus morhua*) (Greer-Walker, 1970), rainbow trout (*O. mykiss*) (Stickland, 1983; Kiessling et al., 1991) and gilthead sea bream (*Sparus auratus*) (Rowlerson et al., 1995). The present study describes patterns of muscle growth in Atlantic salmon (*S. salar* L.) over a complete production cycle in seawater, and extends previous work on tank-reared fish involving the freshwater (Higgins and Thorpe, 1990), and early seawater stages (Johnston et al., 1999). Muscle growth in Atlantic salmon is of particular interest due to its commercial importance and because of its flexible life history strategy. Genetic and environmental factors combine to influence life history factors such as the time spent in freshwater and age and size at sexual maturity, all of which effect growth rate (Thorpe, 1989), and have the potential to modify muscle growth patterns. For example, different patterns of muscle growth have already been described for parr destined to smolt after one or two years in freshwater (Higgins and Thorpe, 1990). Sibling populations of juvenile Atlantic salmon exhibit a bimodal length frequency distribution in late summer. For fish in the upper growth mode, the number of white muscle fibres increased more than 6-fold from the fry to the smolt stage. In contrast, the lower growth mode fish recruited relatively few muscle fibres over the same period, but in their second year resumed a similar pattern of muscle growth to that observed in the upper mode fish the previous year. The decision to smolt in a particular year is known to vary between families, but is strongly influenced by prior feeding opportunity and can be modified by temperature and day-length (Metcalf et al., 1988; Thorpe, 1989).

A major finding of the present study was that Atlantic salmon populations with different growth rates and ages at sexual maturity also showed markedly different patterns of muscle growth. In this case, fish from the two populations were reared together in the same cage and the different growth patterns are probably genetic in

origin. Differences in the average cross-sectional area of white muscle between the populations only became apparent 6 months after seawater transfer (Fig. 2B). Fish from the late maturing population recruited more than twice as many fibres for each square millimeter increase in muscle cross-sectional area between August and July 1997 as the early maturing population (Fig. 4B). As a result, in August the number of white muscle fibres per trunk cross-section was 26% higher in strain Y than strain X (Fig. 4A). In both populations the recruitment of new fibres declined in the period from August to January, as reported for the freshwater stages (Higgins and Thorpe, 1990) (Fig. 4B). For strain X the number of fibres recruited between March and January 1998 ($\sim 30 \text{ mm}^{-2}$ muscle) was only about 10% of that observed between June and July shortly after transfer to seawater. The number of fibres recruited in the high grilising population increased again in the spring and early summer, reaching around five times the winter level ($\sim 150 \text{ mm}^{-2}$ muscle) (Fig. 4B). In marked contrast, there was little fibre recruitment after January in fish from the late maturing population with fibre number remaining relatively constant between 42 and 63 cm fork length (Fig. 5). Thus the increase in muscle girth that was observed between January and the final harvest sample was the result of a combination of fibre recruitment and hypertrophy in the early maturing population but entirely due to fibre hypertrophy in the late maturing population. Since a significant difference in the cross-sectional area of the white muscle between populations was only evident from the January sample onwards our results point to an association between fast muscle growth and fibre recruitment. In contrast, in rainbow trout populations selected for differences in growth rate, no difference was found in the number of white muscle fibres per trunk cross-section at any given body size (Valente et al., 1999).

A similar correlation between fibre recruitment and fast growth rate has been noted previously for the larvae of carp (*Cyprinus carpio*) (Alami-Durante et al., 1997) and Atlantic cod (*G. morhua*) fed different diets (Galloway et al., 1999). In rainbow trout fed ad libitum for 16 weeks growth rate was found to be higher at 12°C than 16°C (Weatherley et al., 1979). The modal muscle fibre diameter was lower in the 12°C than 16°C group, even for fish of similar body length, consistent with a greater contribution of fibre recruitment in the fastest growing fish (Weatherley et al., 1979). Similar relationships between growth characteristics and muscle fibre recruitment have been observed between different species. In a study of 10 teleost fish, the largest, fastest growing species (e.g., smallmouth bass, *Micropterus dolomieu*) were found to sustain recruitment of fibres for longer in the life cycle than smaller, slower growing species (e.g., longnose dace, *Rhinichthys cataractae*) (Weatherley et al., 1988). Meyer-Rochow and Ingram (1993) found that the dwarf lacustrine form of the Southern smelt (*Retropinna retropinna* Richardson) stopped recruiting new white muscle fibres at a significantly shorter fork length than the larger riverine form.

The maximum diameter for white muscle fibres in temperate fish is around 240 μm , perhaps reflecting some constraint on the diffusion of metabolites (Weatherley et al., 1988; Johnston et al., 1999). Small diameter fibres can be expected to grow more quickly than large diameter fibres because of their higher surface to volume ratio and greater capacity to assimilate nutrients (Weatherley et al., 1988). In support of this idea, the white muscle of a fast growing strain of rainbow trout was found to have smaller

diameter fibres than a slow growing strain (Valente et al., 1998). Thus maintaining a high proportion of smaller size classes of muscle fibre through new fibre recruitment aids rapid growth and overcomes the constraint on body size associated with the maximum diameter of the fibres. Although fibre recruitment is critical for growth it should be remembered that most of the increase in muscle girth derives from fibre hypertrophy. The average diameter of a newly formed myotube in the white muscle of Atlantic salmon is only around 5 μm (Johnston et al., 2000b). Thus in strain X, new fibres accounted for only 0.65% and 0.35% of the cross-sectional area of the white muscle in the June 1997 (3.1 mm^2) and June 1998 (41 mm^2) samples, respectively.

Muscle fibre recruitment is presumably dependent on the number of activated satellite cells and their proliferative capacity (Koumans et al., 1991; Koumans and Akster, 1995). Selection for a high body mass in mice (Brown and Stickland, 1993; 1994) and quail (Fowler et al., 1980; Campion et al., 1982) was associated with an increase in the number of satellite cells and muscle fibres present at birth relative to control lines. Fibre number is known to have a strong genetic component, showing moderate to high heritability in rats (Suwa et al., 1996), pigs (Larzul et al., 1997) and cattle (Renand et al., 1995). Triploid Atlantic salmon were also found to have 24% more satellite cells per trunk cross-section than diploid fish and to recruit about one third fewer fibres to reach a given muscle cross-sectional area (Johnston et al., 1999). Whilst differences in satellite cell density might well be responsible for variation in muscle fibre number and recruitment potential between families it is not a likely explanation for the observed population differences in the present study. The most plausible hypothesis to explain variations in the timing of muscle fibre recruitment is a difference in the proliferative capacity of the satellite cells. Activated muscle stem cells are thought to undergo an asymmetric division to regenerate the stem cell and produce a daughter cell that continues to divide several more times before differentiating (Schultz, 1996). Satellite cells that are committed to differentiation express myogenic regulatory factors belonging to the MyoD gene family (Cornelison and Wold, 1997; Sabourin et al., 1999; Johnston et al., 1999). Their behaviour is determined by a complex network of antagonistic proliferation and differentiation signalling pathways that are only just beginning to be investigated in fish (see Johnston, 1999 for a review). Fauconneau et al. (1997) found that growth hormone supplementation in rainbow trout increased the percentage of small diameter fibres suggesting this hormone may have a role in controlling fibre recruitment. Factors that have been implicated in satellite cell activation in mammals include, fibroblast growth factor-2 (FGF2) (Yablonka-Reuveni et al., 1999), FGF6 (Floss et al., 1997), Insulin-like growth factor-1 (IGF-1) (Florini et al., 1991) and hepatocyte growth factor/scatter factor (HGF/SF) (Tatsumi et al., 1998). Interestingly, satellite cells isolated from heavy-weight strains of turkey showed a higher proliferative capacity *in vitro* than cells from light-weight strains (Merly et al., 1998). This also provides evidence that the proliferative capacity of satellite cells is under genetic as well as environmental control.

Another major finding in the present study was for population differences in muscle fibre hypertrophy. From the October 1997 sample onwards the smoothed distribution of muscle fibre diameter was bimodal (Figs. 6, 9–11). In all cases the right-hand peak of maturing fibres was shifted to higher diameters in the early maturing than the late

maturing population (Figs. 6, 9–11). This was not simply a function of differences in body size since for fish of equivalent fork length the 95th percentile of muscle fibre diameter was at higher values in the early than late maturing strain towards the end of the trial (Fig. 8). Evidence for genetic differences in muscle fibre size has been obtained in farm animals. For example, studies in pigs have found heritable variation in the maximum diameter of different muscle fibre types for a given body mass between strains selected for differences daily weight gain and lean and fat content of the carcass (Ruusunen et al., 1996; Larzul et al., 1997). In contrast, the frequency distributions of muscle fibre diameter in populations of rainbow trout selected for fast and slow growth rate were found to be rather similar, particularly for the larger size classes (see Fig. 6 in Valente et al., 1999). The population differences in muscle fibre diameter found in the present study were not directly related to sexual maturation since within strain X greater fibre hypertrophy was observed for immature than mature individuals (Fig. 9B).

Insulin-like growth factors have been broadly implicated in regulating hypertrophic growth (Florini et al., 1991). Induced expression of the non-circulating IGF-1 isoform in a post-mitotic rat myocyte line was shown to result in hypertrophy (Musrò and Rosenthal, 1999). There is evidence that IGF-1 mediated muscle fibre hypertrophy involves the activation of the calcineurin signal transduction pathway (Dunn et al., 1999; Musrò et al., 1999; Semsarian et al., 1999). Calcineurin is a calcium-activated serum phosphatase comprising catalytic (CnA) and regulatory subunits (CnB) and the calcium sensor molecule, calmodulin. Transfected IGF-1 gene expression in a post-mitotic cell line resulted in increased expression of CnA but not CnB transcripts (Musrò et al., 1999). Cell cultures stably transfected with a calcium-independent form of CnA driven by a myogenin promoter had a pronounced hypertrophic phenotype. IGF-1 is thought to act in skeletal muscle by mobilising intracellular calcium stores, which activate calcineurin, and induce the nuclear translocation of the transcription factor NF-ATc1 (Musrò et al., 1999; Semsarian et al., 1999). Three isoforms of NF-AT (nuclear factor of activated T cells) are present in mammalian muscle, and one of them, NF-ATc1 can preferentially translocate to a sub-set of nuclei within a single multi-nucleated myotube (Abbot et al., 1998). IGF-1 and/or activated calcineurin have also been implicated in the expression of GATA-2, which also accumulates in a subset of myocyte nuclei (Musrò et al., 1999). Although the details have not been established CnA and GATA-2 expression may be involved in a signalling cascade associated with the up-regulation of genes involved in muscle fibre hypertrophy (Musrò et al., 1999; Semsarian et al., 1999). In adult mammals, dependence on calcineurin signalling is associated with relative increases in muscle activation characteristics (Dunn et al., 1999). Exercise is known to be a powerful stimulus for muscle fibre hypertrophy in fish (Greer-Walker and Pull, 1973; Johnston and Moon, 1980). Totland et al. (1987) found that the average diameter of white muscle fibres in Atlantic salmon forced to swim in a flume was greater than controls. The differences in fibre hypertrophy observed between populations could therefore be related to behavioural differences in foraging activity and/or reflect genetic differences in the regulatory machinery.

Muscle structure has a major impact on the textural characteristics of fish products (Hurling et al., 1996; Johnston et al., 2000a). Since patterns of muscle fibre recruitment and hypertrophy vary between salmon populations this opens up the possibility of using

selective breeding programs to manipulate muscle structure and hence the textural characteristics of the fillet.

Acknowledgements

This work was supported by an Aquaculture Link Programme from the Natural Environment Research Council. We are grateful to Dr. Marti L. McCracken of the Institute of Mathematical Sciences for statistical advice and for programming.

References

- Abbot, K., Friday, B.B., Thaloor, D., Murphy, T.J., Pavlath, G.K., 1998. Activation and cellular localization of the cyclosporine A-sensitive transcription factor NF-AT in skeletal muscle cells. *Mol. Biol. Cell* 9, 2905–2916.
- Alami-Durante, H., Fauconneau, B., Rouel, M., Escaffre, A.M., Bergot, P., 1997. Growth and multiplication of white skeletal muscle fibres in relation to somatic growth rate. *J. Fish Biol.* 50, 1285–1302.
- Bone, Q., 1978. Locomotor muscle. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology* vol. 7 Academic Press, New York, pp. 361–424.
- Bowman, A.W., Azzalini, A., 1997. Applied smoothing techniques for data analysis. In: *The Kernel Approach with S-Plus Illustrations*. Oxford Science Publications, Oxford Univ. Press, Oxford, p. 193.
- Brown, S.C., Stickland, N.C., 1993. Satellite cell content in muscles of large and small mice. *J. Anat.* 183, 91–96.
- Brown, S.C., Stickland, N.C., 1994. Muscle at birth in mice selected for large and small body size. *J. Anat.* 184, 371–380.
- Campion, D.R., Marks, H.L., Richardson, L.R., 1982. An analysis of satellite cell content in the *semimembranosus* muscle of Japanese quail (*Coturnix coturnix japonica*) selected for rapid growth. *Acta Anat* 112, 9–13.
- Carpenè, E., Veggetti, A., Mascarello, F., 1982. Histochemical fibre types in the lateral muscle of fishes in fresh, brackish and salt water. *J. Fish Biol.* 20, 346–379.
- Cornelison, D.D.W., Wold, B.J., 1997. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Dev. Biol.* 191, 270–283.
- Davison, A.C., Hinkley, D.V., 1997. In: *Bootstrap Methods and their Applications*. Cambridge Univ. Press, Cambridge, p. 257.
- Driedzic, W.R., Hochachka, P.W., 1976. Control of energy metabolism in fish white muscle. *Am. J. Physiol.* 230, 579–582.
- Dunn, S.E., Burns, J.L., Michel, R.N., 1999. Calcineurin is required for skeletal muscle hypertrophy. *J. Biol. Chem.* 274, 21908–21912.
- Fauconneau, B., Andre, S., Chmaitilly, LeBail, P.-Y., Krieg, F., Kaushik, S.J., 1997. Control of skeletal muscle fibres and adipose cells in the flesh of rainbow trout. *J. Fish Biol.* 50, 296–314.
- Florini, J.R., Ewton, D.Z., Magri, K.A., 1991. Hormones, growth factors, and myogenic differentiation. *Annu. Rev. Physiol.* 53, 201–216.
- Floss, T., Arnold, H.-H., Braun, T., 1997. A role for FGF-6 in skeletal muscle regeneration. *Genes Dev.* 11, 2040–2051.
- Fowler, S.P., Campion, D.R., Marks, H.L., Reagan, J.O., 1980. An analysis of skeletal muscle response to selection for rapid growth in Japanese quail (*Coturnix coturnix Japonica*). *Growth* 44, 235–252.
- Galloway, T.F., Kjørsvik, E., Kryvi, H., 1999. Muscle growth and development in Atlantic cod larvae (*Gadus morhua* L.) related to different somatic growth rates. *J. Exp. Biol.* 202, 2111–2120.
- Greer-Walker, M., 1970. Growth and development of the skeletal muscle fibres of the cod (*Gadus morhua* L.). *J. Cons. Int. Expl. Mer.* 33, 421–426.

- Greer-Walker, M., Pull, M.G., 1973. Skeletal muscle function and sustained swimming speeds in the coalfish (*Gadus virens* L.). Comp. Biochem. Physiol. 44A, 495–501.
- Hansen, T., Steffansson, S., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquacult. Fish. Manage. 22, 275–280.
- Higgins, P.J., Thorpe, J.E., 1990. Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, *Salmo salar* L. J. Fish Biol. 37, 505–519.
- Hudson, R.C.L., 1969. Polyneuronal innervation of the fast muscles of the marine teleost *Cottus scorpius* L. J. Exp. Biol. 50, 47–67.
- Hurling, R., Rodell, J.B., Hunt, H.D., 1996. Fibre diameter and fish texture. J. Texture Stud. 27, 679–685.
- Johnston, I.A., 1980. Contractile properties of fish fast muscle fibres. Mar. Biol. Lett. 1, 323–328.
- Johnston, I.A., 1999. Muscle development and growth: potential implications for flesh quality in fish. Aquaculture 177, 99–115.
- Johnston, I.A., Patterson, S., Ward, P.S., Goldspink, G., 1974. The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. Can J. Zool. 52, 871–877.
- Johnston, I.A., Ward, P.S., Goldspink, G., 1975. Studies on the swimming musculature of the rainbow trout: I. Fibre types. J. Fish Biol. 7, 451–458.
- Johnston, I.A., McLay, H.A., 1997. Temperature and family effects on muscle cellularity at hatch and first feeding in Atlantic salmon (*Salmo salar* L.). Can. J. Zool. 75, 64–74.
- Johnston, I.A., Moon, T.W., 1980. Exercise training in skeletal muscle of the brook trout (*Salvelinus fontinalis*). J. Exp. Biol. 87, 177–194.
- Johnston, I.A., Davison, W., Goldspink, G., 1977. Energy metabolism of carp swimming muscles. J. Comp. Physiol. 114, 203–216.
- Johnston, I.A., Strugnell, G., McCracken, M.L., Johnstone, R., 1999. Muscle growth in normal sex ratio and all-female diploid and triploid Atlantic salmon. J. Exp. Biol. 202, 1991–2016.
- Johnston, I.A., Alderson, D., Sandham, C., Dingwall, A., Mitchell, D., Selkirk, C., Nickell, D., Baker, R., Robertson, B., Whyte, D., Springate, J., 2000a. Muscle cellularity in relation to flesh quality in fresh and smoked Atlantic salmon (*Salmo salar* L.). Aquaculture.
- Johnston, I.A., McLay, H.A., Abercromby, M., Robbins, D., 2000b. Phenotypic plasticity of early myogenesis and satellite cell numbers in Atlantic salmon spawning in an upland and a lowland tributary of a river system. J. Exp. Biol., in press.
- Kiessling, A., Storebakken, T., Åsgård, T., Kiessling, K.-H., 1991. Changes in the structure and function of the epaxial muscle of rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age: I. Growth dynamics. Aquaculture 93, 335–355.
- Koumans, J.T.M., Akster, H.A., 1995. Myogenic cells in development and growth of fish. Comp. Biochem. Physiol. 110A, 3–20.
- Koumans, J.T.M., Akster, H.A., Brooms, G.H.R., Lemmens, C.J.J., Osse, J.W.M., 1991. Numbers of myosatellite cells in white axial muscle of growing fish: *Cyprinus carpio* L. (Teleostei). Am. J. Anat. 192, 418–424.
- Larzul, C., Lefaucheur, L., Ecolan, P., Gogu  , J., Talmant, A., Sellier, P., Le Roy, P., Monin, G., 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in Large White pigs. J. Anim. Sci. 75, 3126–3127.
- Merly, F., Magras-Resch, C., Rouaud, T., Fontaine-Perus, J., Gardahaut, M.F., 1998. Comparative analysis of satellite cell properties in heavy- and lightweight strains of turkey. J. Muscle Res. Motil. 19, 257–270.
- Metcalfe, N.B., Huntingford, F.A., Thorpe, J.E., 1988. Feeding intensity, growth rates and the establishment of life history patterns in juvenile Atlantic salmon. J. Anim. Ecol. 57, 463–474.
- Meyer-Rochow, V.B., Ingram, J.R., 1993. Red–white muscle distribution and fibre growth dynamics: a comparison between lacustrine and riverine populations of the Southern smelt *Retropinna retropinna* Richardson. Proc. R. Soc. London, Ser. B 252, 85–92.
- Musar  , A., Rosenthal, N., 1999. Maturation of the myogenic program is induced by post-mitotic expression of IGF-1. Mol. Cell. Biol. 19, 3115–3124.
- Musar  , A., McCullagh, K.J.A., Naya, F.J., Olson, N., Rosenthal, N., 1999. IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-Atc1. Nature 400, 581–585.
- Nicieza, A.G., Reyes-Gavi  n, F.G., Bra  a, F., 1994. Differentiation in juvenile growth and bimodality patterns

- between northern and southern populations of Atlantic salmon (*Salmo salar* L.). Can. J. Zool. 72, 1603–1610.
- Ramírez-Zarzosa, G., Gil, F., Vázquez, A., Arencibia, A., Latorre, R., López-Albors, O., Ortega, A., Moreno, F., 1998. The post-larval development of lateral musculature in gilthead sea bream *Sparus aurata* (L.) and sea bass *Dicentrarchus labrax* (L.). Anat. Histol. Embryol. 27, 21–29.
- Renand, G., Jurie, C., Robelin, J., Picard, B., Geay, Y., Ménissier, 1995. Genetic variability of muscle biological characteristics of young Limousin bulls. Genet. Sel. Evol. 27, 287–296.
- Riddell, B.E., Leggett, W.C., 1981. Evidence of an adaptive basis for geographic variation of body morphology, and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 38, 308–320.
- Rowe, R.W.E., Goldspink, G., 1969. Muscle growth in five different muscles in both sexes of mice: I. Normal mice. J. Anat. 132, 557–579.
- Rowlerson, A., Mascarello, F., Radaelli, G., Veggetti, A., 1995. Differentiation and growth of muscle in the fish *Sparus aurata* (L.): II. Hyperplastic and hypertrophic growth of lateral muscle from hatching to adult. J. Muscle Res. Cell Motil. 16, 223–236.
- Ruusunen, M., Sevon-Aiomen, M.L., Puolanne, E., 1996. Composition and cross-sectional area of muscle fibre types in relation to daily gain and lean and fat content of carcass in Landrace and Yorkshire pigs. Agric. Food Sci. Finl. 5, 593–600.
- Sabourin, L.A., Girgis-Gabardo, A., Seale, P., Asakura, A., Rudnicki, M.A., 1999. Reduced differentiation potential of primary MyoD^{-/-} myogenic cells derived from adult skeletal muscle. J. Cell Biol. 144, 631–643.
- Schaffer, W.M., Elson, P.F., 1975. The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. Ecology 56, 577–590.
- Schultz, E., 1996. Satellite cell proliferative compartments in growing skeletal muscles. Dev. Biol. 175, 84–94.
- Semsarian, C., Ming-Jie, W., Yue-Kun, J., Marciniak, T., Yeoh, T., Allen, D.G., Harvey, R.P., Graham, R.M., 1999. Skeletal muscle hypertrophy is mediated by a Ca²⁺-dependent calcineurin signalling pathway. Nature 400, 576–580.
- Silverman, D., 1986. Density Estimation for Statistics and Data Analysis. Chapman & Hall, New York, 175 pp.
- Stickland, N.C., 1983. Growth and development of muscle fibers in the rainbow trout (*Salmo gairdneri*). J. Anat. 137, 323–333.
- Suresh, A.V., Sheehan, R.J., 1998. Muscle fibre growth dynamics in diploid and triploid rainbow trout. J. Fish Biol. 52, 570–587.
- Suwa, M.T., Nakamura, T., Katsuta, S., 1996. Heredity of muscle fiber composition and correlated response of the synergistic muscle in rats. Am. J. Physiol., Regul., Integr. Comp. Physiol. 271 (40), 432–440.
- Taranger, G.L., Haux, C., Stefansson, S.O., Björnsson, B.T., Walther, B.T., Hansen, T., 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17 β in Atlantic salmon, *Salmo salar*. Aquaculture 162, 85–98.
- Tatsumi, R., Anderson, J.E., Nevoret, C.J., Harvey, O., Allen, R.E., 1998. HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. Dev. Biol. 194, 114–128.
- Taylor, E.B., 1991. A review of local adaptation in Salmonidae, with special reference to Pacific and Atlantic salmon. Aquaculture 98, 185–207.
- Thorpe, J.E., 1989. Developmental variation in salmonid populations. J. Fish Biol. 35 (Suppl. A), 295–303.
- Thorpe, J.E., Mitchell, K.A., 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness, and current management. Can. J. Fish. Aquat. Sci. 38, 1576–1590.
- Thorpe, J.E., Morgan, R.I.G., Talbot, C., Miles, M.S., 1983. Inheritance of developmental rates in Atlantic salmon, *Salmo salar* L. Aquaculture 33, 119–128.
- Totland, G.K., Kryvi, H., Jødestøl, K.A., Christiansen, E.N., Tangerås, A., Slinde, E., 1987. Growth and composition of the swimming muscle of adult Atlantic salmon (*Salmo salar* L.) during long-term sustained swimming. Aquaculture 66, 299–313.
- Valente, L.M.P., Gomes, E.F.S., Fauconneau, B., 1998. Biochemical growth characterisation of fast and slow-growing rainbow trout strains: effect of cell proliferation and size. Fish Physiol. Biochem. 18, 213–224.

- Valente, L.M.P., Rocha, E., Gomes, E.F.S., Silva, M.W., Oliveira, M.H., Monteiro, R.A.F., Fauconneau, B., 1999. Growth dynamics of white and red muscles in fast and slow growing strains of rainbow trout. *J. Fish Biol.* 55, 675–691.
- Verspoor, E., 1997. Genetic diversity among Atlantic salmon (*Salmo salar* L.) populations. *ICES J. Mar. Sci.* 54, 965–973.
- Weatherley, A.H., Gill, H.S., 1985. Dynamics of increase in muscle fibers in fishes in relation to size and growth. *Experientia* 41, 353–354.
- Weatherley, A.H., Gill, H.S., Rogers, S.C., 1979. Growth dynamics of muscle fibres, dry weight, and condition in relation to somatic growth rate in yearling rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* 57, 2385–2392.
- Weatherley, A.H., Gill, H.S., Lobo, A.F., 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *J. Fish Biol.* 33, 851–859.
- Yablonka-Reuveni, Z., Rivera, A.J., 1994. Temporal expression of regulatory and structural muscle proteins during myogenesis of satellite cells on isolated adult rat fibers. *Dev. Biol.* 164, 588–603.
- Yablonka-Reuveni, Z., Seger, R., Rivera, A.J., 1999. Fibroblast growth factor promotes recruitment of skeletal muscle satellite cells in young and old rats. *J. Histochem. Cytochem.* 47, 23–42.