

Family and Population Differences in Muscle Fibre Recruitment in Farmed Atlantic Salmon (*Salmo salar*)

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Abstract

The number of muscle fibres recruited to reach a given body weight in Atlantic salmon (*Salmo salar* L.) showed significant variation between two strains with different growth rates and life history characteristics. Patterns of fibre recruitment also varied between two-year classes of the same strain grown under different production conditions. The maximum diameter of fast muscle fibres was around 240 μm in all cases. The density of muscle fibres decreased with increasing body weight, although average values were relatively constant for fish of 3.5 to 6.5 kg. The variation in muscle fibre density within a family, often 65 to 140 fibres mm^{-2} muscle, was significantly greater than that between families and/or strains. The significance of this variation in fibre recruitment for the textural and processing characteristics of the flesh is discussed.

Key words: Atlantic salmon, fibre recruitment, gaping, muscle growth, *Salmo salar*, texture.

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The majority of the myotomal muscle of teleosts is composed of fast twitch muscle fibres. In the fast muscle of salmonid species, two embryonic and one postembryonic phase of growth have been identified [8]. The postembryonic phase of growth involves the proliferation of a population of myogenic precursor cells [15]. The division products of the myogenic precursor cells become committed to differentiation and express one or more members of the MyoD family of transcription factors [4, 10]. These cells are either absorbed by fibres as they increase in diameter (hypertrophy) or else they fuse to form new muscle fibres (fibre recruitment). Thus growth is associated with the continuous recruitment and hypertrophy of muscle fibres, such that the muscle contains fibres with a wide range of diameters. Johnston *et al.* [11] investigated muscle fibre recruitment in the saltwater stages of the Lochy and Namsen strains of farmed Atlantic salmon (*Salmo salar* L.) reared in 5 m diameter pens. The relative importance of fibre recruitment and hypertrophy to growth varied considerably between the strains. Shortly after transfer to seawater in June 1997 the number of fast fibres was around 150,000 in both populations and there was a unimodal distribution of fibre size with a broad peak at around 50 μm diameter

[11]. However, between August and September, fish from the Namsen strain recruited significantly more fibres per mm^{-2} muscle cross-sectional area than the Lochy strain, resulting in a higher fibre density [11]. The recruitment of fibres slowed during the cooler winter months in the Lochy strain to 50 fibres $\text{d}^{-1} \text{mm}^{-2}$ muscle before increasing to 120 fibres $\text{d}^{-1} \text{mm}^{-2}$ muscle between March and May. In contrast, fibre recruitment in the Namsen salmon ceased altogether in January of the first seawinter, such that subsequent muscle growth was entirely by fibre hypertrophy until harvest at 2-3 kg in September.

In the present study, we have extended our investigation of growth in the 1997-year class of Namsen salmon until they reached sexual maturity, 14 months later, in order to determine whether fibre recruitment restarted later in the life-cycle. We have also compared muscle growth in two successive year-classes of the Lochy strain and investigated family differences in fibre recruitment between strains which have not been reported previously.

Materials and methods

Fish

The Namsen and Lochy strains of Atlantic salmon (*Salmo salar*) that were studied corresponded to a low

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grilising stock of Norwegian origin and a high grilising stock of West Coast Scottish origin respectively. Each population had been maintained for at least five generations in Marine Harvest Scotland's genetic improvement programme. Fish husbandry for the 1997 and 1998-year classes is described in detail in Johnston *et al.* [11] and Johnston *et al.* [14] respectively. Salmon were reared in duplicate 5m sea pens at Loch Duich (1997-year class) and Loch Eil (1998-year class) and fed a standard diet from Biomar Ltd containing approximately 28% oil and 46% protein with 21.4 MJ/kg digestible energy. The 1997-year class was fed to satiation by hand whereas the 1998-year class was fed to demand using an adaptive feeding system (AquaSmart UK Ltd). The Namsen fish studied from the 1997-year class were sexually mature individuals sampled in November 1999. The mean body weight was 8.68 ± 0.87 kg with an average fork length of 94.8 ± 3.6 cm (Mean \pm SE, $n = 11$). Information on the other fish sampled is given in the original publications [11, 14]. The fish were individually PIT tagged (passive integrated transponder, supplied by Fish Eagle Co., Gloucestershire, England), allowing individual families to be identified.

Muscle analysis

A 1cm steak through the trunk was made with a sharp knife at the level of the first dorsal fin ray and the total cross-sectional area of fast muscle determined by digital planimetry from a projected image. A total of 12 to 14 blocks of muscle were prepared so as to sample all areas of the cross-section. Blocks were frozen in isopentane cooled in liquid nitrogen. $8\mu\text{m}$ frozen sections were cut from each block and stained with Mayer's haematoxylin. The outlines of a minimum of 100 fibres per block were digitised using an Image Analysis System (Tema2, ScanBeam, Denmark) and fibre diameters computed (a minimum of 1200 per fish). The number of fibres sampled was plotted against the accumulative estimate of the total number until a stable value was obtained ($\pm 3\%$) [11].

Statistics

The distribution of fibre diameters was analysed using in house software. A random sample of 1000 fibres was taken and nonparametric statistical techniques used to fit smoothed probability density functions (pdfs) to the measured diameters using a kernel function, as described in detail elsewhere [9]. The programs were written in the PC based language R, which is a dialect of Splus [7] and are available on request from the first author. Differences in muscle fibre number between families and populations were analysed using a 2-way ANOVA with fish fork length or muscle cross-sectional area as a covariate.

Results

The group of 1997-year class of Namsen salmon sampled in November 1999 comprised 6 fish between 97

and 109 cm fork length (all males) and 4 fish between 80 and 89 cm fork length (one male and three females). The relationship between the total cross-sectional area of fast muscle and fork length is illustrated in Fig. 1A. There were significant differences between the total cross-sectional area of fast muscle and fork length, both between strains and between the two Lochy year-classes ($P < 0.001$). The number of fast muscle fibres per trunk cross-section in the heaviest group of Namsen salmon ($636, 200 \pm 45,000$; Mean \pm SE, $n = 6$) was not signifi-

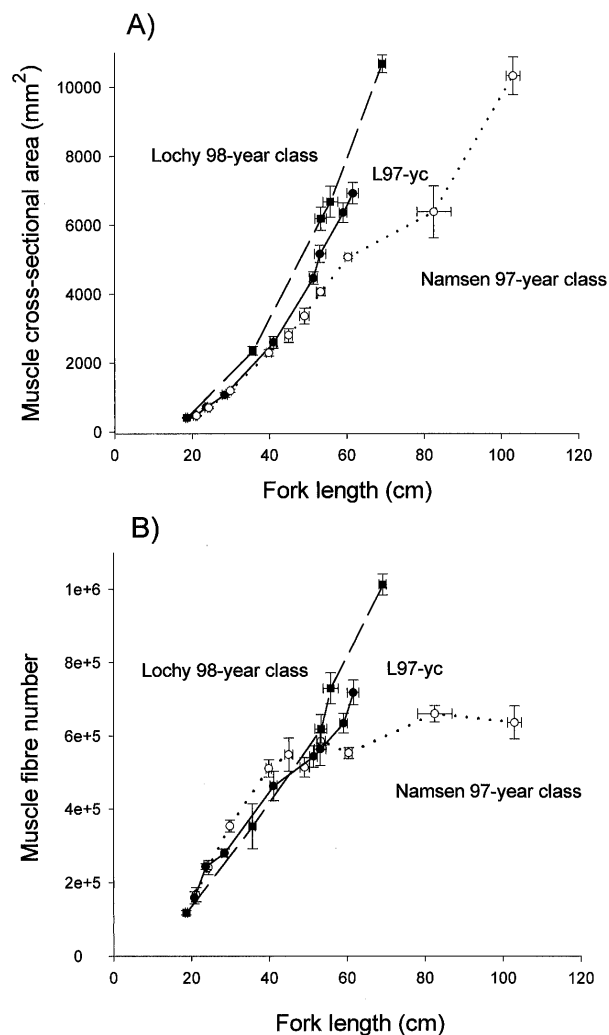


Figure 1. The relationship between fork length and (A) the total cross-sectional area of fast muscle and (B) fibre number at the level of the first dorsal fin ray in the 1997-year class of the Namsen (open circles) and 1997- and 1998-year classes of the Lochy (filled circles and squares respectively) strains of Atlantic salmon (*Salmo salar* L.). Values represent Mean \pm SE of 6 to 10 fish per sample point. The data for Namsen salmon of average body weight 82.5 and 103.0 cm are from the present study and the remaining data from Johnston *et al.* [11] and Johnston *et al.* [14].

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cantly higher than that for fish sampled in September the previous year after just 1 sea-winter. Thus for the Namsen stock it was confirmed that fibre recruitment ceased around 45 cm fork-length (Fig. 1B), about 9 months after seawater transfer. In contrast, fibre recruitment continued for at least 13 months after seawater transfer in the Lochy strain, such that fibre number exceeded 1 million per cross-section in the 1998-year class (Fig. 1B). The data for the two Lochy year classes indicates that the number of fibres recruited during this period varied with production conditions. The average body weight of the fish at harvest in May was around 2.0 to 3.5 kg for the 1997-year class and 4.5 to 6.5 kg for the 1998-year class [11, 14]. However, the two growth trials were different in several respects other than growth rate including, temperature, feeding method, incidence of sea lice infestation and farm site and therefore it is not possible to establish causality for the different recruitment patterns.

The fast muscle of the largest group of male Namsen salmon studied contained no fibres less than 50 μm diameter, consistent with the early cessation of recruitment (Fig. 2). The maximum fibre diameter was around 240 μm and the peak probability density was around 150 μm diameter (Fig. 2). The fibre diameters for the 5th, 10th, 50th, 95th and 99th percentiles of fibre diameter, calculated from the smoothed distributions, are shown

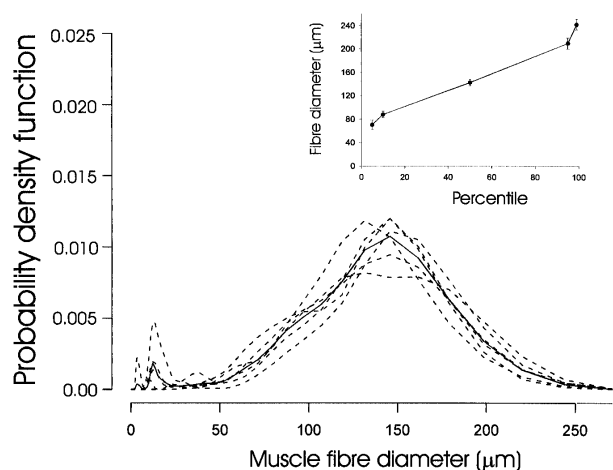


Figure 2. The probability density functions (pdfs) of diameter for fast muscle fibres from 6 sexually mature Namsen salmon from the 1997-year class of average body weight 10.59 kg sampled in November 1999. The dotted lines represent the pdfs of individual fish and the solid line the average pdf for the group. The inset shows the 5th, 10th, 50th, 95th and 99th percentile of fibre diameter calculated from the smooth distributions. The smoothing factor h in the kernel function used to fit the smoothed distribution had a value of 0.086 (see Johnston et al. [9] for further details on statistical methods).

as an inset to Fig. 2. Five percent of the fibres were below 70.4 μm in diameter whereas 5% were close to the maximum fibre diameter (210 to 240 μm) (inset Fig. 2). The average fibre diameter for the 4 fish of mean body weight 5.82 ± 0.23 kg was 106.8 ± 4.6 μm , compared to 140.7 ± 1.9 μm for the 6 fish of 10.59 ± 0.64 kg body weight (Mean \pm SE). The distribution of fibre diameters was unimodal at sexual maturity (Fig. 2). In contrast, in September of the previous year, when the immature fish were harvested for market the distribution of fibre diameters was markedly bimodal [see Fig. 11C in 11].

The relationship between the number of muscle fibres and total muscle cross-sectional area is shown in Fig. 3. The density of fibres declined with increasing body weight, but was relatively constant for fish of a typical harvest weight of 3.5 to 6.5 kg (Fig. 4). An ANCOVA with fibre density as dependent variable and body weight as a covariate ($P = 0.035$) revealed significant differences between strains ($F_{2,76} = 4.18$; $P = 0.018$). Box plots showing the median, mean, 10th, 25th, 75th and 90th percentiles for body weight and muscle fibre density in the different families studied are shown in Fig. 5. The variation in muscle fibre density within a family, often 65 to 140 fibres mm^{-2} muscle, was significantly greater than that between families and/or strains. Mean values of fibre density (number mm^{-2} muscle) in the nine families studied ranged from 81 (Mowi RH) to 128 (Namsen R+). The

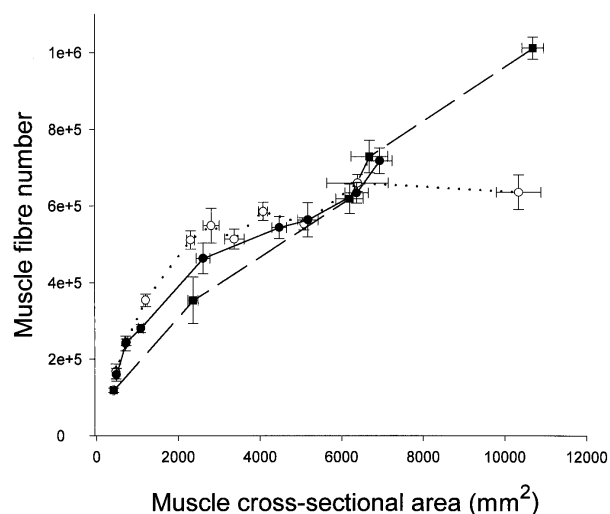


Figure 3. The relationship between the number and total cross-sectional area of fast muscle fibres at the level of the first dorsal fin ray in the 1997-year class of the Namsen (open circles) and 1997- and 1998-year classes of the Lochy (filled circles and squares respectively) strains of Atlantic salmon (*Salmo salar* L.). Values represent Mean \pm SE of 6 to 10 fish per sample point. The data for Namsen salmon of average body weight 82.5 and 103.0 cm are from the present study and the remaining data from Johnston et al. [11] and Johnston et al. [14].

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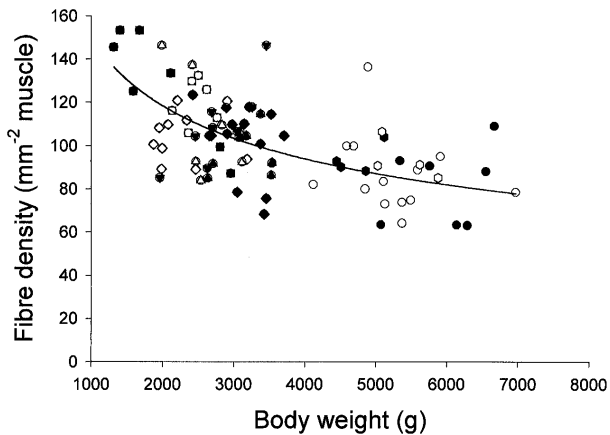


Figure 4. The relationship between the density of muscle fibres (number per mm^2 muscle cross-sectional area) at the level of the first dorsal fin ray and body weight for different strains and families of Atlantic salmon (*Salmo salar* L.). Mowi strain (1998- year class): families RH (filled circle) and LDU (open circle); Lochy strain (1997-year class): families LL (filled triangles), Li (open triangles), LH (filled diamonds), RN (open diamonds), RH (inverted filled triangles); Lochy strain 1998- year class: families RO (filled hexagonal) and RE (open hexagonal); Namsen strain (1997-year class): R+ (filled squares) and R3 (open squares). Data reanalysed by family using data from Johnston et al. [11] and Johnston et al. [14] and unpublished observations. The solid line represents a power function of the form: $\text{Fibre density} = a * \text{Body weight}^b$, where a and b are constants. $\text{Adj Rsqr} = 0.36$; $F_{1,78} = 44.86$; $P < 0.0001$.

range of values was from 26 (106 to 132) in Namsen R3 to 72 in Mowi LDU (64 to 136) (Fig. 5).

Discussion

The present study has shown that the 1997-year class of Namsen salmon recruited only 650,000 fast muscle fibres per trunk cross-section, such that the majority of seawater growth was entirely by fibre hypertrophy. In sexually mature 10.6 kg Namsen salmon, the peak density of fibre diameter was around $150\mu\text{m}$ (Fig. 2). However, only around 5% of fibres had reached the maximum diameter of $240\mu\text{m}$, indicating significant further growth potential. In contrast, the 1998-year class of Lochy salmon had recruited more than 1 million fibres in 5 kg fish. The ultimate fibre number in this strain is unknown. The growth performance of Lochy salmon from the 1998 year-class was superior to that of the 1997 year-class, with fish reaching an average harvest weight of 5.5 kg and 2.5 kg in May respectively [12, 14]. Initially, fish from the 1997-year class recruited more fibres per mm^2 muscle cross-sectional area than the 1998-year class, although this

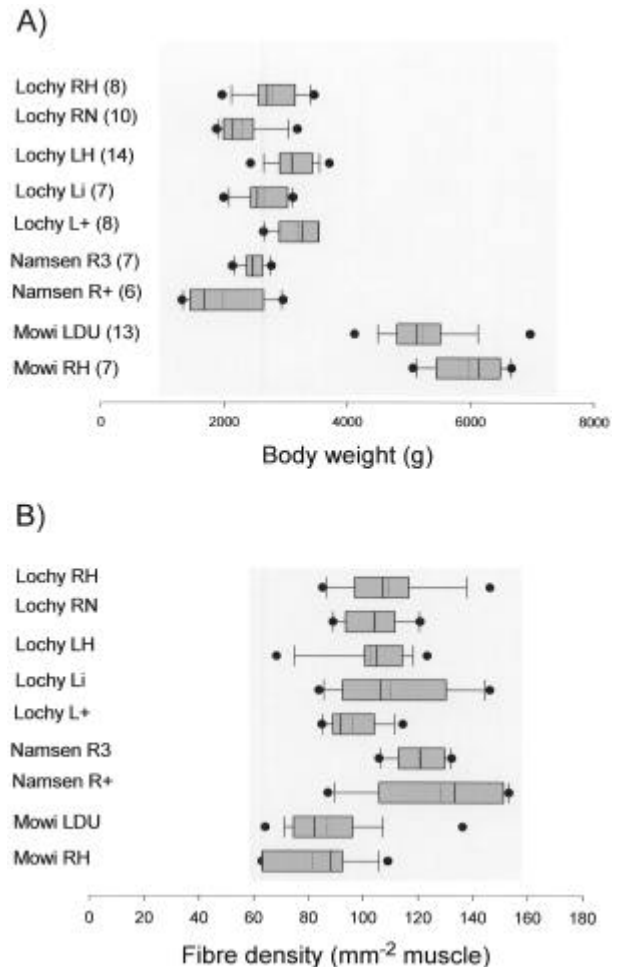


Figure 5. Box plots for (A) body weight and (B) muscle fibre density for different families of Atlantic salmon (*Salmo salar* L.). The median, mean (dotted line), 10th, 25th, 75th and 90th percentiles are plotted as boxes. Outliers are shown as solid symbols. The number of fish analysed in each family is shown in brackets in A.

pattern was reversed in the few months leading to harvest (Fig. 3). Interestingly the 1998-year class had recruited around 20% more fibres by 60 cm fork length than the 1997-year class (Fig. 1B). The superior growth performance of the 1998-year class was probably related to lower levels of sea-lice infestation and the use of an adaptive feeding system, which allowed the fish to feed to appetite between dawn and dusk [14]. It is not possible to attribute differences in fibre recruitment between the year-classes to differences in growth rate since the fish were farmed at different sites and experienced different temperatures and hydrographical conditions. However, this data does illustrate that the relative importance of fibre recruitment to growth is not simply fixed by genetic make-up [11] and/or environmental influences during embryogenesis [10, 12] but can also vary with production conditions.

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The mechanisms underlying differences in fibre number are unknown but probably involve differences in either the number or proliferative capacity of the myogenic precursor cells. Triploid Atlantic salmon have around 25% fewer myogenic precursor cells than diploid fish and recruited one third fewer fibres to reach a given muscle mass [9]. Fish myogenic precursors are analogous to the satellite cells found beneath the basal lamina of mammalian muscle fibres that have a role in repair from injury [3]. However, in salmon, mononuclear myogenic cells expressing muscle-specific markers are not restricted to the basal lamina at least in juvenile fish [10]. Another key regulator of muscle mass is myostatin, a member of the transforming growth factor (TGF- β) superfamily of growth factors. Myostatin “knockouts” in mice have considerably more muscle mass and higher numbers of fibres than wild-type controls [17]. The so-called doubled-muscle cattle breeds (Belgium Blue, Piedmontese and Asturiana de los Valles) have been associated with deletions, transitions or transversions within the coding region of the myostatin gene [2]. Genetic variation in myostatin or differences in its expression patterns are other potential candidates for explaining variation in fibre recruitment between families and populations of farmed salmon.

In Scotland, Quality assurance schemes operated by trade associations such as the “Scottish Quality Salmon” kite mark result in up to 20% of production being downgraded as unsuitable for premium products such as smoked salmon. The causes of downgrading losses are numerous and are related to both the intrinsic quality of the stock and factors involved in the slaughtering, storage, transporting and processing of the carcass [18]. Among the important causes of downgrading are poor and uneven colour, soft flesh and gaping of the fillet. Gaping is the opening up of breaks between the myoseptal sheets that separate blocks of muscle fibres resulting in the most extreme cases in the fillet falling apart. In most markets consumers show a preference for a firm texture such that fibre density can be regarded as a highly desirable quality attribute. A firm texture is also required during the processing of fillets for high value added products such as smoked salmon and gravadlax.

The textural characteristics of the flesh are a function of three components: muscle fibres, connective tissue and fat cells. Scaling considerations dictate that the size distribution of muscle fibres should have an impact on the amount and distribution of connective tissue. The relative amount of connective tissue per unit cross-sectional area is likely to be positively correlated with the muscle fibre density. The connective tissue matrix surrounding individual muscle fibres and bundles of muscle fibres is largely composed of collagen fibres. Collagen exists as a number of different isoforms,

which are subject to extensive posttranslational modifications, which are important to their mechanical properties [1, 15]. The collagen in fish muscle is much less cross-linked than that in red meat and is readily destroyed by cooking [5]. It is likely that in cooked flesh the muscle fibres themselves make the greatest contribution to textural properties. Hurling *et al.* [6] found that sensory estimates of firmness obtained using trained taste panels were inversely related to the average muscle fibre diameter in seven species of marine fish. Thus the firmest flesh was found in the species with the highest density of fibres. In cold-smoked salmon both the muscle fibre and connective tissue compartments probably contribute to the textural characteristics. A positive relationship between fibre density and four measures of texture was found for cold-smoked salmon from the 1997-year class [13]. The best correlation was found for the attribute of “chewiness” for which fibre density explained around 40% of the total variation between samples [12].

Numerous causes of gaping have been identified associated with the production, harvesting, transport and storage of the flesh [19]. However, in the 1998 year-class of Lochy and Mowi salmon a strong relationship between the propensity for gaping and fibre density was observed. No fish with a fibre density exceeding 95 mm^{-2} showed gaping whereas variable amounts of gaping were found in fish with lower fibre densities [14]. Our working hypothesis is that the lack of gaping in fish with a high fibre density reflects associated changes in the amount, types, properties and distribution of the connective tissue matrix surrounding the fibres.

Understanding the genetic basis for differences in fibre density will require basic research on the molecular physiology of muscle growth regulation. It is most likely that there are several major causes of variable fibre density providing multiple selection targets for genetic improvement programs. The elimination of individuals with a fibre density less than 95 mm^{-2} from the population at harvest would dramatically reduce processing losses due to gaping and soft flesh and result in a higher quality product for the consumer.

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