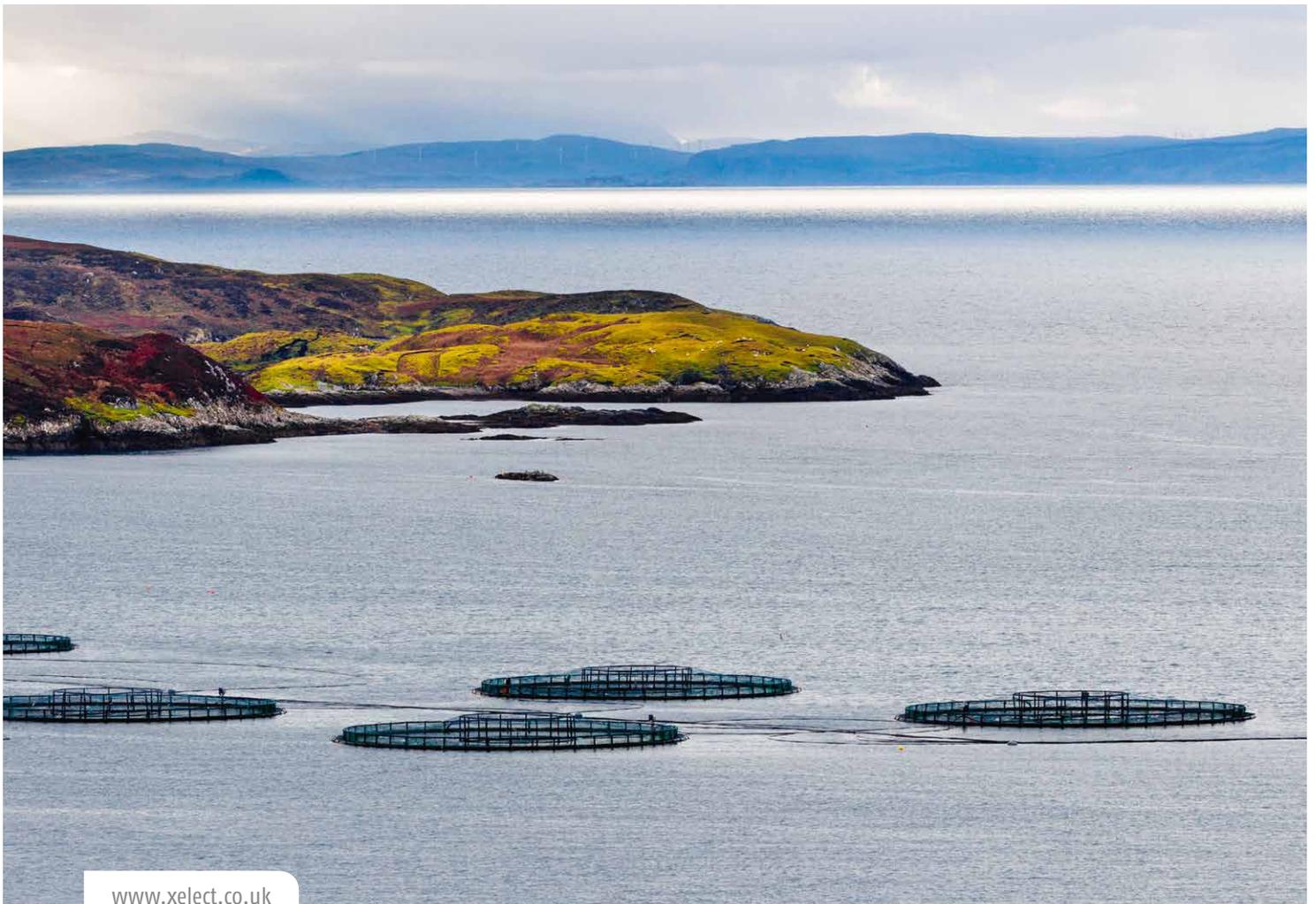


GENETIC MARKERS FOR MUSCLE FIBRE NUMBER IN ATLANTIC SALMON



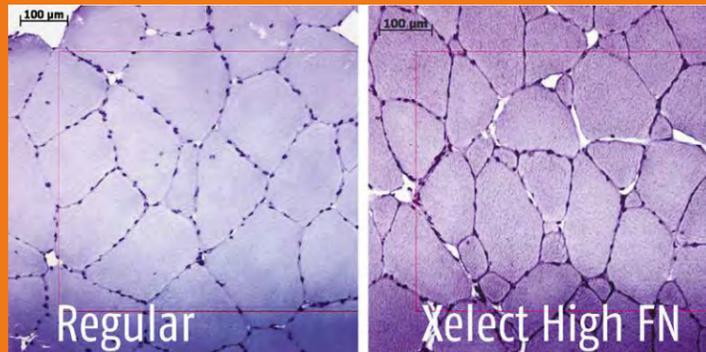
There is a three-fold variation in muscle fibre number (FN) in farmed salmon leading to large differences in fibre density between individuals.

Xelect has discovered genetic markers that select Atlantic salmon with a high muscle fibre density which allows very fast growth whilst preserving optimal carcass quality. The FN markers are available for licensing immediately with a full support package for marker assisted or genomic selection.



MUSCLE FIBRE NUMBER – A CRITICAL TRAIT FOR GROWTH AND CARCASS QUALITY

The difference is clear. Xelect High FN markers select Atlantic salmon with significantly more small muscle fibres, resulting in superior growth and optimal texture.



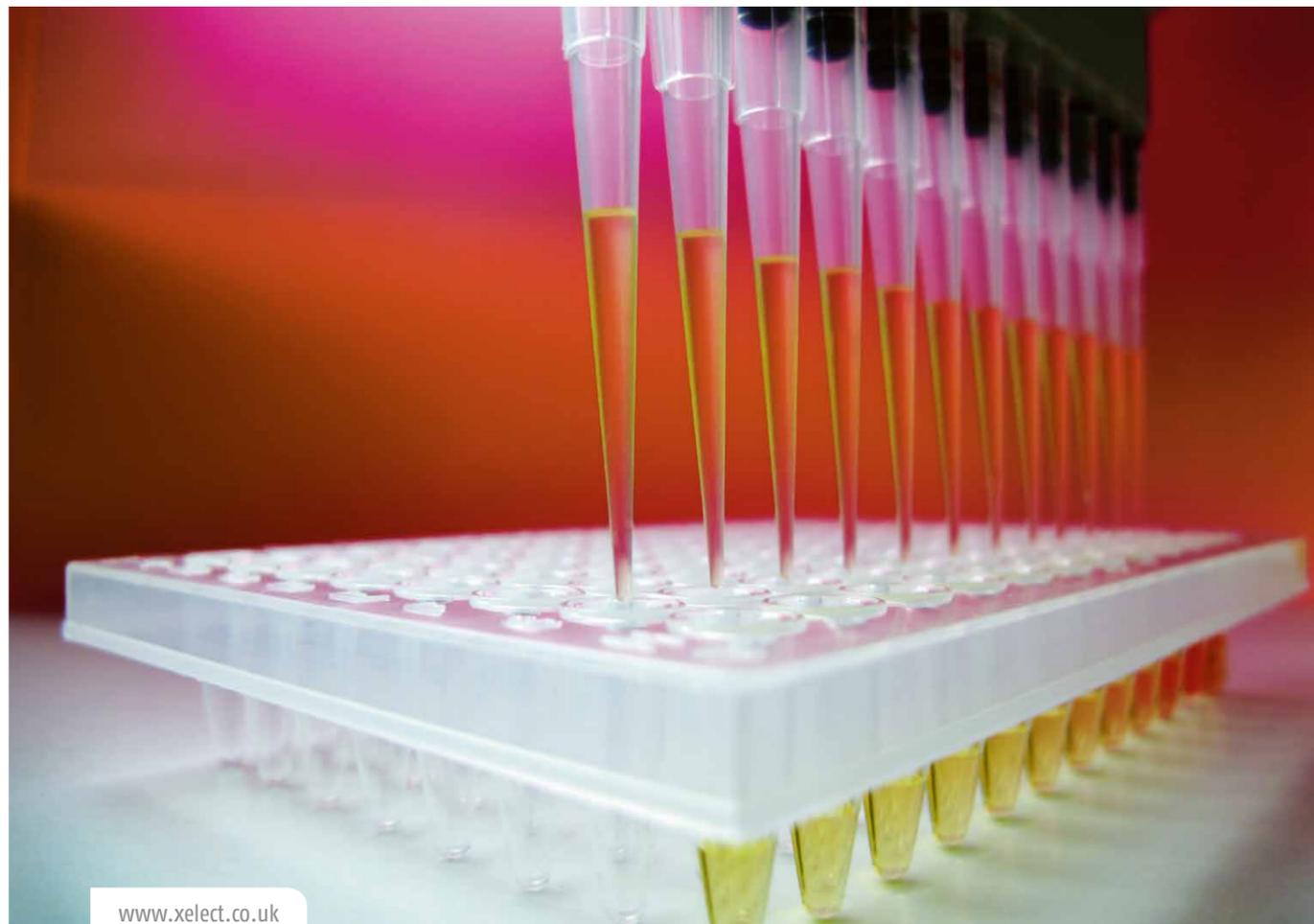
ABOUT XELECT

Based in St Andrews Scotland, Xelect is a leading provider of genetic services to the global aquaculture industry (www.xelect.co.uk). The company was formed in December 2012 as a spin-out from the University of St Andrews, one of the oldest and premier research-intensive universities in the UK. The company specializes in the development of genetic tools to support breeding programs including selection markers and SNP panels for parentage assignment and genomic selection. Xelect has sales offices in Chile and Hong Kong.

BACKGROUND

Fish fillets are composed of serially arranged myomeres. The boundary of each myomere is made up of sheets of connective tissue called myosepta which form points of insertion for the muscle fibres. Growth of muscle fibres involves a population of progenitor cells. These cells form new muscle fibres and provide nuclei for fibres as they expand in diameter and length. In Atlantic salmon, the production of muscle fibres is complete at around 1.5–2.0 kg body weight and growth to the final harvest size of 4–6 kg only involves the expansion of existing fibres¹.

There is a 3-fold variation in the extremes of FN in most salmon populations making this trait one of the most important sources of variation in the farmed product^{2,3}.



EFFECTS OF FN ON GROWTH

The growth benefits of a high FN are expected to be observed when nutrition and environmental conditions are optimal.

Muscle fibres are formed from a population of progenitor cells. Each individual muscle fibre follows a logistic growth curve which means that the highest potential for growth is at intermediate diameters. Theoretically, having a high FN increases the proportion of fibres with intermediate diameters and this gives the potential for very fast growth. This prediction has been validated in a field trial of salmon grown under fast growing conditions, with FN explaining around 35% of the variation in individual growth¹.

EFFECTS OF FN ON CARCASS QUALITY TRAITS

A high FN also results in a high muscle fibre density (FD) which maximizes the amount of connective tissue and cytoskeletal proteins per unit volume increasing the structural integrity of the tissue. Trials have shown that fish with a high FD have firmer fillet, are less prone to post-mortem gaping, and may have a deeper colour for a given level of pigment in the flesh⁴⁻⁸.

The higher the fibre number, the lower the average diameter of fibres, and the higher the density of muscle fibres per unit volume. The relative amount of muscle surface membrane (sarcolemma) is relatively higher in small than large diameter fibres (2/3rds power scaling rule). The sarcolemma is associated with the cytoskeletal proteins (desmin, dystrophin etc.) and extracellular matrix proteins (principally collagens) which provide integrity to the uncooked flesh and are largely responsible for firmness.

Thus, we would expect fibre density to be positively correlated with flesh firmness in raw and smoked product. Negative correlations have been reported between average muscle fibre diameter and the firmness of cooked fish⁹. Since collagen makes little contribution to texture after cooking this suggests that fibre density itself (i.e. fibre architecture) also contributes to firmness. Empirical studies with Atlantic salmon^{5,6} and other fish species^{10,11} have shown a positive correlation between muscle fibre density and firmness in raw and smoked salmon. Taste panel studies in Atlantic salmon found that FD explained 40% of the variation on "chewiness score" and 30% of the variation in "firmness"⁴. Fibre density is also a major factor in the development of post-mortem gaping. Salmon with a FD in excess of 95 fibres mm⁻² showed little or no gaping (FD range 60-140 mm⁻² in 3.8-5.4 kg salmon)⁶. Finally, positive

correlations between FD and flesh colour have been found independent of carotenoid pigment and lipid concentrations⁴. Fish with higher than average FD provide a potential solution to the occasional large financial losses from downgrading that occur due to soft flesh and gaping in fish harvested in the spring.

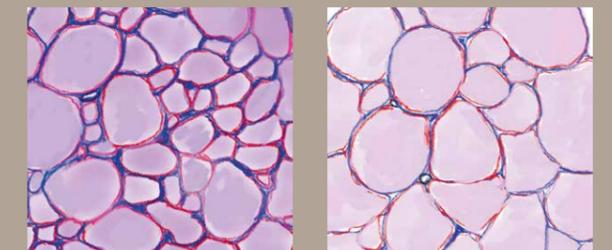


Fig. 1: High FD (left) has a greater amount of connective tissue (blue) and cytoskeletal proteins (red) per unit volume than low FD (right) producing firmer texture and greater tissue integrity.



THE XELECT FN MARKERS

FN exhibits moderately high heritability ($h=0.33$)¹⁰. The Xelect FN markers have been developed on the back of several decades of research into muscle fibre recruitment. The accurate determination of FN is a specialized and labour intensive process in which we are acknowledged global leaders. Using a candidate gene approach based on our research and sequence capture we have discovered 3 SNPs on different chromosomes that are strongly associated with FN.

Following a discovery phase involving 3 different strains of farmed salmon we have conducted extensive field trials over 2 years with a leading integrated farming company meeting all technical expectations. These informant trials involved around 3,000 fish on each occasion.

The frequency of the marker SNPs varies somewhat with strain and between year-classes, but on average is around 5.0% (SNP1), 15.4% (SNP 2) and 7.7% (SNP 3). FN was determined in 4 kg fish at the level of the first dorsal fin ray in fish of known genotype. Meta-analysis of our phenotype data for all three strains is shown in Fig. 2. On average FN was 660,000 in salmon with no marker present. Fish with any one of the favourable genotypes at SNP4, SNP5 or SNP6 (data had an average FN of 840,000 (27% INCREASE). Fish with any two favourable SNPs (1-2% population) had an average FN of 950,000 (44% INCREASE). The average FN with 2 SNPs was 103.8 fibres mm⁻² which is comfortably above the level at which no post-mortem gaping was observed in our earlier study⁶. In fish with the rare combination of 3 favourable SNPs FN was 1.2 million, around double that for fish with none of the markers.

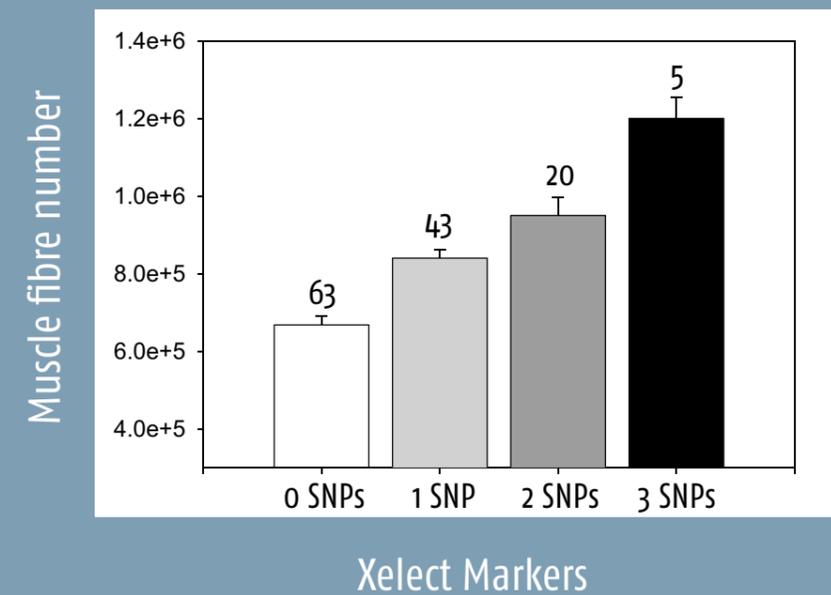


Fig. 2: The effect of single and multiple SNP combinations on muscle fibre number. Mean ± SE.



SELECTION STRATEGIES USING THE MARKERS

1 To achieve the greatest genetic gain in FN with stand-alone marker assisted selection it would be necessary to select from the 28% of the population that have at least one of the favourable genotypes.

2 To reduce constraints on the selection of other traits the presence of one or more of the favourable FN markers could be used as a discretionary step. This would result in a slower rate of genetic gain in the FN trait, but would likely have minimal adverse effect on current selection strategies.

3 An alternative strategy would be to remove fish with the most unfavourable FN genotypes. Although this would result in a slower genetic gain in FN it would impose the least constraints on the selection for other important traits. For example, retaining fish that were homozygous for 1 or more favourable genotype or heterozygous for 2 favourable genotypes would only eliminate 20% of the population for selection on the basis of other traits. Removing the fish with the lowest FN might prove effective increasing average carcass quality and in reducing large seasonal downgrading losses relatively quickly.

4 Finally, FN markers can also be treated as “high value SNPs” in genomic prediction algorithms. For our customers already utilising genomic selection our geneticists can support the incorporation of the FN markers into your breeding scheme. Xelect can bring you the latest advances in breeding technology through our consultancy agreement with the world-leading aquaculture genetics & genomics group at the Roslin Institute in Edinburgh led by Dr Ross Houston.

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