

DEVELOPMENT OF GENETIC TOOLS FOR SUCCESSFUL MANAGEMENT OF MUSSEL HATCHERY BROODSTOCK

PARTNERS

Xelect Ltd | NAFC Marine Centre | Scottish Shellfish

PROJECT LEADS

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BACKGROUND

In 2019, the Scottish rope-grown mussel farming industry produced about 7,000 tonnes of blue mussels with a value of £6 million. Shetland produced over 5,000 tonnes of this total, contributing almost £5m to the local economy.

Like other bivalves, mussels begin life as planktonic larvae. At the end of this planktonic stage, they settle out and attach themselves to suitable substrates to become 'spat' or 'seed'.

The mussel farming sector currently relies on the spawning of wild mussels to obtain their spat by natural settlement onto collector ropes placed in the sea by the farmer. However, availability of wild spat can be highly variable and presents a risk to the reliable expansion of the industry.

The sector therefore developed a pilot mussel hatchery at the NAFC Marine Centre (UHI) in Shetland to generate a reliable source of hatchery-reared blue mussel spat and to allow steady and planned growth of the industry. The 'Stepping Stone' pilot hatchery was supported by the European Maritime and Fisheries Fund (EMFF), Highlands and Islands Enterprise, the Scottish Shellfish Marketing Group (SSMG) and the Sustainable Aquaculture Innovation Centre (SAIC).

The 'Genetic Tools' research project summarised below was supported by SAIC, UHI, SSMG and XELECT Ltd. Being separate from – but complementing – the pilot hatchery, this research project aimed to develop genetic tools and collect necessary background data that would facilitate any future mussel breeding programme.

When animals are cultured for food purposes, it is possible to realise improvements in the animals through controlled breeding programmes. The two main objectives of a breeding programme are, firstly, to minimise inbreeding by maintaining genetic diversity, and secondly, to improve production traits.

Controlled breeding in terrestrial livestock has occurred for thousands of years. But breeding aquatic animals

such as the blue mussel presents some unique challenges and opportunities:

CHALLENGES:

- Fertilisation is by broadcast spawning, which complicates the selection of individuals.
- The larval stages of aquatic animals are usually microscopic in size.
- Understanding signals for reproduction requires research: common factors include day length and water temperature.
- Aquatic animals require aquaria systems to maintain and control breeding.

OPPORTUNITIES:

- Aquatic animals are highly fecund (many gametes produced by individuals). The advantage of this is that once breeding programmes are established, they do not need to be as large as for terrestrial mammals.
- Breeding programmes in shellfish are relatively novel. Therefore, there is great potential to make progress in key production traits, such as growth rate, meat yield and disease resistance.

There are three mussel species found in UK waters and they are known to hybridise. These are *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus*. *M. edulis* is the most abundant and preferred species for farming.

Selective breeding programmes aim to bring genetic improvements to the species being farmed. To successfully breed and improve mussels, the breeding programme must be capable of analysing the available genetic variation. It must also be able to select parent animals to drive genetic improvement in the next generation.

There are many ways to approach a breeding programme. Selection can be based purely on phenotype (a characteristic measured in an individual)

or by genotyping individuals and relating the phenotype back to a particular genotype. The latter method can be more precise, but the data requirements are higher because of the need to establish the linkages between genes and phenotypes. Many traits are not controlled by simple genotypes, but rather by thousands of genetic influences acting together, so this approach may be extremely complicated and impractical. Neutral genetic markers can be used to calculate relatedness and pedigree between individuals, allowing phenotypic selection on traits while avoiding the mating of close relatives.

Several methods are available for genotyping. The example used in this study was Single Nucleotide Polymorphisms (SNPs), which are single changes in base pairs between individuals. Many SNPs can be analysed simultaneously and can assign parentage to a high degree of accuracy (e.g. 99%). To establish SNP panels, a sample of individuals need to have their genome fully or partially sequenced to be able to discover and establish the SNPs. Geneticists use a technique called RAD sequencing for this task (Restriction site Associated DNA sequencing), which effectively provides a reduced representation of the genome, highlighting thousands of SNPs from which the optimal panel may be selected.

AIMS

- Develop SNP-based stock management tools for the blue mussel suitable for broodstock selection to facilitate the establishment of future family selection programmes.
- Testing 'first-cross' hatchery protocols and validating parentage assignment using Shetland-farmed mussels of known pedigree.

1 DEVELOPING SNP-BASED STOCK MANAGEMENT TOOLS FOR THE BLUE MUSSEL SUITABLE FOR BROODSTOCK SELECTION TO FACILITATE THE ESTABLISHMENT OF FUTURE FAMILY SELECTION PROGRAMMES

WORK DONE

Mussels were sampled at 18 farming sites around Shetland for genetic analyses and to describe the occurrence of hybridisation.

Mytilus species and hybridisation was determined using species-specific microsatellite markers.

Thirty pure blue mussel were selected for SNP panel development and these individuals genomes were investigated by sequencing (Restriction site associated DNA sequencing).

SNP panel was developed from the sequence libraries.

OUTCOMES

The three *Mytilus* species are all found around Shetland and they readily hybridise. There are about 61% *M. edulis*, 11% *M. Galloprovincialis* and 1% *M. trossulus* (Figure 1).

A panel of 96 SNPs was developed for use in blue mussel breeding, a range of criteria were used to find suitable SNPs in the libraries, these were, minor allele frequency, genome distribution and assay design suitability.

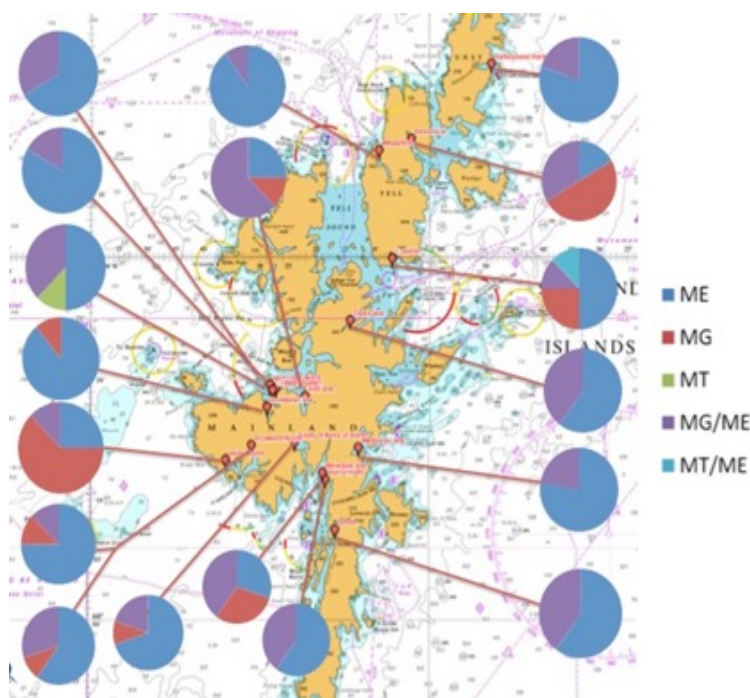


Fig 1. Distribution of pure *Mytilus* species and their hybrids from samples used in the project's microsatellite study. ME = *Mytilus edulis*, MG = *Mytilus galloprovincialis*, MT = *Mytilus trossulus*.

2 TESTING 'FIRST-CROSS' HATCHERY PROTOCOLS AND VALIDATING PARENTAGE ASSIGNMENT USING SHETLAND FARMED MUSSELS OF KNOWN PEDIGREE

WORK DONE

Mytilus edulis were crossed in first-cross experiments, where one female and male were used to create a family of progeny.

The parents and offspring were analysed using the SNP panel.

Based on the SNP data computational parental assignments were made using the software Colony 2.

OUTCOMES

Six single-cross trials were carried out and the families maintained separately, until they were large enough for genetic sampling (2 – 7 mm).

Analysis of the SNP panel showed that 42% of the selected SNPs were not sufficiently variable (did not yield polymorphic data). This indicates that the SNP panel should be further refined before exploitation, using the experience obtained in this project.

Despite some of the SNP markers not being useful, the SNP panel was used to successfully calculate the pedigree and identified all but two of the progeny that were assayed, across the six families.

IMPACT

This project has facilitated the development of more rapid genotyping and sequencing protocols for blue mussels that will help reduce analytical costs and be of future benefit to both farmers and geneticists.

Although some further refinement to the SNP panel is required before it can be exploited within a breeding programme the researchers established the protocols and gained valuable experience working with the blue mussel. Bivalve genomes are typically more variable than vertebrates. They have more frequent SNPs and higher mutation rates, which makes accurate genotyping more challenging using traditional probe-based tools.

Exploitation of these results is possible but depends on the mussel industry developing hatchery production of the blue mussel as the primary source of spat. This is desirable because it offers the possibility for genetic selection for mussels with stronger production traits. However, the challenges encountered when rearing this species mean that, for the time being, these results will not be fully exploited.

Some challenges were encountered when working with the mussel genome because there are many SNPs and these can be located very close to one another. This can interfere with the methods used to detect the SNP markers which, in future, could be overcome using sequence-based genotyping. Genotyping by sequencing allows the determination of which alleles are connected

together on each chromosome copy, not simply which are present. This extra level of information is called the haplotype, and it can further strengthen the calculations of pedigree and relatedness, while solving the issue of densely located SNPs interfering with each other.

Tom Ashton, Director at Xelect said:

“The development of modern genetic tools and hatchery protocols in blue mussels is a vital step towards selective breeding in mussels when the time is right for the industry to take that step.”

A post-doc was trained in new techniques and gained valuable industry exposure and experience. The project findings have been presented at several international conferences.

ADDITIONAL INFORMATION

PRESENTATIONS

Ashton, T. Association of Scottish Shellfish Growers, Annual Meeting (2016). *'Genetic Selection Opportunities for the Shellfish Industry – Are we Cultivating the Right Stock?'*

Smiljanič, B. International Symposium on Genomics in Aquaculture, GIA (2018). *'Developing and Validating SNP-Based Broodstock Management Tools for Mussel Hatcheries'*.

Ashton, T. Aquaculture UK, 'Aquavation – the Ripple Sessions' (2018). *'Developing Hatchery Management Tools for Mussel Hatcheries'*.

Shields, R. (2017). *'Scottish Shellfish Innovation: SAIC project updates.'* Association of Scottish Shellfish Growers, Annual Conference, 26-27 October 2017.