

COMPLETED PROJECT CASE STUDY

INVESTIGATING INFLUENCES ON SHELL BREAKAGE IN SCOTTISH MUSSELS

PARTNERS

University of Stirling's Institute of Aquaculture

CO-FUNDERS

Sustainable Aquaculture Innovation Centre (SAIC),
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BACKGROUND

Mussels are a cornerstone of the Scottish shellfish industry, supporting coastal economies and communities. In the West of Scotland, the predominant species of shellfish is the blue mussel (*M. edulis*), which can hybridise with other species such as the Mediterranean mussel (*M. galloprovincialis*) and the Pacific blue mussel (*M. trossulus*). Shell breakage, often believed to be linked to hybridisation with the Pacific blue mussel, has posed a persistent commercial challenge to industry growth. However, the biological, genetic and environmental factors causing shell breakage remain poorly understood.

This research aims to uncover these contributing factors, offering the potential to mitigate this issue and boost the sector's financial performance. To address this industry-wide challenge, a collaborative team was formed, representing a typical production chain. This project was led by researchers at the University of Stirling's Institute of Aquaculture. The study includes Fassfern Mussels Ltd, which operates in diverse environmental conditions, and the Scottish Shellfish Marketing Group, which manages downstream processing.

AIMS

This project sought to establish whether there were links between shellfish genotype, phenotype, or environment and shell breakage incidents. In particular, the project had four objectives:

1. Determine whether the presence of *M. trossulus* within a shellfish population is correlated with weaker shells;
2. Identify phenotypes within each species that are linked to shell breakage during and after harvest;
3. Analyse the genetic basis for the phenotypes laid out in objective 2 and its impact on selective breeding;
4. Establish the role of the environment in influencing phenotypes.

The team organised the project into four work packages, each focussed on one objective. The project team anticipated that the objectives would result in several outputs: the establishment of a molecular tool for early detection of weaker shells in farmed stock, improved identification of mussel species and hybrids in farmed stock using genotype testing, improvement of site selection, and improvement of harvesting and commercialisation processes.

DETERMINING SPECIES COMPOSITION OF SHELLFISH USING GENETIC TOOLS

To assist farmers with rapid genetic stock identification and to form the basis of the work within the work packages, a cost-effective DNA extraction protocol requiring minimal specialised equipment was developed. DNA extracted with this method was compatible with ME15/16, the most consistent and reliable DNA markers for identifying *Mytilus* species, and Kompetitive Allele Specific PCR (KASP) analysis, a cost-effective genotyping technology used to detect variations in DNA.

Researchers analysed 596 mussels from Loch Sunart to determine species composition. Using ME15/16 markers and KASP assays, the shellfish population was identified as 72% pure *M. edulis*, 19% *M. edulis*/*M. galloprovincialis* hybrid, 7% *M. edulis*/*M. trossulus* hybrid, 1% *M. edulis*/*M. galloprovincialis*/*M. trossulus* hybrid, 1% pure *M. galloprovincialis*, and 0% *M. trossulus*, as shown by the species totals in Table 1. Sampling was undertaken at mid-water farm sites in a seawater-dominated loch system.

Species	# mussels (total 596)
<i>Mytilus edulis</i>	428
<i>M.edulis/ M. galloprovincialis</i>	113
<i>M. edulis/ M. trossulus</i>	41
<i>M. edulis/ M. galloprovincialis/ M. trossulus</i>	5
<i>M. galloprovincialis</i>	8
<i>M. trossulus</i>	1

Table 1: Loch Sunart mussel population species composition using KASP assays to determine the percentage of *M. edulis/ M. galloprovincialis/ M. trossulus* introgression.

Shells were analysed for thickness, aragonite and calcite layers (596 samples), hardness, and fracture toughness (467 samples). Further, these phenotypes were compared against the six species complexes identified above to determine if there were genetic properties causing shell thickness, hardness or fracture toughness.

To understand the environmental factors associated with shell breakage, the team also sought to test mussel populations at a second site, Loch Eil, to establish whether site-specific parameters such as salinity or hydrodynamics play a role in shell breakage.

RESULTS

The genotyping analysis demonstrated that while useful for identifying pure *M. edulis*, *M. galloprovincialis* and *M. trossulus* in the population, the ME15/16 analysis struggled to correctly identify introgressions where the ratio of the introgression was less than 0.3. For this reason, KASP may have a better ability to distinguish between the species of the complex and their hybrids.

The team's findings revealed that only shell thickness and hardness correlated with breakage. Broken shells were significantly thinner (GLM $P < 0.001$, $F = 10.46$, $DF = 1$, $N = 592$) and had lower hardness (GLM $P < 0.001$, $F = 24.30$, $DF = 1$, $N = 467$) than unbroken shells. Fracture toughness showed no significant correlation with shell breakage (GLM $P = 0.184$, $F = 1.77$, $DF = 1$, $N = 467$).

No correlation was found between species genotype and shell characteristics – such as thickness, hardness, or fracture toughness – that could explain the breakages. This suggests that species composition has no effect on any of the shell phenotypes relating to shell strength and therefore cannot be used for selective breeding to combat shell breakages.

More broken shells were collected after processing (145 broken, 150 unbroken) than after harvesting (71 broken, 217 unbroken). This difference might be due to site-specific selection bias. However, no significant relationship was found between shell breakage and shell thickness when evaluating harvesting or processing data.

Due to sampling challenges within the project's timeframe, the analysis of shellfish at the second site, Loch Eil, was not completed. Therefore the team could not investigate potential environmental factors of shell breakage. Thus, the authors' original anticipated outcomes – improved site selection and commercial processes – were not achieved.

IMPACT

While recognising that all objectives were not achieved, the project has led to an understanding of the key shell phenotypes that correlate with shell breakages. Shell thickness and hardness has been found to significantly correlate with shell breakages, with thinner and less hard shells resulting in breakages.

Furthermore, the project has resulted in additional collaboration between academic and industry partners, including a PhD research project to complete the exploration into environmental causes of mussel shell breakages. Understanding the interaction between genotype and environment may lead to developed genomic resources, such as selective breeding, as tools for stock selection.

While species composition cannot be used for selective breeding to combat shell breakages, other potential genetic markers or methodologies might support selective breeding, leaving this avenue underdeveloped. Even though hybridisation with *M. trossulus* has been previously identified as a commercially damaging issue, this study's findings do not demonstrate its direct impact on shell breakage.

The genotyping work in the early stages of the project has led to improved insight into the identification of mussel species and hybrids within farmed stock – and potentially wild stocks – which could support long-term insight into shellfish health. Future research building on this project's conclusions could provide a more holistic understanding of the factors contributing to mussel shell breakage and practical solutions for mitigating this issue in the Scottish shellfish industry.