COMPLETED PROJECT CASE STUDY

INVESTIGATING TUBEWORM BIOFOULING AND MONITORING STRATEGIES IN SCOTLAND

PARTNERS

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BACKGROUND

During the spring and summer, farmed mussels in Scotland are vulnerable to biofouling by organisms such as barnacles and tubeworms (*Pomatoceros triqueter*). Tubeworms are immobile polychaetes that build calcareous tubes and attach to hard surfaces, including mussel shells.

While barnacles can be removed relatively easily, tubeworm infestations are more difficult and costly to manage. Affected mussels (approximately 500 tonnes per year, which equates to ~5% of the total annual harvest) are often discarded due to concerns over vacuum packaging damage and reduced consumer appeal. Although tubeworms do not make mussels inedible, their appearance and smell after cooking can make them unappetising to consumers. Some studies suggest biofouling may also lead to growth and weight reductions in mussels, further impacting their commercial viability.

This case study explores the development of a rapid and targeted monitoring system to help mussel farmers optimise shell cleaning strategies based on local fouling conditions, to prevent tubeworms from attaching to mussel shells, and ultimately improve stock management and product quality.

AIMS

The specific objectives of this project were to:
Develop a rapid diagnostic test to quantify the presence and abundance of *P. triqueter* DNA in plankton and shell swab samples;

- Validate effective field sample collection methods
- Apply the newly developed test to identify the optimal conditions for shell cleaning at commercial mussel farms
- Verify that implementing shell cleaning operations following the test results will significantly reduce biofouling on mussel shells.

OVERVIEW

The project was separated into three work packages:

WORK PACKAGE 1: UNDERSTANDING SETTLEMENT DYNAMICS

This work package focused on understanding tubeworm settlement dynamics at two commercial mussel farming sites in Shetland, operated by Shetland Mussels Ltd. Site 1 (East of Linga, Vaila Sound) had historically high tubeworm settlement. Site 2 (Linkaward, Weisdale Voe) experienced lower settlement levels (Figure 1).\

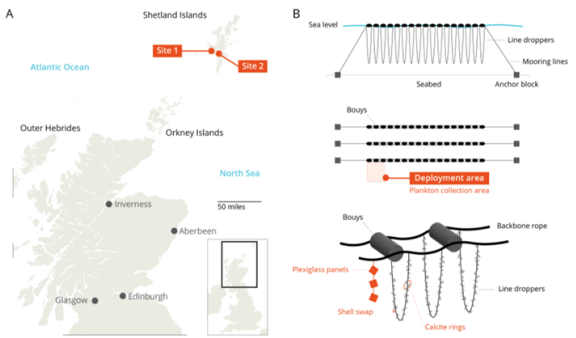

Figure 1. (A) Site locations. (B) At each site, temperature and salinity sensors were deployed. Each site also had white Plexiglass panels (40×40cm) deployed at 3m, 5m, and 10m, as well as a calcite ring attached to a dropped line at 5m. Photos of the Plexiglass panels, calcite rings, mussel shell swabs, and water column plankton were collected every fortnight.

Environmental parameters, including temperature and salinity, were continuously monitored at both sites between June 2021 and June 2022 using multi-parametric sensors (HOBO®). Data collected from June to October 2021 aligned with plankton-sampling efforts to assess peak tubeworm larval settlement. Historical studies suggested settlement peaks in mid-September and mid-April, with minimal activity in early August.

However, this study found peak larval presence in early August, approximately five weeks before peak settlement was detected. Site 1 showed consistently higher tubeworm DNA abundance than Site 2.

Larval settlement was further analysed using gridded artificial substrates (white Perspex tiles) suspended from mussel lines. These tiles were sampled fortnightly, photographed, and replaced.

WORK PACKAGE 2: ASSAY OPTIMISATION
This work package aimed to refine molecular and visual methods for detecting tubeworm larvae. Plankton samples collected between July and October 2021 were analysed using qPCR, which provided relative DNA abundance data for both sites. As Site 1 had the highest recorded tubeworm larvae presence, it became the primary focus for further testing.

Traditional detection methods, such as plankton nets and shell swabs, were found to be labour-intensive. Researchers tested artificial settlement substrates, including calcite rings, white Perspex and dark untreated slates, to visually quantify *P. triqueter* abundance (Figure 1B).

WORK PACKAGE 3: DEMONSTRATION OF IMPACT
Findings from the first year of research informed the selection of slates for visual larval detection and emphasised the importance of qPCR-based plankton sampling for accurate abundance assessments.

Plankton samples from 2021 and 2022 were analysed using environmental DNA (eDNA) metabarcoding and paired-end sequencing to compare biofouling abundance with temperature and salinity data.

RESULTS

Results showed the highest tubeworm abundance occurring in late July 2022, compared to August 2021 (Figure 2). Satellite data confirmed that sea surface temperatures exceeded 12°C in 2022, whereas temperatures only reached 11°C in 2021. This temperature threshold appeared to influence larval settlement timing, with a delayed peak observed in the cooler year.

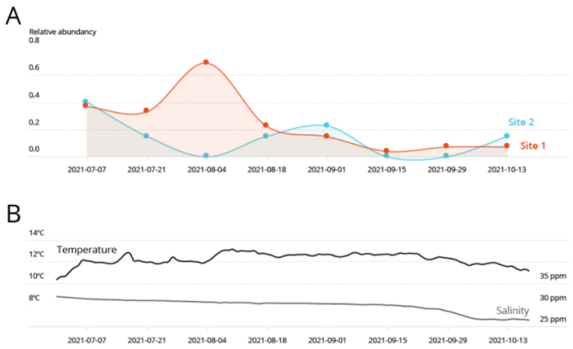

Figure 2. (A) The monitoring data identify a peak of larvae in the water column in early August, five weeks before the reported peak of detectable settlement. Site 1 presented more detectable tubeworm DNA than Site 2. (B) Moate (1985) also reports that above 6°C, growth is significantly associated with temperature. Similarly, salinity above 24ppm has a significant impact on growth of the worms.

The correlation between rising temperatures and tubeworm settlement suggests that temperature could be a predictive indicator for biofouling management. As the abundance of these organisms appears to increase in response to increased sea surface temperatures, mussel aquaculture in the Shetland Islands could be negatively impacted by climate change.

Although a DNA extraction protocol was validated, DNA accessibility remained inconsistent. DNA from plankton samples at peak concentrations was successfully extracted to compare the relative abundance of *P. triqueter.* However, qPCR protocols did not detect eDNA in all samples, necessitating correlation with visual identification via compound microscopy to assess seasonal patterns and biofouling presence.
Swab sampling proved inconclusive, while seawater (plankton) sampling emerged as the preferred method for obtaining larval samples.

Initial tests using calcite rings in year one were unsuccessful and were not repeated in year two. Similarly, visual evaluation with white Perspex was inconclusive, leading to the deployment of dark untreated slates, which also failed to yield reliable results. As a result, a combination of environmental monitoring for temperature and salinity, plankton sampling for DNA extraction, and visual identification via compound microscopy, is required to determine the optimal timing for shell cleaning operations.

IMPACT

Although the cleaning efforts in this project were unsuccessful, valuable insights were gained in designing cleaning and monitoring trials, paving the way for future studies to explore earlier interventions. Additionally, this research ruled out certain tubeworm larvae monitoring techniques and contributed to the development of a qPCR assay for detecting *P. triqueter*, which could be beneficial for future research.

The use of molecular diagnostic tools within global shellfish production is highly innovative. However, further research is needed to enhance PCR extraction techniques for greater reproducibility, or to refine the eDNA approach by targeting a smaller genetic subunit that is capable of identifying polychaetes at the species level.

Establishing 'intervention thresholds' based on temperature data will help mussel farmers determine optimal shell-cleaning schedules, improving farm efficiency and reducing economic losses.