COMPLETED PROJECT CASE STUDY

FEASIBILITY STUDY: PRE-EMPTIVE AND NON-INVASIVE PATHOGEN DIAGNOSTICS TO PREVENT THE SPREAD OF SHELLFISH DISEASE

PARTNERS

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PROJECT LEADS

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FUNDERS

Seafood Innovation Fund (SIF), with support from SAIC

The UK Seafood Innovation Fund supports new ideas to deliver cutting-edge technology and innovation to the UK's fishing, aquaculture and seafood industries. Administered by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) on behalf of the Department for Environment, Food and Rural Affairs (Defra), the programme encourages sustainable and innovative ideas to bring about seafood security, new partnerships across seafood and technology sectors, and to contribute to strong evidence-based management.

BACKGROUND

Disease is the single biggest issue facing oyster aquaculture and restoration. Regulation and monitoring aim to classify the health of a particular area by pathogen presence or absence for key listed pathogen and host species.

Measures to stop the spread of oyster diseases in the UK rely entirely on the prevention of animal movement from disease-positive to disease-free sites. However, most current centralised diagnostic tests are reactive, taking place once a disease is already present. These measures are not entirely successful and have seen recurrent failures in recent years, resulting in gradual spread of pathogens across the UK. Most recently, the deadly notifiable pathogen *Bonamia ostreae* has spread to key farming and restoration sites in Scotland.

There are a few options currently available for farmers to take proactive measures in pathogen control. As well as direct biosecurity measures, it is also possible to survey stock before moving them, by taking a subsample of animals and sending these to a professional or government organisation for testing.

However, these options have several limitations, such as high costs, the requirement for highly trained staff, destructive testing taking place over an extended period of time, and only small numbers of animals being tested; resulting in cases of pathogen slipping through the net. As such, it is not often practical for small-scale shellfish farmers to use these methods proactively. Therefore, to prevent the spread of disease, the development of a system with which oyster farmers can perform their own affordable non-invasive pathogen diagnostic prior to moving animals is required, enabling positive action above and beyond legislative requirements, and contributing to the prevention of disease spread.

> *Our project will tip the way we currently diagnose diseases that affect oysters on its head – taking a pre-emptive rather than reactive approach. We are bringing together the right technology with the right people to solve some of the shellfish sector's biggest health challenges and potentially make significant improvements to oyster health.*
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> **Dr Tim Bean, Roslin Institute**

AIMS

The aim of this study was to create a portable and rapid tool that would enable the aquaculture practitioner to perform diagnostic analysis on site by through a pre-emptive, non-invasive and affordable diagnostics method. This would allow the testing of large numbers or batches of animals in single assays, facilitating disease identification prior to oysters being moved, and introducing proactivity into the management of disease risk.

EXPERIMENTAL STUDY

Two experimental disease challenges with two oyster species – *Crassostrea gigas* with OsHV-1 (Oyster Herpesvirus type 1) and *Ostrea edulis* with parasite *Bonamia ostreae* – were completed over the course of the study.

The OsHV-1 challenge was done in the Roslin Institute. Five experimental holding tanks were used; two control tanks and three disease tanks. Each tank held ten *Crassostrea gigas*. In each of three experimental tanks, one individual oyster was injected with OsHV-1 directly into the adductor muscle. All oysters were incubated for 24h. After the incubation time, the water in each tank was mixed and filtered, and any remaining sediment in

the tanks collected. All samples were stored at -20°C until analysis.

The *Bonamia ostreae* experiment was adapted from initial proposal to fit the availability of animals likely to have been exposed to the pathogen. As such, the oyster holding and incubation was completed on site, in the Mersea area of Essex, a *Bonamia*-positive location. Eighty *Ostrea edulis* were collected, with no prior knowledge of the infection status of each animal. The eighty animals were split between eight buckets and held for 24 hours. After this period, a water sample was filtered and the sediment remaining in the buckets collected. In this case, each oyster was also dissected and sampled. All samples were stored at -20°C until analysis.

The equipment required for this feasibility study included the Biomeme 'Franklin' portable qPCR machine, the handheld MPBio fastprep (tissue homogeniser), and the reagents associated with DNA extraction and PCR for both laboratory and field-based analysis.

During the research, several processes and qPCR assays on both Applied Biosystems 7500 (laboratory-based, full-size qPCR machine) and a Biomeme Franklin qPCR machine were tested, with positive and negative material behaving as expected in duplicate (AB-7500) or triplicate (Biomeme Franklin) reactions. Additionally, DNA was extracted for all substrates with both "gold standard" commercial laboratory kits and the Biomeme M1 portable kit.

Oyster tissue samples were lysed by physical disruption and/or proteinase K digestion. DNA was then extracted from this buffer.

Filter samples were extracted by the Qiagen Power water kit in the laboratory, and for a field protocol, the filter was backwashed with DNA lysis buffer and run through a M1 DNA extraction cartridge.

For sediment extraction, samples were mixed and a subsample was extracted with the Qiagen Power soil kit in the laboratory. For field extraction, a subsample was added to DNA lysis buffer and run through a M1 DNA extraction cartridge.

All laboratory samples were analysed by qPCR and, where required for validation, also by the new portable system.

All samples from OsHV-1 challenges were analysed for the presence of *Crassostrea gigas* and OsHV-1, and all samples from the *Bonamia* experiment were analysed for the presence of *Ostrea edulis* and *Bonamia ostreae.* In this case, samples were prepared and analysed on-site as it was run in a field scenario, as mentioned above.

OUTCOMES

During the study, operational assays were demonstrated on Biomeme Franklin and standard qPCR machines.

It was confirmed that *Bonamia ostreae* DNA can be detected from the sediment left in oyster holding tanks, following a 24h incubation, using portable equipment. Presence of *Bonamia ostreae* DNA in the sediment from these tanks is consistent with the presence of at least one individual oyster in that tank being infected with the parasite. However, *Bonamia* DNA cannot be reliably detected in water alone from these holding tanks. Thus, it is suggested that sediment is the most appropriate proxy for assessing infection. OsHV-1 can be reliably detected in the water of tanks that hold artificially infected (lab-challenged) *Crassostrea gigas* after a 24h incubation period, as well as from sediment. Likewise, relevant oyster DNA can be detected in water and/or sediment from tanks that have held either *Ostrea edulis* or *Crassostrea gigas*, demonstrating effective DNA extraction.

Hence, successfully demonstrating the capacity for DNA extraction and amplification by PCR is possible in field scenarios, meaning it is possible to run the analyses on site.

For *Bonamia* in particular, this process appears to be very sensitive. The initial work has demonstrated accurate detection of a 4% infection rate in the population, and it is predicted from the data collected that the actual limits-of-detection are considerably more sensitive. This process now requires refinement and further validation.

This project has demonstrated the ability of a new non-invasive diagnostic process, which can be used prior to moving oysters between sites, delivering a scale of effective and timely testing and disease control otherwise unachievable. This new tool and methodology will enable proactive action beyond legislative requirements, to keep oyster farms and restoration sites disease-free and prevent further spread of diseases.

The project has a natural continuation and expansion, which will be conducted and supported through a further SIF R&D call.

ADDITIONAL INFORMATION

The project has involved work and close engagement with Loch Creran Oysters, Lochnell Oysters, and the Association for Scottish Shellfish Growers (ASSG). These sector collaborations will continue beyond the initial project in further testing of the systems.

A summary of the work was presented to the annual meeting of the Association for Scottish Shellfish Growers (ASSG) 2022.

Pre-emptive and non-invasive pathogen diagnostics to prevent the spread of shellfish disease (RD135) - Seafood Innovation Fund

Pre-emptive and non-invasive pathogen diagnostics to prevent the spread of shellfish disease — University of Edinburgh Research Explorer

Watch this short animation to get an overview of the follow-on R&D project: