COMPLETED PROJECT CASE STUDY

PROLIFERATIVE KIDNEY DISEASE (PKD): MODELLING PARASITE EXPOSURE AND IMMUNE RESPONSE TO SAFEGUARD FARMED RAINBOW TROUT IN SCOTLAND

PARTNERS

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BACKGROUND

Proliferative kidney disease (PKD), caused by the parasite *Tetracapsuloides bryosalmonae*, can negatively affect rainbow trout farming. In the absence of an effective vaccine, trout producers rely on management strategies that enable fish to develop natural immunity following controlled exposure to the parasite. One such approach is the use of 'pre-exposure programmes', which are already widely applied on trout farms in southern England. These programmes rely on carefully timed introduction of previously unexposed fish to farming sites during periods when parasite exposure is sufficient to induce immunity without causing severe disease.

However, pre-exposure strategies have historically been implemented without detailed knowledge of parasite levels in the environment or how environmental conditions influence infection dynamics and immune responses. This lack of data limits the ability of producers to determine whether fish have received sufficient exposure during the first production year to protect them during subsequent re-exposure.

Recent research has developed new monitoring tools capable of measuring the presence of parasites in the environment, the prevalence of infections in fish, and parasite-specific immune responses. These include quantitative PCR (qPCR) assays for parasite detection and serological assays that measure adaptive immune responses in fish. When combined with environmental monitoring, such as water temperature and other site-specific variables, these tools offer the potential to build predictive models that inform optimal exposure timing.

Building on earlier research conducted on trout farms in southern England, the Modelling PKD Exposure to Safeguard Farmed Rainbow Trout (MPERT) project applied this monitoring framework to Scottish trout farms operated by Kames Fish Farming Ltd.

The project brought together industry and academic partners from the University of Aberdeen and the University of Nottingham to investigate PKD exposure dynamics in Scottish production systems and develop tools to support improved disease management.

AIMS

The project had two principal objectives.

The first objective was to apply a validated monitoring toolkit to measure environmental parasite load, infection prevalence, and immune responses on PKD-affected trout farms in Scotland. This work aimed to support the development of evidence-based stock management strategies capable of mitigating disease outbreaks.

The second objective was to optimise pre-exposure management strategies for broodstock and to initiate development of a practical exposure model that could ultimately support the rapid field testing of vaccine candidates and improved disease management approaches.

OVERVIEW AND METHODS

The project combined environmental monitoring, fish health diagnostics, and assay development to investigate PKD exposure dynamics across several trout farms operated by the industry partner. Sampling and data collection were integrated into routine fish health monitoring carried out by farm staff, while laboratory analysis and assay development were conducted by the academic partners.

WORK PACKAGE 1: MONITORING ENVIRONMENTAL EXPOSURE, INFECTION PREVALENCE, AND IMMUNE RESPONSES

The first work package focused on applying an established PKD monitoring toolkit to characterise parasite exposure and host responses in farmed rainbow trout.

Environmental DNA (eDNA) sampling was used to detect parasite DNA in water samples collected during routine farm operations. Sampling was conducted between September and December 2024 and again between May and July 2025, reflecting periods when parasite presence is most likely to be detectable.

A total of 67 water samples were analysed using qPCR assays targeting the parasite's 18S genetic region. Sampling covered four farm sites operated by the industry partner. Environmental monitoring was combined with water temperature measurements and fish condition surveys to provide additional environmental context for parasite dynamics.

Fish infection prevalence was assessed through molecular analysis of kidney tissue samples. Seventy-seven samples were collected across seven sampling points during the project. These were analysed using qPCR assays to detect parasite DNA within fish tissues.

To evaluate the development of adaptive immunity following parasite exposure, the project also used an indirect enzyme-linked immunosorbent assay (ELISA) designed to detect antibodies against the parasite. This assay targeted a recombinant parasite antigen known as P14G8, which had previously been identified as a virulence-associated secretory factor. Serum samples were collected repeatedly from tagged fish across the trial period, generating a total of 290 samples.

Two cohorts of fish were monitored throughout the study. These cohorts differed in the timing of their initial exposure to the parasite, allowing researchers to compare immune responses and infection dynamics associated with different exposure histories. The monitoring programme extended over approximately sixteen months and included both initial exposure and subsequent re-exposure phases.

Histological analysis of kidney tissues was also undertaken to assess tissue pathology associated with infection. Sixty-five samples were examined by an external laboratory to identify pathological changes consistent with PKD.

WORK PACKAGE 2: DEVELOPMENT OF A RAPID ON-FARM PARASITE DETECTION ASSAY

The second work package focused on developing a rapid diagnostic tool that could allow farmers to detect PKD parasites directly on site without laboratory-based analysis.

The approach combined recombinase polymerase amplification (RPA) with CRISPR-Cas12a detection. DNA extracted from fish tissues or environmental samples was first amplified using an isothermal RPA reaction, which can operate at a constant temperature and therefore avoids the need for conventional PCR thermocyclers.
Following amplification, parasite DNA was detected using a CRISPR-Cas12a system guided by a sequence-specific RNA molecule. When the guide RNA binds to the amplified parasite DNA, the Cas12a enzyme activates and cleaves a reporter molecule. This reaction produces either a fluorescent signal or a visual readout on a lateral flow test strip.

The full process can be completed in approximately 30-45 minutes at approximately 37 °C, enabling the possibility of rapid, field-based detection using minimal equipment.

RESULTS

ENVIRONMENTAL PARASITE MONITORING

Analysis of environmental DNA samples confirmed the presence of *T. bryosalmonae* at several monitored farm sites. qPCR analysis detected parasite DNA concentrations ranging from 24 to 12,822 copies per litre of water.

Parasite presence was clearly detected at two farm sites associated with the monitored fish cohorts. Environmental monitoring also detected parasite DNA at an additional site between October and December 2024, while parasite detection at another monitored site remained at or near the assay's detection limit.

Because eDNA sampling at one exposure site began in October 2024, parasite levels before this point could not be determined. However, parasite detection at a second site between May and July 2025 confirmed that fish transferred there earlier in the year were re-exposed to the parasite.

INFECTION PREVALENCE IN FISH

Kidney tissue analysis revealed relatively low levels of infection in the cohort introduced to the farm site in July. Approximately 10–20 per cent of fish from this group tested positive for parasite DNA during both sampling years.

In contrast, fish from the second cohort showed no evidence of infection during 2024 sampling, indicating that they likely remained previously unexposed before exposure in 2025. After exposure the following year, most fish in this group tested positive for parasite DNA.

Development of parasite-specific immune responses
Serological analysis indicated that parasite-specific antibody responses developed gradually during the study period.

No detectable antibody response was observed during the first exposure season or at the start of the second season in either cohort.

By the end of the sampling period in September 2025, however, up to 33 per cent of fish in the first cohort and 25 per cent of fish in the second cohort had developed measurable antibody responses. Sampling constraints during the summer of 2025 prevented the assessment of antibody levels during part of the re-exposure period.

Comparisons with samples collected from trout farms in southern England indicated that Scottish samples produced broadly similar but generally lower and more variable antibody signal-to-noise ratios when tested against different recombinant antigen variants.

HISTOLOGICAL OBSERVATIONS

Histological examination of kidney tissue samples identified mostly mild pathological changes consistent with low-level PKD infection. Samples collected between November 2024 and May 2025 exhibited occasional mild interstitial cell proliferation associated with PKD, but parasites were not observed in these tissues.

Some fish showed mineralised deposits in kidney tubules consistent with nephrocalcinosis, alongside minimal to mild tubular changes and nephritis. These changes were generally not considered severe enough to compromise kidney function.

Later samples collected in September 2025 showed similarly mild pathological changes in most fish. Only one individual exhibited more advanced pathology, including widespread tissue hyperplasia and numerous parasites within the kidney tissue.

RAPID DETECTION ASSAY DEVELOPMENT

Proof-of-concept experiments demonstrated that the combined RPA and CRISPR-Cas12a assay could detect parasite DNA at concentrations as low as approximately 10 DNA copies in controlled reactions. Fluorescence-based detection enabled measurable signals within 15-30 minutes, indicating strong potential for rapid diagnostic applications.

The system was also tested using lateral flow test strips. These produced visible detection signals corresponding to parasite DNA concentrations, although the results highlighted the need for further optimisation to eliminate background noise and improve reliability.

Despite these limitations, the assay demonstrated strong potential as a rapid field-based diagnostic tool capable of detecting parasite DNA in fish tissues or environmental samples without laboratory infrastructure.

IMPACT

The project provided new insights into the interactions between parasite presence in the environment, infection dynamics in fish, and the development of adaptive immune responses. These findings have improved the understanding of PKD risk dynamics on Scottish trout farms and enabled the industry partner to make more informed decisions regarding production planning and stock management.

Environmental DNA monitoring generated site-specific profiles of parasite presence, allowing producers to identify periods of elevated infection risk and adjust farm operations accordingly. This approach provides a practical framework for evidence-based risk management that could be applied across other trout production sites.

The work also advanced the development of rapid on-site diagnostic tools for PKD detection. Although further optimisation is required, the CRISPR-based detection system represents an important step toward providing fish farmers with accessible tools to monitor parasite presence directly on the farm.

The project also demonstrated a relationship between the magnitude of environmental parasite signals and infection intensity in fish stocks. This observation suggests that infection pressure may influence disease severity, offering a more nuanced understanding of PKD dynamics than previously recognised.

Beyond technical outcomes, the project helped establish a broader collaborative network among researchers and industry stakeholders focused on PKD research. This network provides a platform for continued collaboration, data sharing, and the development of coordinated research strategies to address PKD challenges across the aquaculture sector.