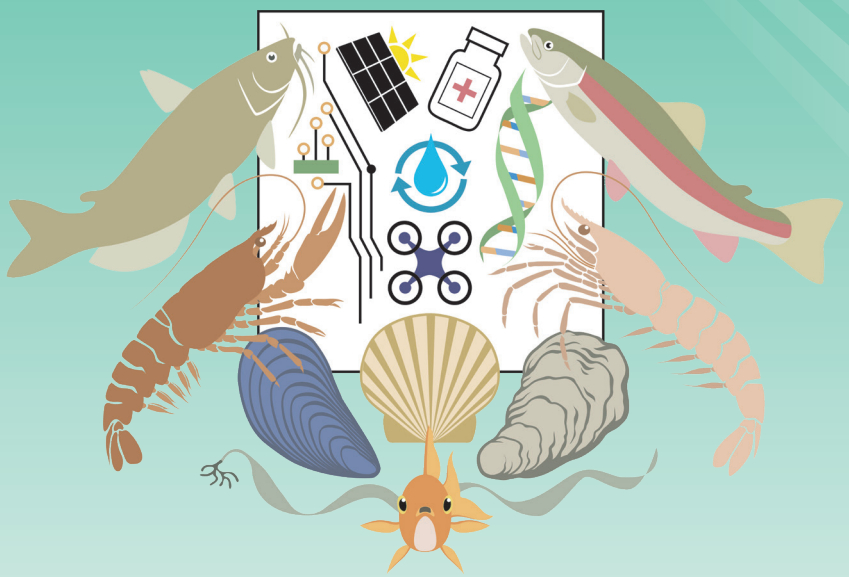
Aquaculture 2025

Innovation Through Technology



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DEVELOPMENT OF DIGITAL DROPLET PCR FOR THE DETECTION OF *Tetracapsuloides bryosalmonae* FROM WATER AND TISSUE OF PKD NATURALLY INFECTED RAINBOW TROUT

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Aim of the project RESILTROUT (Aquaculture resilient to global climate changes: research to support Italian rainbow trout production) is to perform a genome-wide association study (GWAS) to identify DNA markers for resistance to proliferative kidney disease (PKD) to be used in selective breeding programmes. GWAS will be conducted using a medium-density SNP array on dead and survived fish in natural outbreaks. Water samples were also collected to detect the presence of the *Tetracapsuloides bryosalmonae*, the causal agent of PKD.

A first screening on dead fish and water, for *T. bryosalmonae* detection, was performed using end-point PCR described by Kent et al. (1998) amplifying a 435-bp segment of the SSU-rDNA gene. Digital droplet PCR (ddPCR) was, instead, developed for absolute quantification and phenotype definition, establishing a ranking of infestation to support the role of *T. bryosalmonae* as primary cause of mortality. Specific primers and probes described for real-time PCR by Sieber et al. (2023) were used.

All dead fish analysed so far tested positive from kidney samples, with some spleen samples initially testing negative but confirmed positive by ddPCR. Water samples also tested positive for the pathogen. Figure 1 reports end-point PCR tested (spleen and kidney) while Figure 2 reports ddPCR plots for spleens negative in end-point PCR but confirmed positive, undiluted, with ddPCR (1S and 2S; B02 and G01); a kidney positive in end-point and confirmed diluted (1:100) in ddPCR (4K and F02); D01 was a kidney negative in end-point PCR tested undiluted in ddPCR and still negative. Results confirmed the high sensitivity of dd-PCR and its useful application for establishing a ranking of infestation.

Figure1. End-Point PCR.

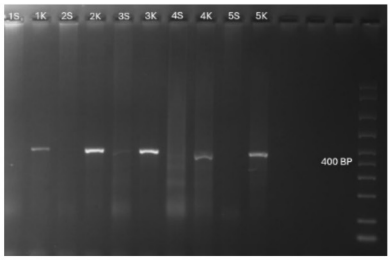

Figure 2. dd-PCR.