
Detecting Trouble Before It Spreads: Optimising Environmental DNA Detection of *Bonamia ostreae* in Flat Oysters

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Résumé

The protozoan parasite *Bonamia ostreae* poses a persistent threat to the restoration and aquaculture of the European flat oyster (*Ostrea edulis*), necessitating scalable and non-lethal diagnostic tools. This study refines environmental DNA (eDNA)-based surveillance methodologies for *B. ostreae* by standardising quantitative PCR (qPCR) workflows and benchmarking passive sampling strategies. Across six experimental trials in static and flow-through systems, we evaluated the effects of filter material, water volume, incubation temperature, and exposure duration on detection performance. Filtration using 0.2 μ m mixed cellulose ester membranes consistently yielded the highest target DNA recovery and outperformed other passive materials, including 0.45 μ m membranes, glass microfiber filters, and cellulose sponges. While longer incubations and warmer temperatures (12 - 20°C) enhanced detectability in both water and passive samples, detection success sharply declined under cold conditions (-1°C) and in oysters with low infection intensities, where false negatives were most frequent. Sponges exhibited poor retention across all conditions. Detection accuracy was strongly influenced by the timing between tissue and eDNA sampling: concordance dropped significantly beyond 60 days, highlighting challenges in monitoring chronic infections and the need for synchronised sampling to validate disease status. Furthermore, deployment of these methods to evaluate wastewater treatment in aquaculture systems revealed inconsistent removal of *B. ostreae* DNA by microfiltration units, raising concerns about biosecurity reliability. Collectively, these findings advance non-lethal diagnostic approaches by identifying key parameters that influence detection sensitivity. The study supports the development of standardised eDNA surveillance protocols to enhance early disease detection, system monitoring, and sustainable management of flat oyster aquaculture.

Mots-Clés: *Bonamia ostreae*, *Ostrea edulis*, eDNA, qPCR, passive sampling, protozoan, parasite, aquaculture, low trophic, disease monitoring, waterborne pathogens, molecular diagnostics

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