HIGH-THROUGHPUT NON-INVASIVE ENVIRONMENTAL MONITORING IN RECIRCULATING AQUACULTURE SYSTEMS: ASSESSING FISH HEALTH

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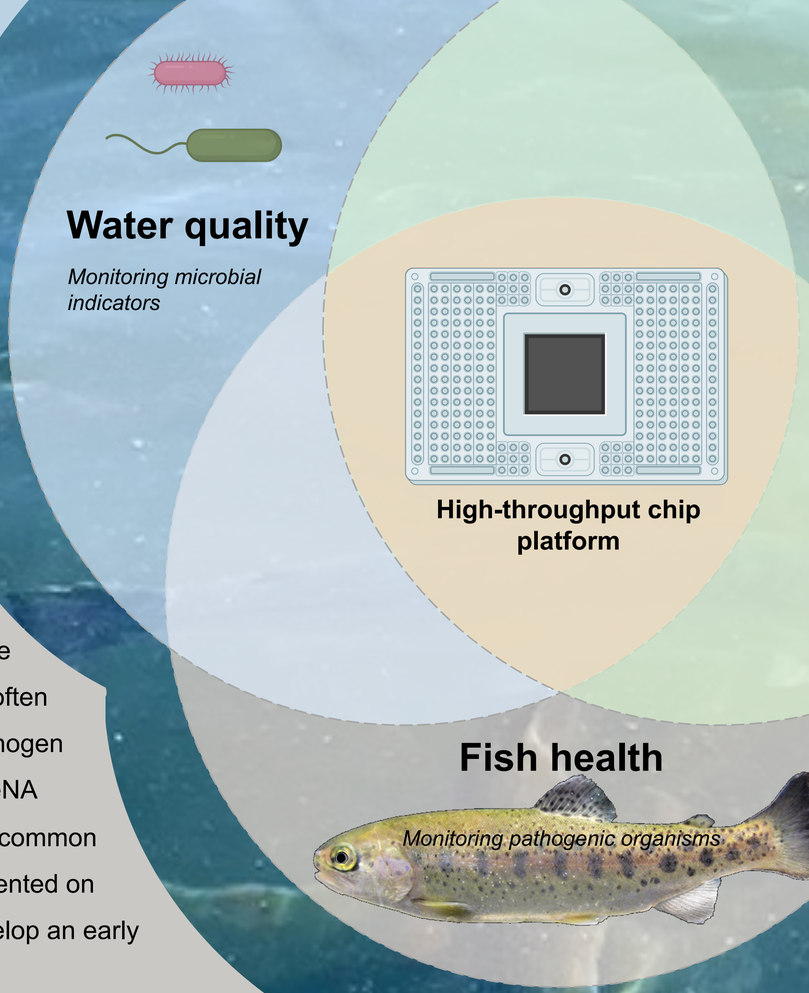
INTRODUCTION

The RASOPTA project aims to address and bridge critical knowledge gaps in the production of key aquaculture species essential to human nutrition and the European economy. It also serves as a training platform, educating 12 Early Stage Researchers (ESRs) in cutting-edge RAS technologies. The program is structured into three interconnected work packages (WPs), integrating academic knowledge with practical experience from the RAS industry. Research efforts focus on species of high economic importance in Europe, including salmon, rainbow trout, pike-perch, and sturgeon (for caviar production). Focus is on 1) water quality, 2) off-flavour, and 3) fish health. Central to its research objectives is the development of a novel chip platform tailored for the Recirculating Aquaculture Systems (RAS) industry, which we will be focusing on here. The chip utilizes environmental nucleic acid (eNA) to detect microbial indicators important for water quality, off-flavour genes and pathogenic organisms critical for fish health.

WP1: RAS enable long-term water reuse, but accumulating fine solids and dissolved organic matter can degrade water quality, favor fast-growing heterotrophs, and harm fish health. Effective system design, treatment, and management are essential, yet microbial dynamics in RAS remain poorly understood. This water quality focus aims to advance RAS technologies through biofilter optimization. Nitrifying biofilters—key microbial hotspots—are studied for their role in improving microbial water quality, preventing bacterial invasion, and serving as potential pathogen reservoirs. Another goal is to identify key microorganisms affecting water quality and integrate them as targets on the chip platform, providing insights into RAS microbiome balance.

WP2: RAS farms can produce high-quality seafood but often suffer economic losses from “musty” or “earthy” off-flavours, mainly caused by geosmin and 2-methylisoborneol (2-MIB). Conventional mitigation methods like ozonation and depuration are costly and stressful for fish. Advances in molecular and sensory techniques now allow earlier detection of off-flavour-producing microbes, yet knowledge gaps remain in their biology, chemistry, absorption pathways, and consumer perception. RASOPTA tackles these gaps with novel strategies to identify dominant off-flavours in water and fish. Targets for gene abundance and activation (geosmin & 2-MIB) were also developed for monitoring on the chip platform.

WP3: Maintaining fish health and welfare is crucial to preventing infectious disease outbreaks in RAS. Understanding the host–pathogen–environment triad is key to moving from reactive to proactive man-agement. This work package focuses on early detection through water sampling and eNA moni-toring as indicators of pathogen presence and disease risk—an improve-ment over traditional methods that often detect disease too late. Work included assessing pathogen diversity, identifying entry points, and characterizing eNA profiles during outbreaks. Assays for a wide range of common and economically significant pathogens were implemented on the high-throughput qPCR-based chip (Fig. 1) to develop an early warning system for RAS.



METHODS

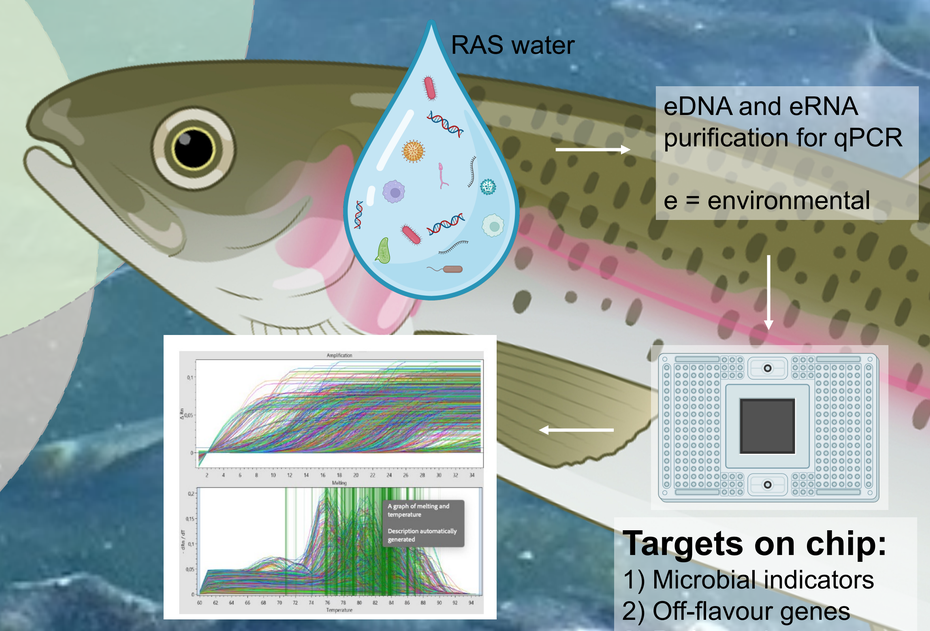


Fig. 1. qPCR data from a chip run and below melting curves

RESULTS

As part of the RASOPTA project, datasets were generated on water quality, off-flavour, and fish health. This report focuses specifically on the eNA chip-based data. Analyses were performed for both eDNA and eRNA (Fig. 2) collected from RAS facilities in Denmark and revealed distinct profiles of infectious agent composition between eDNA and eRNA, with eDNA detecting a broader spectrum of agents. eDNA captures signals from both viable and non-viable organisms, whereas eRNA reflects live organisms. Data also showed pathogen co-occurrence pat-terns within the systems (Fig. 3). A greater number of statistically significant correlations were identified in eDNA datasets compared to eRNA, indicating eDNA's potential to reflect broader historical and environmental interactions. Notably, eRNA data showed strong concordance with veterinary field diagnoses, particularly in the identification of active infections caused by specific pathogens. In addition, the biosynthesis of the main off-flavour compounds—geosmin and 2-methylisoborneol (2-MIB)—was assessed across water, air, and fish tissue samples (Fig. 4). Among these matrices, a high detection rate was evident in air samples, suggesting volatilization dynamics may play a key role in off-flavour dissemination within RAS environments.

Fig. 2. *Relative abundance of pathogens detected with eDNA (top) and eRNA (bottom) purified from RAS water samples.*

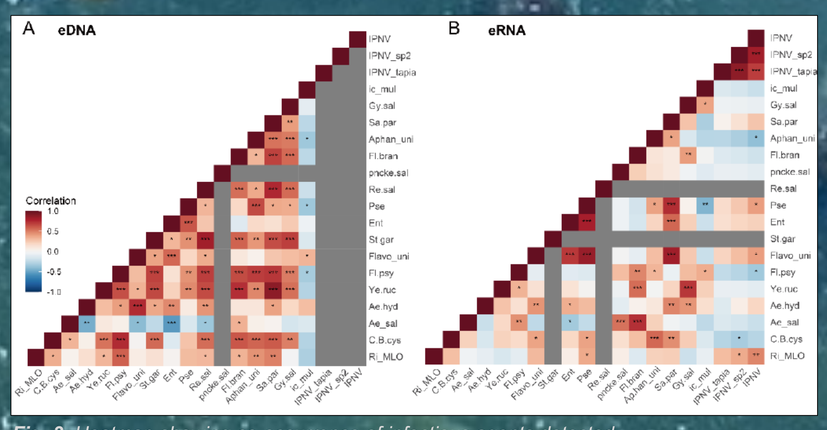


Fig. 3. *Heatmap showing co-occurrence of infectious agents detected with eDNA (A) and eRNA (B) purified from RAS water samples.*

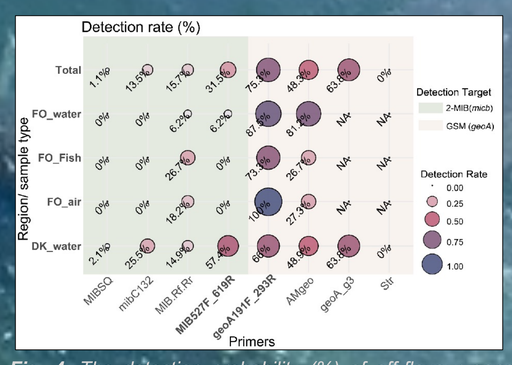


Fig. 4. *The detection probability (%) of off-flavour gene activation across water, air and fish tissue samples from Denmark and Faroe Island. Geosmin and 2-MIB were targets.*

DISCUSSION

With the increasing application of eNA-based technologies in aquatic research, our findings further support the utility of this approach. Specifically, eRNA-based profiling offers a more accurate snapshot of real-time biological activity compared to eDNA-based methods, which, in contrast, capture a broader taxonomic spectrum, including both current and legacy signals. The distinct co-occurrence patterns observed between eDNA and eRNA suggest potential co-infections reflective of both historical and ongoing farm conditions. This dual approach holds significant promise for aquaculture, particularly for the early detection of pathogenic organisms and off-flavour-producing microbes, thereby enabling timely and targeted management interventions.

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