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Continuous nitrite and nitrate monitoring of recirculating aquaculture systems using a deployable ion chromatography-based analyser

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Abstract
Water quality and the management of nitrogenous compounds are of key importance within aquaculture. In this paper, automated and portable analysers for in situ analysis of nitrite and nitrate in water were deployed in freshwater and saline recirculating aquaculture systems (RAS). The analysers were based upon ion chromatography (IC) and employed a NaCl eluent with an anion exchange guard column for low backpressure anion separation, in combination with selective 235 nm ultra-violet light-emitting diode (UV-LED) based absorbance detection. The analysers were monitored and delivered real-time concentration data using a cellular internet of things (IoT) module and cloud-based dashboard. Overall performance and chromatographic repeatability were tested across various temperature profiles and 500 sequential runs within the laboratory. Deployments in freshwater and saline RAS, with concentrations ranging between 0.1–3.6 mg L^{-1} nitrite and 0.6–392 mg L^{-1} nitrate, were successful and the analytical performance was comparable to that of accredited lab-based instrumentation.

Keywords Recirculating aquaculture system · Nutrients · Nitrite · Nitrate · Biofilter · Real-time water quality monitoring

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Introduction

On a global scale, aquaculture is the fastest-growing food-production technology with 87.5 million tonnes of aquatic animal product produced in 2020 (Hough, 2022). Due to environmental concerns associated with cage and freshwater pond aquaculture, recirculating aquaculture systems (RAS) are expected to minimise the environmental impact caused by the sector (Yogev and Gross, 2019). RAS refer to those aquaculture systems in which water exchange is limited and water is recirculated between the culture and water treatment stages. Benefits brought by RAS include no major water loss, minimal impact on local biodiversity by reduced disease outbreak, and control of water quality while reducing water discharge (Ahmed and Turchini, 2021). RAS include fresh, brackish, and fully saline water (Hüpeden et al., 2020).

Water quality in RAS is important since only 20–30% of the applied feed is recovered as fish biomass while the rest of the unassimilated nitrogen is released into water (Yogev et al., 2017). RAS can recycle 90–99% of water (Badiola et al., 2012) with nitrification and denitrification bioreactor technology promoting total nitrogen removal, which is part of water quality management in RAS (Preena et al., 2021). Saline RAS present further challenges since the salinity effect on nitrification kinetics is not well understood and can impact biofilter performance, jeopardising water quality (Kinyage et al., 2019). Hüpeden et al. (2020) observed reduced nitrification performance from freshwater RAS nitrifiers when exposed to increased salinity, highlighting the sensitivity of the biofilter and its performance towards salinity shifts. The removal of nitrogenous compounds is also important for fish welfare as nitrite (NO_2^-) tolerance limits in freshwater aquaculture range from 0.005–1 mg L^{-1} (Ciji and Akhtar, 2020) Also, excessively high levels of nitrate (NO_3^-) can have an impact on fish health and growth (Yogev and Gross, 2019; Davidson et al., 2014) with toxicity levels ranging from 50–500 mg L^{-1} depending on fish species (Li et al., 2023). Nitrate levels have been reported to increase from 100–1000 mg L^{-1} due to minimal water renewal with long-term nitrate exposure becoming a concern in RAS production (Yu et al., 2021). For saline waters, an inverse relationship between salinity and toxicity has been reported (Kir and Sunar, 2018) leading to higher nitrite toxicity tolerance in fish found in high-salinity waters (Tomasso, 1994). For nitrate toxicity, marine species have been reported to have a tolerance above 500 NO_3-N mg L^{-1} (Pierce et al., 1993). If fish welfare is compromised due to poor water quality, the productivity of the system will also be jeopardised, therefore monitoring of nitrogenous compounds in RAS is critical.

Nitrite and nitrate monitoring within aquaculture systems is typically carried out through grab sampling followed by colorimetric analysis (Guerdat et al., 2010; Liu et al., 2017; Tadda et al., 2021). The most commonly applied colorimetric assay for nitrite/nitrate detection is the Griess reaction followed by spectrophotometric detection at a wavelength of 540 nm (APHA, AWWA, WEF, 1998; García-Robledo et al., 2014; Nesterenko et al., 2016). Using a grab sample approach has multiple drawbacks. It is labour-intensive, costly, and results are only provided for a specific point in time. In addition to these drawbacks, grab samples which are sent to a laboratory need to be preserved and transported. According to APHA standard methods (APHA, AWWA, WEF, 1998), incorrect or delayed sample preservation can cause a decreased NO_2^- result because of bacterial conversion of NO_2^- to NO_3^- within the sample. To minimise fish exposure to toxic NO_2^- levels by quickly responding to a change in concentration, to obtain a complete picture of the nitrification/denitrification processes, and to maximise production, real-time, high frequency, and accurate concentration information is essential (Badiola et al., 2012).

Several commercially available direct UV (ultraviolet) detection probes exist for in situ nitrate analysis in water systems (HACH 2023; OTT HydroMet, OTT ecoN, 2019; SEA·BIRD SCIENTIFIC, SUNA V2 Nitrate Sensor, 2023; TriOS Optical Sensor, Nico, 2023; YSI Xylem Brand, EXO NitraLED UV Nitrate Sensor, 2023). However, these UV probes do not selectively and directly detect for nitrate and nitrite and are often majorly impacted by matrix effects and interferences present within natural waters, as a range of dissolved constituents absorbs light within the UV region. These include inorganic constituents, such as bromide, iodate, and hydrogen sulphide, as well as dissolved organic matter (Pellerin et al., 2013). For these reasons, such probes are not well suited for robust, continuous in situ analysis within RAS.

In recent years, new technologies have been developed for in situ analysis of nitrate and nitrite based upon lab-on-a-chip (LOC) technology employing colorimetry. These systems have shown promise for environmental monitoring and several successful field deployments have been performed (Beaton et al., 2017; Catini et al., 2022; Nightingale et al., 2019). However, these technologies remain poorly implemented or adopted due to a range of drawbacks. Firstly, in situ colorimetry-based systems are often complex and require the use of hazardous, toxic reagents. On-board internal standards are required to account for the analytical drift of the system. Sample turbidity and high-salinity content can also severely impact the accuracy of analyte detection. Finally, costs and technical challenges arise when considering large-volume production, mass manufacture of complex microfluidic components, and assembly of LOC systems (Ríos et al., 2012). In a recent study, Altahan et al. (2022) reported an optimised multi-macronutrient analyser for seawater deployments which employed automated colorimetry, again highlighting the potential of colorimetric-based analysers when considering real-time monitoring. Another example of a microfluidics approach is paper-based microfluidic devices employing colorimetry for the detection of NO_2^- and NO_3^- in water samples (Tesfaye and Hussen, 2022; Rajasulochana et al., 2022). Charbaji et al. (2022) focused on the optimisation of a paper-based microfluidic device and have demonstrated inexpensive lightboxes that can be obtained through 3D printing for the application of this technology in the field (Charbaji et al., 2022). Although promising for cost-effective on-site testing, these solutions do not deliver continuous in situ concentration data which is remotely accessible.

When considering the determination of inorganic anions such as nitrite and nitrate in complex water matrices like wastewater or process water, ion chromatography (IC) is a widely used technique within a laboratory setting (Michalski, 2018). IC methods have numerous advantages over colorimetric methods, including no requirement for multiple reagents, simple or no sample pre-treatment, and high selectivity in complex samples (Michalski, 2018). Developments in automation techniques, separation phases, and advancements in UV light-emitting diodes (LEDs) for optical detection, have collectively enabled the development and deployment of portable chromatographic analysers (Elkin, 2014; Fitzhenry et al., 2021; Kiplagat et al., 2010; Lace et al., 2022; Murray et al., 2020). In 2020, Murray et al. (2020) described a portable and deployable IC-based analyser for nitrite and nitrate determination in both freshwater and wastewater. Selective detection of these anions was achieved through rapid ion chromatography in combination with a 235 nm LED coupled with a photodiode for direct UV detection (Murray et al., 2019). During field deployments, the analyser demonstrated high precision and accuracy comparable to accredited laboratory-based methods. This portable analyser has been further developed since then and is now a commercially available analyser and platform.

Within this work, this portable in situ nitrate and nitrite IC-based analyser, integrated with an internet of things (IoT) software platform, was employed for the first time to deliver

real-time continuous monitoring within freshwater and fully saline RAS. The unique capability of the system to provide continuous and real-time nitrate and nitrite levels within both freshwater and saline RAS facilities is explored and presented. Through the generation of accurate real-time nitrite and nitrate concentration information, biofilter status and condition is informed along with information associated with feeding patterns. The analytical performance and repeatability of the analyser were demonstrated using freshwater and seawater samples, and the impacts of temperature on system performance were investigated. Two analysers were used within this study. One system was deployed and tested within the freshwater RAS of the Marine Institute, Newport Catchment Facilities, Ireland, and the other system was deployed within a fully saline RAS located in Kingfish Zeeland in Kats, The Netherlands.

Materials and methods

Chemicals and reagents

High-purity deionised water (Milli-Q) was used for the preparation of standards and solutions. Analytical grade chemicals (or higher) were used for the preparation of all solutions. Nitrate and nitrite standard solutions were prepared from dilutions of a 1000 mg L^{-1} NO_2^- and a 1000 mg L^{-1} NO_3^- certified reference standard (CPAchem). Eluent was sodium chloride at 120 mM prepared from NaCl salts (Sigma Aldrich).

Artificial full saline matrix (35 ppt) was prepared with analytical grade chemicals from Sigma Aldrich including sodium chloride (NaCl), magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2 \times 6H_2O$), calcium chloride ($CaCl_2$ x $2H_2O$), and sodium bicarbonate ($NaHCO_3$) (Fitzhenry et al. 2021). To prepare artificial 20 ppt salinity solution, 35 ppt solution was diluted. These solutions were then spiked with CPAchem nitrite (NO_2^-) and nitrate (NO_3^-) standards to achieve desired concentrations.

Automated nitrate and nitrite analyser

The portable nitrate and nitrite analyser (Aquamonitrix Ltd., Ireland) used in this work was based upon the IC platform and a 235-nm LED-based absorbance detector module, previously reported by Murray et al. (2020) and Bluett et al. (2023). Within this work, the portable IC configuration used 120 mM sodium chloride eluent at a flowrate of 0.65 mL min^{-1} and the IC column was a 4 × 50 mm Dionex AG15 guard column (Thermo Fisher Scientific, Sunnyvale, CA, USA). A 10 µL sample loop was used and detection was achieved using the above-mentioned direct absorbance detector. A detailed fluidic schematic and photographs of the analyser are shown in appendix A1 and A2 of the electronic supplementary information (ESI). The nitrite and nitrate calibration curves used for the determination of nitrite and nitrate concentrations in freshwater, brackish, and fully saline matrices are illustrated in Figure B1 and B2 of the ESI. The analytical ranges of the analyser were 0.2–100 mg L^{-1} NO_2^- ($R^2 = 0.999$) and 1–500 mg L^{-1} NO_3^- ($R^2 = 0.999$). The limit of detection (LOD) for nitrite and nitrate was 0.1 mg L^{-1} NO_2^- and 0.5 mg L^{-1} NO_3^-. LOD levels were calculated using a signal-noise-ratio (S/N) = 3 and the procedure as set out within Fitzhenry et al. (2021). The analyser included a FX30 IoT Module (Sierra Wireless, Richmond, Canada) which was connected to the system to transmit the concentration readings generated by the analyser to a cloud platform/database. From this platform, the results could be monitored remotely in real-time. The IoT platform dashboard is visually shown in appendix C1 of the ESI.

Robustness assessment

Robustness of the system was assessed through environmental chamber testing. The analytical system was placed inside an environmental chamber (Vötsch VC0100 Climate Chamber, Balingen, Germany) with controlled temperature and humidity. A freshwater sample matrix with concentrations of 7 mg L^{-1} NO_2^- and 20 mg L^{-1} NO_3^- was used for sequential chromatogram production. A total of two temperature cycles, low temperature (LT) cycle (7 °C to 21 °C) and a high temperature (HT) cycle (19 °C to 40 °C) with 60% humidity were used. LT cycle decreased 1 °C/h, with a 3-h incubation period at 7 °C. HT increased 3 °C/h, with a 3-h incubation period at 40 °C. A camera system was set-up inside the chamber to allow for the monitoring of leaks and air bubbles. Sampling frequency occurred every 18 min.

Deployments

The freshwater RAS was located at the Marine Institute Freshwater Research Facility, Newport, Co. Mayo, Ireland. The RAS (total volume of 44,000 L) consisted of eight 5.5 m^3 square fibreglass tanks complete with a drum filter, biotower, degassing tower, UV filtration, and oxygenation systems. The RAS was stocked with 8598 Atlantic salmon (*Salmo salar*) at an average weight of 39 g on a gradual basis between September 28th and October 6th, 2021. During the trial period from September to November 2021, the fish ranged in weight from 39 to 58 g (average). The fish were sourced as eyed ova from two commercial companies, a mixed-sex Irish strain (Mowi Ireland) and an all-female Icelandic strain (Stofnfiskur, Benchmark Genetics Iceland HF). Fish were fed with a commercial diet (EWOS Harmony 40P, Cargill/EWOS Ltd., Scotland) at a rate of 1.9% body weight per day, using automatic feeders (Arvo-Tec, Finland). Grab samples of 100 mL of water, in a sterile plastic container, were taken from the outflow of the biotower during the trial. All water samples were immediately filtered through 0.45 µm cellulose filter paper and stored at −20 °C. Samples were analysed commercially for nitrate and nitrite (Aquatic Services Unit, University College Cork, Ireland).

The saline RAS (34 ppt) was located at Kingfish Zeeland in Kats, The Netherlands. The RAS consisted of four 1650 m^3 round concrete tanks complete with two disk filters, two degassers, one moving bed and one fixed bed biofilter, one protein skimmer with ozone dosing, UV filtration, and oxygenation systems. The RAS contained yellowtail kingfish (*Seriola lalandi*) with individual weights ranging from 763 to 2341 g during the analyser deployment period. The deployment period extended from August 17th to September 20th, 2022. Fish were sourced from the in-house hatchery and constituted a mixed-sex population. Fish were fed two commercial diets until they appeared satiated. Fish were fed using automatic feeders, with the daily ration being spread out over 18 h. Grab samples of 500 mL of water were collected in sterile plastic containers from the sump after the disk filters. All water samples were filtered through 11 µm cellulose filter paper (Whatman 1001-150) immediately after collection and stored at 4 °C. Samples were analysed commercially for nitrate and nitrite according to NEN-ISO 15923-1 (Normec All Water Services, 's-Hertogenbosch, The Netherlands).

The Aquamonitrix analysers were calibrated in a factory using a total of 8 freshwater standards (0.2–100 mg L^{-1} NO_2^- / 1–500 mg L^{-1} NO_3^-). Linear calibration plots were obtained for both nitrite and nitrate and stored automatically on the analysers. Based on the

sample concentrations estimated within the deployment sites, the relevant calibration range was chosen from this linear range.

Results and discussion

Analytical performance

Repeatability of measurements was assessed by sequential chromatogram generation for freshwater and saline matrix samples with concentrations of 1 mg L^{-1} NO_2^- and 15 mg L^{-1} NO_3^- over 7 days at room temperature. Sampling frequency was set for automatic analysis every 15 min. Figure 1 illustrates the concentration results generated through the sequential testing.

For both sample matrices, % relative standard deviation (RSD) was lower for nitrate than nitrite. The concentrations of nitrate in the samples used for the sequential testing

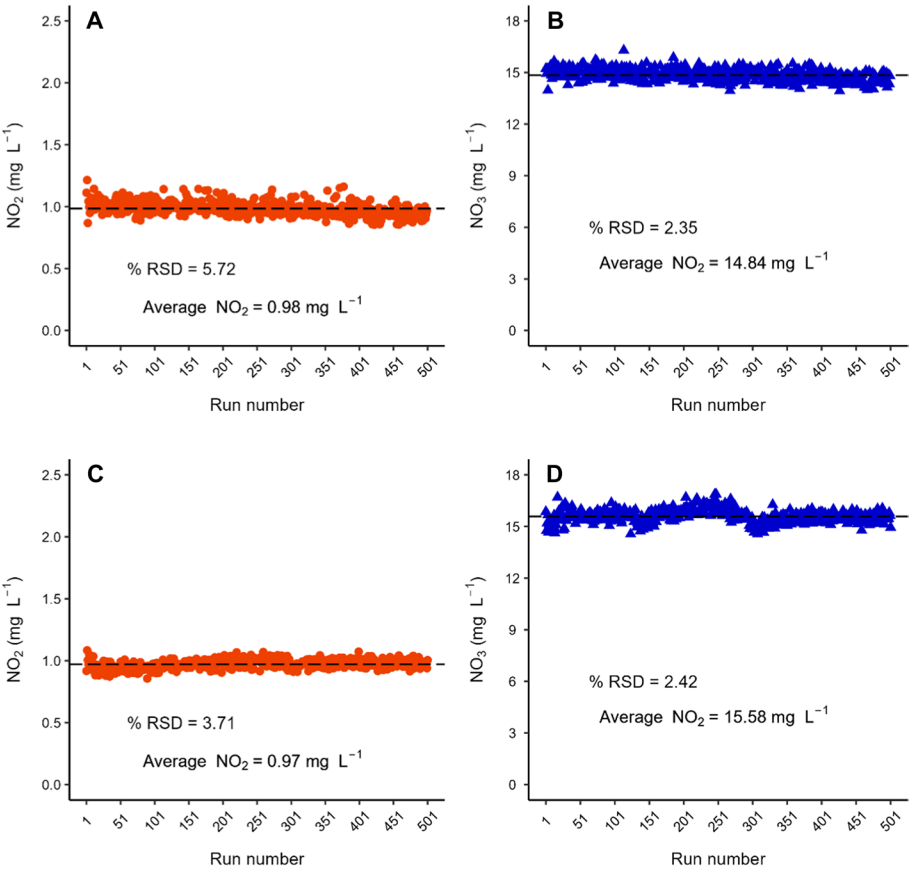

Fig. 1 Freshwater and saline water (35 ppt) sequential test with calculated % RSD and average concentration values over 500 runs (dash line). **A** Freshwater NO_2^- concentrations (orange circle). **B** Freshwater NO_3^- concentrations (blue triangle). **C** Saline matrix NO_2^- concentrations (orange circle). **D** Saline matrix NO_3^- concentrations (blue triangle)

were greater which led to greater precision and therefore lower % RSD values. However, the results obtained were comparable to laboratory-based analytical systems including ion chromatography (IC) systems and were in line with acceptable levels in terms of validation requirements (Jackson, 2015; Shabir 2013). Accuracy was assessed by testing five certified standard quality checks (QCs) of the opposite sample matrix during the sequential analysis. The concentrations of these checks were 1 mg L^{-1} NO_2^- and 15 mg L^{-1} NO_3^-. Throughout the freshwater sequential test, 20 ppt salinity QCs achieved percentage errors of 4% and 0.5% for NO_2^- and NO_3^-, respectively and 35 ppt salinity QCs achieved errors of 7% and 2.5% for NO_2^- and NO_3^-, respectively. Throughout the saline sequential tests, freshwater QCs achieved percentage errors of 7% and 3.6% for NO_2^- and NO_3^-, respectively, and 20 ppt salinity QCs achieved errors of 9% and 3.2% for NO_2^- and NO_3^-, respectively. Through the evaluation of these QC checks accuracy levels > 90% were achieved, despite varying salinity levels. The production of sequential chromatograms demonstrated the repeatability of results in both matrices while only requiring a single calibration prior to testing. These results show the versatility of the system, enabling application in both freshwater and saline matrices. Figure 2 shows examples of chromatograms generated by the system for the analysis of 1 mg L^{-1} NO_2^- and 15 mg L^{-1} NO_3^- standards of varying salinity.

Analytical repeatability at varying temperatures in both LT and HT cycles was successful with no significant variation observed for either analyte concentration (Fig. 3). The % RSD for both temperature cycles were in line with laboratory-based analytical systems and acceptable levels in terms of validation requirements (Jackson, 2015; Shabir 2013) emphasising the precision of the analyser. Air bubbles within system syringes were observed, starting at 34 °C in HT cycle. Bubbles formed in the syringe due to decreased gas solubility with increasing temperatures (Pollack, 1991). However, this did not impact on the performance of the system as no air bubbles were observed in the chromatograms produced by the analyser. These repeatability results across both temperature cycles demonstrate the robustness of the system in relation to temperature and highlighted the applicability of the analyser to the environments of RAS.

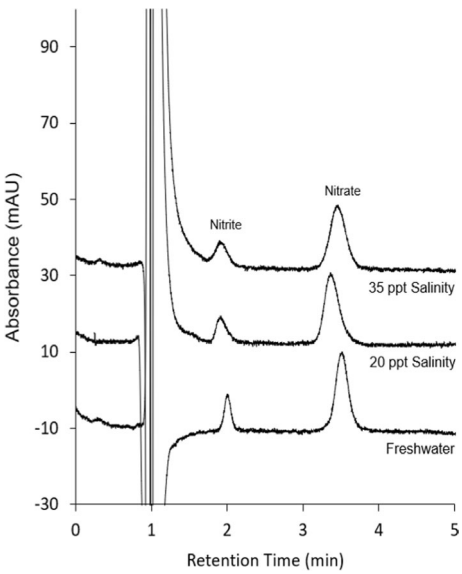

Fig. 2 Chromatograms generated for the analysis of 1 mg L^{-1} NO_2^- and 15 mg L^{-1} NO_3^- freshwater, 20 ppt salinity and 35 ppt salinity sample matrices

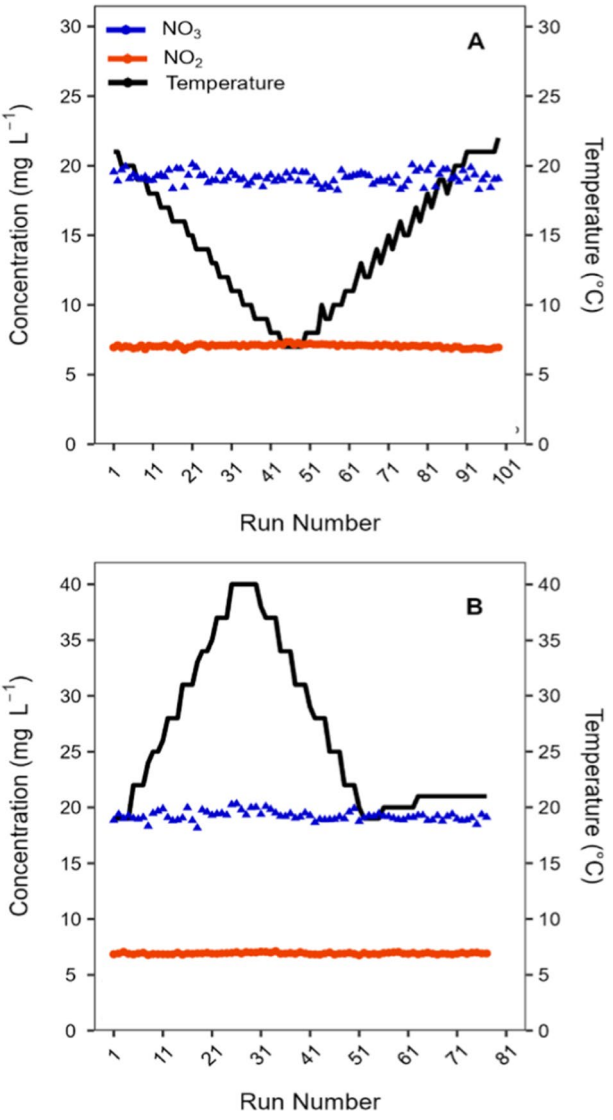

Fig. 3 Temperature cycles showing temperature changes (black line), NO_2^- (orange circle) and NO_3^- (blue triangle) concentrations measured throughout the incubations. **A** Low temperature (LT) cycle; average concentrations were 7.09 mg L^{-1} NO_2^- (% RSD = 1.40) and 19.13 mg L^{-1} NO_3^- (% RSD = 2.18). **B** High temperature (HT) cycle; average concentrations were 6.92 mg L^{-1} NO_2^- (% RSD = 1.08) and 19.23 mg L^{-1} NO_3^- (% RSD = 2.17).

Data generation from RAS

Analysers were deployed in two RAS for a period of 6 weeks. One system was deployed in a freshwater RAS (Marine Institute, Ireland), and a second system was deployed in a fully saline RAS (Kingfish Zeeland, Netherlands). Sampling frequency was set to every 4 h and 2 h for the freshwater and saline RAS deployments, respectively to monitor changes in nitrite and nitrate concentrations.

During the freshwater RAS deployment, a rapid increase of nitrite in the second week of the deployment was observed, which was then followed by a clear and significant

drop in the anion concentration (Fig. 4A). This aligns with the RAS being stocked with fish between September 28th and October 6th, 2021. The nitrite spike reflects the difficulty in maintaining stable nitrite levels which typically fluctuate daily (Mortensen et al., 2022). A gradual increase of nitrate concentration throughout the deployment was also observed (Fig. 4B). Grab samples were taken and analysed using accredited lab-based instrumentation and in situ concentrations generated by the analyser were comparable. The average difference between grab sample and analyser data was 7.1 % for NO_3^- and 3.1% for NO_2^-. The concentration comparison is tabulated in Table D1 of the ESI. While grab samples provided evidence that the analyser was reporting the correct nitrite and nitrate concentrations, the drawbacks of grab samples for the monitoring of nitrite and nitrate in RAS cannot be ignored. The results from this deployment highlight the concentration variation which takes place within RAS. For example, the sharp nitrite increase observed in the second week of the deployment (Fig. 4A) could have been completely missed or detected at its highest point.

Due to the toxic nature of nitrite on fish health (Ciji and Akhtar, 2020), grab sampling cannot safeguard the fish, high sample frequency, real-time data is the only practical solution to provide more immediate monitoring, and thus rapid corrective action when significant nitrite fluctuations occur. During the saline RAS deployment, a clear pattern of nitrite concentrations was observed (Fig. 5A) while nitrate concentrations (Fig. 5B) were less variable during the deployment period. Grab samples were taken and analysed using accredited lab-based instrumentation and again an acceptable correlation was observed for grab sample concentrations and in situ analyser concentrations. The average difference between grab sample and analyser data was 8% for NO_3^- and 3 % for NO_2^-. The concentration comparison is tabulated in Table D2 of ESI.

The results from these deployments highlight the applicability of the analyser technology to sample matrices relevant to RAS. The versatility of the analyser technology to function across sample matrices could allow the system to be used in scenarios where water salinity might shift or change over time. For example, Ytrestøyl et al. (2020) studied the possibility of reducing Atlantic salmon smolt loss by reducing production time in open sea cages by transferring part of the production in land-based RAS, exploring which salinity would be most favourable for optimal growth. Changes in salinity might also aid in the control and treatment of disease outbreaks (Reid et al., 2019). These examples of salinity managements in RAS emphasise the relevance of this nitrite and nitrate analyser technology being reliable and adaptable to those changes.

Matrix type was not the only parameter differing between fresh and saline RAS deployments. The sampling point for the freshwater RAS was post-biofilter while the sampling point for the saline RAS was pre-biofilter. Sampling location might explain the differences in concentration patterns observed between deployments; however, it also highlights another possible application of the analyser. Management of microbes within RAS biofilters can be challenging due to lengthy colonisation and maturation, largely uncharacterised organisms and the possibility of incomplete nitrification leading to a build-up of toxic intermediate nitrite taking place (Bentzon-Tilia et al., 2016). Other factors including tank cleaning, grading, and fish harvesting modify water parameters which in turn will affect the efficiency of the biofilter (Badiola et al., 2012). Access to real-time monitoring of nitrogen anions can inform on RAS biofilter efficiency and stabilisation, ensuring it is functioning at its optimal capacity to provide the required water quality.

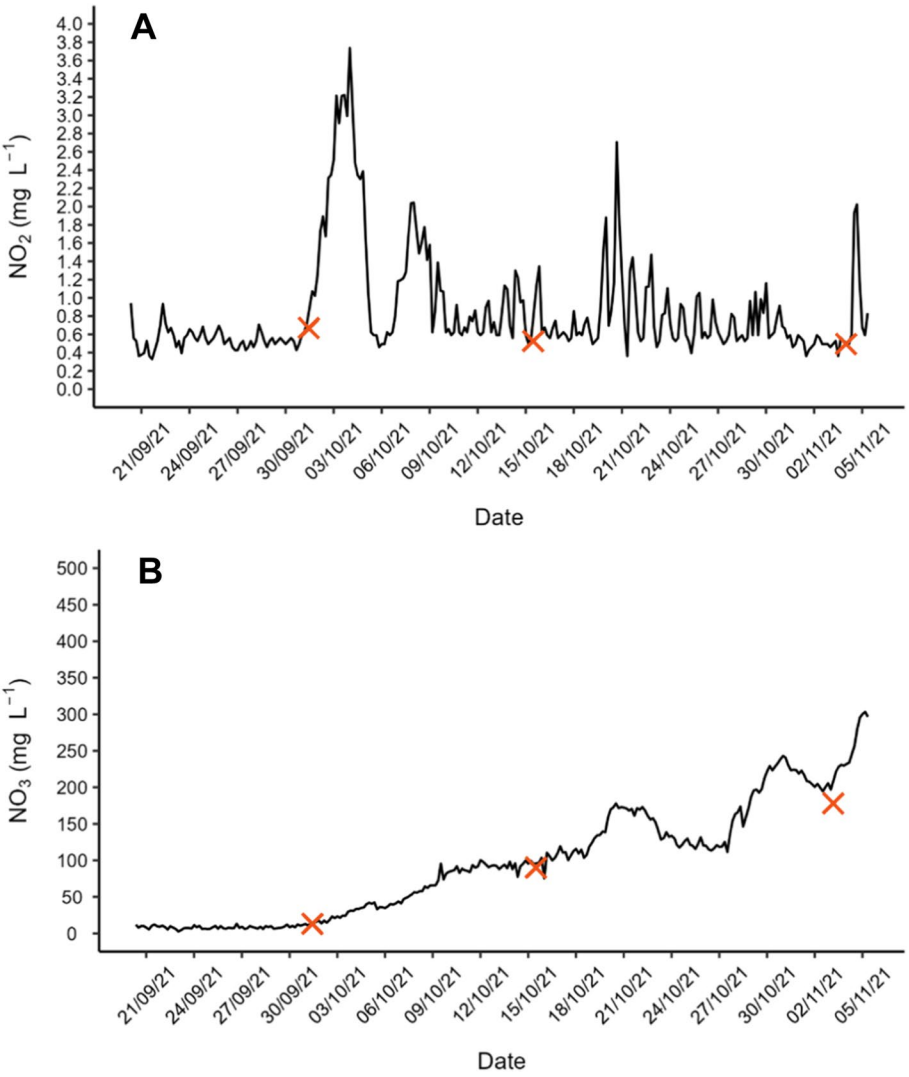

Fig. 4 Nitrite and nitrate concentrations from freshwater aquaculture RAS deployment, Ireland. Grab samples were collected (orange cross) to compare analyser concentrations with laboratory results. **A** Nitrite concentrations from Freshwater RAS deployment. **B** Nitrate concentrations from Freshwater RAS deployment

Conclusions

For the first time a real-time simultaneous nitrite and nitrate analyser, employing rapid ion chromatography, was successfully deployed in two separate RAS of varying salinities. The results from the deployments demonstrate that this analyser technology has the potential to replace the more time-consuming, costly, and inadequate anion monitoring technique of grab and lab analysis, providing in situ monitoring of both nitrite and nitrate concentrations. The analyte concentrations generated by the in situ

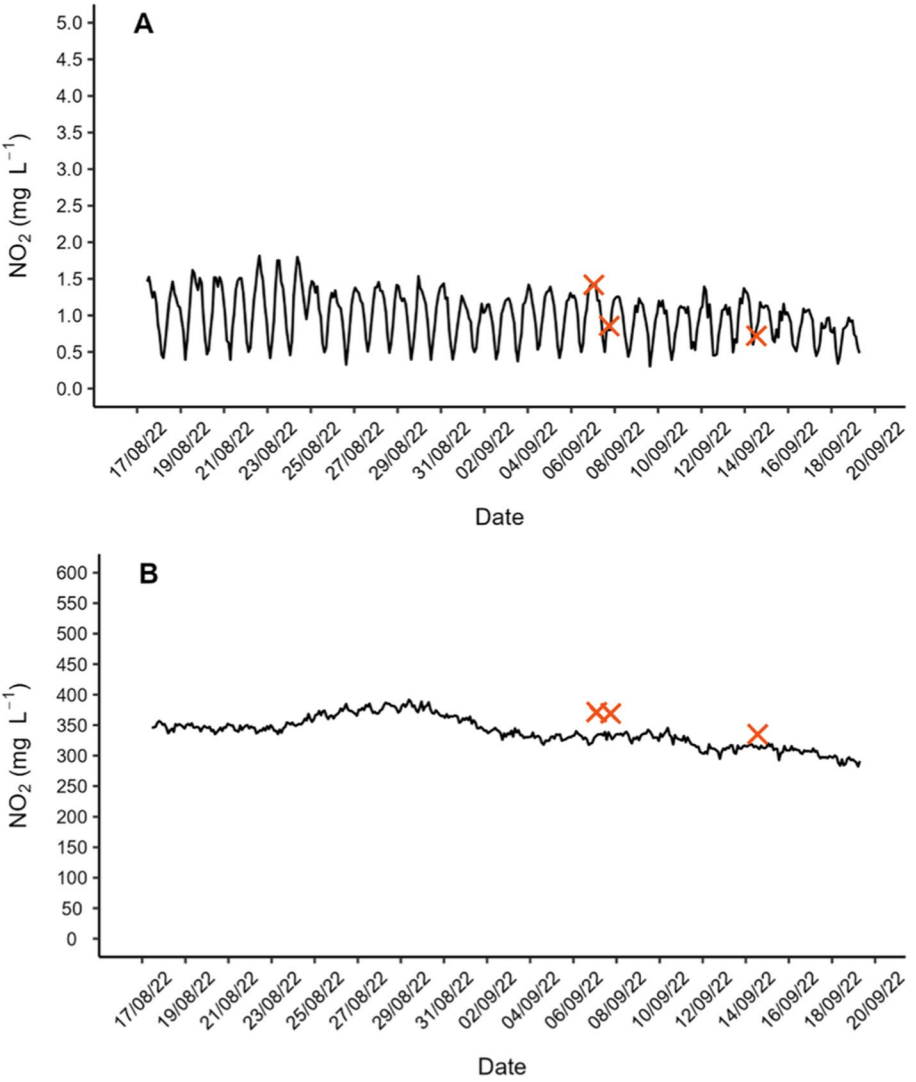

Fig. 5 Nitrite and nitrate concentrations from fully saline aquaculture RAS deployment. Grab samples were collected (orange cross) to compare analyser concentrations with laboratory results. **A** Nitrite concentrations from Netherlands RAS deployment. **B** Nitrate concentrations from Netherlands RAS deployment

analyser for both the freshwater and saline RAS deployments were comparable to grab sample concentrations determined using lab-based instrumentation. Through the application of in situ real-time analysers, monitoring of water quality in RAS could take place to ensure action can be taken before nitrite can build-up above toxicity thresholds of 1 mg L^{-1} NO_2^- (Ciji and Akhtar, 2020). The real-time data could also provide insight on the efficiency and performance of the biofilter within RAS. These RAS deployments demonstrate how this analytical technology could be exploited to add value within the aquaculture sector.

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Availability of data and materials Not applicable

Author contribution M.G.B. and V.A.—methodology, visualisation, writing original draft, data curation; J.D. —investigation, conceptualisation, project administration; N.R. and E.R. —resources, writing review and editing; F.E. —investigation, methodology; T.S. —conceptualisation, writing review and editing, project administration; B.P. —writing review and editing, visualisation; E.M. —conceptualisation, supervision, visualisation, project administration, validation, writing review and editing. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethical approval Not applicable

Competing interests Dr E. Murray receives a salary from Aquamonitrix Ltd. where he is the head of Research and Development.

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