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Protocol for the use of an Optical Nitrate Nitrogen (NO_3–N) sensor for measuring ground and surface water NO_3–N concentrations ☆

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ABSTRACT

Optical nitrate-nitrogen (NO_3–N) sensors are used in environmental monitoring for the real-time detection of dissolved inorganic nitrate and are readily available and increasingly affordable for use by non-experts and may eventually replace the need for expensive laboratory analysis. Many different manufacturers have developed their own instruments for use as permanent *in situ* sensors in groundwater bores, or as portable *ex situ* units. The advantage of these NO_3–N sensors is that they can be deployed to complement traditional discrete sampling programmes and significantly improve temporal data resolution and provide high resolution data that captures the rate that NO_3–N may naturally vary in the environment. However, the potential over dependence on technology i.e. a plug and play approach without careful development of quality assurance protocols can easily lead to poor data outcomes. Thus, the effective use of an optical NO_3–N sensor, especially in community-led science, requires specific sensor protocols for its effective use, including:

- A regimen of cross checks relative to known standards and/or independently verified laboratory results;
- the collection of metadata to contextualise the results; and,
- the need for Quality Assurance and Quality Control (QAQC) protocols to provide confidence in the data.

Specifications table

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☆ **Related research article:** Legg et al. (in press). Nitrate-Nitrogen (NO_3–N) response domains following a large-scale rainfall event on the Hekeao Hinds Plains of Mid Canterbury, New Zealand. Journal of Hydrology.

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Background

The development of optical nitrate -nitrogen (NO_3–N) sensors for permanent *in situ* deployment in groundwater bores as well as *ex situ* portable units has led to several different manufacturers applying the technology to relatively inexpensive stand-alone instruments, which significantly improve the resolution of NO_3–N data collection. NO_3–N sensors can be deployed to complement traditional discrete sampling programmes and allow users to observe high resolution sampling at frequencies in which NO_3–N may actually vary in the environment [1]; albeit at potentially lower precision than traditional analytical methods in low NO_3–N environments. Optical NO_3–N sensors have been used in riverine [2,3], estuarine (e.g., [4,5]) marine [6,7] and groundwater environs [2,8,9] with NO_3–N concentrations between 0.002 mg/L and 50 mg/L [2,10]. The burgeoning use of NO_3–N sensors is now such that it is a readily available technique for environmental monitoring within regulatory organisations but is also being used as a technological widget by community groups, land managers, and consultancies [11–13].

However, the dependence on technology to the point of abdication i.e. a plug and play approach [14,15] and an absence of critical thinking and/or understanding of the results or how they generated is a *"clear prescription for disaster"* [16]. Hence the importance of understanding how data are collected and contextualizing results accordingly, is vital to the success and credibility of any monitoring programme [17,18]. As NO_3–N sensors become the de facto method for observing NO_3–N in the freshwater environmental monitoring space, it is essential that appropriate and cost-effective water quality monitoring protocols are readily available to non-expert users and the data are supported by robust Quality Assurance and Quality Control (QAQC) procedures. Thus, the objective of this paper is to describe the protocols for the effective use of a low-cost optical NO_3–N sensor to inform community-led science, specifically focussed on strategies for monitoring groundwater quality.

Method details

Ultraviolet spectrophotometrically measures NO_3–N content and temperature by measuring the absorption spectra in Ultraviolet (UV) light between 200 and 350 nm [2,19]. UV light is emitted and passes through a measurement cell containing the water sample, after which the intensity, over the whole wavelength range, is measured by a detector and a concentration calculated via a metric calculation from the absorption of the wavelengths (i.e. 220 nm and 270 nm to determine the NO_3–N concentration (Fig. 1a) [6]. The 270 nm waveband is also recorded as a reference to measure the potential effects of contaminants such as chlorine, nitrite, iron (III), and organic matter, that also absorbs UV light and may impede the absorption on the 220 nm waveband [19,20]. The reported outputs are NO_3–N concentration, as well as a dimensionless absorbance unit for the 220 nm and 270 nm readings that can be used for QAQC protocols.

In 2020 MHV (farmer owned irrigation co-operative operating on the Hekeao Hinds Plains of Mid Canterbury New Zealand) purchased a refurbished Hydrometrics GW50 optical NO_3–N probe and converted it to a portable *ex situ* device to reduce water quality monitoring survey time and costs; later in 2022, MHV purchased an additional *in situ* probe and deployed it into a shallow

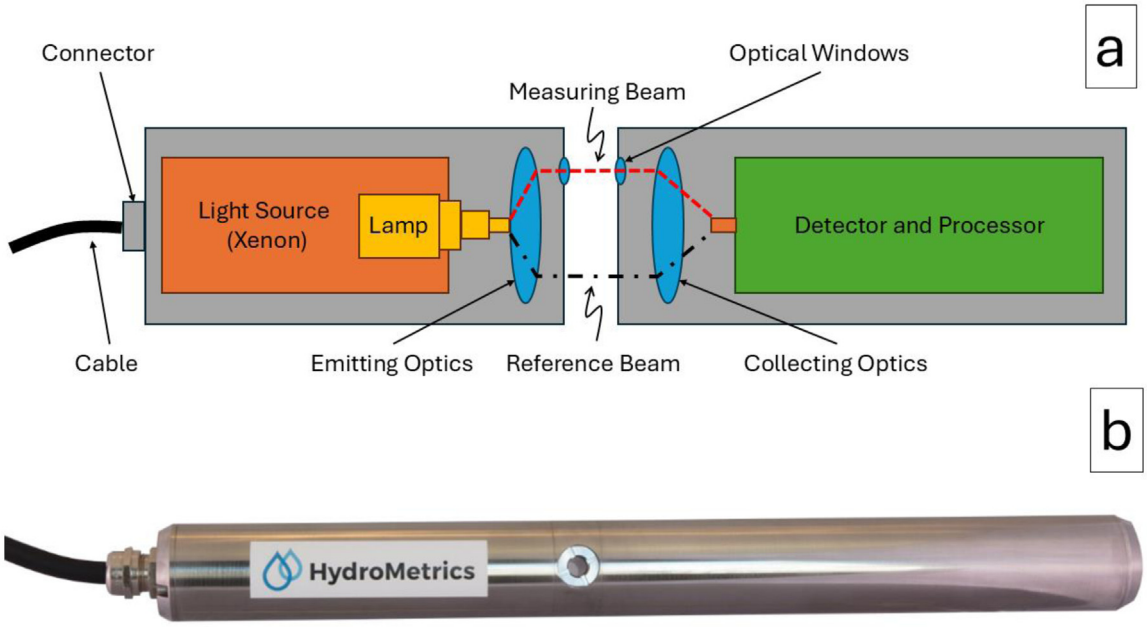

Fig. 1. a) Schematic of an optical NO_3–N Probe [21], b) the GW50 NO_3–N optical probe [22].

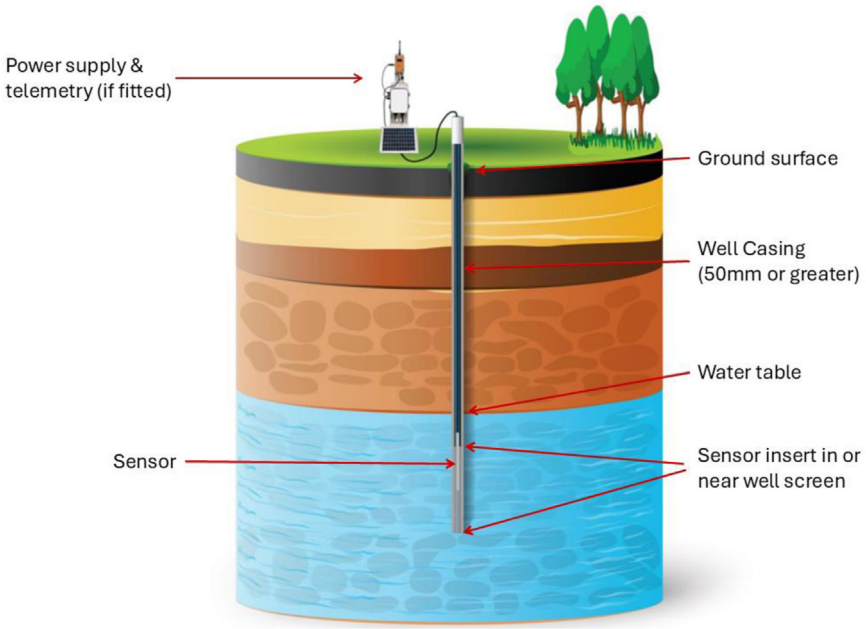

Fig. 2. Schematic *in situ* installation [22].

bore to measure NO_3–N, in real time; hence this paper presents the protocols developed for using both *in situ* and *ex situ* optical NO_3–N probes, especially in community-led monitoring programs.

The GW50 NO_3–N optical probe is a 455 mm long stainless-steel tube with a diameter of 42 mm and an 8 mm measurement cell (Fig. 1b) and uses the 220 nm and 270 nm wave bands as described above. It can be installed *in situ* within a bore or used as an *ex situ* device.

The default analysis configuration is three consecutive readings reporting an arithmetic average, once an hour; however, users can specify higher (or lower) frequency sampling rates. To avoid interference with pumping in production wells the instruments should be installed in dedicated observation wells ≥100 mm diameter where there is a reliable range in water levels. Prior to data monitoring, considerations such as bore construction and well head security, geological logs, seasonal groundwater level fluctuations (from soundings) and hydraulic transmissivity (where data were available) should be evaluated to assess the suitability (and potential variability) of the monitoring well especially its vulnerability to drying up, which may damage the sensor. The sensors are also deployed within the screened interval of the bore (Fig. 2) to ensure regular water exchange and reduced risk of NO_3–N stratification, which may affect readings [2]. Prior to deployment testing for dissolved organic carbon and turbidity levels are measured, because these variables in excess may interfere with the sensor and adversely affect the suitability of the monitoring site. Near-continuous monitoring of NO_3–N-nitrogen using these deployment criteria have been initiated in the case study area of the Hekeao Hinds Plains to assess NO_3–N responses in different physiographic units.

The other use of the NO_3–N probes was as an *ex situ* measurement of discrete grab samples, in lieu of laboratory analysis. MHV converted a refurbished GW50 to a portable sampling platform via a USB to 0–5 V Serial FTDI interface module and a 12 V battery with a wooden crate acting as a stand, which enabled infield near real-time analysis. To avoid spurious results from particulate contaminants, samples were filtered through a single use 0.45 μm filter attached to a syringe and stored in a clean polyethylene sample bottle. If the sample was not immediately tested, it was refrigerated and analysed within 24 h. The NO_3–N concentration was measured 5 times and the arithmetic mean recorded. If the reported measurements were greater than ±0.5 ppm of each other the sample was discarded, the probe cleaned, and the water sample re-analysed. The ± 0.5 ppm tolerance corresponds to the reported instrument precision (±0.3 ppm or ± 5 %) and was confirmed by repeated replicate measurements of water samples of a known concentration (as determined by laboratory analysis). Precision may vary between different sensors and manufacturers (e.g. the TriOS optical nitrate sensors report ± 0.1 ppm or ± 5 %) [23].

Quality Assurance and Quality Control (QAQC) protocols were developed for the NO_3–N sensor probe and included the collection of sample meta-data (See: Supplementary material) and independent laboratory testing of samples. The QAQC protocol is considered de rigour in a laboratory setting, but is often underestimated, forgotten or overlooked as a means of managing and quantifying uncertainty in an environmental sampling context; subsequently without it, potential sources of error(s) (such as cross contamination, faulty field equipment and/or handling errors) cannot be identified or quantified and thus the results cannot be validated [24–26]. Such uncertainties can be particularly problematic in the context of community-led monitoring initiatives where little (or no) training of best practice is available beyond the manufacturer's documentation [27,28].

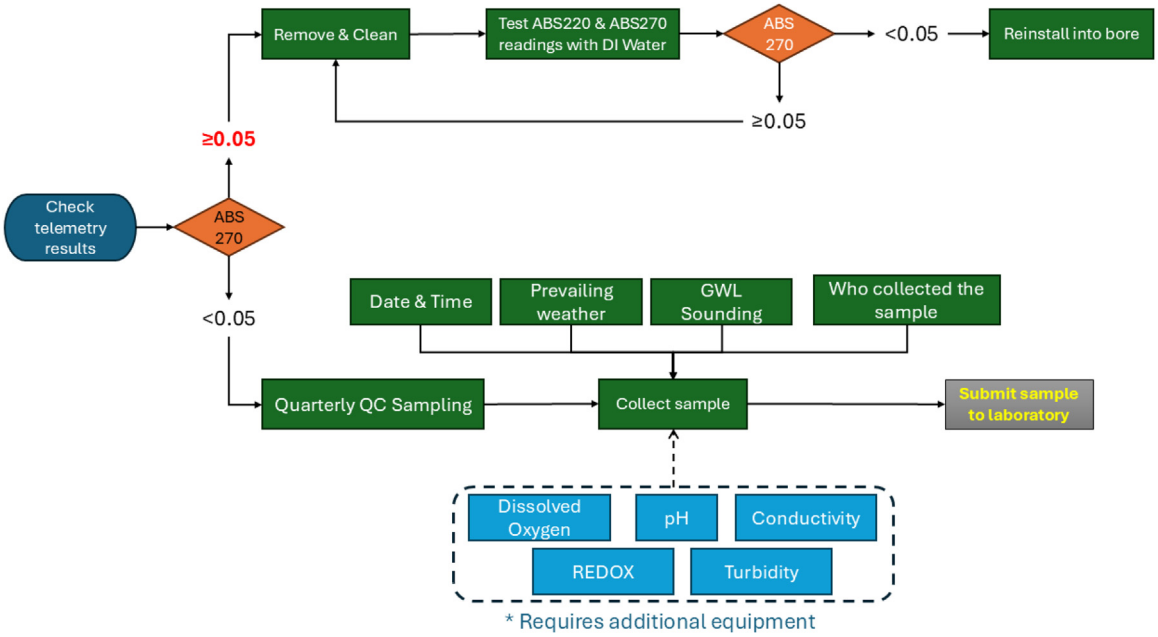

Fig. 3. Process flow diagram for water sampling for an *in situ* probe.

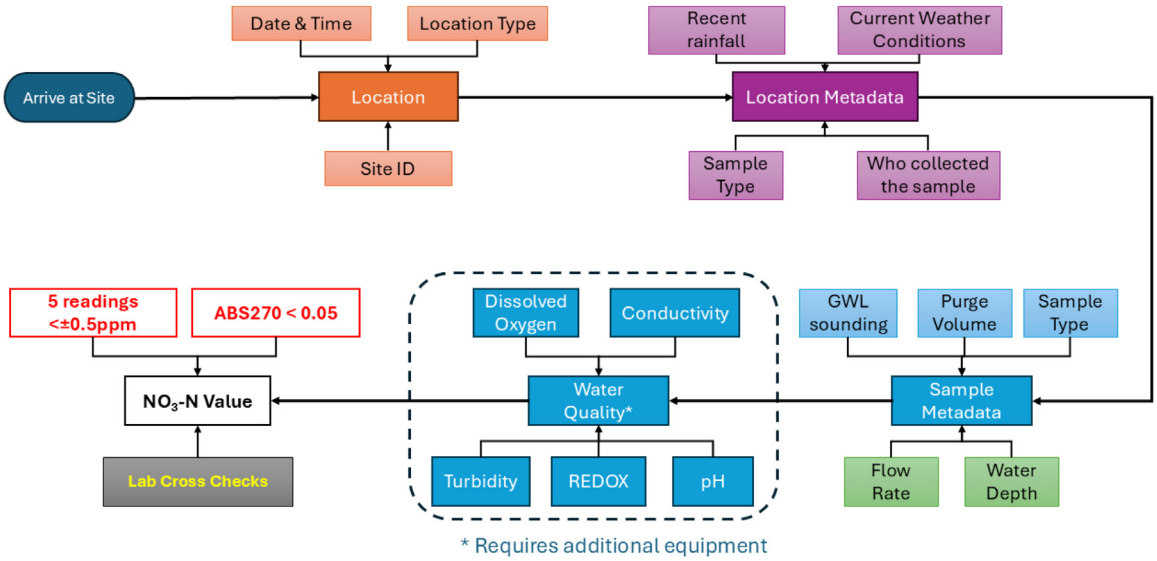

Fig. 4. Process flow diagram for water sampling for an *ex situ* probe.

Quality assurance

Samples were collected in accordance with the locally relevant sampling protocols (i.e., the New Zealand National Environmental Monitoring Standards, NEMS [24,29]. For community groups to develop an effective quality assurance protocol, the following methodology was developed and implemented to maintain data integrity [30,31]:

1. Identify what you are trying to measure and why is it important (NO_3–N for environmental data monitoring to ensure good farmland/ water management). Train the people undertaking the sampling not only how to *do* the sampling but also *why* they are doing it in a particular way, so they understand and have 'buy in' for the need for good quality data;
2. Understand the ability & capacities of the people collecting the data (non-experts in a community);
3. Develop simple standard operating procedures (SOPs) that can be followed and applied to a variety of situations (Fig. 3 and Fig. 4). These SOPs should also specify stage gates and metadata such as:

Table 1
Different types of quality control samples.

Type	Description	Use	Implementation
Singles	A single sample sent to the laboratory	Used as a cross check with the in-house device to ascertain the accuracy of the field measurement.	Standard sample collection for the monitoring programme
Blanks	A sample with an expected value of zero (e.g. de-ionised water)	If the results are significantly greater than zero (e.g. twice the detection limit) this may indicate that the probe is reading inaccurately.	Distilled water blank measured at the beginning and end of each field day
Duplicates	A duplicate of a sample that was collected in the field (i.e. 2 samples from the sample place at the same time) and submitted to a laboratory.	The results should be within <10 % of each other. If not, it suggests cross contamination either in the field or at the laboratory. If the 'blank' value is acceptable, then it would suggest that contamination occurred in the field, and vice versa. Additionally, it quantifies precision or repeatability of the laboratory results	Sample collection method implemented when samples return irregular or noisy results. Implemented on subsequent field collection trips.
Certified reference materials (CRM's)/ Standards	A sample with a known quantity of the solute being reported within an accepted tolerance.	Quantifies precision and accuracy of the results. Often expensive to buy with a limited (i.e. it will be unsuitable to use after 3 months) Standard sample collection for the monitoring programme	Not regularly used due to practicalities of community-led field monitoring programmes.

Table 2
Example of a quality control sampling programme.

Monitoring programme	Frequency	Acceptable Variance	Samples to Lab	Duplicate samples to lab	Blanks	Standards
Drinking Water	weekly	<± 5 %	1:5	1:20	1:5	1:5
Ecological Monitoring	Monthly	<± 10 %	1:10	1:40	1:0	1:10
Groundwater Monitoring	Quarterly	<± 20 %	1:15	1:60	1:15	1:15

a. an ABS 270 ≥ 0.05 is an indication that the sensor may be optically compromised an requires cleaning as per the manufacturers guidelines – see Limitations;
b. temporal metadata such as recent rainfall and local site conditions at the time of sampling – see Table 3 - Supplementary material;
c. if the variance between the instrument readings and the Quality Control (QC) samples submitted to a commercial laboratory are *constantly* >10 %, then the instrument should be checked and re-verified (see Quality Control); as well as,
d. annual equipment maintenance and verification schedules.

4. The inclusion of additional water quality parameters such as pH, requires additional instrumentation. Details about the type and applicability of these metrics are presented in Table 4 - Supplementary material.

SOPs should also specify routine equipment maintenance and calibration schedules as well as QC measures and can be audited and regularly reviewed.

Quality control

Quality control (QC) is essential for identifying potential issues such as instrumentation error, cross contamination, and validation with independent laboratory reporting [30]. This entailed two work streams: (i) a known standard (e.g., blanks) was tested to determine if the instrument was reading accurately; and (ii) a specified number of independent samples (i.e. based on a ratio) were sent to a commercial laboratory and compared to the data obtained from the NO_3–N sensor. Table 1 presents the different types of QC samples were developed for the monitoring programme.

An example of the QC protocol is presented in Table 2 for sampling frequency and used as the underlying principles for the reported work on NO_3–N-nitrogen in groundwater (e.g., [32]). Initially the monitoring programme used singles, duplicates and blanks on a 1:5 ratio to establish a base line of confidence between the GW50 NO_3–N-nitrogen sensor and the commercial laboratory results, as well as to monitor for potential sampling errors. Once established, the monitoring used only singles on a 1:10 ratio to reduce ongoing laboratory costs. Exceptions occurred when discrete samples measured on the GW50 sensor fell outside of the 10 % confidence intervals of laboratory validated samples. When an *ex situ* water sample exceeded these tolerances, the GW50 data were recorded as not meeting the QAQC requirements and discarded from the dataset; and new samples were collected.

Method validation

By implementing cross checks as part of a QAQC protocol, confidence in the data collected can be assured (Fig. 5), which illustrates a good relationship between the optical NO_3–N data and commercial laboratory data. There are small analytical differences between

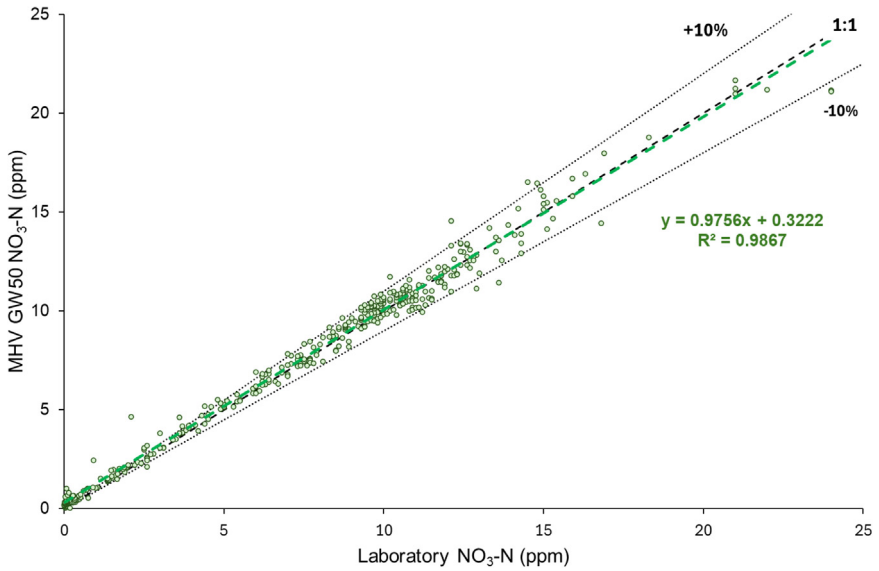

Fig. 5. Comparison of GW50 optical NO_3–N results and Hill Laboratory Azo dye colorimetry results.

the optical UV sensor technique, and the Automated Azo dye colorimetry method used in commercial laboratories, with a flow injection analyser [33]. The laboratory methods had a detection limit of 0.001 ppm and a reported confidence level based on the 95th percentile between a minimum of 2 samples. The validation curve was generated from 400 samples collected between August 2023 and January 2025 and shows a method comparability uncertainty ~ 3 %.

Limitations

NO_3–N optical sensors require maintenance and servicing to ensure they are working correctly and producing reliable results. A maintenance regime specifying regular cleaning, as well as annual calibration verification, must be incorporated into a Quality Assurance SOP (e.g., sensor maintenance, calibration/validation history) as outlined in Quality Assurance [34].

UV spectrometry is susceptible to optical impediments such as dissolved organic matter, biofilm growth and/or suspended material on the sensor which will affect the results. Under normal conditions (i.e. in a bore or a stream sample) an indication that the sensor may be optically compromised and require cleaning is an elevated ABS270 value of >0.05. Cleaning is achieved using a 70 % solution of ethanol or Isopropyl Alcohol (IPA) and fresh (<12 months old) de-ionised or distilled water following the manufacturers guidelines.

It should be noted, however, that at very high concentrations of NO_3–N (i.e., > 300 ppm) there is greater absorption at longer wavelengths (>250 nm). Such concentrations are unlikely in natural freshwater systems, but have been reported in contaminated areas or immediately adjacent to open crop field run off [35]. In these specific instances an ABS270 value of >0.05 may not indicate that the instrument needs cleaning.

Example 1: regular cleaning of bores

In January 2022, a GW50 was installed into an existing abandoned shallow bore (<10 m) near Lowcliffe in Canterbury [32]. As part of the installation process, the bore was pumped continuously for approximately 4 h before installing the GW50. Unfortunately, this process caused the friable material on the inner bore casing to foul the water making NO_3–N readings erratic for several months. In April 2022, the probe was removed, cleaned and re-installed resulting in an initial improvement in the data; however, by early 2023, the results were again reporting markedly different results from the QC grab samples, necessitating it being cleaned again. A review of the NO_3–N results from the *in situ* probe compared to its corresponding QC samples, as well as the ABS270 values (Fig. 6), showed that regular cleaning of the measurement cell on a quarterly basis has improved the reliability of the results. This workflow is now incorporated into the QA Standard Operating Procedures (SOPs) and is indicative of the requirement for regular, and consistent maintenance of *in situ* NO_3–N probes.

Example 2: annual calibration

As part of the QAQC protocols approximately 15 % of samples (n = 233) were cross checked with an independent commercial laboratory. The validation data ranged from 0.6 to 23 ppm NO_3–N, was normally distributed with a mean concentration of 8.6 ppm, and a standard deviation of 4.6. The results from the GW50 were compared to the laboratory QC samples via a scatter plot and tested using least squares regression in SPSS. The NO_3–N regression equation was GW50 = 0.8909 Lab – 0.1628, with a 95 % confidence

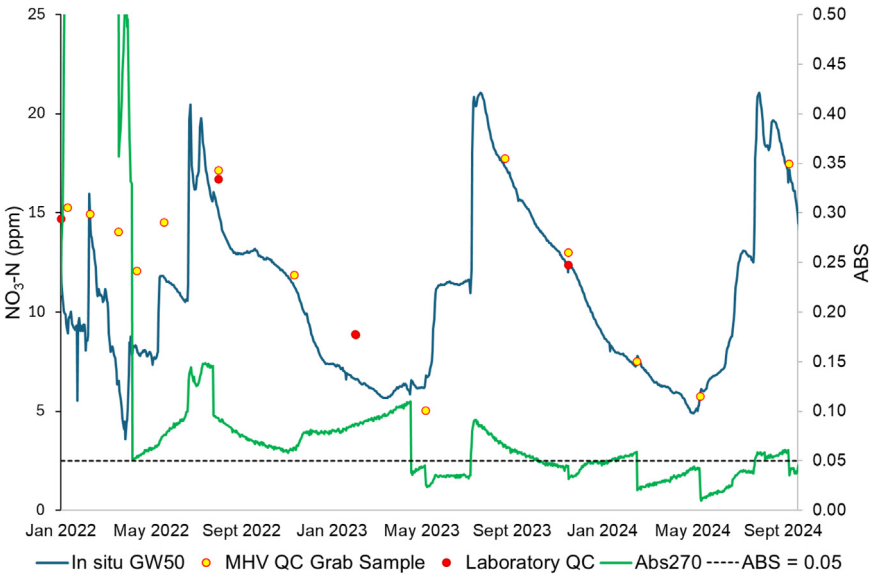

Fig. 6. Realtime NO_3–N data compared to corresponding ABS270 and QC Samples, note the disparity between the probe and QC samples when the ABS > 0.05 nm.

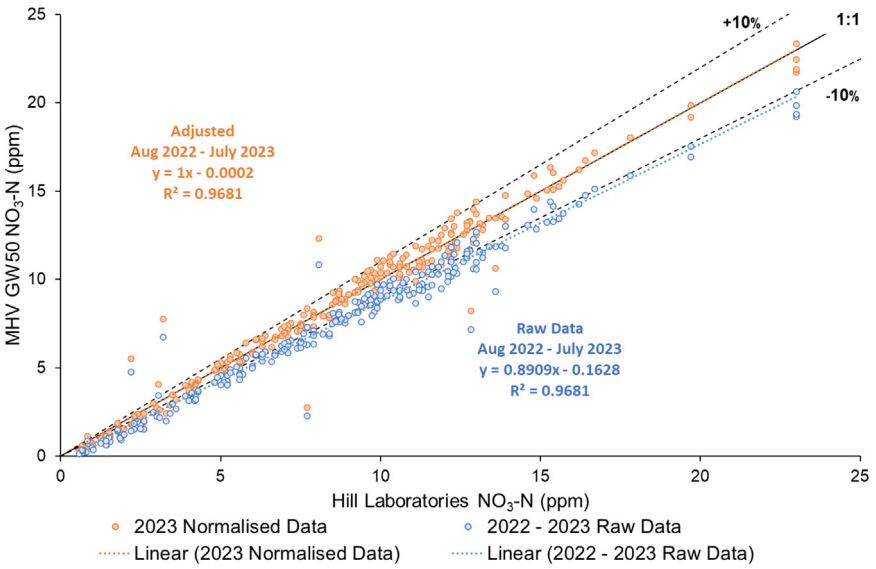

Fig. 7. Scatter plot of GW50 data and corresponding Laboratory results (blue) and the regression correction (orange).

interval of the slope (beta) of ±0.11, adjusted coefficient of determination r^2=0.968, and p = 0.000. As the local accredited laboratory (Hill Laboratories New Zealand) analyses for NO_3–N via Automated Azo dye colorimetry, with a flow injection analyser [36], it was expected that there would be some degree of variation between the laboratory and GW50 field results (Fig. 7 in blue), due to subtle differences in the analytical methods and biases. The difference between the optical probe data and the laboratory results showed a consistent bias towards the lab results of ~12 %, regardless of the NO_3–N concentration for a period of 24 months and was interpreted as a systematic difference in how NO_3–N is detected between optical and laboratory methods. To compensate for this offset all optical concentrations of NO_3–N collected using the GW50 were adjusted by a multiplication factor of 1.1091 and +0.185 so that the data plotted on a slope of 1:1 and intersected the origin (Fig. 7 in orange).

Whilst there are a variety of statistical methods that could be employed to reconcile the differences between the analytical techniques, the use of regression equations is generally considered acceptable [37,38], and easily available without the need for dedicated statistical programs. As the coefficient of determination was high (i.e. >0.95), a regression correction was applied to the GW50 data population to standardise the results to be compatible with a laboratory value (Fig. 7). Where values were appreciably

different to the laboratory values (i.e. >10 % or more than two standard deviations), the data were rejected from the environmental reporting dataset; and further investigation of the metadata interrogated to identify what may be the cause of the discrepancy.

After two years of use, the portable GW50 was returned to the manufacturer for a preventative maintenance check and calibration verification. It was found that the cause of the difference in results (Fig. 7) was not due to differences in analytical methods between the GW50 and the laboratory (as assumed by the data team), but rather the optical sensor was slightly out of alignment (possibly to being knocked during field use) thus producing a constant but biased result. Subsequently, the instrument's calibration is validated annually by the manufacturer as part of the QA programme. Thus, an additional limitation to the use of *ex situ* GW50 probes is that rough handling may result in internal damage that may affect results as shown in Fig. 7.

Example 3: iron-oxidizing bacterium aka irrigation plaque

Gallionella ferruginea is a bean-shaped iron-oxidizing bacterium (FeOB) bacterium that thrives in iron-bearing waters and that acts as a catalyst of iron cycling in wetland environments, but is often confused for 'rust' as the cells are usually mixed with iron precipitates [39,40]. *Gallionella spp*. live in low-oxygen conditions and are associated with redox boundaries and require opposing gradients of oxygen and iron (Fe^{2+}) to grow [41]. Subsequently *Gallionella spp*. are found in environments with reduced iron, and sufficient amounts of carbon, phosphorus and nitrogen [42]. When present the bacterium forms a hydrated gelatinous slime (>90 % water) that varies from yellow to red to in colour, and is colloquially referred to as *Iron ochre* [43] or *Irrigation Plaque* [44], and its presence interferes with irrigation systems and optical NO_3–N probes. There are no sustainable long term methods for mitigating against iron-oxidizing bacterium other than amending the oxidation state, or the removal of iron source material [43].

On the Hekeao Hinds Plains, *Gallionella ferruginea* has been observed on three occasions since 2020 in both new and existing bores spudded into shallow, well drained, silty loams. The presence of *Gallionella ferruginea* in the bores resulting in damage to an *in situ* GW50 being inoperable due to poor water quality, and in another instance the bacterium likely contributed the corrosion of the optical sensor necessitating extensive repairs. From our limited observations in the Hekeao Hinds case study, *Gallionella ferruginea* dissipated after significant rainfall and corresponding changes in groundwater levels, and/or once the bore casing had been *in situ* for more than a year and developed a patina of rust and/or bio film. There is currently no work around to the use of GW50 probes in these conditions.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Justin Legg reports financial support was provided by MHV. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Ethics statements

N/A

Credit author statement

Legg, J: Conceptualization: (100 %). Methodology: (100 %). Formal Analysis: (100 %). Investigation: (100 %). Fieldwork: (100 %). Writing – original draft: (100 %). Writing – review and editing: (70 %). Visualization: (100 %). **Mager, S:** Writing – review and editing: (15 %). Supervision: (50 %). **Horton, S:** Writing – review and editing: (15 %). Supervision: (50 %).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2025.103286.

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