RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	IN2025_E01
Chief Scientist	N/A
Voyage title:	Trial and calibration
Report compiled by:	Dawn Herweynen and Pavie Nanthasurasak

1. Contents

Executive Summary 4
Objectives 4
General Hydrochemistry Information 4
Itinerary 6
Key personnel list 7
Summary 8
Sample Type and Number Assayed 8
CTD samples (Conductivity, Temperature, Density) 8
Thermosalinograph (TSG) samples 8
Data Processing Overview 9
Conventional hydrology data 9
Salinity 10
Salinity Measurement Parameters 10
Salinity Method 11
CTD Salinity vs Bottle Salinity Plot 11
OSIL Salinity Standard Plot 13
Dissolved Oxygen 14
Dissolved Oxygen Measurement Parameters 14
Dissolved Oxygen Method 14
CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot 15
Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction. 15
Nutrients 18
Nutrient Measurement Parameters 18
Nutrient Methods 19
HyPro Processing Summary for Nutrients 20
Refractive Index Blank (RIB) measurement and correction 21
Accuracy - Reference Material for Nutrient in Seawater (RMNS) 22
Nutrient plots of RMNS 25
Measurement Uncertainty 36
Method Detection Limit for Nutrients 36
Sampling Precision 37
Redfield Ratio Plot (14.0) for CTD Deployments. 37

Temperature & Humidity Change over Nutrient Analyses 39
Appendix 40
Salinity: Reference material used 40
Nutrients: RMNS results for each CTD Deployment 40
Lot CQ 40
Lot CR 40
Lot CS 41
Lot CM 41
Missing or Suspect Salinity Data 42
Missing or Suspect Dissolved Oxygen Data 42
Missing or Suspect Nutrient Data 42
Data Quality Flag Key 43
GO-SHIP Specifications 43
Salinity 43
Dissolved Oxygen 44
$Si(OH)_4$ 44
PO_4 44
NO_3 44
Notes 44
References 45

Executive Summary

Objectives

IN2025_E01 serves as a crucial opportunity to conduct system trials and calibrations following the 2024-25 long maintenance period and preparations for upcoming voyages. This voyage encompasses a range of technical assessments, including:

- **Geophysical Survey and Mapping Team** – Gravity meter trials and Kongsberg system backscatter calibration.
- **Seagoing Instrumentation Team** – Heave compensation performance assessments and Triaxus deployments.
- **Field Operations Team** – Testing of multi-net, multi-core, and box-core sampling systems.
- **Data Acquisition and Processing Team** – Verification and testing of onboard science instrumentation and acquisition systems.

For **Hydrochemistry**, the voyage provides two key opportunities:

1. Reassembly and Readiness – Reassembling the hydrochemistry laboratory following the dry dock period to ensure all necessary consumables and instrumentation are present and fully operational for upcoming voyages.
2. Instrumentation Testing:
    - AA500 Nutrient Analyser – Testing the new AA500 nutrient analyser as a potential replacement for the AA3 at sea.
    - Guildline Salinometers – Evaluating two new Guildline salinometers for their suitability in measuring practical salinity, with the goal of replacing aging units in service for over a decade.

General Hydrochemistry Information

Water samples collected during the voyage were analysed in the ship's hydrochemistry laboratory for nutrients, dissolved oxygen, and salinity. For nutrients, this is the first voyage where additional correction for refractive index blank (RIB) was applied post-processing. Pronounced RIB effect was identified and is unique to AA500 nutrient analyser. This RIB correction was not required for previous model nutrient analyser, AA3. RIB measurement and correction procedures will be documented and outlined in the developing AA500 standard operating procedure (SOP). For this voyage, final nutrients dataset was corrected prior to submission. No other significant sample collection, analysis, or data processing issues were encountered.

Five nutrients were analysed silicate, phosphate, nitrate + nitrite, nitrite and ammonium using AA500 autoanalyser. Certified reference materials for nutrients in seawater (RMNS) analysed in runs containing CTD samples were within acceptable limits of accuracy after refractive index blank (RIB) was applied. Missing and suspect hydrology samples are listed in Appendix, Missing or Suspect Nutrient Data.

Please cite the following manuscript when reporting or publishing data for silicate, phosphate, nitrate+nitrite (NOx) and nitrite:

Rees, C., L. Pender, K. Sherrin, C. Schwanger, P. Hughes, S. Tibben, A. Marouchos, and M. Rayner. (2018) "*Methods for reproducible shipboard SFA nutrient measurement using RMNS and automated data processing*."

Limnol. Oceanogr: Methods, 17(1): pp. 25-41.

doi:10.1002/lom3.10294

If publishing ammonium data, please cite the following:

Rees, C., Janssens, J., Sherrin, K., Hughes, P., Tibben, S., McMahon, M., McDonald, J., Camac, A., Schwanger, C. and Marouchos, A., (2021*) "Method for Reproducible Shipboard Segmented Flow Analysis Ammonium Measurement Using an In-House Reference Material for Quality Control."*

Frontiers in Marine Science, 8.

doi:10.3389/fmars.2021.581901

Final hydrology data, analytical methods, related log sheets and processing notes can be obtained from the CSIRO data centre.

For Data, contact: NCMI_DataLibrarians@csiro.au

Itinerary

Table 1: Voyage itinerary

	Depart	Arrive
Port	Hobart	Hobart
Date	19/02/2025	01/03/2025
Time	09:00	13:20

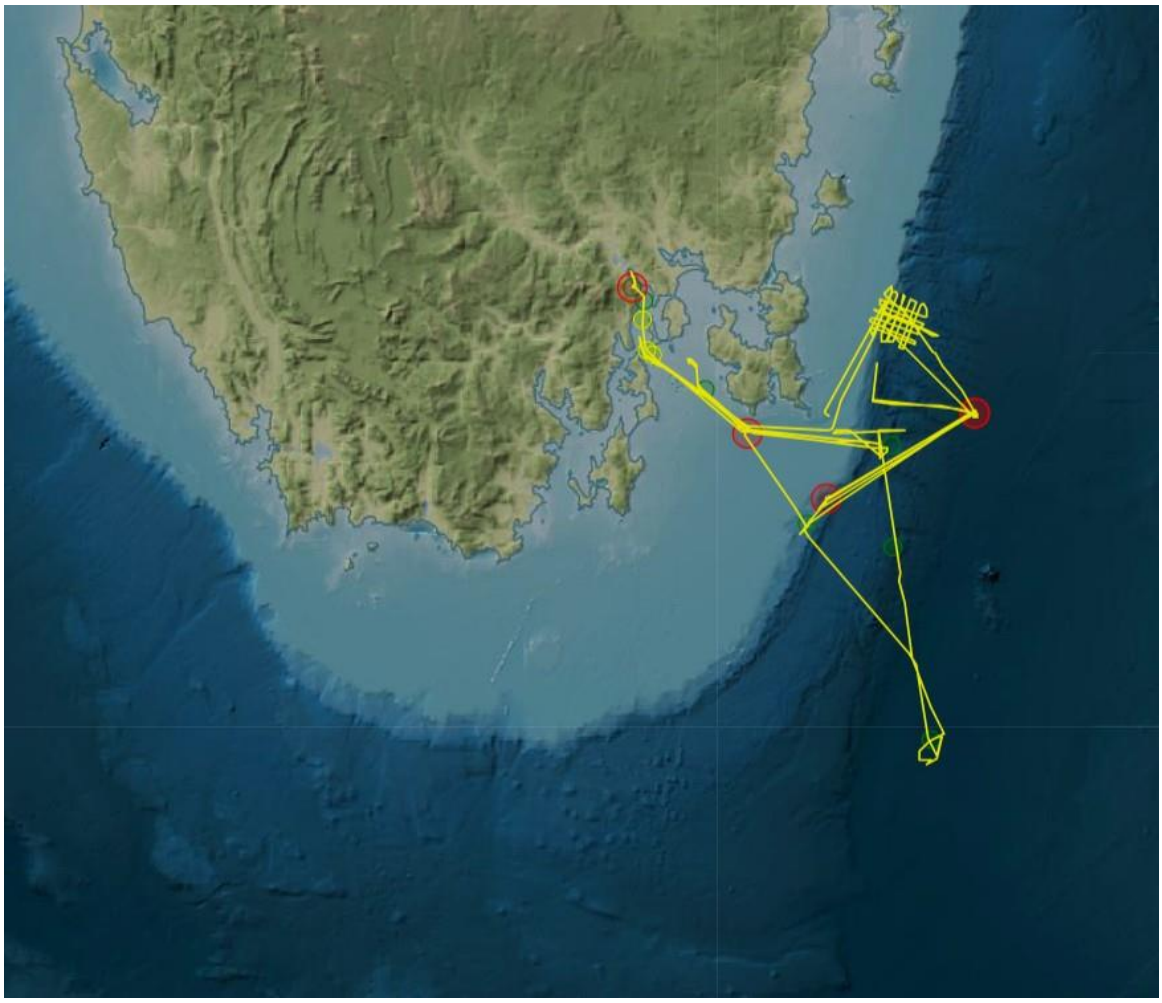


Figure 1. Voyage map of IN2025_E01.

Key personnel list

Table 2: Key Personnel list

Name	Role	Organisation
N/A	Chief Scientist	N/A
Max McGuire	Voyage Manager	CSIRO
Claire Grubb	Deputy Voyage Manager	CSIRO
Dawn Herweynen	Hydrochemist	CSIRO
Pavie Nanthasurasak	Hydrochemist	CSIRO

Summary

Sample Type and Number Assayed

Table 3: Sample Type and Number Assayed

Analysis (instrument)	Number of Samples	Processing Status at voyage end
Salinity (Guildline Salinometer)	52 CTD	Completed
	9 TSG	Completed
Dissolved Oxygen (automated titration)	52 CTD	Completed
Nutrients (Seal AA500)	52 CTD	Completed

CTD samples (Conductivity, Temperature, Density)

- Taken from the 12 L Ocean Test Equipment bottles on the CTD rosette that is deployed at depth for water collection.
- A total of 2 CTD deployments were conducted over the duration of the voyage. Of the 2 deployments, 2 were sampled by:
    - Hydrochemistry: Dawn Herweynen and Pavie Nanthasurasak
    - CSIRO workshop: Josh Wiggins and Luke Holloway

Thermosalinograph (TSG) samples

- Taken from the underway instrument clean seawater line supplying the pCO_2 instrument in the underway laboratory.
- TSG samples collected by hydrochemistry. Results emailed to Vito Dirita (CSIRO) at the completion of the voyage.
- TSG sampling team: Dawn Herweynen and Pavie Nanthasurasak

Refer to voyage EVERLog for TSG sample information.

Data Processing Overview

Conventional hydrology data

The sample meta-data, measured bottle salinity results, dissolved oxygen assay results and the nutrient assay raw data are processed by the CSIRO program HyPro. The final output is the hydrology data set for dissolved oxygen and salinity. For nutrients, processed data from HyPro was further corrected with refractive index blank (RIB) offset prior to being collated into final hydrology dataset. An overview of this process is illustrated below (fig.2).

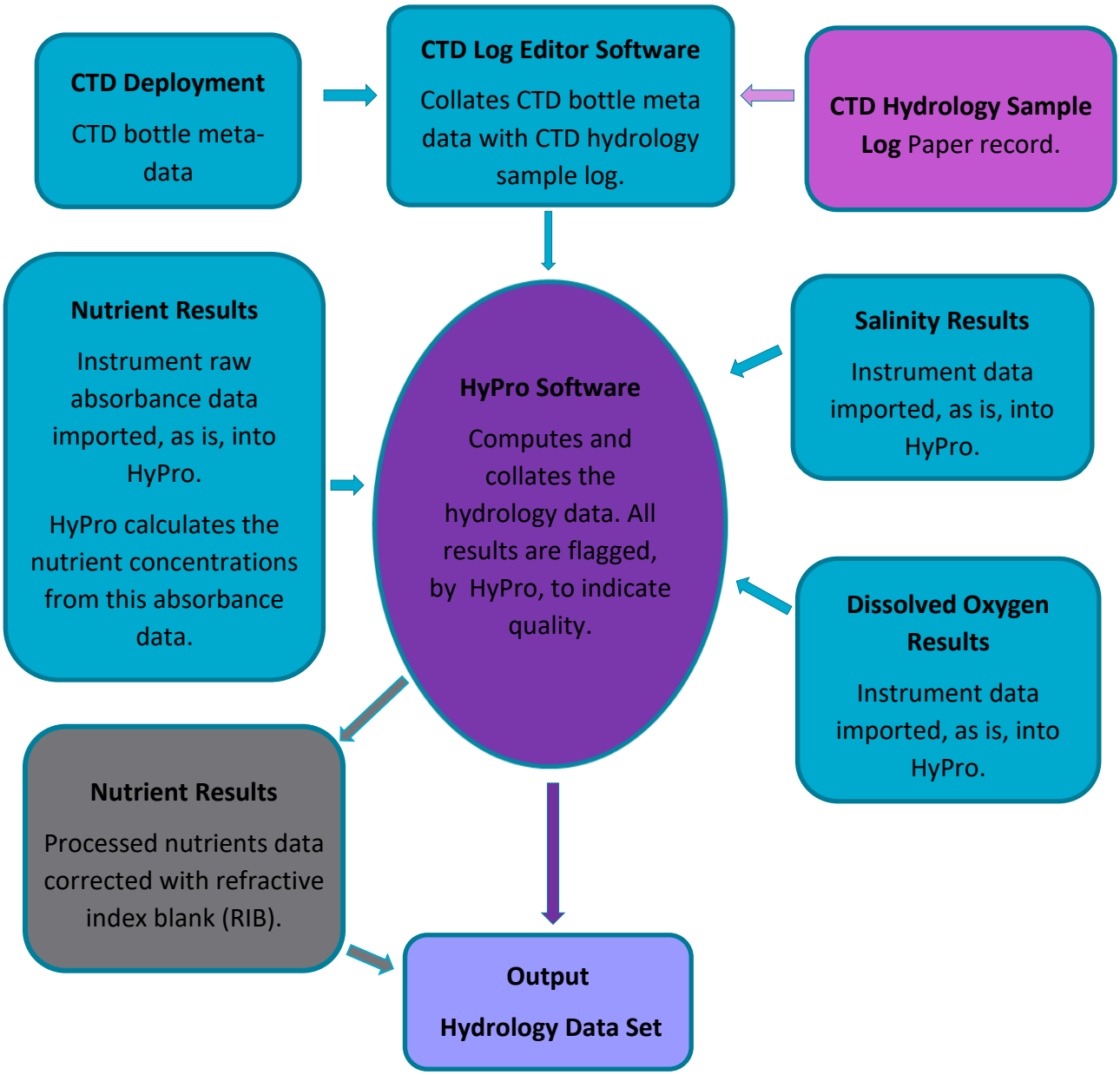


Figure 2. Conventional Hydrology Data Processing Flow Diagram.

Salinity

Salinity Measurement Parameters

Table 4. Salinity Measurement Parameters

Details	
HyPro Version	5.7
Instruments	Guildline Autosal Laboratory Salinometer 8400(B) – SN 74680. Bath temperature 24.0°C
Software	Ocean Scientific International Ltd (OSIL) Data Logger ver 1.2
Hydrochemistry Methods	Sampling: WI_Sal_002 Analysis: SOP 006
Accuracy	± 0.001 practical salinity units
Reference Material	OSIL IAPSO – Batch P167, use by 21/02/2026, K_{15} = 0.99988
Sample Container	200 mL volume OSIL bottles made of type II glass (clear) with disposable plastic insert and plastic screw cap.
Sample Storage	Stored in salinometer lab for minimum of 8 hrs before measurement. All samples were analysed within 48 hours of reception.
Lab Temperature	Mean 22.0°C SD 0.5°C
Analysts	Dawn Herweynen and Pavie Nanthasurasak
Comments	See DAP report for CTD calibration details.

Salinity Method

Salinity samples were measured on a Guildline Autosal 8400B instrument operated in accordance with its technical manual. The measured value is recorded with an OSIL data logger.

Practical salinity (S) is defined in terms of the ratio (K_{15}) of the electrical conductivity measured at 15°C 1 atm of seawater to that of a potassium chloride (KCl) solution of mass fraction 32.4356 x 10^{-3}.

Before each lot of sample measurements, the Autosal is calibrated with standard seawater (OSIL, IAPSO) of known K_{15} ratio. A new bottle of OSIL standard is used for each calibration. The frequency of calibration is at least one per run.

Method Summary: The salinity sample is collected in a 200 mL OSIL bottle. The bottle is rinsed then filled from the bottom, via a polytetrafluoroethylene (PTFE) straw, untill overflowing. The bottle is removed from the straw and the sample is decanted to allow a headspace of approximately 25cm^3. A dry plastic insert is fitted, the bottle inverted and rinsed with water then capped and stored cap-down until measured. To measure, the salinometer cell is flushed three times with the sample and then measured twice. The OSIL data logger software captures the conductivity ratio and calculates the practical salinity.

The output from the data logger is imported into HyPro and collated with the CTD deployment meta-data.

CTD Salinity vs Bottle Salinity Plot

For this voyage, the difference between the unprocessed (uncorrected) CTD value and the measured bottle value is generally less than 0.02 PSU (Figure 3). Generally, larger differences are for shallow samples across the sudden changes in the thermohaline profile. Bad salinity data is tabulated in Appendix, Missing or Suspect Salinity Data.

The unprocessed CTD values are adjusted (corrected) by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the NCMI_DataLibrarians@csiro.au for corrected CTD data.

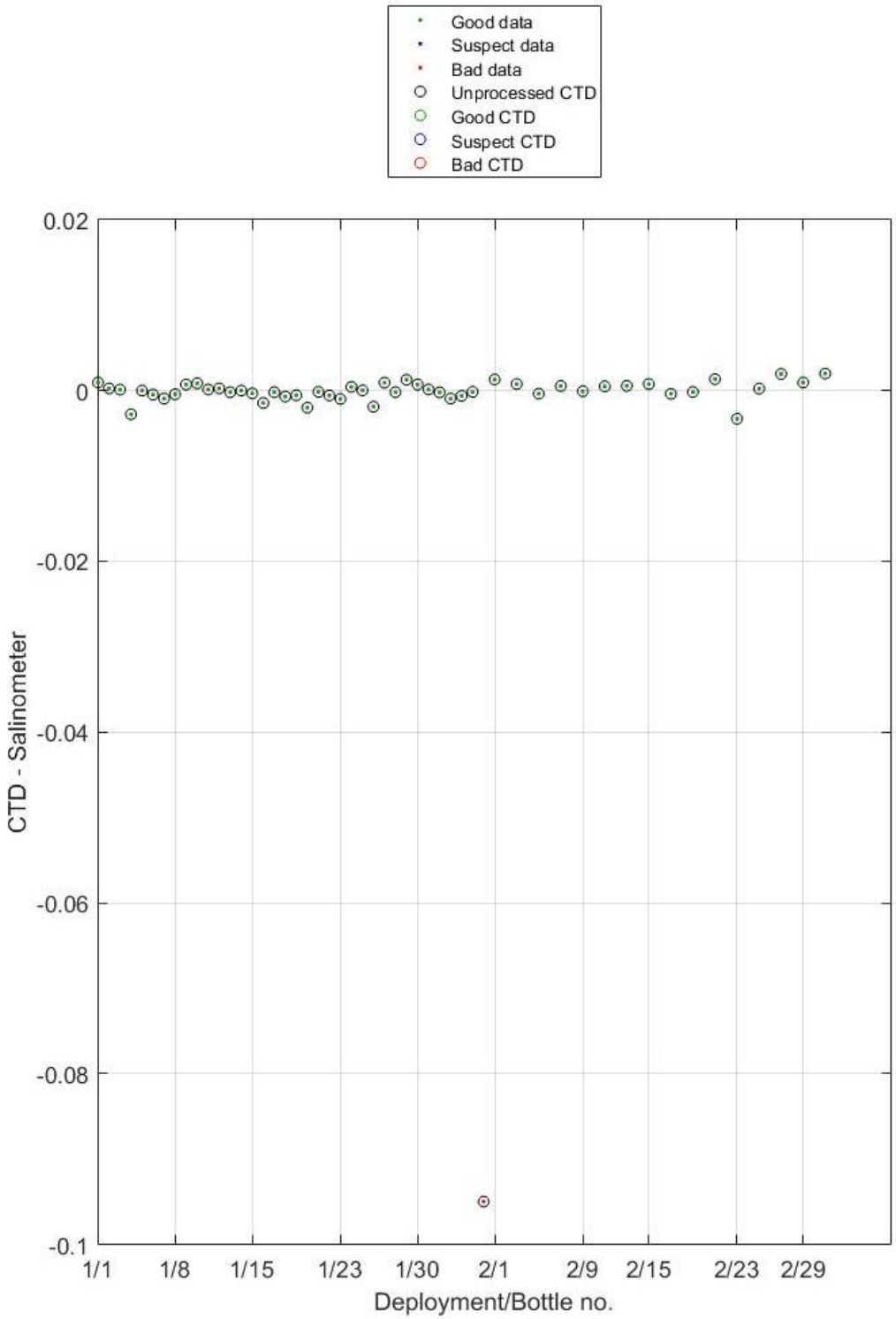


Figure 3. CTD Salinity - Bottle Salinity vs CTD deployment plot. The data quality is coded by colour and delineated by a dot for the bottle salinity and a circle for the CTD salinity. Green = GOOD. Blue = SUSPECT. No fill = BAD. Black = UNPROCESSED. Units: PSU (dimensionless). **Note: Bad salinity bottle data is listed in* Appendix, Missing or Suspect Salinity Data.

OSIL Salinity Standard Plot

The instrument is calibrated with OSIL standard seawater lot P167 (PSU = 34.995). The plot below shows the OSIL lot P167 measured results for each run on this voyage. The blue line represents the mean of all standards measured for standardisation.

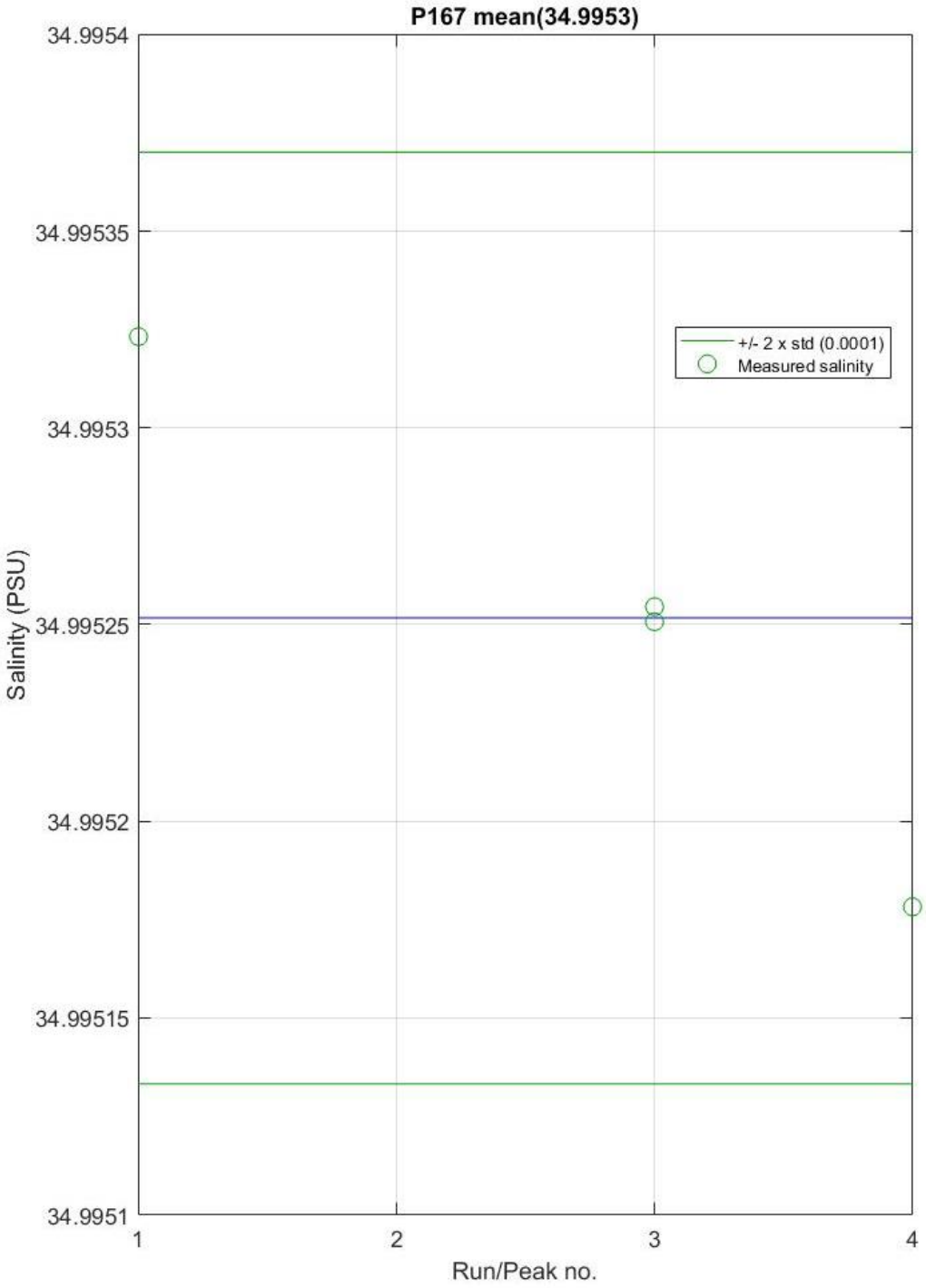


Figure 4. Measured OSIL standard for each salinity run and average value (P167 mean) across IN2025_E01.

Dissolved Oxygen

Dissolved Oxygen Measurement Parameters

Table 5. Dissolved oxygen measurement parameters.

Details	
HyPro Version	5.7
Instrument	Scripps Automated Photometric Oxygen System (SIO)
Software	Scripps Institution of Oceanography (SIO)
Hydrochemistry Methods	Sampling: WI_DO_001 Analysis: SOP 005
Accuracy	± 0.5 µmol L^{-1}
Analysts	Dawn Herweynen and Pavie Nanthasurasak
Lab Temperature (±1°C)	Mean 20.6°C SD 0.4°C
Titrant	Sodium Thiosulphate – 50 g/L
Sample Container type	140 mL glass iodine determination flasks with glass stopper.
Sample Storage	Samples stored in the hydrochemistry lab until analysis. All analysis performed within 48 hours.
Comments	See DAP report for CTD calibration details.

Dissolved Oxygen Method

Scripps Institution of Oceanography (SIO) method used. The method is based on the whole bottle modified Winkler titration of Carpenter (1965) plus modifications by Culberson *et al* (1991).

Method Summary: The sample is collected in an iodine determination flask of known volume. 1 mL of manganese (II) chloride solution followed by 1 mL of alkaline iodide solution is added to the sample; the flask stoppered and inverted a minimum of 30 times. The dissolved oxygen oxidizes an equivalent amount of Mn (II) to Mn (IV) which precipitates. Just before titration, the sample is acidified, Mn (IV) is reduced to the divalent state liberating iodine. The iodine is titrated with a

standardised thiosulphate solution using a Metrohm 876 Dosimat fitted with a 1 mL burette. The endpoint is determined by measuring the decrease in the UV absorption 365 nm.

The thiosulphate solution is standardised with a 10 mL aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two titres of consecutive additions of 1 mL aliquots of potassium iodate to the same blank sample. The standardisation is done at least once per 12-hour shift, when samples are being assayed.

The output from the SIO instrument software is imported into HyPro and collated with the CTD deployment meta-data.

CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

For this voyage, the difference between the unprocessed CTD value and the measured bottle value is generally less than 18 µmol L^{-1} (Figure 5). The larger differences are for shallow samples across the sudden changes and or mixing in the dissolved oxygen profile.

The unprocessed CTD values are adjusted (corrected) by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the NCMI_DataLibrarians@csiro.au for corrected CTD data.

Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.

The calculated thiosulphate concentration varied by less than 0.0005 N between standardisations. The blank correction is used in the calculation of the thiosulphate normality and is due to redox species in the reagents that are added to the KIO_3 aliquot before the titration. A single batch of thiosulphate reagent was used during the voyage. The mean normality is as follows:

The two CTD deployments analysed that lie within casts 1 and 2:

Mean: 0.197954 SD: 0.000 n=1

For thiosulphate normality plots (Figure 6), the red lines indicate ± 0.0005 N either side of the mean titrant (thiosulfate) concentration. For blank plot (Figure 6), red lines indicate acceptable variation either side of the mean blank concentration. The titrant should not vary more than 0.0005 N between analyses.

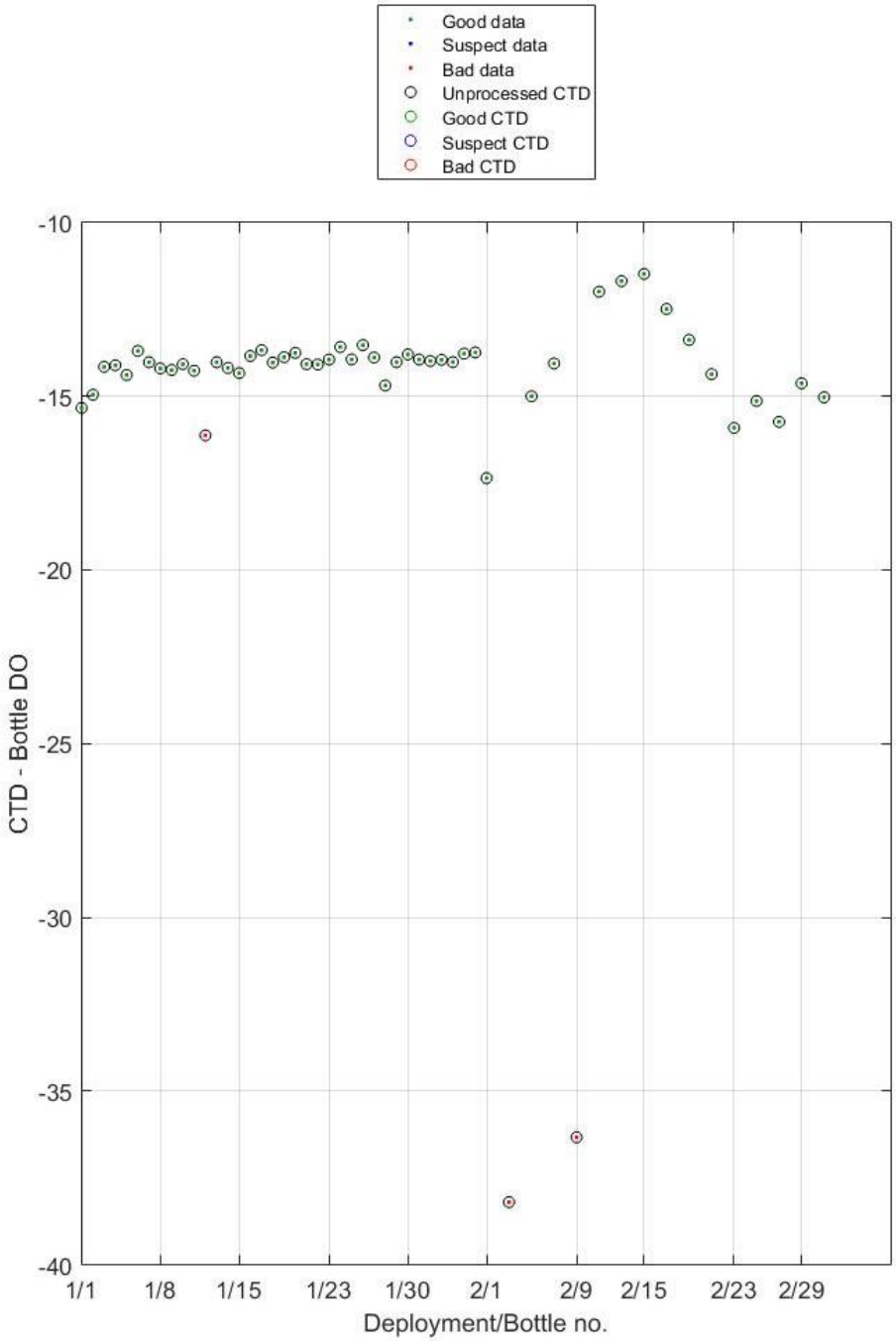


Figure 5. CTD Dissolved Oxygen - Bottle Dissolved Oxygen vs Deployment Plot. The data quality is coded by colour and delineated by a dot for the bottle DO and a circle for the CTD DO. Green = GOOD. Blue = SUSPECT. No fill = BAD. Black = UNPROCESSED. Units: µmol L^{-1}. **Note: Bad oxygen bottle data is listed in* Appendix, Missing or Suspect Dissolved Oxygen Data.

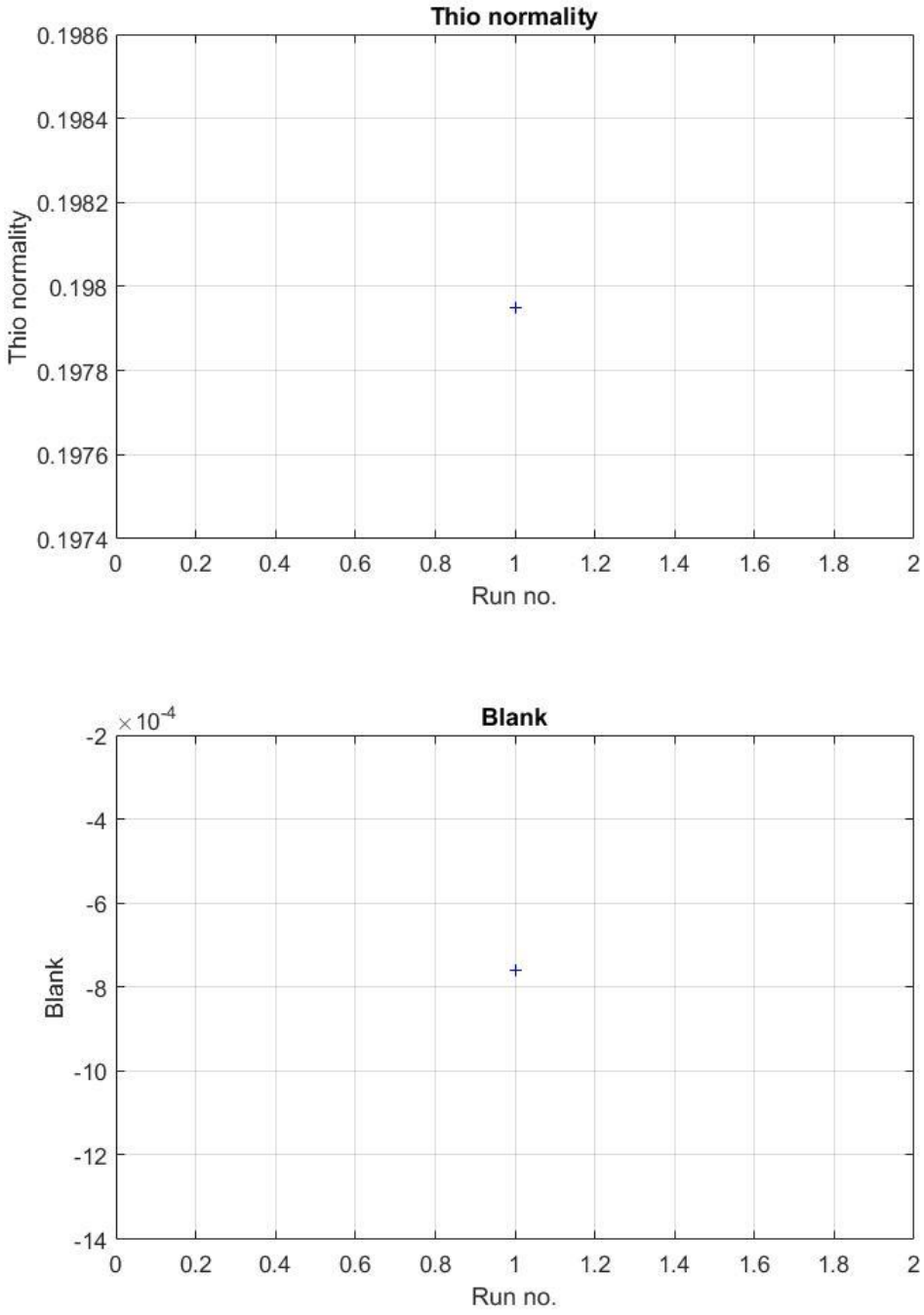


Figure 6. Thiosulphate standardisation (top) and blank correction (bottom) plots.

Nutrients

Nutrient Measurement Parameters

Table 6. Nutrient measurement parameters analysed with Seal AA500 segmented flow analyser. All instrument parameters, reagent batches, and instrument events are logged for each analysis run. This information is available on request.

Instrument	**Seal AA500 segmented flow analyser**				
HyPro version	5.7				
Operating Software	AACE 8.03				
Hydrochemistry Sampling Method	WI_Nut_001				
Hydrochemistry analysis method	SOP001	SOP002	SOP003	SOP003	SOP004
Nutrients	Silicate SiO_4^{4-}	Phosphate PO_4^{3-}	Nitrate + Nitrite $NO_3^- + NO_2^-$	Nitrite NO_2^-	Ammonia NH_4^+
Top concentration (µmol L^{-1})	112.0	3.0	36.4	1.4	2.0
Method detection limit (µmol L^{-1})	0.2	0.02	0.02	0.02	0.02
Reference Material	KANSO RMNS lot CQ, CR, CS, and CM				
Sample Container	CTD: 50 mL HDPE with screw cap lids. Reused after acid wash with 10% hydrochloric acid solution.				
Sample Storage	< 4 hours at room temperature after collection or < 12 hours at 4°C after collection				
Sample preparation	Assayed as neat. No filtration. The lids were kept on the samples until all the standards and QC samples were analysed, then they were removed and covered individually with foil.				
Lab Temperature (°C)	Mean 20.6°C SD 0.4°C				
Analysts	Dawn Herweynen and Pavie Nanthasurasak				

Nutrient Methods

Nutrient samples are assayed on a Seal AA500 segmented flow auto-analyser fitted with 1 cm flow-cells for colorimetric measurements and a Shimadzu RF-10AXL fluorescence instrument as the ammonium detector.

Silicate (SOP001): colourimetric, molybdenum blue method. Based on Armstrong et al. (1967). Silicate in seawater is reacted with acidified ammonium molybdate to produce silicomolybdic acid. Tartaric acid is added to remove the phosphate molybdic acid interference. Tin (II) chloride is then added to reduce the silicomolybdic acid to silicomolybdous acid and its absorbance is measured at 820 nm.

Phosphate (SOP002): colourimetric, molybdenum blue method. Based on Murphy and Riley (1962) with modifications from the NIOZ-SGNOS[1] Practical Workshop 2012 optimizing the antimony catalyst/phosphate ratio and the reduction of silicate interferences by pH. Phosphate in seawater forms a phosphomolybdenum complex with acidified ammonium molybdate. It is then reduced by ascorbic acid and its absorbance is measured at 880 nm.

Nitrate (SOP003): colourimetric, Cu-Cd reduction – naphthylenediamine method. Based on Wood et.al (1967). Nitrate is reduced to nitrite by first adding an ammonium chloride buffer then sending it through a copper - cadmium column. Sulphanilamide is added under acidic conditions to form a diazo compound. This compound is coupled with 1-N-naphthly-ethylenediamine di-hydrochloride to produce a reddish purple azo complex, and its absorbance is measured at 540 nm.

Nitrite (SOP003): colourimetric, naphthylenediamine method. As per nitrate method without the copper cadmium reduction column and buffer. Absorbance measured at 540 nm.

Ammonium (SOP004): fluorescence, ortho-phtaldiadehyde method. Based on Kérouel and Aminot (1997). Ammonium reacted with ortho-phtaldialdehyde and sulphite at a pH of 9.0-9.5 to produce an intensely fluorescent product. Its emission is measured at 460 nm after excitation at 370 nm.

SOP methods can be obtained from the CSIRO Oceans and Atmosphere Hydrochemistry Group.

[1] Royal Netherlands Institute for Sea Research – Study Group on Nutrient Standards.

[1] Royal Netherlands Institute for Sea Research – Study Group on Nutrient Standards.

HyPro Processing Summary for Nutrients

After a run, the raw absorbance/ fluorescence data is exported from the instrument and processed by HyPro. For each analyte, HyPro re-creates the peak traces, defines the region on the peak's plateau (peak window) used to determine the peak heights, constructs the calibration curve, applies corrections for carry-over, baseline, and sensitive drifts then, derives the nutrient concentrations for each sample. The corrections are quantified using dedicated solutions included in every run.

HyPro identifies suspect calibration points, noisy peaks, method detection limits that are above the nominal limit assigned to each analyte, and duplicates sample results that do not match.

Suspect calibration points are weighted less when fitting the calibration curve. The cut-off limits for good calibration data are:

- ±0.5% of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE[1]).
- 0.02 µmol L^{-1} for phosphate, nitrite, and ammonium.

HyPro classifies the quality of data as good, suspect, or bad and flags accordingly. The Flag key is in Appendix, Data Quality Flag Key. Missing or suspect nutrient data is tabulated in Appendix, Missing or Suspect Nutrient Data.

[1] World Ocean Circulation Experiment

Table 7. HyPro 5.7 Processing Parameters. All instrument parameters and reagent batches and operation events are logged for each analysis run. This information is available on request.

Result Details	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Data Reported as	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}
Calibration Curve fit	Linear	Linear	Linear	Quadratic	Quadratic
# of points in Calibration	6	6	6	6	6
Forced through zero	N	N	N	N	N
Matrix correction	N	N	N	N	N
Blank correction	N	N	N	N	N

Refractive index blank (RIB) correction	N	N	N	N	N
Peak window defined by	HyPro	HyPro	HyPro	HyPro	HyPro
Carryover correction (HyPro)	Y	Y	Y	Y	Y
Baseline drift correction (HyPro)	Y	Y	Y	Y	Y
Sensitivity drift correction (HyPro)	Y	Y	Y	Y	Y
Data Adj for RMNS variance.	N	N	N	N	N
Medium of Standards	Low nutrient seawater (LNSW, bulk on PW1 wharf, CSIRO Hobart) collected in November 2022. Sub-lot passed through a 5-micron filter (filtered in October 2024) and stored in 20 L carboys in the hydrochemistry laboratory at 20°C.				
Medium of Baseline	18.2 Ω water. Dispensed from the Milli Q IQ 7010 system.				
Duplicate samples	CTD: Niskins fired at the greatest depth were analysed in duplicate. Single samples were analysed for remaining depths.				
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data are tabulated in Appendix, RMNS results for each CTD Deployment				

Refractive Index Blank (RIB) measurement and correction

The refractive index blank (RIB) is an optical interference caused by the difference in refraction between seawater and ultrapure water. This effect alters light transmission and scattering within the flow cell, resulting in baseline shifts and potential errors in absorbance measurements during colorimetric analysis (Kirkwood, D.S., 1996). In the AA500 system, a negative RIB effect has been observed, where the apparent absorbance decreases when low-nutrient seawater (LNSW) is analyzed compared to ultrapure water used as baseline.

To quantify RIB, the non-coloured reagent method was employed. In this approach, ultrapure water and LNSW were analyzed using the complete reagent set described in the Nutrients Method section above, except for the colour-generating chemicals. Specifically, 1-N-naphthyl-ethylenediamine

dihydrochloride was omitted for NOx and NO_2, while ammonium molybdate was excluded for silicate and phosphate. No RIB effect was observed for ammonium channel.

At the end of each analysis run, the full suite of reagents was switched to the non-coloured reagent setup, and the percentage difference between ultrapure water and LNSW was recorded for each channel using AACE software. This percentage was then converted into concentration values using a proportional ratio calculation, where the highest calibrant's nominal concentration and its corresponding peak height percentage were used as reference points. The average RIB values from multiple measurements are presented in Table 8. After processing in HyPro, nutrient data was corrected for RIB by applying the calculated offset to all samples.

Table 8. Average refractive index blank (RIB) offset value for each channel used for correction on this voyage (Units: µmol L^{-1}, average n = 18).

Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
0.0650 ± 0.0595	0.0271 ± 0.0092	0.0084 ± 0.0045	0.0130 ± 0.0147

Accuracy - Reference Material for Nutrient in Seawater (RMNS)

Descriptive statistics are used to ascertain the accuracy and precision of the analysis from the repetitive measurement of the RMNS for silicate, phosphate, NOx, and nitrite in seawater.

For this voyage, Japanese KANSO certified RMNS lot CQ, CR, CS and CM were assayed at least 5 times in each run to monitor accuracy. An internal bulk quality control (BQC) was also analysed in each analysis run.

The GO-SHIP criteria (Hyde *et al*., 2010), Appendix, GO-SHIP Specifications, specifies using 1-3% of full scale (depending on the nutrient) as acceptable limits of accuracy. However, for hydrochemistry, certain RMNS-certified values are sufficiently low that 1% of the certified value falls below the instrument's method detection limit (MDL) stated in table 6. In such cases, an alternative accuracy threshold of 1–3 times the MDL is applied to ensure measurement reliability for each nutrient.

KANSO publishes the RMNS nutrient values in µmol kg^{-1}. These are converted to µmol L^{-1} at 21°C (table 9). The RMNS CM is not certified for ammonium. NO_x is derived by summing the NO_3 and NO_2 values. The assayed RMNS values per CTD deployments are listed in the Appendix, RMNS results for each CTD Deployment.

For RMNS lot CQ, measurement for NOx is generally within 3 MDL, phosphate is within 2 MDL, nitrite is within 1 MDL, ammonia is slightly below 3 MDL, and silicate is within 2 MDL of its certified mean concentration.

For RMNS lot CR, measurement for NOx is generally within 3%, phosphate is within 1 MDL, nitrite is within 1 MDL, ammonia is within 2 MDL, and silicate is generally within 1 MDL% of its certified mean concentration.

For RMNS lot CS, measurement for NOx is generally within 2%, phosphate is within 2 MDL, nitrite is within 1 MDL, ammonia is within 2 MDL, and silicate is within 1% of its certified mean concentration.

For RMNS lot CM, measurement for NOx is within 2%, phosphate is within 1%, nitrite is within 1 MDL, and silicate is within 2% of its certified mean concentration.

Table 9. RMNS certified concentrations ± expanded uncertainty (U) at 21°C. Units: µmol L^{-1}

RMNS	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonium (NH_4)
Lot CQ	2.2534 ± 0.0717	0.0307 ± 0.0092	0.0717 ± 0.0717	0.1332 ± 0.1024	1.8027 ± 0.0717
Lot CR	14.3389 ± 0.3073	0.4035 ± 0.0143	0.9935 ± 0.0717	6.5856 ± 0.2356	0.9730 ± 0.1536
Lot CS	34.6156 ± 0.4097	1.0948 ± 0.0195	0.1843 ± 0.0717	17.2361 ± 0.2355	0.7476 ± 0.0614
Lot CM	102.9169 ± 0.5120	2.4372 ± 0.0307	0.0184 ± 0.0061	34.0169 ± 0.3134	N/A

Table 10. RMNS statistics for of this voyage. Units: µmol L^{-1}

RMNS CQ	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonium (NH_4)
Minimum	2.00	0.00	0.059	0.13	1.72
Maximum	2.20	0.03	0.074	0.21	1.81
Median	2.10	0.02	0.068	0.19	1.73
Mean	2.10	0.02	0.068	0.18	1.75
Repeatability	0.07	0.01	0.004	0.03	0.04

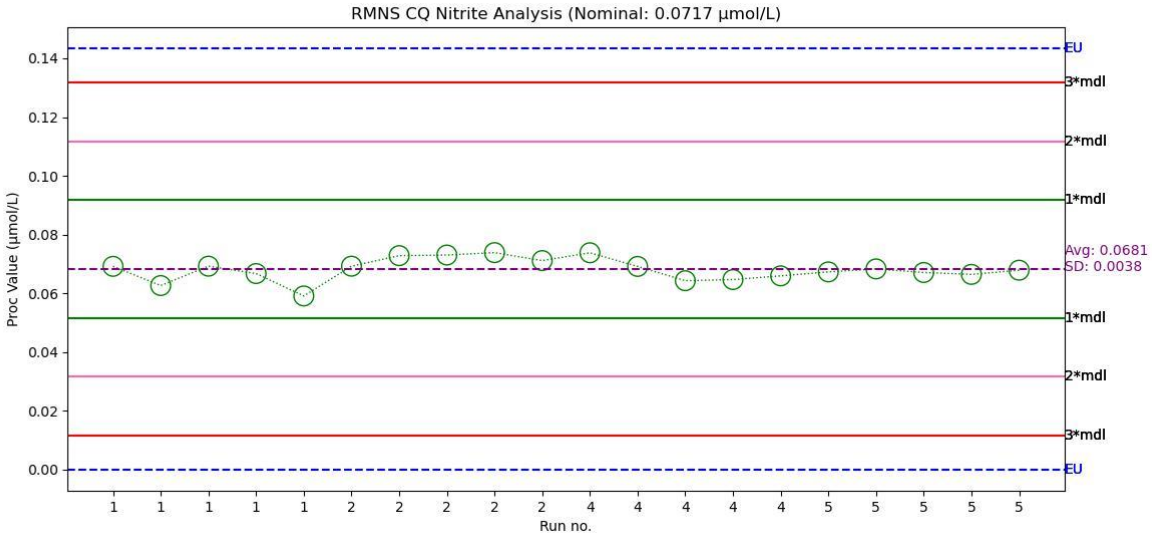
RMNS CR	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonium (NH_4)
Minimum	14.10	0.38	0.980	6.31	0.930
Maximum	14.20	0.42	0.998	6.43	0.950
Median	14.20	0.40	0.993	6.39	0.940
Mean	14.20	0.40	0.991	6.38	0.941
Repeatability	0.04	0.01	0.006	0.04	0.007

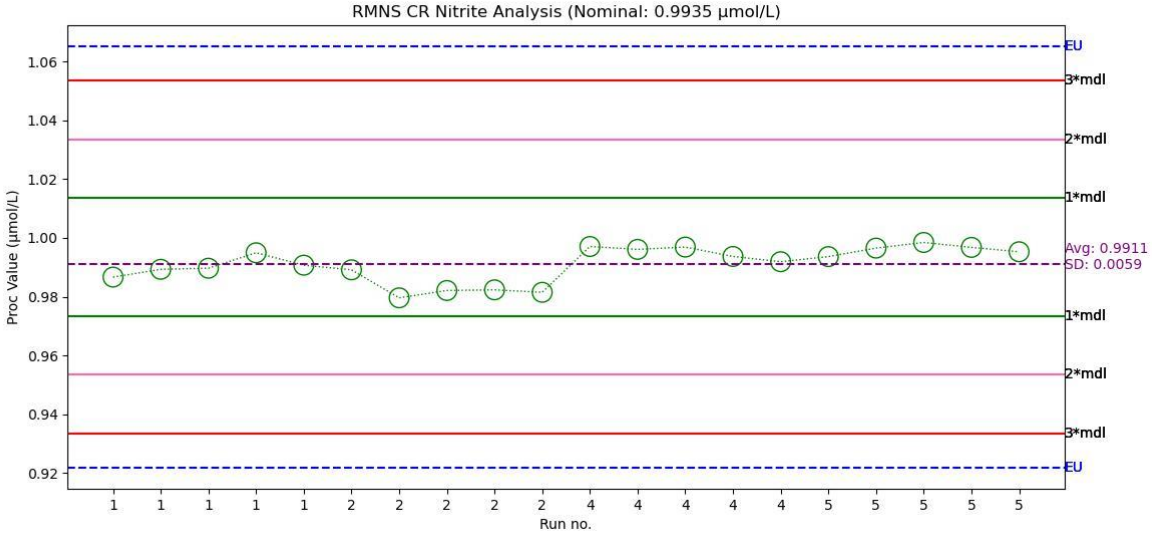
RMNS CS	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonium (NH_4)
Minimum	34.5	1.07	0.171	16.9	0.71
Maximum	34.9	1.11	0.19	17.3	0.79
Median	34.7	1.09	0.178	17.1	0.72
Mean	34.7	1.09	0.179	17.1	0.74
Repeatability	0.1	0.01	0.005	0.1	0.04

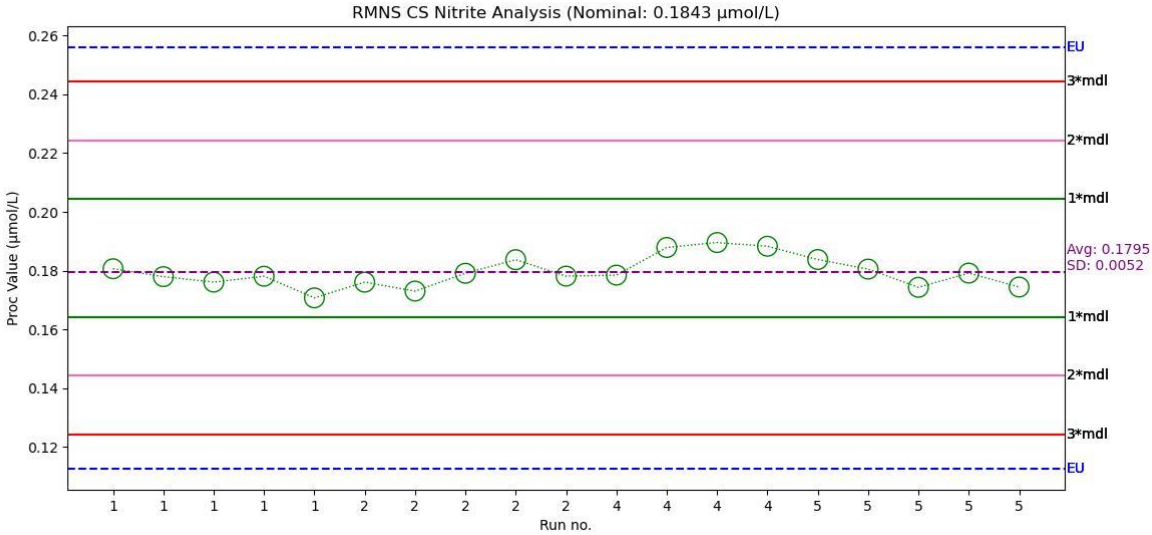
RMNS CM	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_x)	Ammonium (NH_4)
Minimum	101.6	2.41	0.011	33.6	N/A
Maximum	103.6	2.45	0.030	34.4	N/A
Median	102.9	2.43	0.024	34.0	N/A
Mean	102.7	2.43	0.022	34.0	N/A
Repeatability	0.6	0.01	0.006	0.3	N/A

Nutrient plots of RMNS

The green, pink, and red contours are at 1%, 2% and 3% or 1 MDL, 2 MDL, and 3 MDL from the RMNS certified mean value. The blue line is the certified value's expanded uncertainty. Purple dashed line is the average and standard deviation value of each RMNS across all runs on this voyage. Plots are RMNS value versus instrument run number.




RMNS CR Nitrite Analysis (Nominal: 0.9935 µmol/L)
Proc Value (µmol/L)
Run no.
EU
3*mdl
2*mdl
1*mdl
Avg: 0.9911
SD: 0.0059
1*mdl
2*mdl
3*mdl
EU


RMNS CS Nitrite Analysis (Nominal: 0.1843 µmol/L)
Proc Value (µmol/L)
Run no.
EU
3*mdl
2*mdl
1*mdl
Avg: 0.1795
SD: 0.0052
1*mdl
2*mdl
3*mdl
EU

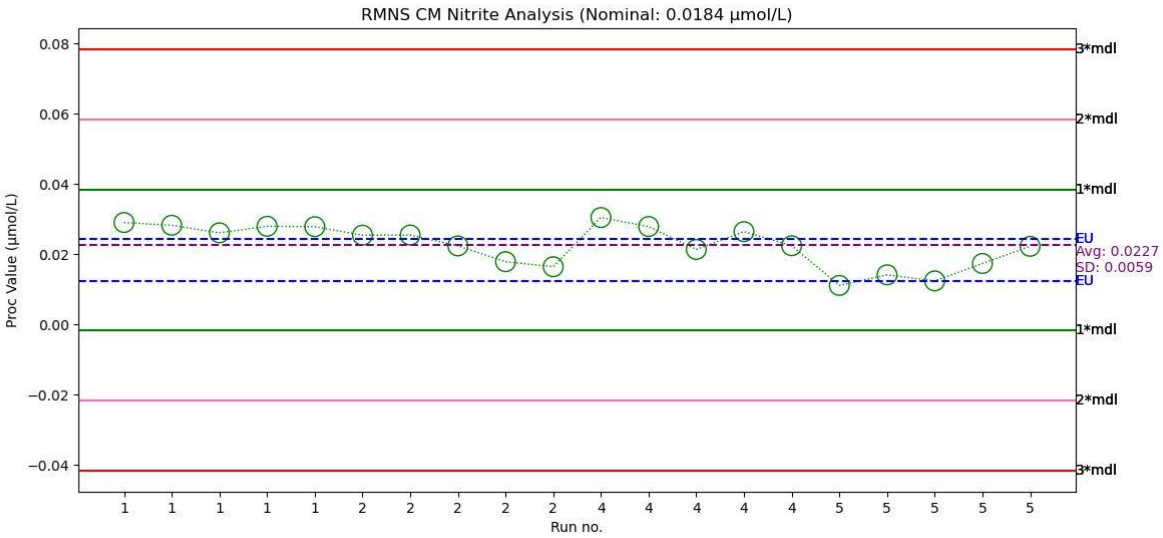
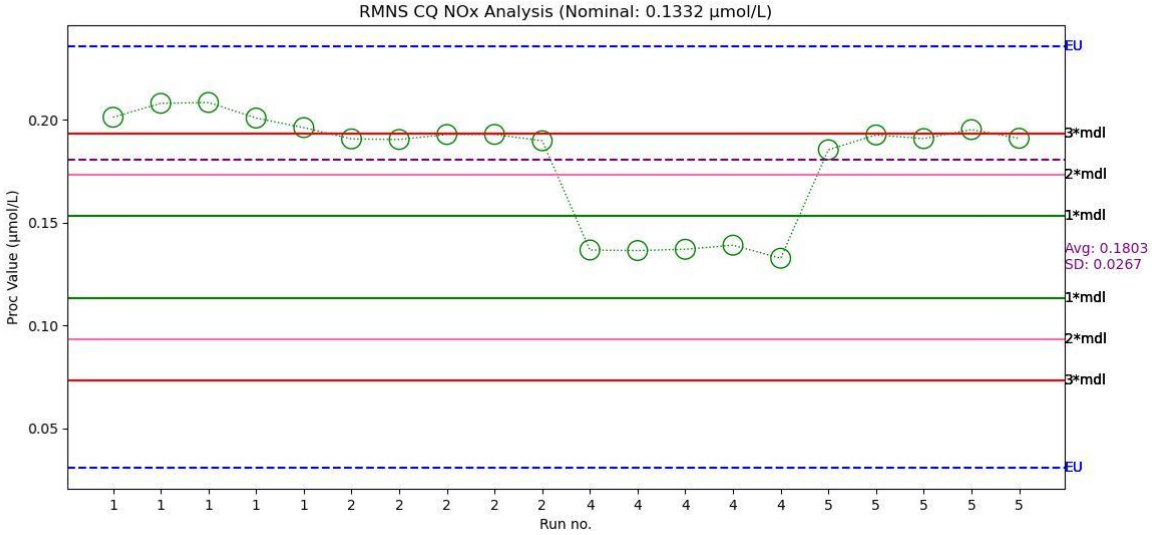
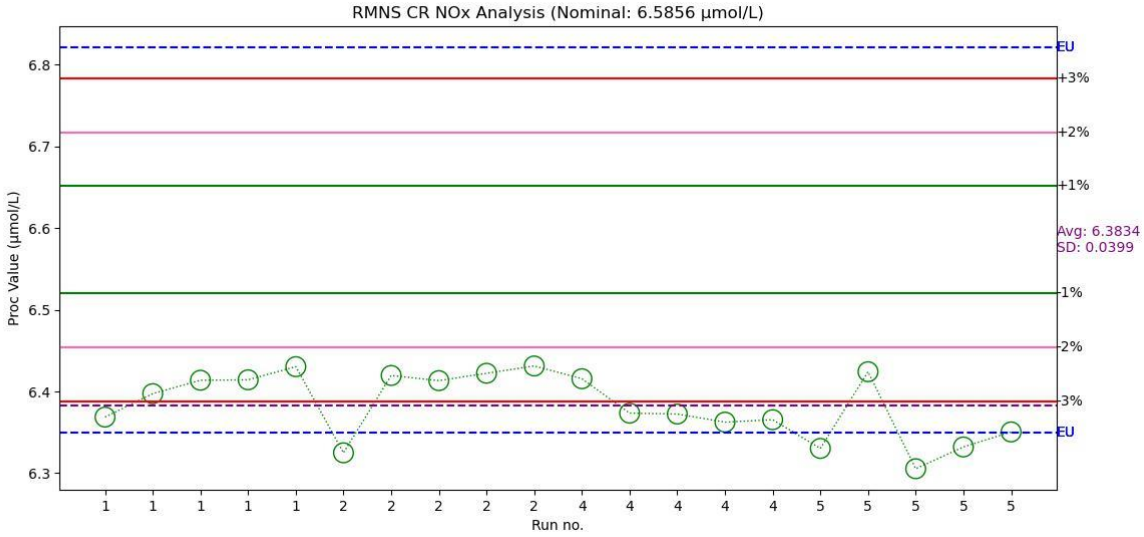


Figure 7. Nitrite RMNS lot CQ, CR, CS, and CM. Units: µmol L^{-1}.


RMNS CQ NOx Analysis (Nominal: 0.1332 µmol/L)
Proc Value (µmol/L)
0.20
0.15
0.10
0.05
EU
3*mdl
2*mdl
1*mdl
Avg: 0.1803
SD: 0.0267
1*mdl
2*mdl
3*mdl
EU
1 1 1 1 1 2 2 2 2 2 4 4 4 4 4 5 5 5 5 5
Run no.


RMNS CR NOx Analysis (Nominal: 6.5856 µmol/L)
Proc Value (µmol/L)
6.8
6.7
6.6
6.5
6.4
6.3
EU
+3%
+2%
+1%
Avg: 6.3834
SD: 0.0399
-1%
-2%
-3%
EU
1 1 1 1 1 2 2 2 2 2 4 4 4 4 4 5 5 5 5 5
Run no.

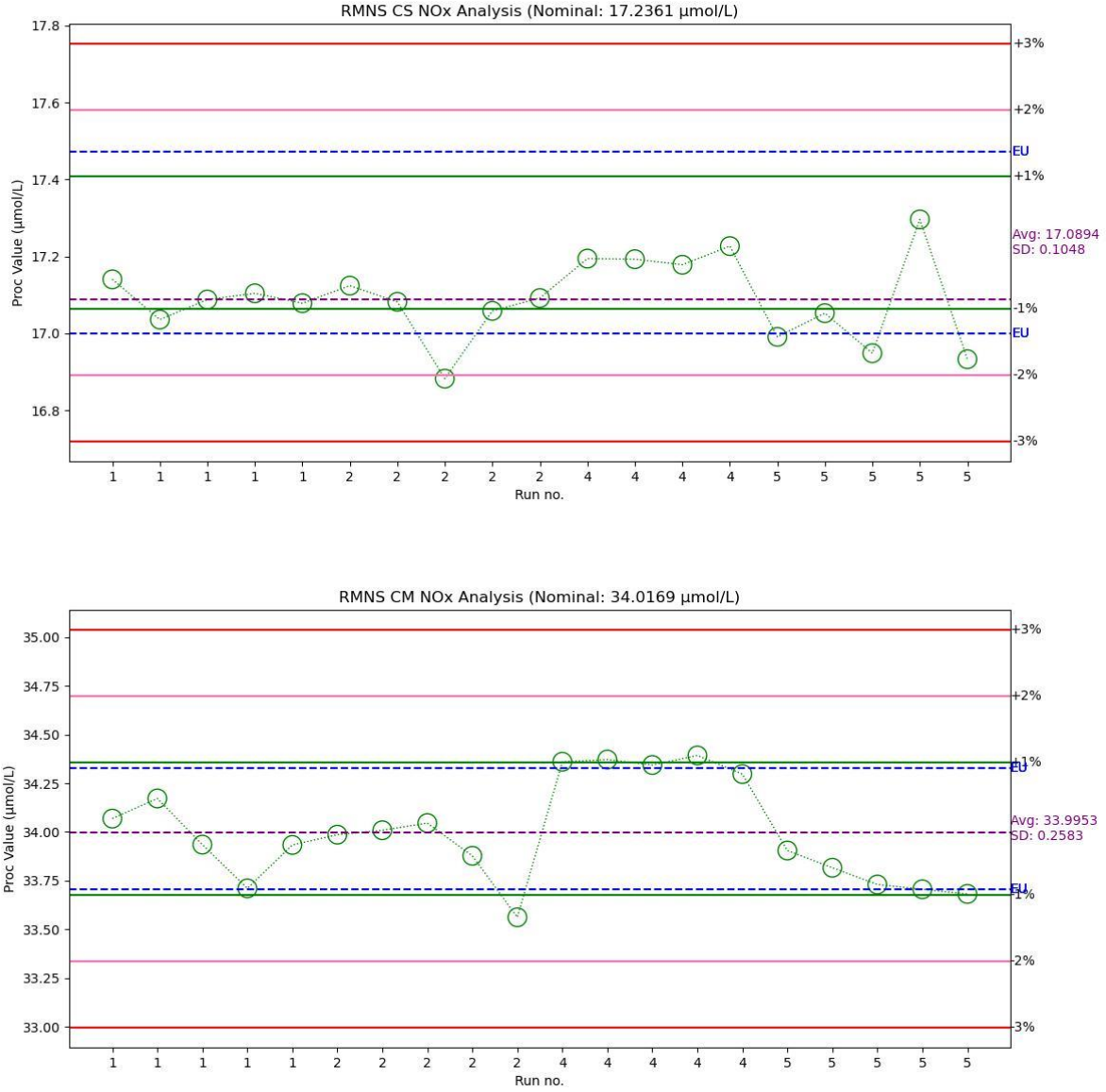
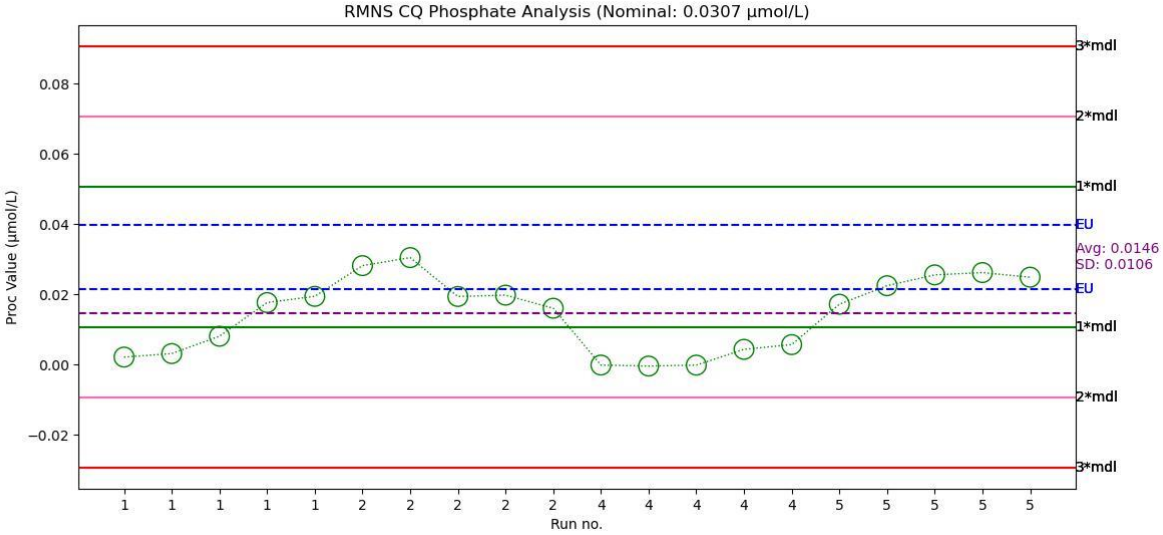
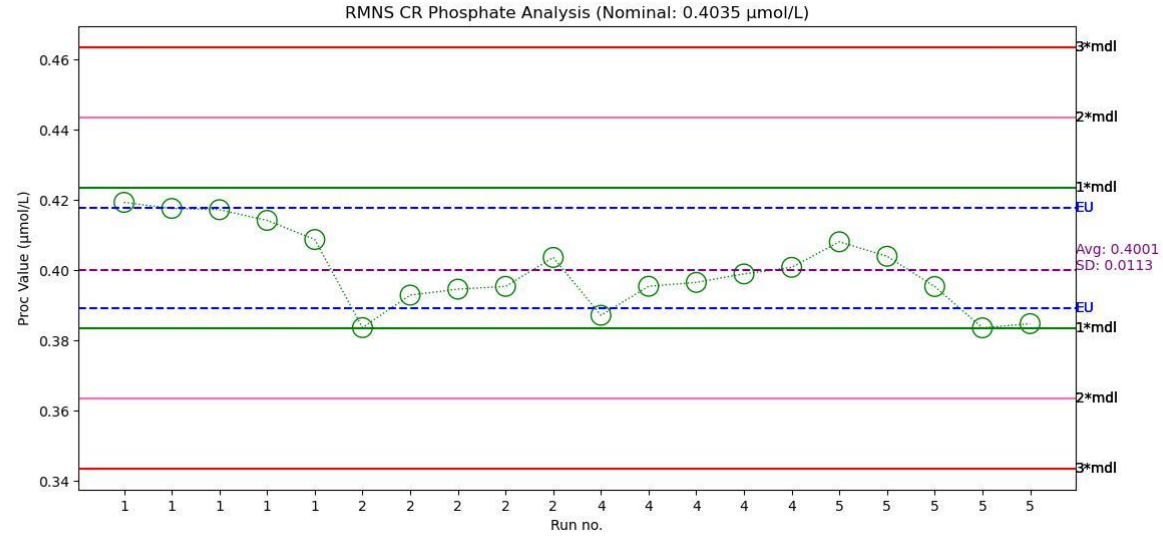


Figure 8. Nitrate + Nitrite (NOx) RMNS lot CQ, CR, CS, and CM. Units: µmol L^{-1}.


RMNS CQ Phosphate Analysis (Nominal: 0.0307 µmol/L)
Proc Value (µmol/L)
Run no.
3*mdl
2*mdl
1*mdl
EU
Avg: 0.0146
SD: 0.0106


RMNS CR Phosphate Analysis (Nominal: 0.4035 µmol/L)
Proc Value (µmol/L)
Run no.
3*mdl
2*mdl
1*mdl
EU
Avg: 0.4001
SD: 0.0113

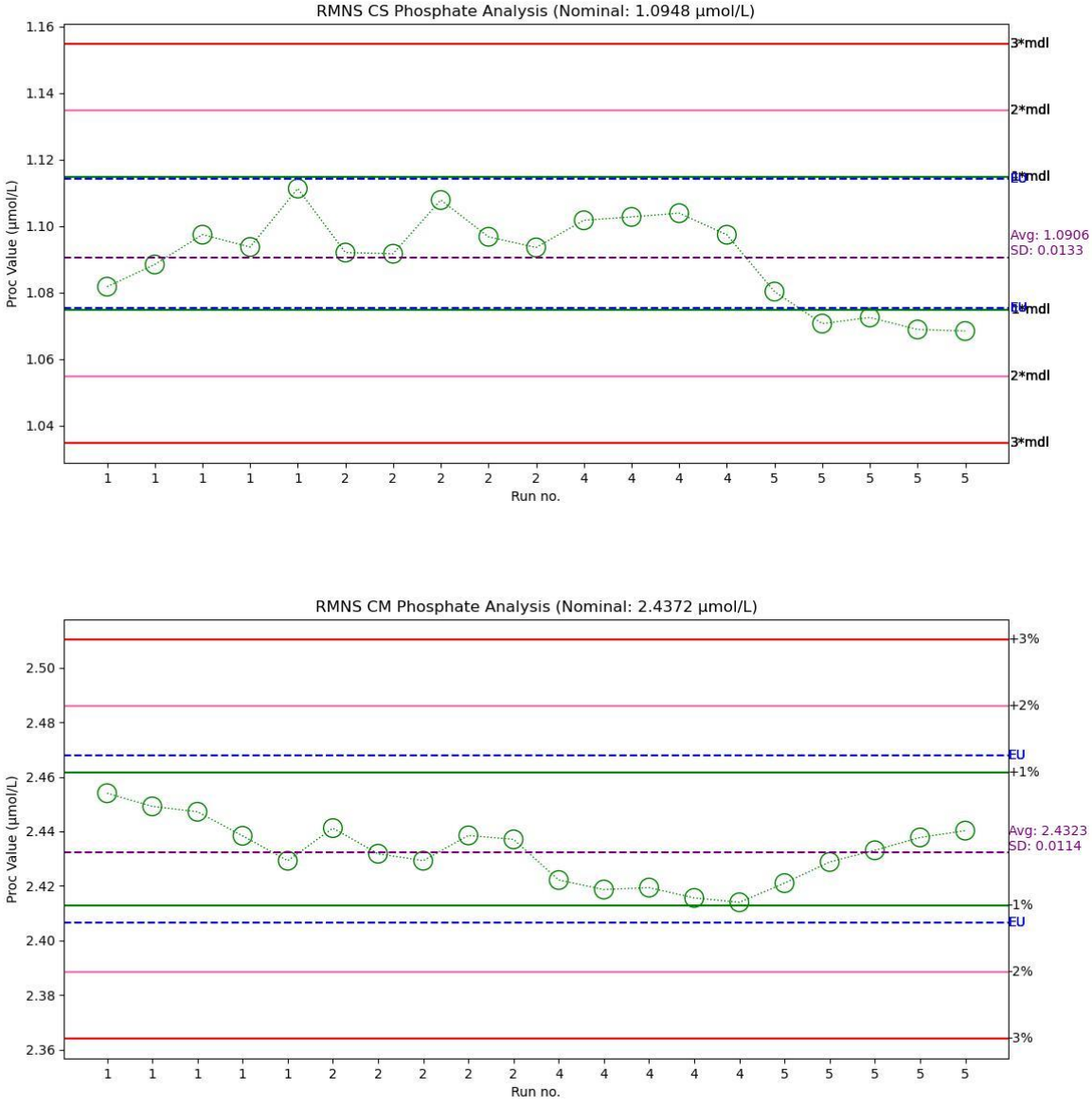
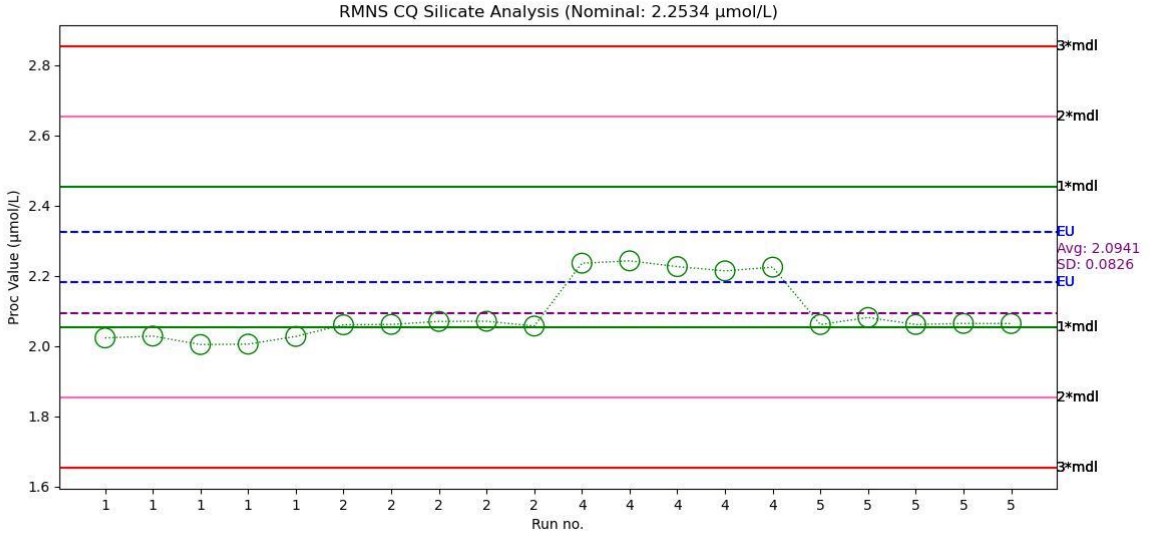
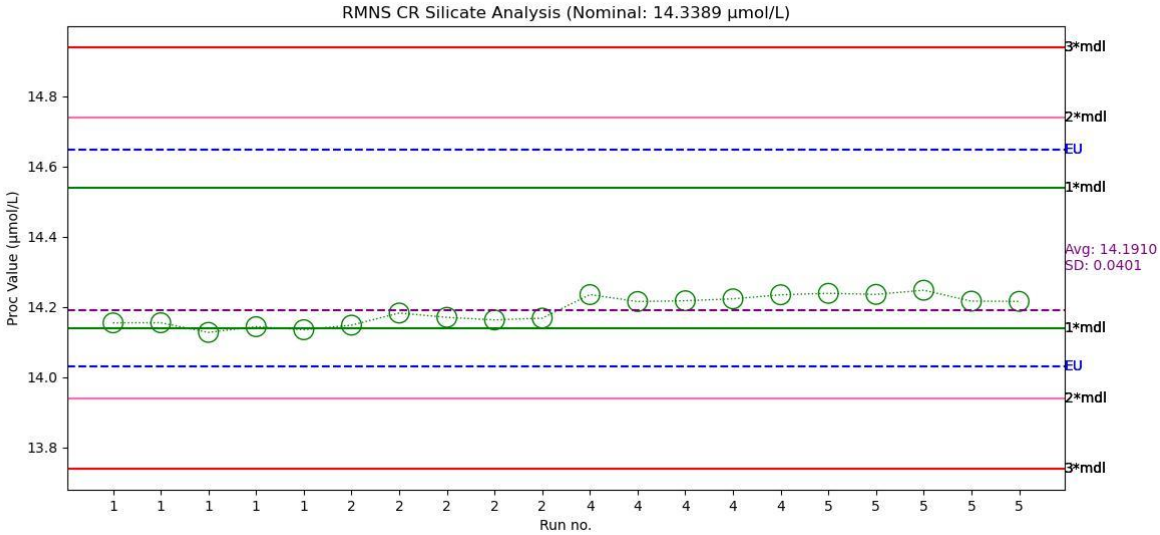


Figure 9. Phosphate RMNS lot CQ, CR, CS, and CM. Units: µmol L^{-1}.


RMNS CQ Silicate Analysis (Nominal: 2.2534 µmol/L)
Proc Value (µmol/L)
Run no.
3*mdl
2*mdl
1*mdl
EU
Avg: 2.0941
SD: 0.0826


RMNS CR Silicate Analysis (Nominal: 14.3389 µmol/L)
Proc Value (µmol/L)
Run no.
3*mdl
2*mdl
EU
1*mdl
Avg: 14.1910
SD: 0.0401

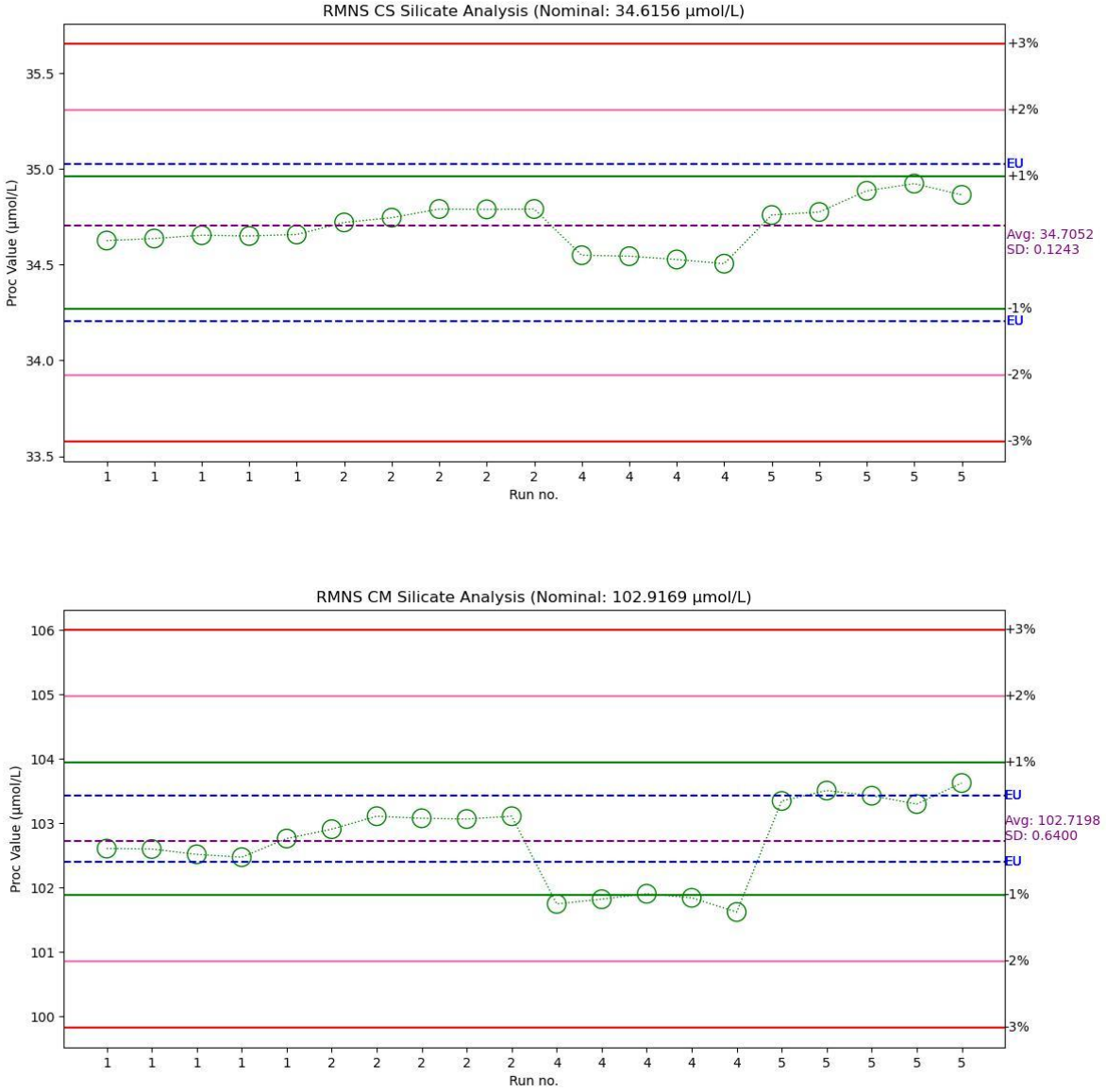
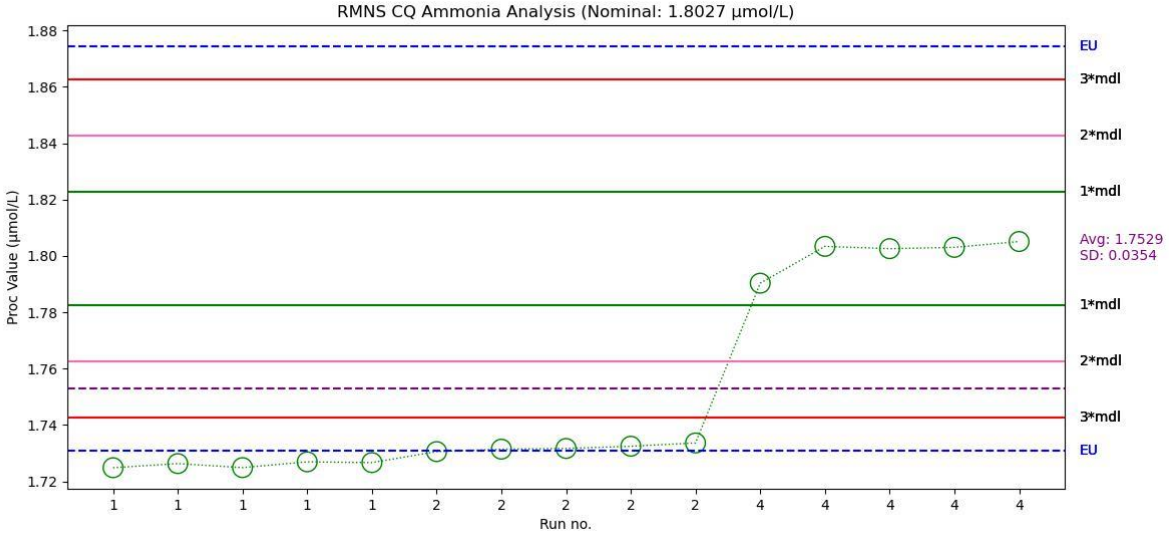
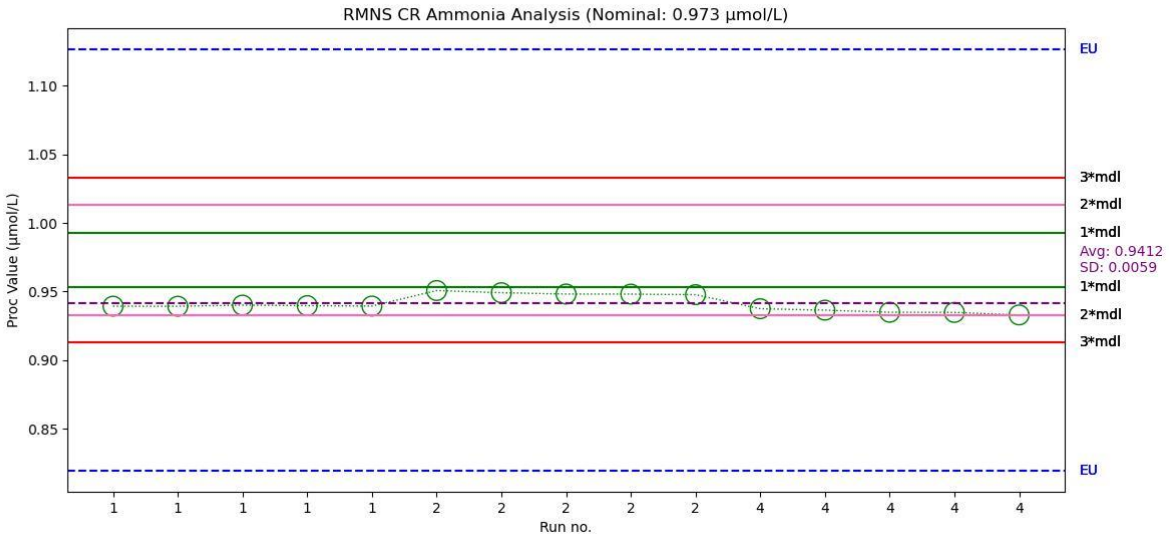


Figure 10. Silicate RMNS lot CQ, CR, CS, and CM. Units: µmol L^{-1}.


RMNS CQ Ammonia Analysis (Nominal: 1.8027 µmol/L)
Proc Value (µmol/L)
Run no.
EU
3*mdl
2*mdl
1*mdl
Avg: 1.7529
SD: 0.0354
1*mdl
2*mdl
3*mdl
EU


RMNS CR Ammonia Analysis (Nominal: 0.973 µmol/L)
Proc Value (µmol/L)
Run no.
EU
3*mdl
2*mdl
1*mdl
Avg: 0.9412
SD: 0.0059
1*mdl
2*mdl
3*mdl
EU

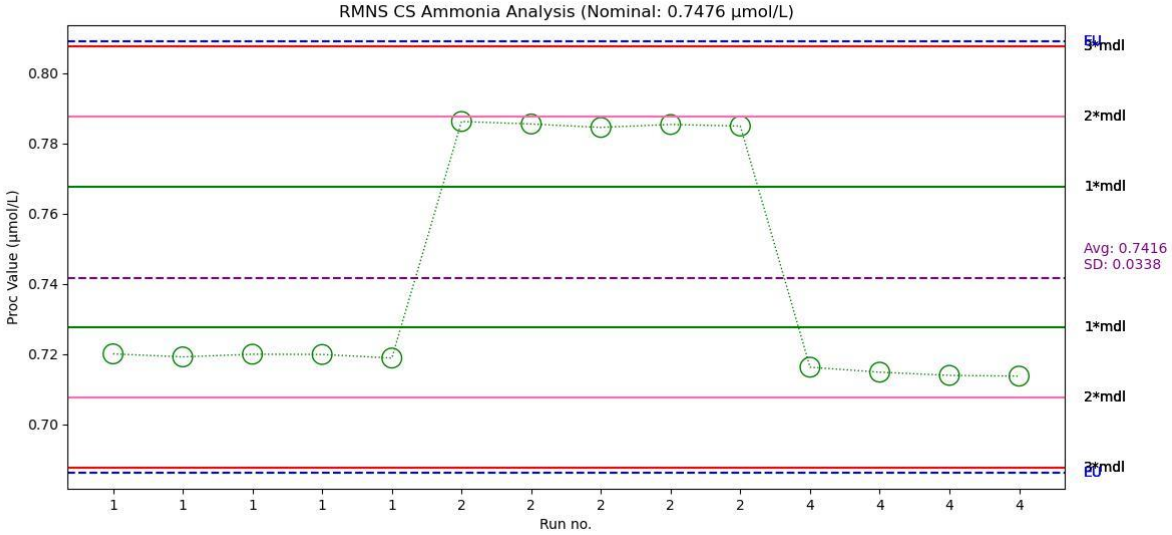


Figure 11. Ammonia RMNS lot CQ, CR, and CS. Units: µmol L^{-1}.

Measurement Uncertainty

The CSIRO hydrochemistry method measurement uncertainty (MU) has been calculated for each nutrient based on the variation in the calibration curve, calibration standards, pipette and glassware calibration, and precision of the RMNS over time (Armishaw 2003).

Table 11. CSIRO Hydrochemistry nutrient analysis uncertainty values. Units: µmol L^{-1}

Calculated Measurement Uncertainty at 1 µmol L^{-1}				
Silicate	Phosphate	Nitrite	Nitrate + Nitrite (NOx)	Ammonia
±0.017	±0.024	±0.140	±0.019	±0.30[¥]

The reported uncertainty is an expanded uncertainty using a coverage factor of 2 giving a 95% level of confidence.

[¥]The ammonia MU precision in this table does not include data for the RMNS.

Method Detection Limit for Nutrients

The method detection limit (MDL) is set to three times the standard deviation (SD) of the LNSW results (National Association of Testing Authorities 2013). The resultant MDL was used to assess the analysis precision at low concentrations.

Table 12. AA500 auto analyser MDL statistics for this voyage. The minimum, maximum, mean, median, and reproducibility (standard deviation) are calculated from every analytical run performed over the voyage. Units: µmol L^{-1}.

MDL	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonia (NH_4)
Nominal MDL	**0.2**	**0.02**	**0.02**	**0.02**	**0.02**
Standard Dev. Min	0.00	0.006	0.001	0.000	0.000
Standard Dev. Max	0.04	0.009	0.005	0.010	0.012
Standard Dev. Median	0.02	0.007	0.003	0.002	0.000
Standard Dev. Mean	0.02	0.007	0.003	0.004	0.004

Precision of MDL (stdev)	0.02	0.007	0.003	0.004	0.004

Sampling Precision

The sampling precision for this voyage is GOOD.

Initial sampling precision is determined with the CTD test deployment (CTD 1) where multiple bottles are fired the same depth, each of which is then sampled for hydrochemistry.

Table 13. Test cast deployment at 1000 dbar bottles RP1-36. Units: µmol L^{-1}.

Duplicates	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonia (NH_4)
Minimum	41.0	2.13	-0.013	31.02	-0.05
Maximum	41.2	2.16	0.001	31.34	-0.03
Mean	41.1	2.14	-0.003	31.18	-0.04
StDev	0.1	0.01	0.004	0.07	0.01

Duplicate nutrient samples are also collected from the greatest depth of subsequent CTD deployments. For nutrients, the sampling precision is good if the difference from the mean of duplicate measurements is less than the nominal method detection limit (Table 6). The exception: NOx (nitrate+nitrite) which uses the limit 0.06 µmol L^{-1}

Duplicate samples that exceed this limit are flagged 69 (suspect). These are tabulated in Appendix, Data Quality Flag Key.

Redfield Ratio Plot (14.0) for CTD Deployments.

The Redfield ratio for this voyage: **14.66**

The Redfield Ratio is a check for the accuracy of phosphate and nitrate + nitrite (NOx) analysis. The ratio is the required amount of P to N for marine phytoplankton growth. Redfield Ratio plot is shown in figure 12.

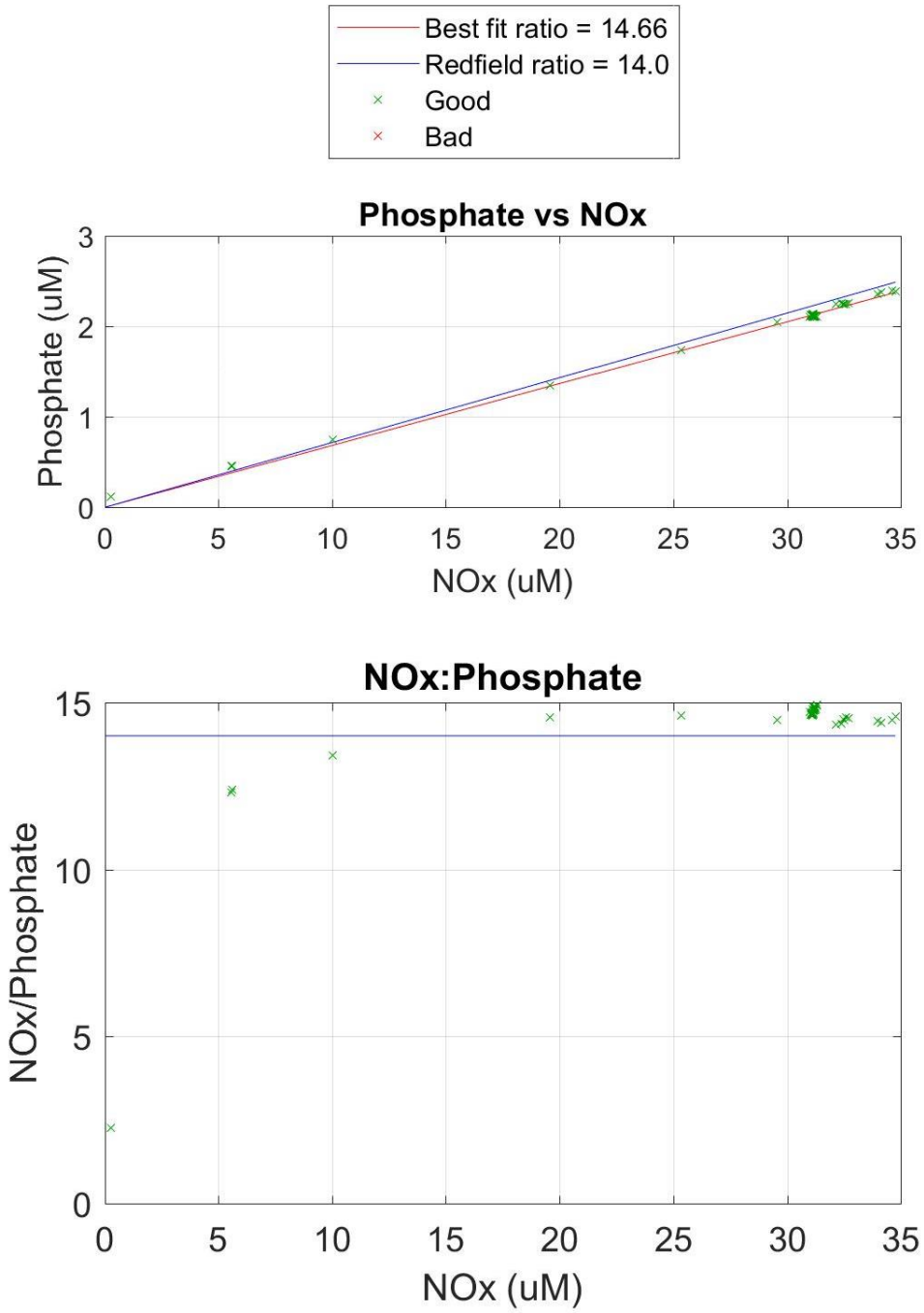


Figure 12. Redfield ratio plots. Red = Data below nominal detection limit.

Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry laboratory and within the segmented flow analyser were monitored before and after each run to ensure no major temperature drift in the laboratory and instrument heater (channel specific) during the analysis run. The temperature was logged using Ruuvi temperature logger and displayed through Grafana. For IN2025_E01, no significant drift in temperature during each analysis run was observed.

Appendix

Salinity: Reference material used

OSIL IAPSO Standard Seawater	
Batch	P167
Use by date	21/02/2026
K_{15}	0.99988
PSU	34.995

Nutrients: RMNS results for each CTD Deployment.

Lot CQ

Run analysis #	CTD Deployment #	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)	Ammonia (NH_4)
1	1	2.0	0.010	0.20	0.065	1.73
2	2	2.1	0.024	0.19	0.072	1.73
4		2.2	0.002	0.14	0.068	1.80
5		2.1	0.024	0.19	0.067	

Lot CR

Run analysis #	CTD Deployment #	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)	Ammonia (NH_4)
1	1	14.1	0.42	6.40	0.990	0.94
2	2	14.2	0.39	6.40	0.983	0.95
4		14.2	0.40	6.38	0.995	0.93

5		14.2	0.39	6.35	0.996	

Lot CS

Run analysis #	CTD Deployment #	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)	Ammonia (NH_4)
1	1	34.7	1.09	17.09	0.177	0.72
2	2	34.8	1.10	17.05	0.178	0.79
4		34.5	1.10	17.20	0.186	0.71
5		34.9	1.07	17.04	0.178	

Lot CM

Run analysis #	CTD Deployment #	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)
1	1	102.6	2.44	33.96	0.028
2	2	103.1	2.44	33.90	0.021
4		101.8	2.42	34.35	0.024
5		103.4	2.43	33.77	0.015

The submitted nutrient results do <u>NOT</u> have RMNS corrections applied.

<u>How to use the RMNS for Correction</u>

Ratio = Certified RMNS Concentration/Measured RMNS Concentration in each run

Corrected Concentration = Ratio x Measured Nutrient Concentration

<u>Or for smoothing data</u>

Ratio = Average RMNS Concentration across voyage/Measured RMNS Conc. in each run

Corrected Concentration = Ratio x Measured Nutrient Concentration

Missing or Suspect Salinity Data

Data is flagged based on CTD sampling log notes, observations during analysis, and examination of depth profile plots (Appendix, Data Quality Flag Key).

CTD	RP	Flag	Reason for Flag
1	36	133	Data is bad, marked by operator. Obvious outlier - potential contamination

Missing or Suspect Dissolved Oxygen Data

Data is flagged based on CTD sampling log notes, observations during analysis, and examination of the depth profile (Appendix, Data Quality Flag Key).

CTD	RP	Flag	Reason for Flag
1	12	133	Data is bad, marked by operator. Obvious outlier - bad endpoint was picked during the analysis
2	3	133	Data is bad, marked by operator. Obvious outlier - bad endpoint was picked during the analysis
2	9	133	Data is suspect, marked by operator. Obvious outlier, potential contamination

Missing or Suspect Nutrient Data.

Data is flagged based on CTD sampling log notes, observations during analysis, and examination of the depth profile (Appendix, Data Quality Flag Key)

CTD	Analyte	RP	Flag	Reason for Flag
2	NH4	5	133	Data is suspect, marked by operator. Obvious outlier, potential contamination

Data Quality Flag Key

Flag	Description	
0	Data is GOOD	
63	Nutrients only.	Data below nominal detection limit.
65	Data is SUSPECT.	Nutrients only: Absorbance peak shape, measured by the instrument, is marginally outside set limits.
69	Data is SUSPECT.	Duplicate data is outside of set limits (software). Data point is an outlier on the depth profile plot (operator). Tagged by software or operator
79	Data is SUSPECT.	Nutrients only. Measured Method Detection Limit (MDL) for the analysis run is greater than the nominal MDL. All samples in that run tagged.
129	Data is BAD.	Nutrients Only. Absorbance peak exceeds the maximum value that can be measured by the instrument.
133	Data is BAD.	Set by operator.
134	Data is BAD.	Nutrients Only. Absorbance peak shape of calibrants, measured by the instrument, is outside of set limits (software).
141	NO Data.	Used in netcdf results file. Not used in csv results file.

GO-SHIP Specifications

Salinity

Accuracy of 0.001 is possible with Autosal™ salinometers and concomitant attention to methodology. Accuracy with respect to one particular batch of Standard Sea Water can be achieved at better than 0.001 PSS-78. Autosal precision is better than 0.001 PSS-78. A precision of approximately 0.0002 PSS-78 is possible following the methods of Kawano with great care and experience. Air temperature stability of ± 1°C is very important and should be recorded[2].

Dissolved Oxygen

Target accuracy is that 2 sigma should be less than 0.5% of the highest concentration found in the ocean. Precision or reproducibility (2 sigma) is 0.08% of the highest concentration found in the ocean.

$Si(OH)_4$

Approximately 1-3% accuracy[1], 0.2% precision[3], full scale.

PO_4

Approximately 1-2% accuracy[1], 0.4% precision[3], full scale.

NO_3

Approximately 1% accuracy[1], 0.2% precision[3], full scale.

Notes

[1] If no absolute standards are available then accuracy should be taken to mean the reproducibility presently obtainable in the better laboratories.

[2] Keeping constant temperature in the room where salinities are determined greatly increases their quality. Also, room temperature during the salinity measurement should be noted for later interpretation if queries occur. Additionally, monitoring and recording the bath temperature is also recommended. The frequent use of IAPSO Standard Seawater is endorsed. To avoid the changes that occur in Standard Seawater, the use of the most recent batch is recommended. The bottles should also be used in an interleaving fashion as a consistency check within a batch and between batches.

[3] Developments of reference materials for nutrients are underway that will enable improvements in the relative accuracy of measurements and clearer definition of the performance of laboratories when used appropriately and the results are reported with the appropriate meta-data.

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