

RV INVESTIGATOR

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	IN2024_V05
Chief Scientist	Richard Little
Voyage title:	South-East Australian Marine Ecosystem Survey (SEA-MES) III
Report compiled by:	Maddy Lahm and Narendra Pati

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Executive Summary

Objectives

The marine waters of southeast Australia are one of a series of global ocean-warming hotspots. In this region, the ocean surface is warming at a rate four times the global average and many species have extended their distributions southward, with apparent changes in local abundance. The region is home to a range of important economic activities such as fisheries, oil and gas production, and emerging renewable energy industries. It also contains nationally important amenities such as marine parks.

Fishery and ecosystem assessments were last conducted in the region 25 years ago. This project will repeat the surveys to document changes and establish a new biological and environmental baseline. This will help us better understand what is changing in the region and why, and the impacts from climate change.

The main activities on this voyage were to collect data on the demersal fish community, benthic habitat, water column, and prey fields. Equipment used includes: multibeam echosounders for seafloor habitat mapping, deep towed camera, demersal and multinet trawls for sampling marine life in the water column and close to the seafloor, and CTD (conductivity, temperature and depth) casts. Projects onboard included carbonate chemistry sampling in Bass Strait via the collection of water samples as part of an initiative to investigate ocean alkalinity enhancement in Bass Strait.

94 CTDs were deployed in total. Of these 94, 75 CTD deployments were sampled and analysed by the on-board Hydrochemistry team for nutrients, dissolved oxygen and salinity.

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, 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 autoanalyzer. 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.

RMNS were within 3% of their certified values. Missing and suspect hydrology samples are listed in [Appendix, Missing or Suspect Nutrient Data](#).

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Please cite the following manuscript when reporting or publishing data for silicate, phosphate, nitrate+nitrite (NO_x) 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 trawler](#).

For Data, contact: NCMI_DataLibrarians@csiro.au

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Itinerary

Table 1: Voyage itinerary

	Depart	Arrive
Port	Hobart	Hobart
Date	12/11/2024	12/12/2024
Time	10:00	20:00

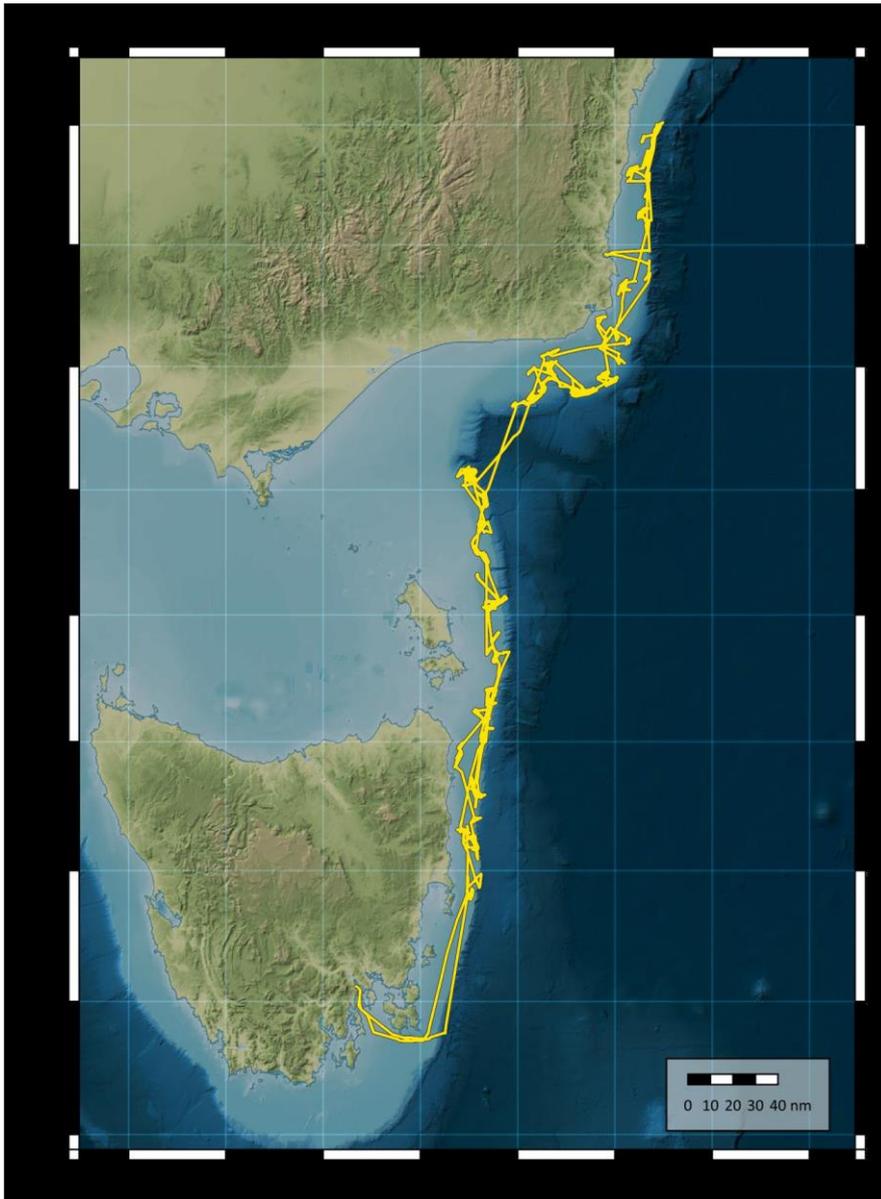


Figure 1. Voyage map of IN2024_V05.

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Key personnel list

Table 2: Key Personnel list

Name	Role	Organisation
Richard Little	Chief Scientist	CSIRO
Tegan Sime	Voyage Manager	CSIRO
Maddy Lahm	Hydrochemist	CSIRO
Narendra Pati	Hydrochemist	CSIRO

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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)	287 CTD	3 Not Completed
	30 TSG	3 Not Completed
Dissolved Oxygen (automated titration)	287 CTD	Completed
Nutrients (Seal AA500)	287 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 94 CTD deployments were conducted over the duration of the voyage. Of the 94 deployments, 75 were sampled by:
 - Hydrochemistry: Maddy Lahm and Narendra Pati
 - Science party: Claire Davies, Sahan Jayasinghe, Jackson Griffin, Amelia Jensen, and Clothilde Langlais.

Thermosalinograph (TSG) samples

- Taken from the underway instrument clean seawater line supplying the pCO₂ 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: Maddy Lahm and Narendra Pati

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).

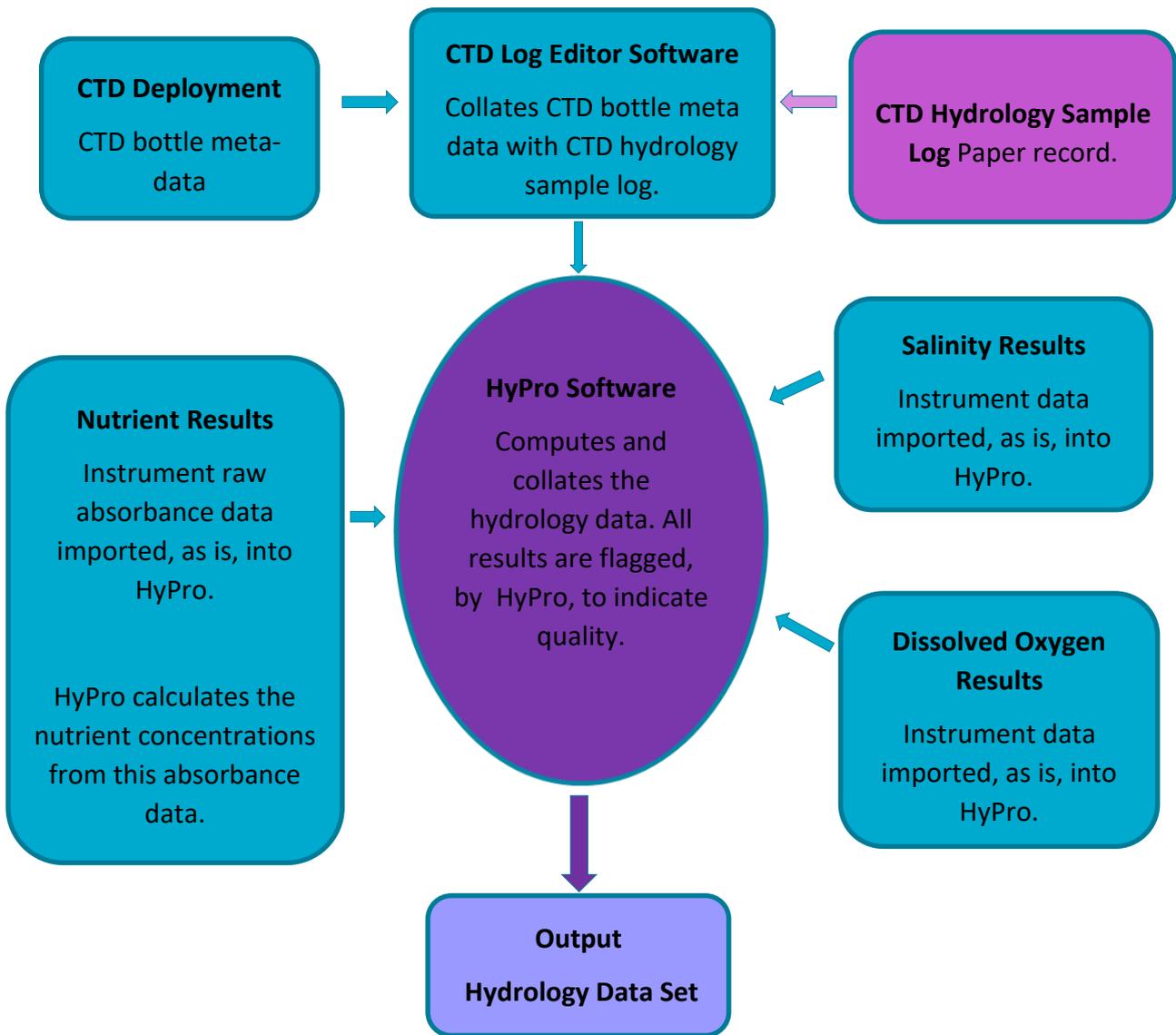


Figure 2. Conventional Hydrology Data Processing Flow Diagram.

Salinity Measurement Parameters

Table 4. Salinity Measurement Parameters

Details	
HyPro Version	5.7
Instruments	Guildline Autosal Laboratory Salinometer 8400(B) – SN 72089. 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.
Lab Temperature	Mean 21.9°C SD 0.7°C
Analysts	Maddy Lahm and Narendra Pati
Comments	See DAP report for CTD calibration details.

OFFICIAL Salinity Method

Salinity samples were measured on a Guildline Autosol 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 32.4356×10^{-3} .

Before each lot of sample measurements, the Autosol 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: 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, till overflowing. The bottle is removed from the straw and the sample is decanted to allow a headspace of approximately 25cm³. 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 after the fourth and fifth flush. 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 0.0045 PSU (Figure 3). The larger differences are for shallow samples across the sudden changes in the thermohaline profile. The two major outliers were checked for comments during analysis but neither of these had any issues that could attribute to them being outliers.

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](mailto:NCMI_DataLibrarians@csiro.au) for corrected CTD data.

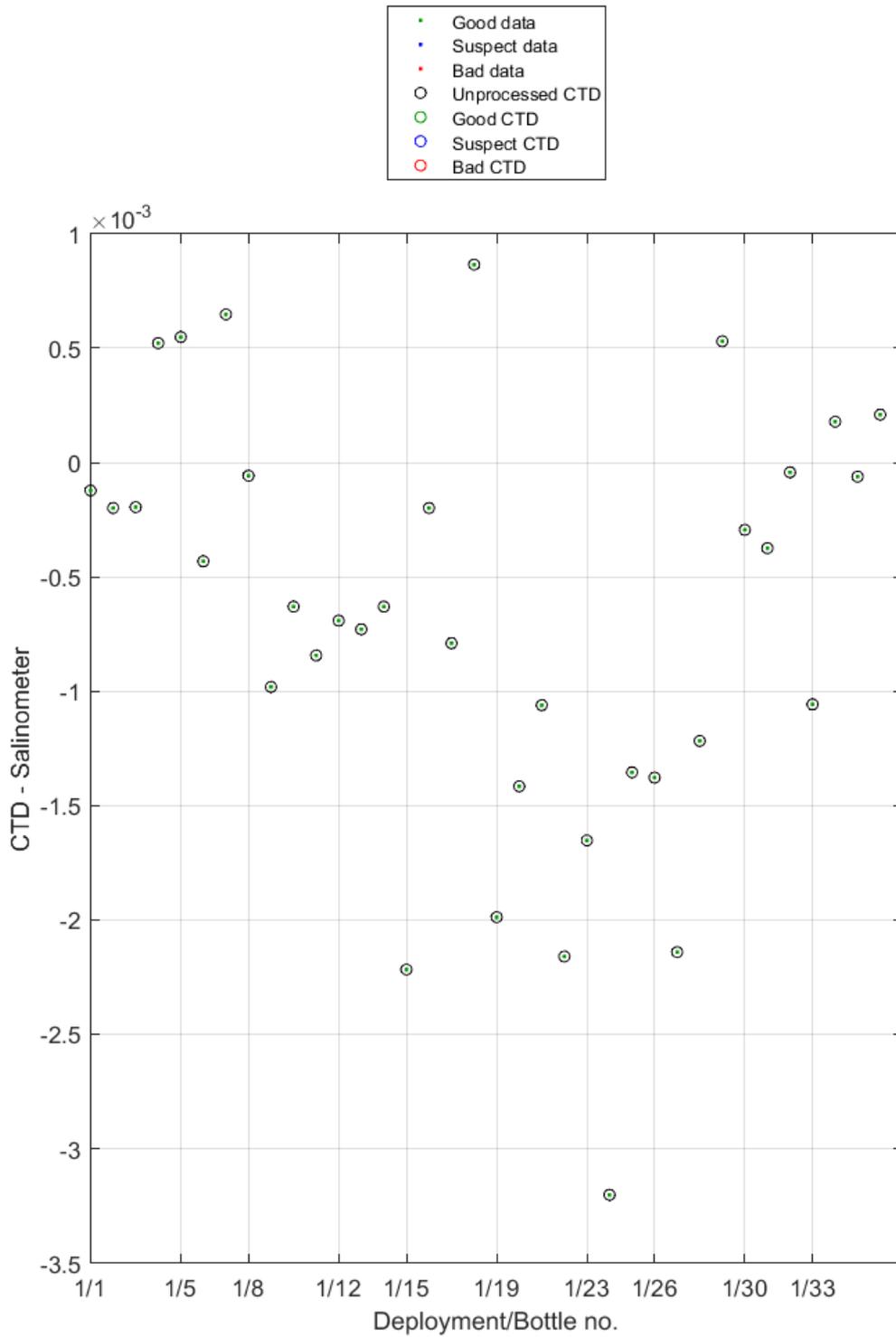


Figure 3. CTD Salinity - Bottle Salinity vs CTD deployment plot. The data quality is coded by colour and delineated by a dot for the bottle salinity and a circle for the CTD salinity. Green = GOOD. Blue = SUSPECT. No fill = BAD. Black = UNPROCESSED. Units: PSU (dimensionless). *Note: Bad salinity bottle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

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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.

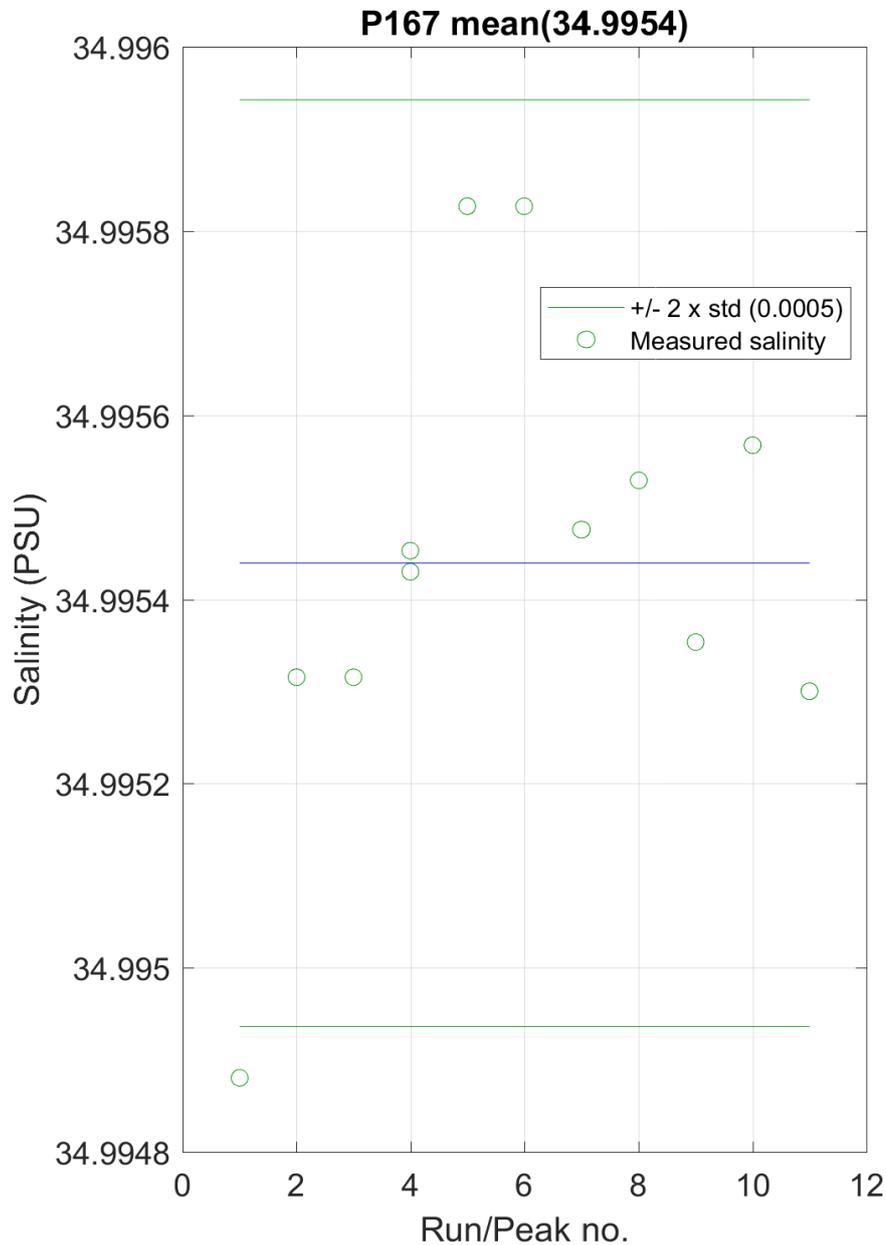


Figure 4. Measured OSIL standard for each salinity run and average value (P167 mean) across IN2024_V05

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OFFICIAL 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	$\pm 0.5 \mu\text{mol L}^{-1}$
Analysts	Maddy Lahm and Narendra Pati
Lab Temperature ($\pm 1^\circ\text{C}$)	Mean 20.8°C SD 1.6°C
Sample Container type	140 ml glass iodine determination flasks with glass stopper.
Sample Storage	Samples stored in the hydrochemistry lab until analysis.
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 Culbertson *et al* (1991).

Method: 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 665 Dosimat fitted with a 1 ml burette. The endpoint is determined by measuring the decrease in the UV absorption 365 nm.

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The thiosulphate solution is standardised by 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 $10 \mu\text{mol L}^{-1}$ (Figure 7). 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](mailto:NCMI_DataLibrarians@csiro.au) for corrected CTD data.

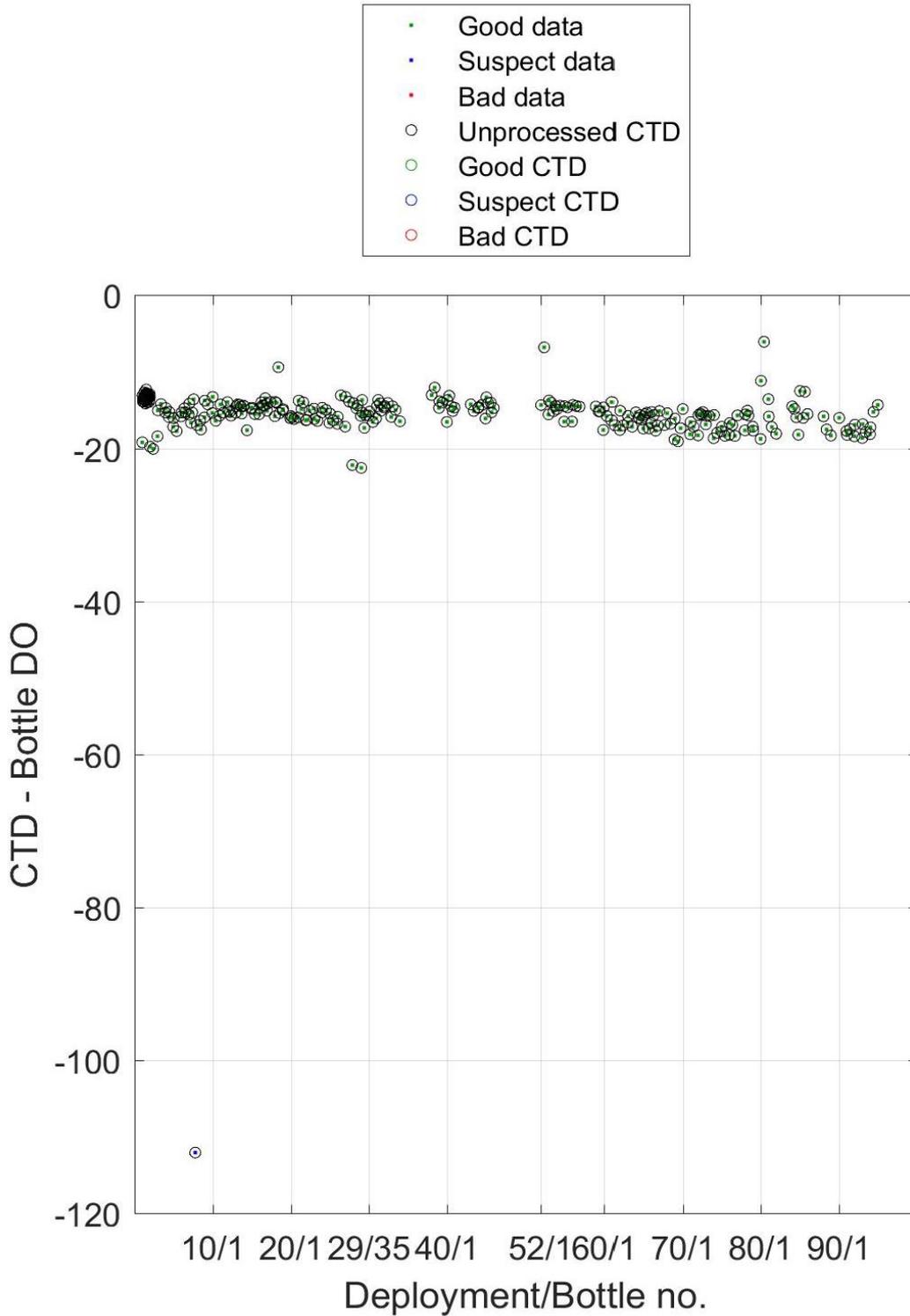


Figure 5. CTD Dissolved Oxygen - Bottle Dissolved Oxygen vs Deployment Plot. The data quality is coded by colour and delineated by a dot for the bottle DO and a circle for the CTD DO. Green = GOOD. Blue = SUSPECT. No fill = BAD. Black = UNPROCESSED. Units: $\mu\text{mol L}^{-1}$. *Note: Bad oxygen bottle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

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Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.

The variation in thiosulphate concentration is within our QC parameter of less than 0.0005 N between standardisations. The blank correction is used in the calculation of the thiosulphate normality and is due to oxidisable species in the reagents and in the MQ water that is 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 75 CTD deployments analysed that lie within casts 1 to 94:

Mean: 0.203545 SD: 0.000245 n= 8

For thiosulphate normality plots, the red lines indicate ± 0.0005 N either side of the mean titrant (thiosulfate) concentration. For blank plot, red lines indicate acceptable variation either side of the mean blank concentration. Blanks out of range but within acceptable limits due to reagents tested and approved by Hydrochemistry prior to voyage in reflection of the consistent blank range coverage within accepted the Thio normality range across all runs. The titrant does not vary more than 0.0005 N between analyses.

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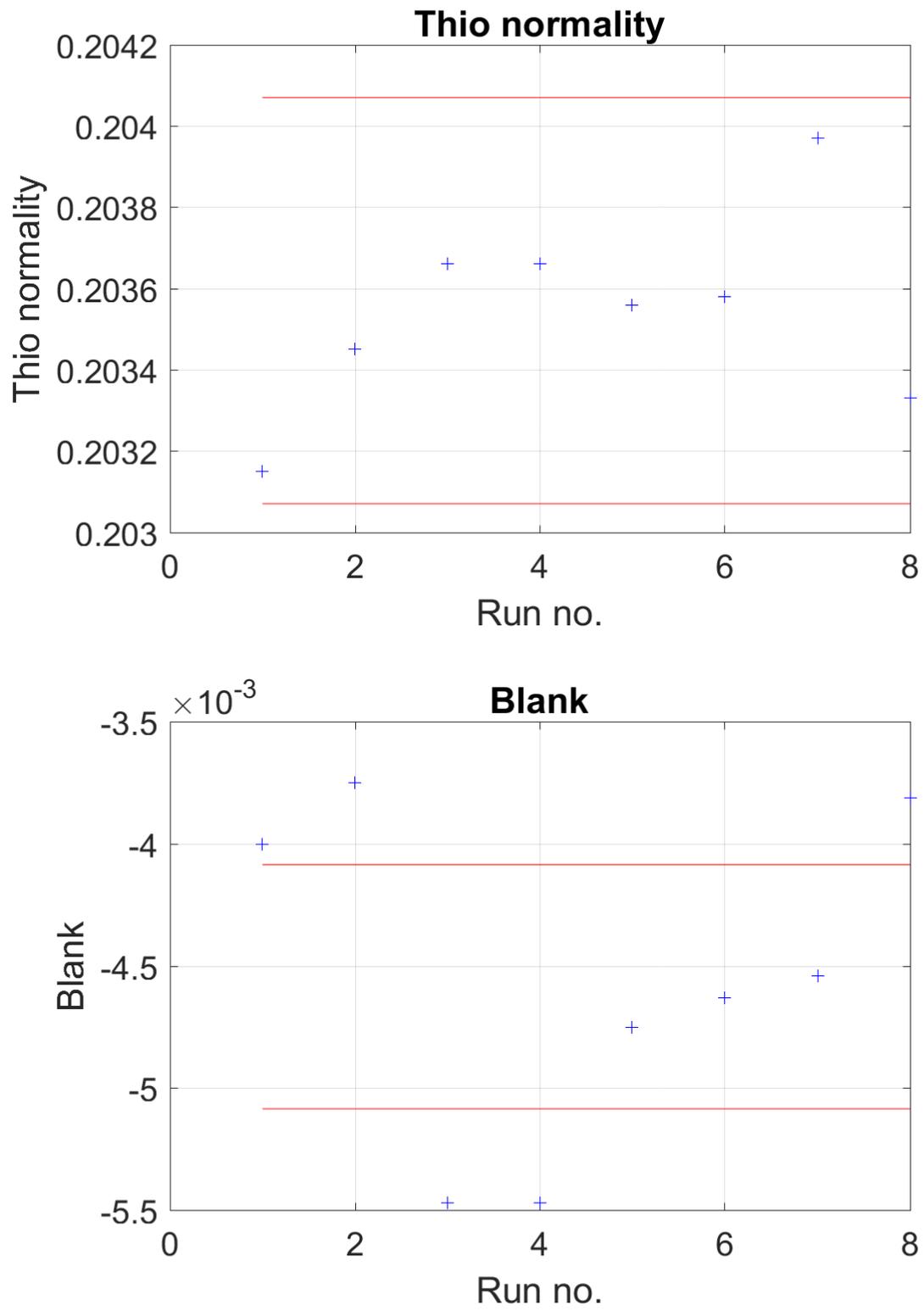


Figure 6. Thiosulphate standardisation (top) and blank correction (bottom) plots.

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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)	140.0	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 CO				
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.				
Lab Temperature (°C)	Mean 21.0°C SD 1.7°C				
Analysts	Maddy Lahm & Narendra Pati				

OFFICIAL 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 JASCO FP2020 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-SGNOS¹ 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-naphthyl-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-phtaldialdehyde method. Based on K erouel 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.

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.

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HyPro uses criteria to identify suspect calibration points, noisy peaks, method detection limits that are above the nominal limit, and duplicate 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:

- $\pm 0.5\%$ of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE¹).
- $0.02 \mu\text{mol L}^{-1}$ 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 (NO _x)	Nitrite	Ammonia
Data Reported as	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$
Calibration Curve fit	Linear	Linear	Linear	Quadratic	Quadratic
# of points in Calibration	7	6	7	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	Y	Y	Y	Y	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 June 2021. Sub-lot passed through a 5-micron filter (filtered in 2023 & 2024) and stored in 20 L carboys in the clean dry laboratory at 22°C.				
Medium of Baseline	18.2 MΩ 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 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 NO_x and NO₂, 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

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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: $\mu\text{mol L}^{-1}$, average $n = 23$).

Silicate (Si(OH) ₄)	Phosphate (PO ₄)	Nitrite (NO ₂)	NO ₃ + NO ₂ (NO _x)
0.030943 ± 0.094	0.02293 ± 0.012	0.01746 ± 0.0047	0.01484 ± 0.032

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, NO_x, and nitrite in seawater.

For this voyage, Japanese KANSO certified RMNS lot CO was assayed in triplicates in each run to monitor accuracy (Table 8). RMNS CP was only analysed in the characterisation run as additional accuracy monitoring. RMNS CL was only analysed in run 5, and CR was only analysed for runs 2, 4, and 15 for additional accuracy monitoring. An internal bulk quality control (BQC) was also analysed in each analysis run.

For RMNS lot CO for NO_x, nitrite and phosphate are generally within 1% of their respective certified mean concentration, whereas silicate is generally within 2% of their respective certified mean concentration.

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.

KANSO publishes the RMNS nutrient values in $\mu\text{mol kg}^{-1}$. These are converted to $\mu\text{mol L}^{-1}$ at 21°C. The RMNS is not certified for ammonium. NO_x is derived by summing the NO₃ and NO₂ values. The assayed RMNS values per CTD deployments are listed in the [Appendix, RMNS results for each CTD Deployment](#).

Table 9. RMNS certified concentrations \pm expanded uncertainty (U) at 21°C. Units: $\mu\text{mol L}^{-1}$

RMNS	Silicate (Si(OH) ₄)	Phosphate (PO ₄)	Nitrite (NO ₂)	NO ₃ + NO ₂ (NO _x)
Lot CO	35.552 \pm 0.164	1.205 \pm 0.014	0.041 \pm 0.041	16.281 \pm 0.195

Table 10. RMNS CO statistics for of this voyage. Units: $\mu\text{mol L}^{-1}$

RMNS CO	Silicate (Si(OH) ₄)	Phosphate (PO ₄)	Nitrite (NO ₂)	NO ₃ + NO ₂ (NO _x)
Minimum	24.6	0.88	0.031	16.16
Maximum	36.3	1.25	0.063	16.45
Mean	35.4	1.22	0.047	16.325
Median	35.9	1.23	0.047	16.33
Repeatability	2.00	0.05	0.007	0.064

Nutrient plots of RMNS

The green, pink, and red contours are at 1%, 2% and 3% from the RMNS certified mean value. Exception: nitrite, the contours are at 0.02 $\mu\text{mol L}^{-1}$ increments from the certified value. The blue line is the certified value's expanded uncertainty. Plots are RMNS value versus instrument run number. Please note that plots for lot CP, CL, and CR are representing only certain runs.

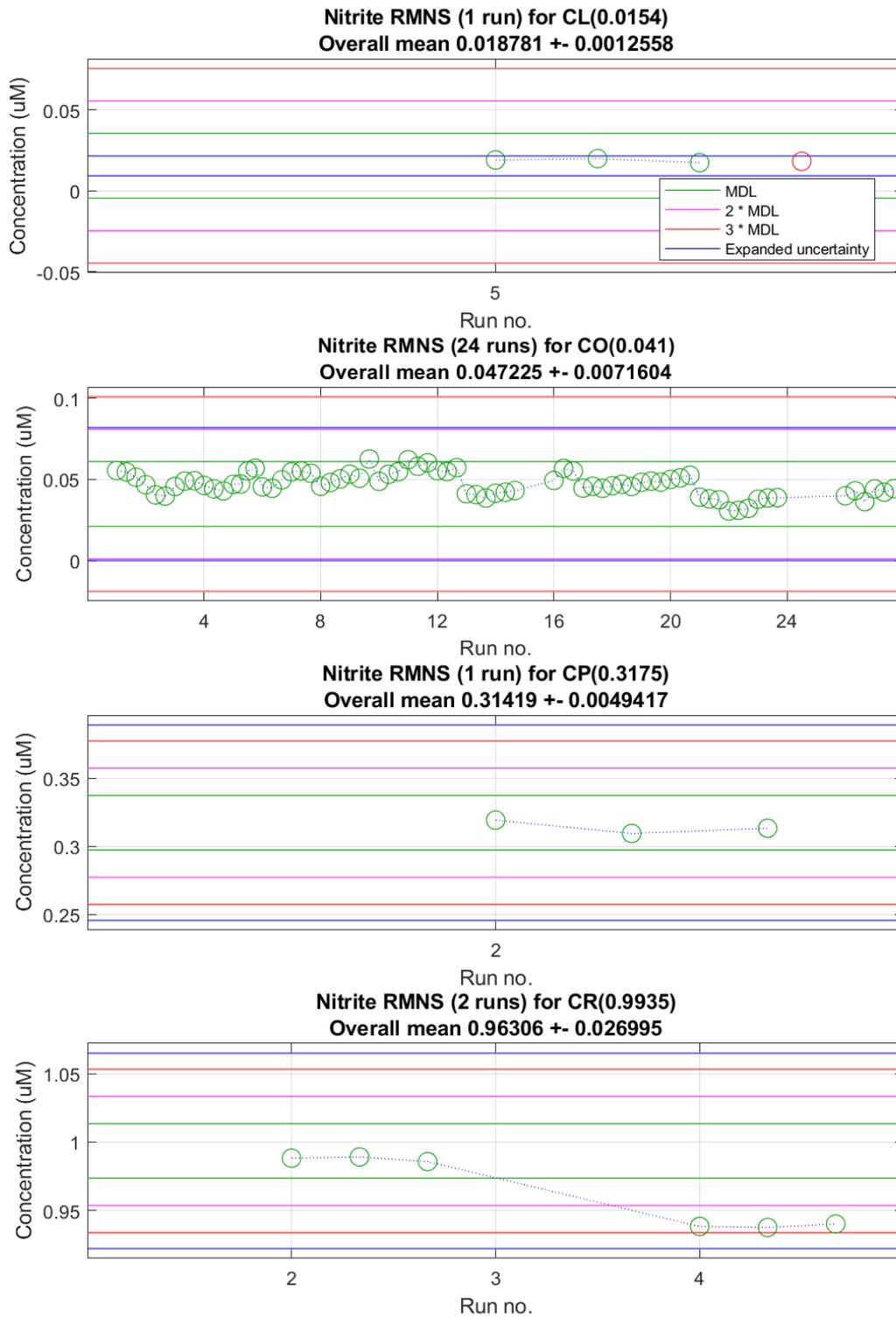


Figure 7. Nitrite RMNS lots CL, CO, CP, CR plots. Units: $\mu\text{mol L}^{-1}$.

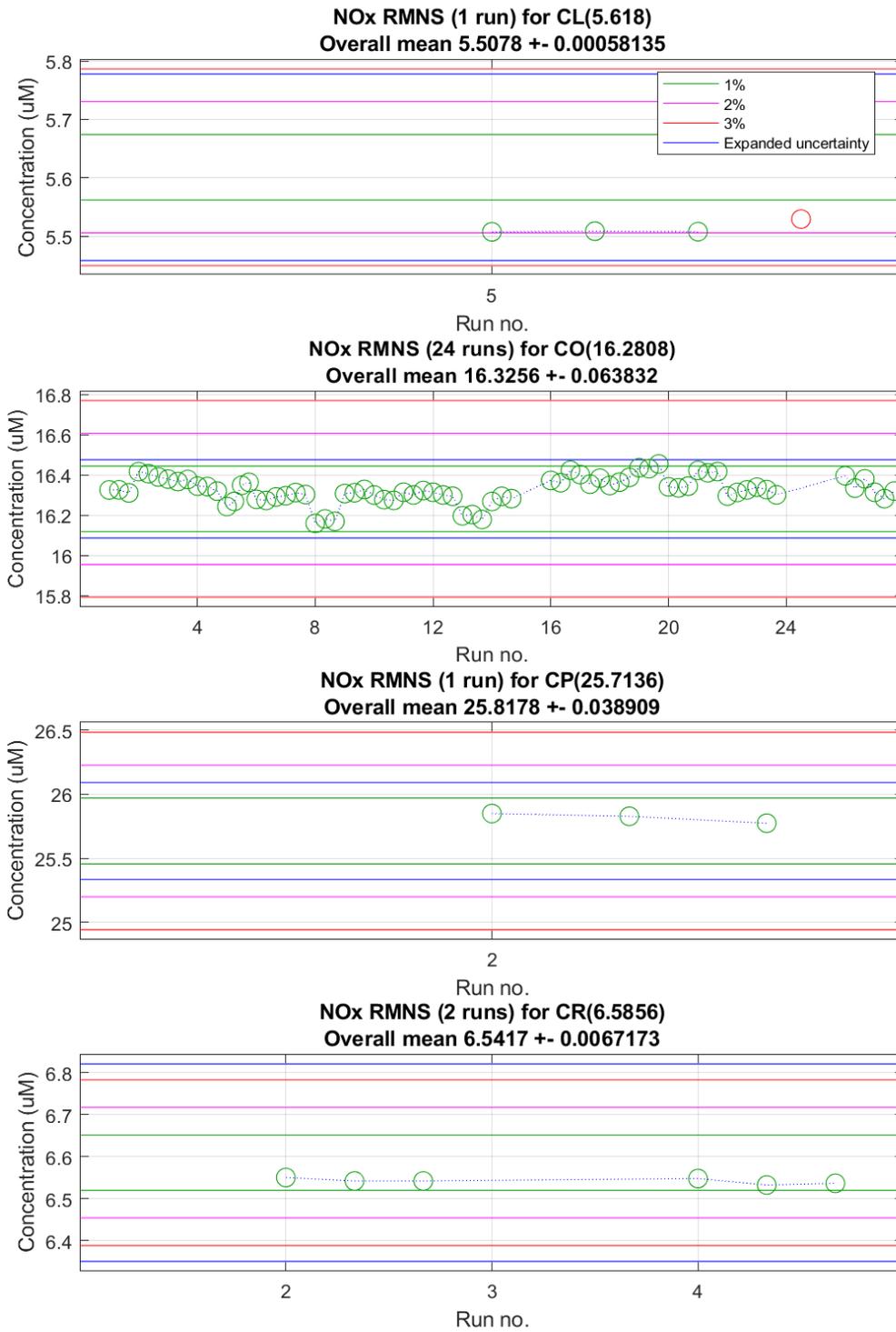


Figure 8. Nitrate + Nitrite (NOx) RMNS lots CL, CO, CP, CR plots. Units: $\mu\text{mol L}^{-1}$.

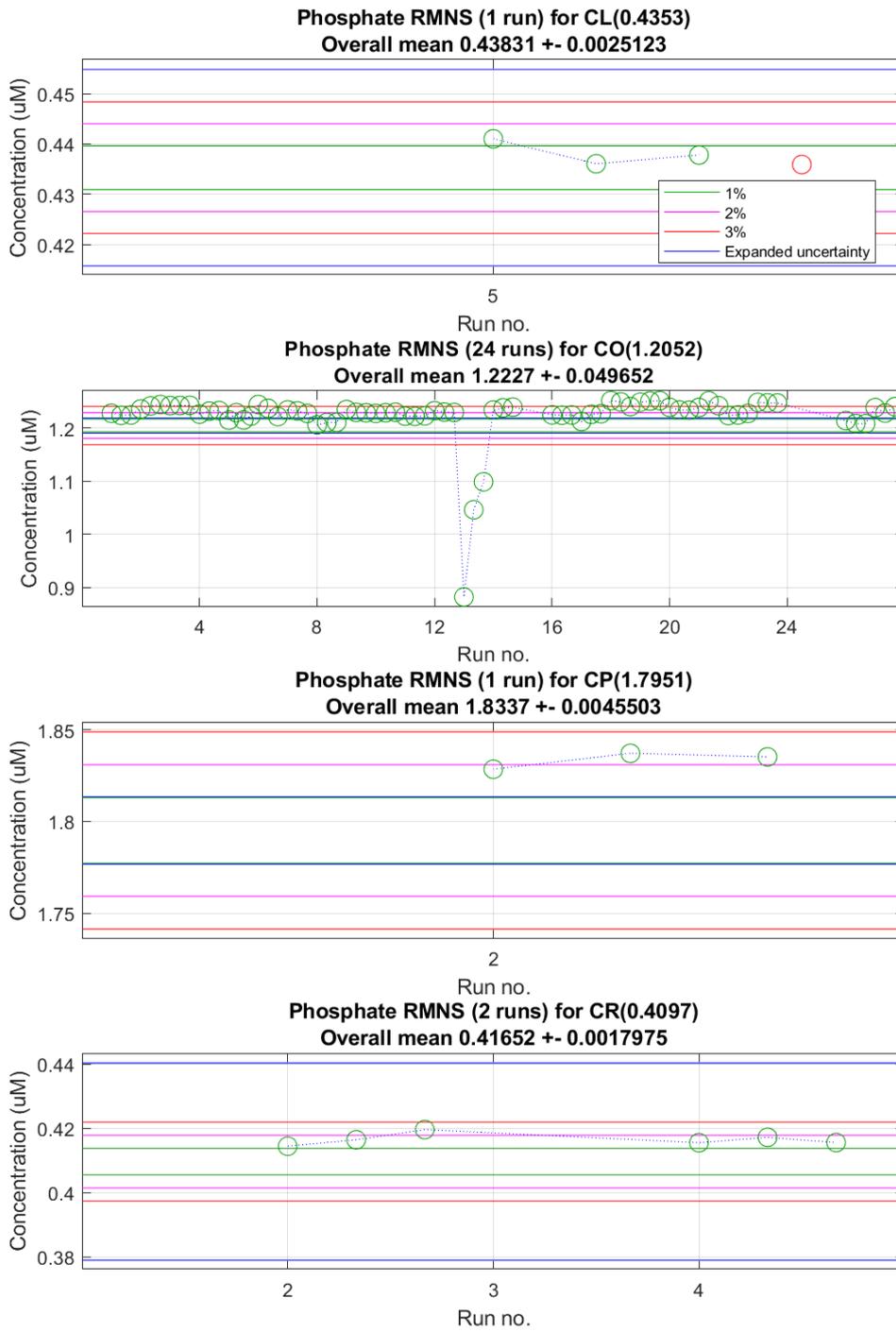
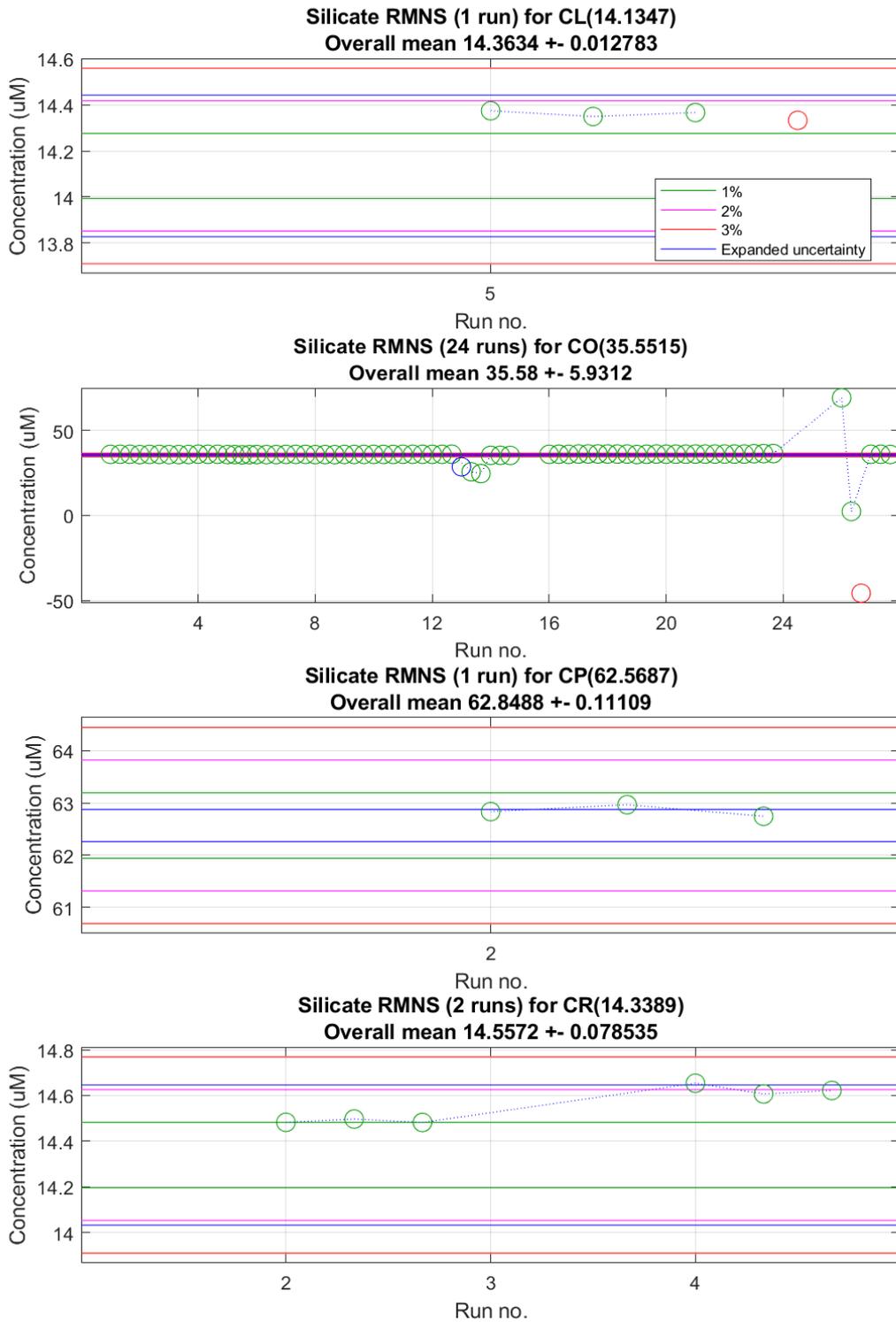


Figure 9. Phosphate RMNS lots CL, CO, CP, CR plot (top to bottom). Units: Units: $\mu\text{mol L}^{-1}$.



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: $\mu\text{mol L}^{-1}$

Calculated Measurement Uncertainty at 1 $\mu\text{mol L}^{-1}$				
Silicate	Phosphate	Nitrite	Nitrate + Nitrite (NO _x)	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 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: $\mu\text{mol L}^{-1}$.

MDL	Silicate (Si(OH) ₄)	Phosphate (PO ₄)	Nitrate + Nitrite (NO _x)	Nitrite (NO ₂)	Ammonia (NH ₄)
Nominal MDL	0.707	0.053	0.028	-0.001	0.075
Standard Dev. Min	0.000	0.0	0.000	0.000	0.000
Standard Dev. Max	9.34	0.11	0.012	0.008	0.006
Standard Dev. Mean	0.462	0.008	0.002	0.002	0.001
Standard Dev. Median	0.100	0.006	0.000	0.002	0.000
Precision of MDL (stdev)	0.462	0.008	0.002	0.005	0.001

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Sampling Precision

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: $\mu\text{mol L}^{-1}$.

Duplicates	Silicate (Si(OH)_4)	Phosphate (PO_4)	Nitrite (NO_2)	$\text{NO}_3 + \text{NO}_2$ (NOX)	Ammonia (NH_4)
Minimum	44.7	2.16	-0.042	30.61	0
Maximum	45.1	2.21	0.006	31.5	0.43
Mean	44.9	2.20	-0.020	31.0	0.013
StDev	0.107	0.009	0.011	0.242	0.072

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 16). The exception: NOx (nitrate+nitrite) which uses the limit $0.06 \mu\text{mol L}^{-1}$.

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: **13.81**

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.

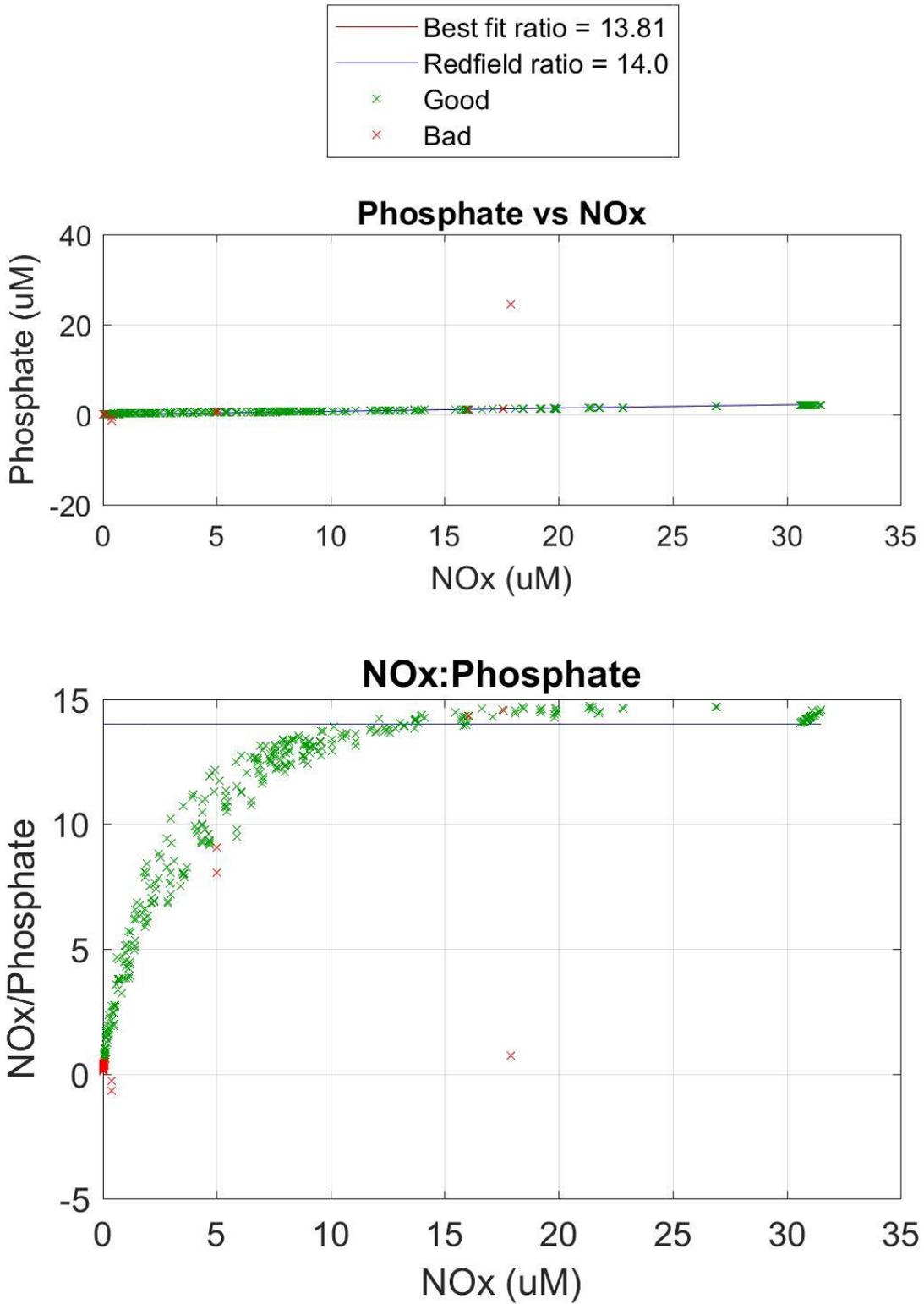


Figure 11. Redfield ratio plots. Red = Data below nominal detection limit.

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Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry laboratory and within the segmented flow analyser were measured and logged in the following locations:

- Hydrobox - Above the DO dosimat station
- Hydrobox – Above the Portasal station
- Hydrobox AA500 - On the chemistry module deck, post heater
- Hydrochemistry Salts Lab – below window.

Data was measured using Ruuvi temperature logger and humidity sensor and logged and monitored in Grafana, with the exception of data from 13-23/11/2024. Mark Brunton of DAP confirmed the Raspberry Pi for Ruuvi data collection failed between 13-23/11/2024 but the rest of temperature data is reported per standard reporting. Measurements were recorded every 1 second for the duration of the voyage. If required, this data will be provided on request.

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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 CO

Run analysis #	CTD Deployment #	Silicate (Si(OH) ₄) (μmol L ⁻¹)	Phosphate (PO ₄) (μmol L ⁻¹)	NO _x (NO ₂ + NO ₃) (μmol L ⁻¹)	Nitrite (NO ₂) (μmol L ⁻¹)
1	-	35.9	1.227	16.323	0.054
2	1, 2, 3	35.8	1.24	16.407	0.043
3	4	35.767	1.24	16.377	0.048
4	5, 6, 7	35.933	1.23	16.333	0.044
5	5, 6, 7, 8, 9, 10	35.725	1.222	16.305	0.052
6	11, 12, 13	35.767	1.233	16.28	0.047
7	14, 15, 16	35.9	1.23	16.303	0.055
8	17, 18, 19	35.7	1.21	16.17	0.048
9	20, 21	35.8	1.233	16.317	0.056
10	22, 23, 24	35.833	1.23	16.283	0.052

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11	25, 26, 27	35.9	1.22	16.131	0.06
12	28, 29, 30	35.933	1.23	16.3	0.056
13	39	26.267	1.01	16.193	0.04
14	31, 32, 33, 38, 39, 40	35.267	1.237	16.28	0.042
16	43, 44, 45, 52, 53, 54, 55, 56, 59, 60	35.933	1.227	16.383	0.054
17	61, 62, 63	36.1	1.223	16.377	0.045
18	64, 65, 66	36.1	1.247	16.367	0.046
19	67, 68, 69	35.933	1.25	16.44	0.048
20	70, 71, 72, 73	36.0	1.233	16.34	0.051
21	74, 75, 76	36.033	1.243	16.417	0.038
22	77, 78, 79	36.067	1.227	16.31	0.031
23	80, 81, 84	36.3	1.25	16.323	0.039
27	90, 91, 92, 93, 94	35.9	1.237	16.303	0.43

The submitted nutrient results do NOT have RMNS corrections applied.

How to use the RMNS for Correction

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

Corrected Concentration = Ratio x Measured Nutrient Concentration

Or for smoothing data

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

Corrected Concentration = Ratio x Measured Nutrient Concentration

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Missing or Suspect Salinity Data

No data is flagged based on CTD sampling log notes, observations during analysis, and examination of depth profile plots ([Appendix, Data Quality Flag Key](#)).

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
7	29	69	Data point is an outlier on the depth profile plot (operator). Tagged by operator.

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	RP	Analyte	Flag	Reason for Flag
28	1	Ammonia, NOx, Phosphate, Silicate	69	Duplicate data is outside of set limits (software).
29	1	NOx, Phosphate, Silicate	69	Duplicate data is outside of set limits (software).
39	1	Phosphate, Silicate	69	Duplicate data is outside of set limits (software).
39	35	NOx	65	Nutrients only: Absorbance peak shape, measured by the instrument, is marginally outside set limits.
45	1	Ammonia	69	Duplicate data is outside of set limits (software).
52	1	Ammonia, Phosphate	69	Duplicate data is outside of set limits (software).
54	1	Ammonia	69	Duplicate data is outside of set limits (software).
90	1	Silicate	69	Duplicate data is outside of set limits (software).
91	1	Silicate	69	Duplicate data is outside of set limits (software).
92	1	Ammonia, Silicate	69	Duplicate data is outside of set limits (software).
93	1	Silicate	69	Duplicate data is outside of set limits (software).

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 Autosol™ 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 $\pm 1^\circ\text{C}$ is very important and should be recorded².

Dissolved Oxygen OFFICIAL

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% accuracy¹, 0.2% precision³, full scale.

PO₄

Approximately 1-2% accuracy¹, 0.4% precision³, full scale.

NO₃

Approximately 1% accuracy¹, 0.2% precision³, 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.

References

Armishaw, P. (2003) *“Estimating measurement uncertainty in an afternoon. A case study in the practical application of measurement uncertainty.”* Accred Qual Assur, 8: pp. 218-224

- Armstrong, F.A.J., Stearns, C.A., and Strickland, J.D.H. (1967) "The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment," *Deep-Sea Res.*, 14: pp.381-389. doi: 10.1016/0011-7471(67)90082-4
- Hood, E.M. (2010). "Introduction to the collection of expert reports and guidelines." The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. IOCCP Report No 14, ICPO Publication Series No. 134, Version 1, 2010.
- Hydes, D., Aoyama, M., Aminot, A., Bakker, K., Becker, S., Coverly, S., Daniel, A.G., Dickson, O., Grosso, R., Kerouel, R., van Ooijen, J., Sato, K., Tanhua, T., Woodward, E.M.S., and Zhang, J.Z. (2010). "Determination of dissolved nutrients (N, P, Si) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers." The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. IOCCP Report No 14, ICPO Publication Series No. 134, Version 1, 2010. (UNESCO/IOC)
- Kirkwood, D. S. (1996) "Nutrient Analyses in Seawater Using Autoanalyzers: Refractive Index and Other Sources of Error", *Marine Chemistry*, 52(1), 1-10. [DOI: 10.1016/0304-4203(95)00086-0]
- K erouel, R., and Aminot, A. (1997) "Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis". *Mar. Chem.*, 57: pp. 265-275. doi: 10.1016/S0304-4203(97)00040-6
- Murphy, J. And Riley, J.P. (1962)"A Modified Single Solution Method for the Determination of Phosphate in Natural Waters", *Anal. Chim. Acta*, 27: p.30. doi: 10.1016/S0003-2670(00)88444-5
- 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
- Wood, E.D., Armstrong, F.A.J., and Richards, F.A. (1967) "Determination of nitrate in seawater by cadmium-copper reduction to nitrite." *Journal of the Marine Biological Association of U.K.* 47: pp. 23-31.