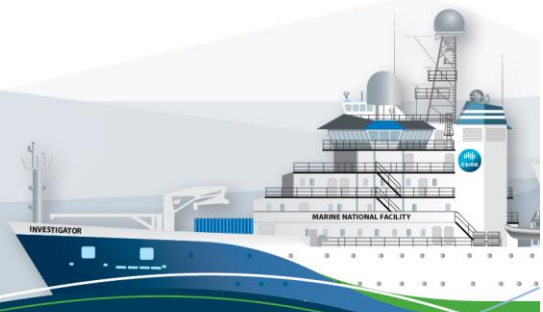
RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	in2021_v01
Chief Scientist:	So Kawaguchi
Voyage title:	TEMPO – Trends in Euphausiids off Mawson, Predators and Oceanography
Report compiled by:	Peter Hughes & Merinda McMahon



Contents

1 Executive Summary

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

1.1 Objectives/Proposal

The primary goal of the voyage was to collect data to estimate krill biomass with a view to update the precautionary catch limit for krill in CCAMLR's Division 58.4.2-East. The survey was designed to improve the understanding on the connectivity of the krill population and overlap between krill and predators. A deeper understanding of these factors will allow the design of a sustainable long-term monitoring plan and spatial management (small-scale management unit) of the krill fishery in East Antarctica.

A further goal was to undertake biological oceanography measurements to characterise bottom-up effects on krill populations. Phytoplankton food sources will be related to krill distribution and abundance, and the physical oceanography characteristics within the survey area defined. Sources of Antarctic bottom water and exchanges between the Indian and Pacific Oceans was also examined.

1.2 General Hydrochemistry Information

Water samples collected during the voyage were analysed in the ship's hydrochemistry laboratory for nutrients, dissolved oxygen, and salinity.

Five nutrients were determined: silicate, phosphate, nitrate + nitrite, nitrite and ammonium. Certified reference materials for nutrients in seawater (RMNS) were within 3% of their certified values. CTD deployment versus measured RMNS values are listed in Appendix 8.2

Missing and suspect hydrology data are listed in Appendix 8

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

2 Itinerary

Hobart to Hobart, January 29th, 2021 – March 24th, 2021.

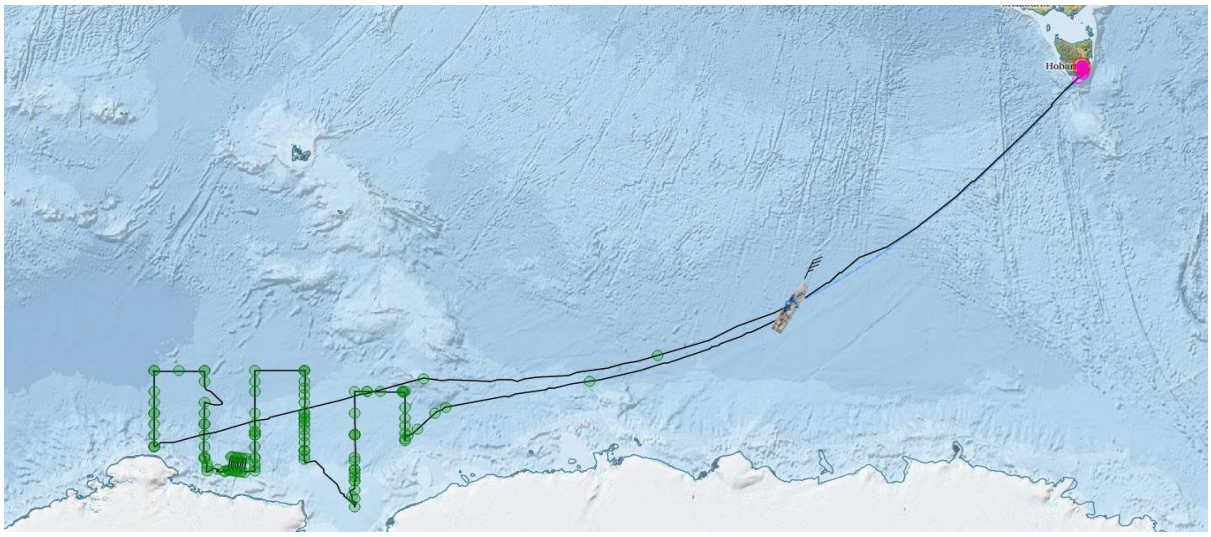


Figure 1: Voyage track

3 Key personnel list

Table 1: Key Personnel list

Name	Role	Organisation
So Kawaguchi	Chief Scientist	AAD
Linda Gaskell	Voyage Manager	CSIRO
Peter Hughes	Hydrochemist	CSIRO
Merinda McMahon	Hydrochemist	CSIRO

4 Summary

4.1 Sample Type and Number Assayed

Table 2: **Sample Type and Number Assayed**

Analysis	Samples Assayed	Type
Salinity	1118 27 3	CTD TSG UWY
Dissolved Oxygen	1142 3	CTD UWY
Nutrients	1195 3	CTD UWY

4.1.1 CTD Samples (Conductivity, Temperature, Density)

- Taken from the 12L Ocean Test Equipment bottles on the CTD rosette that is deployed at depth for water collection.
- A total of 66 CTD deployments were sampled by
    - hydrochemistry, Peter Hughes and Merinda McMahon
    - science party: Sophie Bestley, Annie Foppert, Clara Rodriguez Vives, Andreas Klocker. With additional help, when available, from Luke Edwards, Paul Chant, Jeremy Bird, Derek Hamer, Abigail Smith, Chris Berry and Linda Gaskell.

4.1.2 TSG Samples (Thermosalinograph)

- 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) during the transit back to port.
- Results for samples closest to the SOTS-9 mooring site, TSG028, TSG029, TSG030, were also emailed to Elizabeth Shadwick (CSIRO) & Sophie Bestley (UTAS).

4.1.3 UWY (Underway)

- Taken from the same sampling point as per the TSG samples.
- Triplicate nutrient and dissolved oxygen (DO) samples were collected closest to the SOTS-9 mooring site. DO results were emailed to Elizabeth Shadwick (CSIRO) & Sophie Bestley (UTAS). Nutrients to be assayed on voyage in2021_v02.

4.2 Data Processing Overview

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. An overview of this process is illustrated below (fig.2).

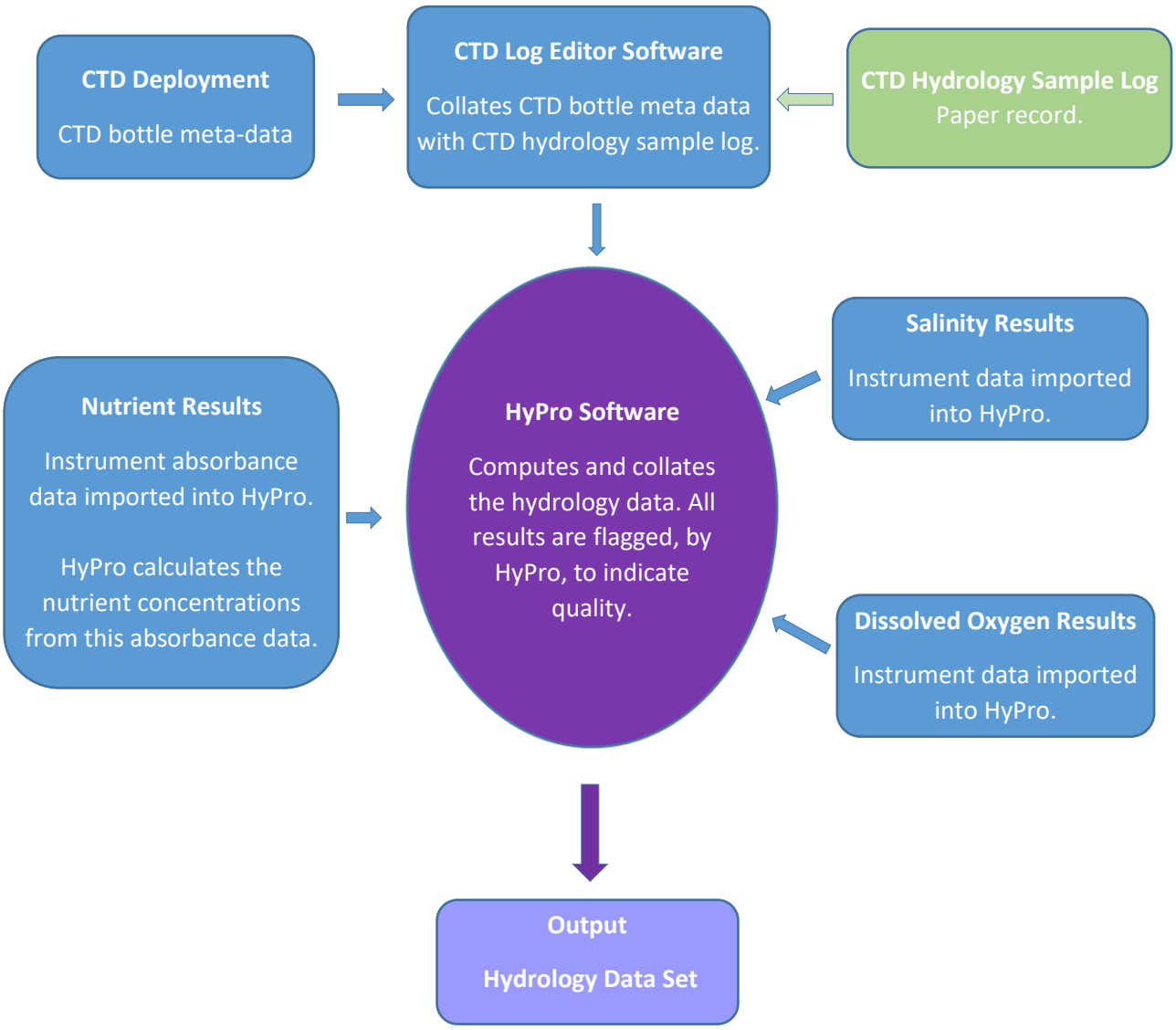


Figure 2: Hydrology Data Processing Flow Diagram.

5 Salinity

5.1 Salinity Measurement Parameters

Table 3: Salinity Measurement Parameters

Details	
HyPro Version	5.7
Instruments	Guildline Autosal Laboratory Salinometer 8400(B) – SN 72151 and 72088. Bath temperature 24.0°C
Software	Ocean Scientific International Ltd (OSIL) Data Logger ver 1.2
Hydrochemistry Methods.	Sampling: WI_Sal_002 Measurement: SOP006
Accuracy	± 0.001 practical salinity units
Reference Material	OSIL IAPSO - Batch P163, use by 10/04/2022, K_{15} = 0.99985
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 > 8 hrs before measurement.
Lab Temperature	Mean 21.0°C SD 0.9
Analysts	Peter Hughes, Merinda McMahon
Comments	Good agreement between bottle salinity and CTD salinity results. See DAP report for CTD calibration details. Autosal 72151 lost stability requiring additional measurements for each sample due to fluctuating salinity values in the order of 0.005 PSU. Autosal 72088 used exclusively for CTD 40 samples on.

5.2 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.

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 (one run consists of samples from up to two CTD deployments).

Method: The salinity sample is collected in a 200ml 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 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 Autosal 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.

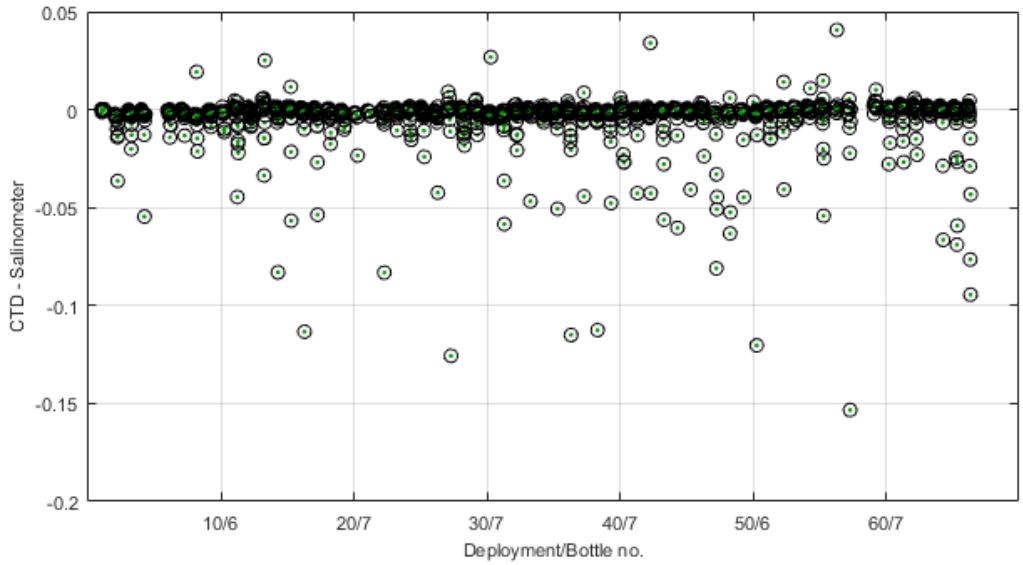
The output from the data logger is imported into HyPro and collated with the CTD deployment meta-data.

5.3 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 0.002 PSU. The larger differences are for shallow samples across the sudden changes in the thermohaline profile.

Missing and suspect salinity data are listed in Appendix 8.4.

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. Black = UNPROCESSED. Units: PSU (dimensionless).



6 Dissolved Oxygen

6.1 Dissolved Oxygen Measurement Parameters

Table 4: Dissolved oxygen measurement parameters.

Details	
HyPro Version	5.7
Instrument	Automated Photometric Oxygen System
Software	Scripps Institution of Oceanography (SIO)
Hydrochemistry Methods	Sampling: WI_DO_001 Assay: SOP005
Accuracy	± 0.5 µmol L^{-1}
Analysts	Peter Hughes and Merinda McMahon
Lab Temperature (±1°C)	Mean 20.4°C SD 1.3
Sample Container type	140 mL glass iodine determination flasks with glass stopper.
Sample Storage	Samples stored in the hydrochemistry lab until analysis.
Comments	Good agreement between bottle and CTD results for deeper samples. See DAP report for CTD calibration details. Draw temperature probes were faulty resulting in sampling delays as probes were changed or attempts to fix. Faults: temperatures out by 10C or more to not working. Krill temperature probe (slow response) used for CTD 24 to 27. Thermo TL-1 probe (fast response) used for remainder. Accuracy of probes checked in the Autosal baths.

6.2 Dissolved Oxygen Method

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: The sample is collected in an iodine determination flask of known volume. 1mL 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 15 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.

The thiosulphate solution is standardised by with a 10ml 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.

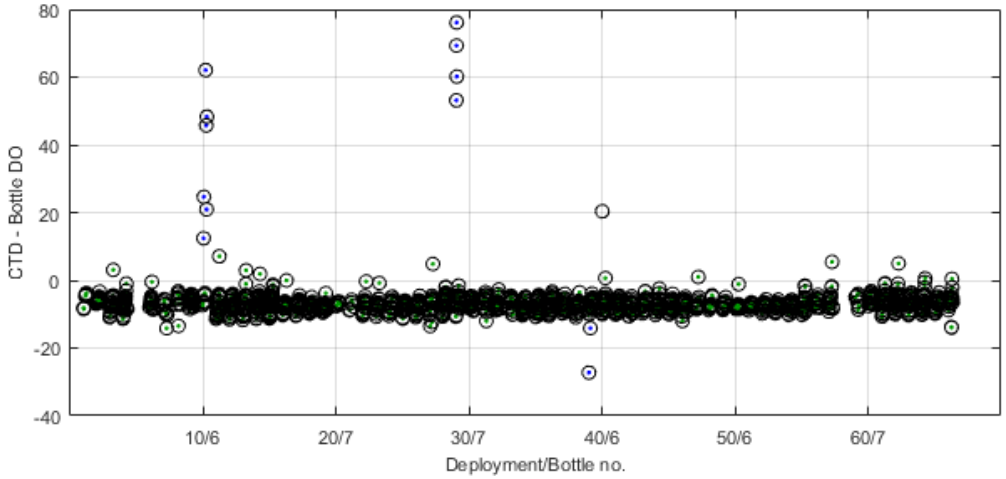
6.3 CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

For this voyage, the difference between the unprocessed CTD value and the measured bottle value is generally less than 10 µmol L^{-1}. The larger differences are for shallow samples across the sudden changes in the dissolved oxygen profile.

Deployments 10, 29 and 39 have values which are outliers on the depth profile when compared to the CTD instrument. Cause unknown. These values are flagged as suspect.

Missing and suspect dissolved oxygen data are listed in Appendix 8.5.

Figure 4. 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. Black = UNPROCESSED. Units: µmol L^{-1}



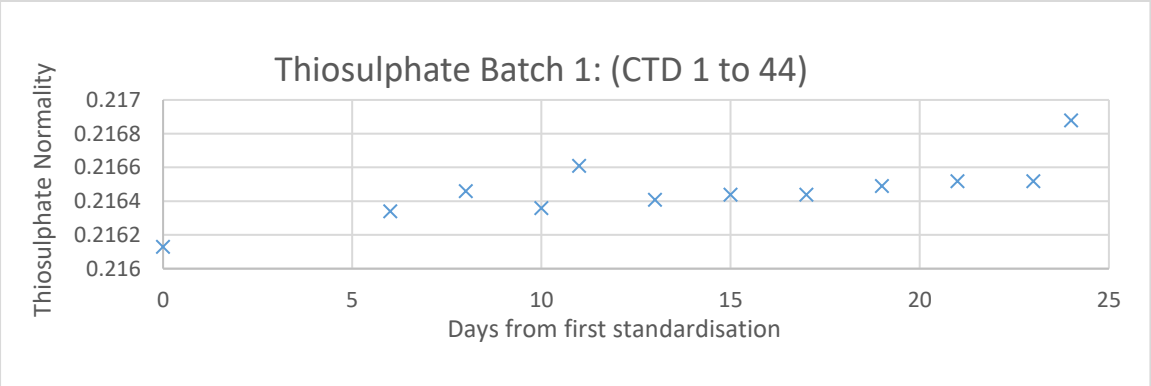
6.4 Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.

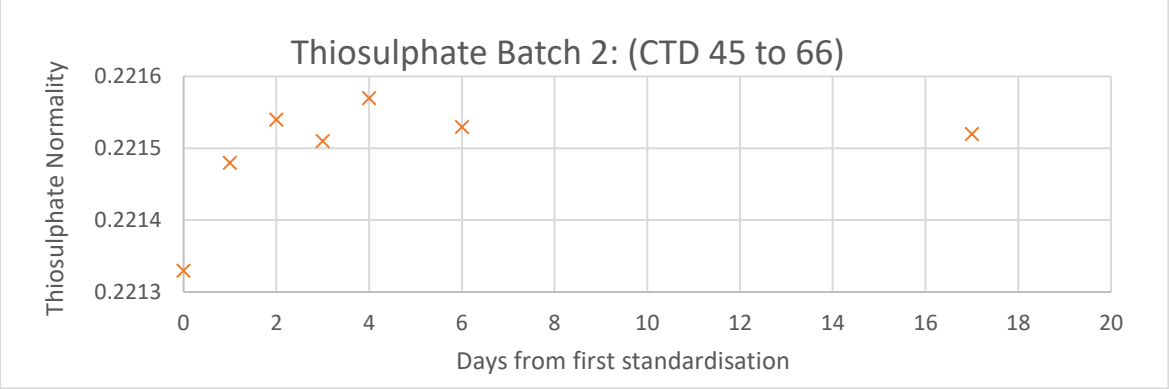
The variance in thiosulphate concentration is within our QC parameter of less than 0.0005N between standardisations. Two batches of thiosulphate reagent were used during the voyage. Batch 1 was prepared in Hobart. Batch 2 was made during the voyage. Their mean normality as follows:

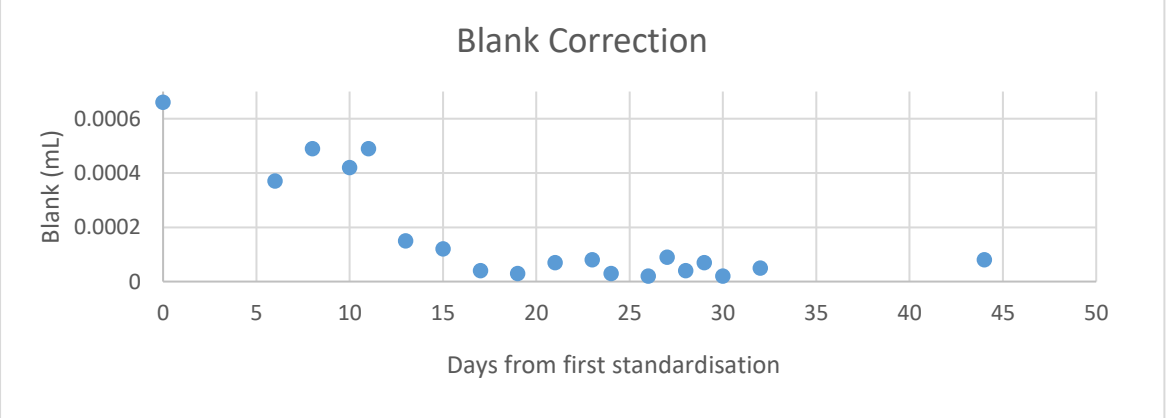
CTD Deployment 1 to 44:	Batch 1	0.21647 (N) SD 0.00017 (n=12)
CTD Deployment 45 to 66:	Batch 2	0.22150 (N) SD 0.00007 (n=7)

The blank correction is used in the calculation of the thiosulphate normality and is due to oxidisable species in the MQ water that is added to the KIO_3 aliquot before the titration. The reduction in the blank correction, from day 13 (fig 5.) is due to a change in procedure from using freshly dispensed MQ water to MQ water that has been given time to out-gas and reach room temperature by storing it in a 30L carboy for at least 24 hours prior to use.

Figure 5. Thiosulphate standardisation and blank correction plots.







7 Nutrients

7.1 Nutrient Measurement Parameters

Table 5: Nutrient measurement parameters. All instrument parameters, reagent batches and instrument events are logged for each analysis run. This information is available on request.

Details					
Processing Software	CSIRO HyPro 5.7				
Instrument	Seal AA3HR segmented flow analyser.				
Operating Software	AACE 7.10				
Hydrochemistry. Methods	Sampling: WI_DO_001				
	Assay:				
	SOP001	SOP002	SOP003	SOP004	SOP005
	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonia
Top concentration (µmol L^{-1})	161	3.0	42	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 CC, CI				
Sample Container	50 mL HDPE with screw cap lids. Reused after acid wash with 1M HCl				
Sample Storage	< 4 hrs at room temperature or < 12 hrs @ 4°C				
Sample preparation	Assayed as neat. No filtration.				
Lab Temperature (°C)	Mean 20.4°C SD 1.3				
Analysts	Peter Hughes, Merinda McMahon				
Comments	Issues with nitrite assays for some CTD deployments. Only for shallow depths. Peak shapes exhibit raised plateaus typical of bubble interference. Outcome: higher concentration than actual, up to 0.1 µmol L^{-1}. Unable to isolate cause. Sample assays repeated.				

7.2 Nutrient Methods

When using silicate, phosphate, nitrate+nitrite (NOx) and nitrite data set for publication, please cite the paper:

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

Nutrient samples are assayed on a Seal AA3HR segmented flow auto-analyser fitted with 1cm 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 660nm.

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 880nm.

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 520 nm.

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

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 460nm after excitation at 370nm.

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.

7.3 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 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:

- ±0.5% of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE[1]).
- 0.02umol^{-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 8.7. Missing or suspect nutrient data are listed in Appendix 8.6.

[1] World Ocean Circulation Experiment

Table 6: HyPro 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 degree	Linear	Linear	Quadratic	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
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 deck of Investigator) collected on in2019_v05. Sub-lot passed through a 10-micron filter and stored in 20 L carboys in the clean dry laboratory at 22°C.				
Medium of Baseline	18.2 Ω water. Dispensed from the Milli Q Integral 10 unit.				
Duplicate samples.	CTD: Niskin fired at the greatest depth sampled in duplicate. Single samples collected for remaining depths.				
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in appendix 8.3.				

7.4 Accuracy - Reference Material for Nutrient in Seawater (RMNS)

Japanese KANSO certified RMNS lot assayed in triplicate in each run to monitor accuracy. The certified values are in Table 7.

For in2021_v01, the certified reference material results for NOx and silicate are within 2%, phosphate is within 3% and nitrite within 0.06 µmol L^{-1} of their certified mean concentration.

The GO-SHIP criteria (Hyde *et al*., 2010), appendix 8.8, specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy.

The assayed RMNS values per CTD deployments are listed in the appendix 8.3.

Table 7: RMNS certified concentrations ± expanded uncertainty (U) at 21°C. Units: µmol L^{-1}

RMNS	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	Nitrate (NO_3)	NO_3+ NO_2 (NO_X)
Lot CC	88.228 ± 0.492	2.130 ± 0.019	0.119 ± 0.006	31.62 ± 0.246	31.740 ± 0.252
Lot CI	8.46 ± 0.09	0.97 ± 0.01	0.420 ± 0.04	14.11 ± 0.133	14.53 ± 0.170

KANSO publishes the RMNS nutrient values in µmol kg^{-1}. These are converted to µmol L^{-1} at 21°C. The RMNS is not certified for ammonium. NO_x is derived by summing the NO_3 and NO_2 values.

Table 8: RMNS CC statistics for of this voyage. Units: µmol L^{-1}

RMNS CC	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	87.7	2.13	0.107	31.65
Maximum	89.7	2.18	0.188	32.33
Mean	88.7	2.15	0.149	32.00
Median	88.7	2.15	0.149	31.98
Repeatability	0.4	0.01	0.010	0.10

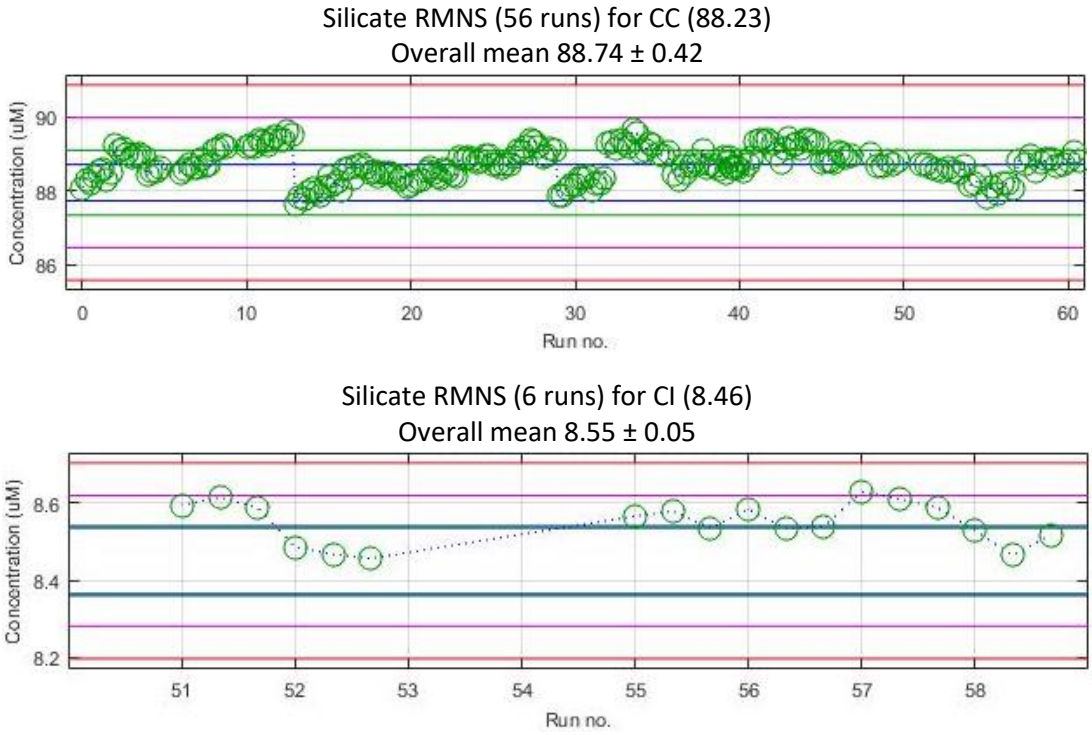
Table 9: RMNS CI statistics for of this voyage. Units: µmol L^{-1}

RMNS CI	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	8.5	0.96	0.438	14.48
Maximum	8.6	0.97	0.455	14.59
Mean	8.55	0.97	0.445	14.55
Median	8.55	0.97	0.442	14.55
Repeatability	0.05	0.005	0.006	0.03

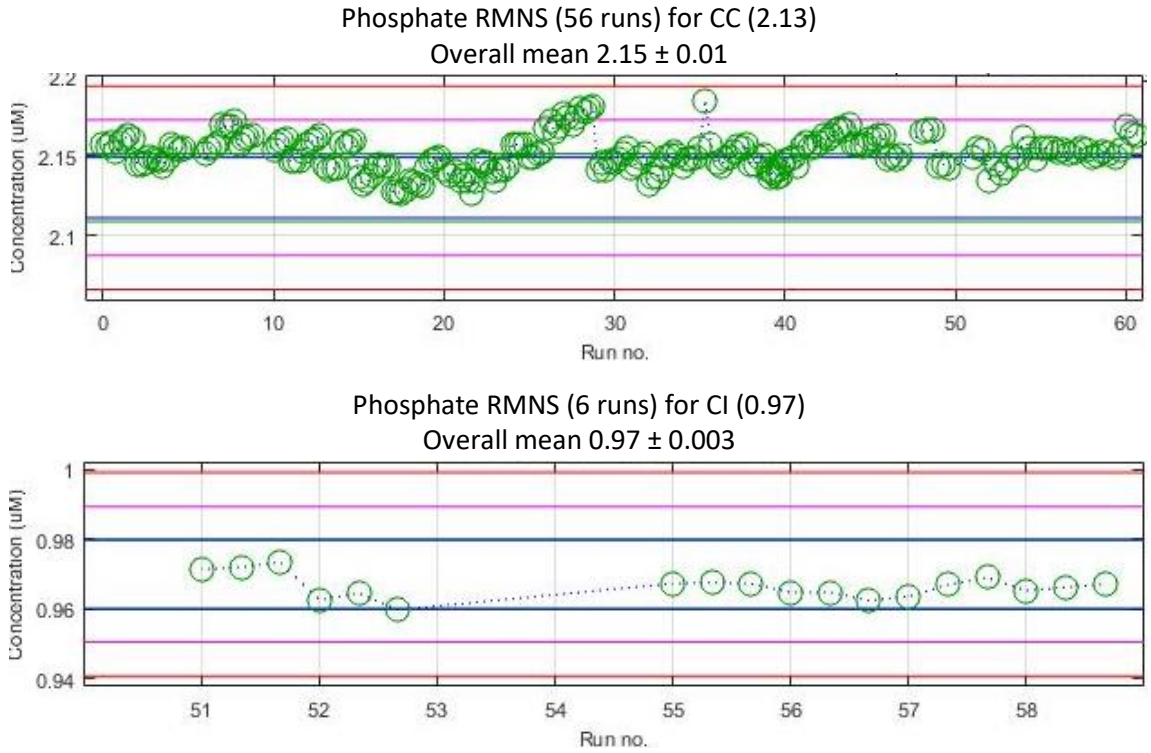
7.5 Nutrient plots of RMNS

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 µmol 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.

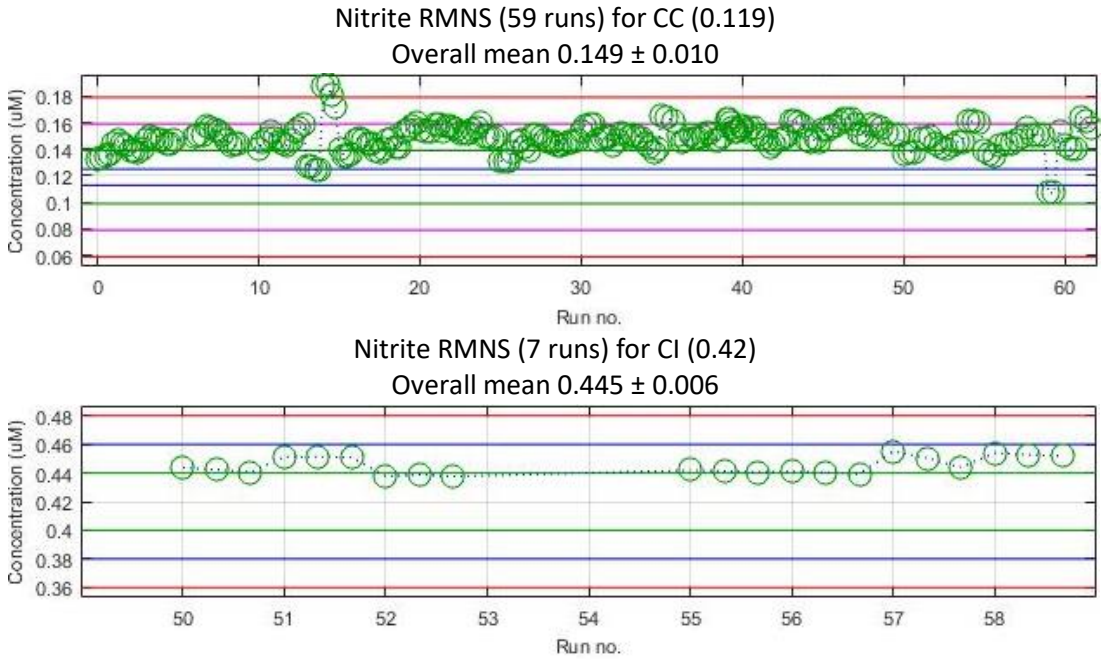
7.5.1 **Figure 6:** Silicate RMNS Plot (µmol L^{-1})



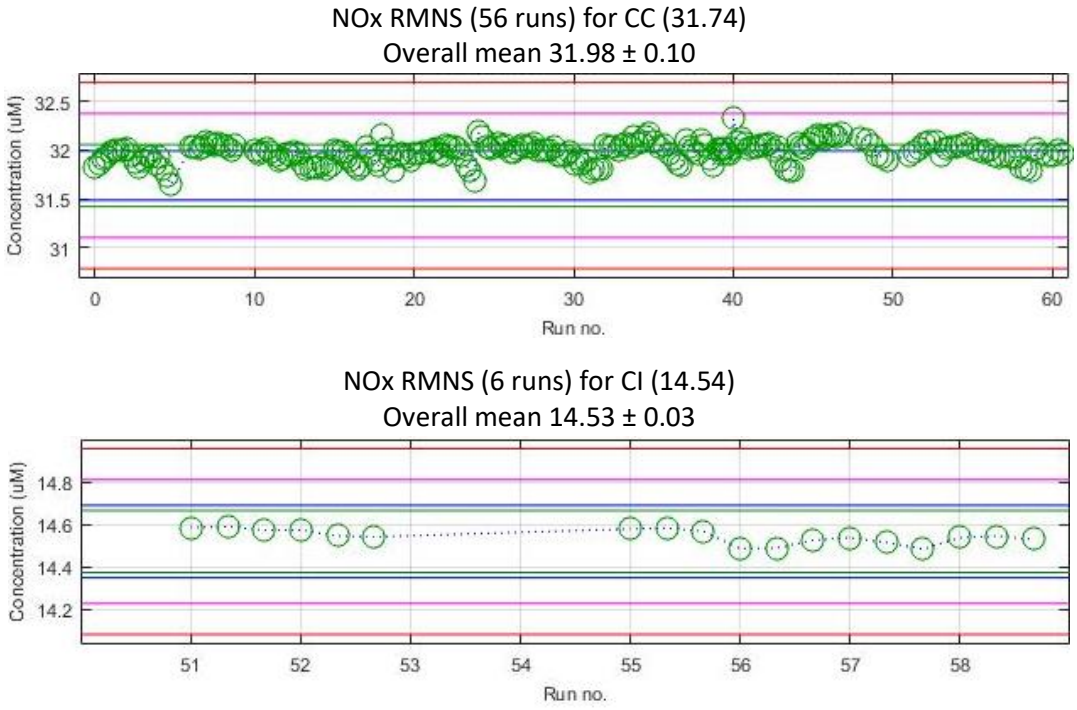
7.5.2 **Figure 7:** Phosphate RMNS Plot (µmol L^{-1})



7.5.3 **Figure 8:** Nitrite RMNS Plot (µmol L^{-1})



7.5.4 **Figure 9:** Nitrate + Nitrite (NOx) RMNS Plot (µmol L^{-1})



7.6 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 10: CSIRO Hydrochemistry nutrient analysis uncertainty values. Units: µmol L^{-1}

Calculated Measurement Uncertainty @ 1 µmol L^{-1}				
Silicate	Phosphate	Nitrite	Nitrate + Nitrite (NOx)	Ammonia
±0.017	±0.024	±0.14	±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.

7.7 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 12, 13). Duplicate nutrient samples are also collected from the greatest depth of subsequent CTD deployments (Table 11).

For nutrients, the sampling precision is good if the difference from the mean of duplicate measurements is less than the nominal method detection limit. 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 8.6.

Table 11: Difference between duplicate results. CTD 2 – CTD 66 Units: 1 µmol L^{-1}

	Silicate ($Si(OH)_4$)	**Phosphate (PO_4)**	**Nitrite (NO_2)**	**NO_3+ NO_2 (NO_X)**	**Ammonia (NH_4)**
Minimum	0.0	0.00	0.00	0.00	0.00
Maximum	0.8	0.01	0.05	0.20	0.05
Mean	0.2	0.00	0.00	0.04	0.00
Variance	0.1	0.00	0.00	0.04	0.00

Table 12: CTD deployment 1. 6 bottles at 501 dbar. Units 1 µmol L^{-1}

	Salinity (PSU)	Dissolved Oxygen	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	34.7139	198.4	92.7	2.23	0.022	32.34
Maximum	34.7145	199.0	93.0	2.23	0.025	32.51
Mean	34.7141	198.6	92.9	2.23	0.024	32.45
SD	0.0002	0.2	0.1	0.00	0.001	0.06

Table 13: CTD deployment 1. 6 bottles at 2600 dbar. Units 1 µmol L^{-1}

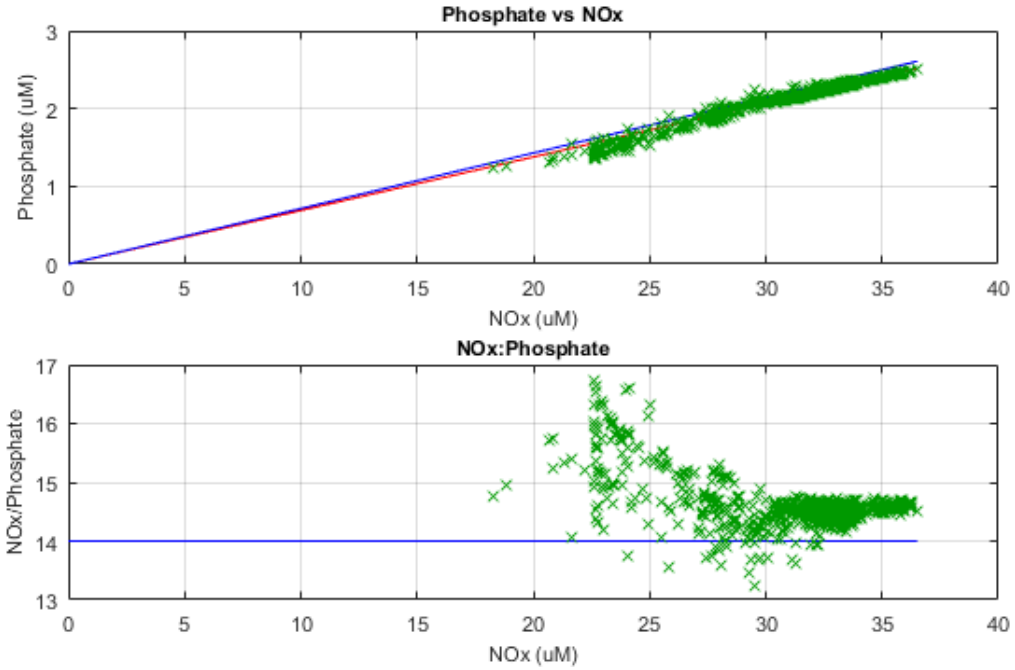
	Salinity (PSU)	Dissolved Oxygen	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	34.6768	225.9	129.5	2.31	0.013	33.32
Maximum	34.6783	226.3	130.2	2.31	0.017	33.48
Mean	34.6775	226.0	129.8	2.31	0.015	33.40
SD	0.0005	0.2	0.2	0.00	0.001	0.05

7.8 Redfield Ratio Plot (14.0) for CTD Deployments.

The Redfield ratio for this voyage: 14.53

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 10. Redfield ratio plots.



7.9 Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry laboratory and within the AA3HR instrument were measured and logged as follows:

(1) Above the AA3HR instrument, temperature only. Mean 20.4°C SD 1.3

(2) On the deck of the nitrate & nitrite AA3HR chemistry module, temperature and humidity. Data on request.

(3) On the outboard bulkhead, Temperature, humidity and pressure. Data on request.

8 Appendix

8.1 Salinity: Reference Material Used

OSIL IAPSO Standard Seawater	
Batch:	P163
Use by date:	10/04/2022
K_{15}:	0.99985
PSU:	134.994

8.2 Nutrients: Reference Material Used

RMNS	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	Nitrate (NO_3)	NO_3+ NO_2 (NO_X)
Lot CC	88.228 ± 0.492	2.130 ± 0.019	0.119 ± 0.006	31.62 ± 0.246	31.740 ± 0.252
Lot CI	8.46 ± 0.09	0.97 ± 0.01	0.420 ± 0.04	14.11 ± 0.133	14.53 ± 0.170

8.3 Nutrients: RMNS results for each CTD Deployment.

8.3.1 RMNS Lot CC Results per CTD Deployment

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	88.5	2.16	32.00	0.145
2	89.1	2.15	31.92	0.139
3	89.0	2.15	31.91	0.148
4	88.5	2.16	31.78	0.146
6	88.6	2.16	32.03	0.153
7	88.7	2.17	32.07	0.153
8	89.2	2.16	32.03	0.145
9	89.4	2.15	31.93	0.146
10	89.5	2.16	31.96	0.154
11	87.8	2.14	31.83	0.126
12	88.0	2.16	31.83	0.182
13	88.2	2.14	31.97	0.137
14	88.6	2.14	31.85	0.147
15	88.6	2.13	31.93	0.140
16	88.5	2.13	31.97	0.145
17	88.3	2.15	31.93	0.156
18	88.2	2.14	31.98	0.154
19	88.5	2.13	31.98	0.157
20, 21	88.5	2.15	32.04	0.154
22, 23	88.9	2.14	31.82	0.156
24	88.9	2.16	32.09	0.149
25	88.7	2.15	32.05	0.133
26	88.9	2.17	32.01	0.143
27	89.3	2.17	32.04	0.150
28	89.1	2.15	32.00	0.144
29	88.0	2.15	31.96	0.146
30	88.3	2.15	31.88	0.157
31	88.2	2.15	31.80	0.149
32, 33	89.3	2.14	32.03	0.149
34	89.5	2.15	32.07	0.150
35	89.2	2.15	32.12	0.141
36	89.0	2.16	32.06	0.163
37	88.5	2.15	31.90	0.148
38	88.8	2.16	32.03	0.150
39	88.7	2.15	31.98	0.149
40, 41	88.7	2.14	32.00	0.158
42	88.8	2.15	32.15	0.156
43, 44	89.4	2.16	32.03	0.147
45, 46	89.1	2.16	32.04	0.147
47, 48	89.3	2.17	31.82	0.147

50, 51	88.8	2.16	32.13	0.156
52, 53	89.0	2.15	32.16	0.153
54	88.8	2.17	32.09	0.158
55	88.8	2.14	31.93	0.138
56	88.7	2.15	31.98	0.149
57	88.6	2.14	32.07	0.142
59	88.6	2.15	32.00	0.145
60	88.3	2.16	32.05	0.161
61	87.9	2.15	32.00	0.137
62	88.2	2.15	31.96	0.144
63	88.8	2.15	31.94	0.151
64	88.7	2.15	31.81	0.150
65	88.8	2.15	31.98	0.160
66	88.9	2.16	31.97	0.141

8.3.2 RMNS Lot CI Results per CTD Deployment

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) ($\mu mol\ L^{-1}$)
56	8.6	0.97	14.52	0.442
57	8.5	0.96	14.55	0.451
61	8.6	0.97	14.58	0.438
62	8.5	0.96	14.50	0.441
63	8.6	0.97	14.51	0.440
64	8.5	0.97	14.54	0.450

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

Or for smoothing data

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

8.4 Missing or Suspect Salinity Data

Data is flagged based on CTD sampling log notes, observations during analysis, and examination of depth profile plots (Flag key: appendix 8.7)

CTD	RP	Flag	Reason for Flag
12	17	141	No Result. Sample measured but not recorded. Operator error.
13	6, 8	141	No Result. As per CTD 12 above.
15	11	141	No Result. As per CTD 12 above.
18	13	141	No Result. As per CTD 12 above.
36	16	141	No Result. As per CTD 12 above.
38	12	141	No Result. As per CTD 12 above.
43	28	141	No Result. As per CTD 12 above.
44	10	141	No Result. As per CTD 12 above.
44	21	141	No result. Instrument error. Measuring cell blocked during operation.
60	20	141	No Result. As per CTD 12 above.
62	20	141	No Result. As per CTD 12 above.

8.5 Missing or Suspect Dissolved Oxygen Data

Data is flagged based on CTD sampling log notes, observations during analysis, and examination of the depth profile (Flag key: appendix 8.7).

CTD	RP	Flag	Reason for Flag
4	11, 12	141	No Result. Analytical error. Sample assayed; endpoint not well defined. Cause unknown.
8	10	141	No Result. As per CTD 4 above.
10	11, 13, 26, 30, 32, 34	69	Result suspect. Outlier on depth profile plot when compared with the CTD dissolved oxygen results. Cause unknown.
18	36	141	No Result. As per CTD 4 above.
24	32, 34, 36	141	No Result. As per CTD 4 above.
29	10, 11, 12, 13	69	Result suspect. Outlier on depth profile plot when compared with the CTD dissolved oxygen results. Cause unknown
32	16, 17	141	No Result. Sampling error. Sample not collected.
39	6, 16	69	Result suspect. Outlier on depth profile plot when compared with the CTD dissolved oxygen results. Cause unknown

40	6	141	Result Bad. Niskin bottle premature closure at shallower depth. Indicated by higher draw temperature than expected. (0.9°C vs CTD -0.1°C). Confirmed by outlier on depth profile compared with the CTD dissolved oxygen results. Result not reported.
47	33	141	No Result. As per CTD 4 above.

8.6 Missing or Suspect Nutrient Data.

Not included, Data flagged 63 (below detection limit). Data flagged 133 is not reported in the final hydrology dataset. (Flag key: appendix 8.7)

CTD	RP	Analyte	Flag	Reason for Flag
1	6	NOx	69	Duplicate sample results do not match. Results 33.33, 33.48 µmol L^{-1}
1	22	Nitrite	133	No result. Sample assayed but measurement peak's shape compromised with an air bubble spike. Sample not repeated due to operator error.
12	11	All	141	No Result. Sample collected according to CTD log sheet. Sample not assayed. Cause of discrepancy unknown.
14	20	All	141	No Result. As per CTD12 above
17	24	All	141	No Result. As per CTD12 above
19	16	All	141	No Result. As per CTD12 above
21	7	Silicate	69	Duplicate sample results do not match. Results 111.8, 112.6 µmol L^{-1}
26	7	Silicate	69	Duplicate sample results do not match. Results 116.9, 117.4 µmol L^{-1}
31	6	Silicate	69	Duplicate sample results do not match. Results 101.8, 102.2 µmol L^{-1}
32	6	Silicate	69	Duplicate sample results do not match. Results 124.9, 125.3
36	6	Silicate	69	Duplicate sample results do not match. Results 152.2, 152.6 µmol L^{-1}
42	19	Ammonia	0	Outlier on depth profile plot.
43, 44	All	Ammonia	0	Analytical baseline offset. Deep samples around -0.03uM. Cause Unknown.
45	7	Silicate	69	Duplicate sample results do not match. Results 127.9, 128.3 µmol L^{-1}
46	21, 22	Nitrite	133	No result. Sample assayed but measurement peak's shape compromised with an air bubble spike. Sample not repeated due to operator error.

47	6	NOx	69	Duplicate sample results do not match. Results 31.74, 31.94 µmol L^{-1}
52	22, 24, 28, 31	Nitrite	133	No result. Sample assayed but measurement peak's shape compromised with an air bubble spike. Sample not repeated due to operator error.

8.7 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.

8.8 GO-SHIP Specifications

8.8.1 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].

8.8.2 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.

8.8.3 $Si(OH)_4$

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

8.8.4 PO_4

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

8.8.5 NO_3

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

8.8.6 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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