

RV INVESTIGATOR

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage: in2019_v03 **Chief Scientist** Lynnath Beckley David Antoine (Curtin University), Peter Thompson (CSIRO), Helen Principal Phillips (University of Tasmania), Michael Landry (Scripps, San **Investigators** Diego), Andrew Jeffs (University of Auckland), Martin Ostrowski (Macquarie University), Justin Seymour (University of Technology Sydney), Pilar Olivar (CSIC, Barcelona), Raleigh Hood (University of Maryland) and Anya Waite (Dalhousie, Halifax). Voyage title: A coupled bio-physical, ecosystem-scale, examination of Australia's International Indian Ocean Expedition line 110E **Report compiled by:** Julie Janssens, Peter Hughes



Owned and operated by CSIRO on behalf of the nation.

Contents

1	Exe	Executive Summary						
2	Itin	Itinerary						
3	Key personnel list							
4	Sun	Summary						
	4.1	Sam	ple Type and Number Assayed	6				
	4.1.	1	CTD (Conductivity, Temperature, Density)	6				
	4.1.	2	EXP (experimental), UWY (underway) and TSG (Thermosalinograph)	6				
	4.2	Data	a Processing Overview	7				
5	Sali	nity	Data Processing	8				
	5.1	Saliı	nity Parameter Summary	8				
	5.2	Saliı	nity Method	8				
	5.3	Mis	sing or Suspect Salinity Data	9				
	5.4	CTD	Salinity - Bottle Salinity Plot	9				
	5.4 OS	IL Sa	linity Standard PSU across the Voyage	10				
6	Dis	solve	d Oxygen Data Processing	11				
	6.1	Diss	olved Oxygen Parameter Summary	11				
	6.2	Diss	olved Oxygen Method	11				
	6.3	Mis	sing or Suspect Dissolved Oxygen Data.	12				
	6.4	CTD	Dissolved Oxygen - Bottle Dissolved Oxygen Plot	13				
	6.5	Diss	olved Oxygen Instrument titrant: thiosulphate normality and blank correction	14				
7	Nut	rient	Data Processing	15				
	7.1	Nut	rient Assay Parameter Summary	15				
	7.2	Nut	rient Methods	15				
	7.3	Mis	sing or Suspect Nutrient Data	16				
	7.4	НуР	ro Processing Parameters	17				
	7.5	HyP	ro Data Processing Summary	17				
	7.6	Acc	uracy - Reference Material for Nutrient in Seawater (RMNS)	18				
	7.6.	1	Silicate RMNS Plot (µmol L ⁻¹)	19				
	7.6.	2	Phosphate RMNS Plot (µmol L ⁻¹)	20				
	7.6.	3	Nitrate + Nitrite (NOx) RMNS Plot (μmol L ⁻¹)	21				
	7.6.	4	Nitrite RMNS Plot (μmol L ⁻¹)	22				
	7.7	Ana	lytical Precision	23				
	7.7.	1	Nutrient Measurement Uncertainty	23				
	7.7.2		Nutrient Method Detection Limit	23				

7.7.3		3	Reference Material for Nutrients in Seawater	.24
7	7.8 San		pling Precision	.25
7.8.1		1	Silicate Duplicates Plot	.25
	7.8.	2	Phosphate Duplicates Plot	.25
	7.8.	3	Nitrate + Nitrite (NOx) Duplicates Plot	.26
	7.8.	4	Nitrite Duplicates Plot	.26
	7.8.	5	Ammonia Duplicates Plot	.27
7	.9	Red	field Ratio Plot (14.0) for CTD Deployments	.28
7	.10	Tem	perature & Humidity Change over Nutrient Analyses	.29
8	Арр	bend	x	.30
8	.1	Saliı	nity: Reference Material Used	.30
8	8.2 Nu		rients: RMNS results for each CTD Deployment	.31
	8.2.	1	RMNS Lot CJ Results (μmol L ⁻¹)	.31
	8.2.	2	RMNS lot CB Results (μ mol L ⁻¹)	.32
8	.3	Nut	rients: Measured Detection Limit for each CTD Deployment	.33
8	.4	Flag	Key for Hydrology Data Set	.35
8	.5	G0-	SHIP Specifications	.36
	8.5.	1	Salinity	.36
	8.5.	2	Dissolved Oxygen	.36
	8.5.	3	SiO2	.36
	8.5.	4	PO4	.36
	8.5.	5	NO3	.36
	8.5.	6	Notes	.36
9	Ref	eren	ces	.37

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

This multi-disciplinary voyage studied the coupling of physical, biogeochemical and ecological processes in the pelagic ecosystem of the SE Indian Ocean along the 110°E line at stations originally occupied by Australia during the first International Indian Ocean Expedition in 1962/63. This voyage is part of Australia's contribution to the second International Indian Ocean Expedition (2015-2020).

The objectives of this voyage are:

- 1) Quantification of multi-decadal, ecosystem-scale change from the 1960s benchmark in the physical, chemical and biological properties of the water column along 110°E;
- 2) Characterization of the physical and biological sources of nitrogen to the region and their impacts on regional biogeochemistry and ecology;
- 3) Determination of trophic relationships between nitrogen-fuelled primary production and zooplankton, including the larvae of mesopelagic fishes;
- 4) Relating field information on phytoplankton community composition, primary production and carbon export to bio-optical quantities derivable from satellite ocean colour radiometry.

As part of this, water samples were collected and analysed in the ship's hydrochemistry laboratory for nutrients, dissolved oxygen, and salinity. The samples are from deployments of the conductivity, temperature and depth (CTD) rosette and the underway system (instrument clean seawater supply).

High quality data was produced for the three measured parameters. The nutrients measured where silicate, phosphate, nitrate + nitrite, nitrite and ammonium. Certified reference materials for nutrients in seawater were also analysed and their results where within the specified limits of their certified value.

No samples were collected from CTD deployments 39 to 42, 44 and 46.

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

Contact: <u>DataLibrariansOAMNF@csiro.au</u>

2 Itinerary

Fremantle to Fremantle, May 14th – June 14th, 2019.

Voyage Track:



3 Key personnel list

Name	Role	Organisation	
Lynnath Beckley	Chief Scientist	Murdoch University	
Hugh Barker	Voyage Manager	CSIRO	
Peter Hughes	Hydrochemist	CSIRO	
Julie Janssens	Hydrochemist	CSIRO	

4 Summary

4.1 Sample Type and Number Assayed

Analysis (instrument)	Number of Samples
Salinity (Guildline Salinometer)	745 CTD
	22 TSG
	10 EXP
Dissolved Oxygen (SIO automated titration)	747 CTD
	59 UWY
Nutrients (Seal AA3HR segmented flow)	788 CTD
	37 UWY

4.1.1 CTD (Conductivity, Temperature, Density)

- Sampling point, 36 bottle rosette with 12L Ocean Test Equipment bottles (Niskin) deployed at depth for water collection.
- 54 CTD deployments in total. Deployments 39 to 42, 44 and 46 not sampled.

4.1.2 EXP (experimental), UWY (underway) and TSG (Thermosalinograph)

- UWY and TSG samples were collected from the instrument clean seawater supply upstream from the Thermosalinograph and pCO₂ instruments in the underway laboratory.
- EXP salinity samples were supplied by Dr Andrew Jeffs.
- TSG samples were collected by the hydrochemistry team.
- UWY samples were collected by the science party and the hydrochemistry team.
- Sampling meta-data was recorded in the voyage eLog. Available on request.

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 in figure 1.



Figure 1: Hydrology Data Processing Flow Diagram.

5 Salinity Data Processing

5.1 Salinity Parameter Summary

Details					
HyPro Version	5.7				
Instrument	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71613				
Software	Ocean Scientific International Ltd (OSIL) Data Logger ver 1.2				
CSIRO Hydrochem Method.	Sampling: WI_Sal_002 Measurement: SOP006				
Accuracy	± 0.001 practical salinity units (PSU)				
Analysts	Julie Janssens, Peter Hughes				
Lab Temperature (±0.5°C)	21 -23°C during analysis.				
Bath Temperature	24.007°C				
	OSIL IAPSO ¹ - Batch P161, use by 03/05/2020, K ₁₅ = 0.99987				
Reference Material	OSIL IAPSO ¹ - Batch P162, use by 16/04/2021, K ₁₅ = 0.99983				
Sampling Container type	200 ml volume OSIL bottles made of type II glass (clear) with disposable plastic insert and plastic screw cap.				
Sample Storage	Samples stored in the Salt lab for a minimum of 8 hrs before measurement.				
Comments	None.				

5.2 Salinity Method

Salinity samples are measured on a Guildline Autosal 8400B salinometer operated in accordance with its technical manual.

Before each batch of sample measurements, the Autosal is calibrated with a standard seawater (OSIL, IAPSO¹) of known K_{15} ratio. A new bottle of OSIL solution is used for each calibration. The frequency of calibration is once per every 36 samples.

Method synopsis: Salinity samples are collected into 200ml OSIL bottles, 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 plastic insert is fitted, the bottle inverted and rinsed with water, capped, and stored cap-down until measured. To measure, the Autosal's conductivity cell is flushed three times with the sample and then measured after the fourth and fifth flush. The conductivity measurements are captured by the OSIL data logger software which then calculates the practical salinity.

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

¹ International Association for the Physical Sciences of the Oceans

5.3 Missing or Suspect Salinity Data

Data is flagged based on notes from CTD sampling log sheet, observations during analysis, and examination of depth profile plots (Flag key in appendix 8.4).

CTD	RP	Flag	Reason for Flag or Action			
8	21	-	No Result. Operator error. Sample measured but the software request to record the measurement was cancelled when it should have been accepted.			
27	12	-	No Result. Operator error. As per CTD 8 RP 21			
33	13	-	No Result. Operator error. As per CTD 8 RP 21			
36	5	69	Result Suspect. Flagged by operator. Outlier in depth profile plus large offset from CTD salinity. Cause unknown. Data, bottle: 35.172, CTD(raw): 34.965. Units PSU.			

5.4 CTD Salinity - Bottle Salinity Plot

For in2018_v03, the difference between the raw CTD salinity values and the measured bottle salinities is generally less than 0.01 PSU. Note, CTD36 RP5 point not included in plot.

The CTD salinity values are not reported in the hydrology set. Please contact the <u>DataLibrariansOAMNF@csiro.au</u> for this data.

Note: dots = bottle samples, circles = CTD instrument (raw)





5.4 OSIL Salinity Standard PSU across the Voyage

The instrument was calibrated with OSIL standard seawater lots P161 (PSU = 34.995) and P162 (PSU = 34.993). After calibration, the same solution was measured as a sample at the start of the run. These measurements are plotted below. Indicates minimal instrument drift after calibration.

6 Dissolved Oxygen Data Processing

6.1 Dissolved Oxygen Parameter Summary

Details					
HyPro Version	5.7				
Instrument	Automated Photometric Oxygen System				
Software	Scripps Institution of Oceanography (SIO)				
CSIRO Hydrochem. Method	Sampling: WI_DO_001 Assay: SOP005				
Accuracy	± 0.5 μM				
Analyst(s)	Peter Hughes, Julie Janssens				
Lab Temperature (±1°C)	Variable, 21 - 23°C				
Sample Container type	Pre-numbered 140 mL glass iodine determination flasks with glass stopper. 18 flasks per light-proof container.				
Sample Storage	Samples stored in the hydrochemistry lab until analysis. All samples were analysed within ~48 hrs				
Comments	None.				

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 synopsis: The sample is collected in an iodine determination flask of known volume. 1mL of manganese (II) chloride solution then 1 mL of alkaline iodide solution is added to the sample, the flask stoppered and inverted a minimum of 20 times. The dissolved oxygen oxidizes Mn (II) to Mn (IV) which precipitates as a floc. Just before titration, the sample is acidified, solvating the Mn(II) which then reduces the iodide to iodine. The iodine concentration is determined by titration with a standardised thiosulphate solution using a Metrohm 665 Dosimat fitted with a 1 mL burette. The endpoint is determined by measuring changes in the UV absorption of the iodine at 365 nm. The point at which there is no change in absorbance is the endpoint.

Before each batch of sample assays, the thiosulphate titrant is standardised against a 10ml aliquot of potassium iodate primary standard solution. A blank correction is also determined from the difference between two consecutive titres for 1 mL aliquots of the same potassium iodate solution.

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

6.3 Missing or Suspect Dissolved Oxygen Data.

Data is flagged as Good, Suspect or Bad based on notes from CTD sampling log sheet, observations during analysis, and examination of depth profile and waterfall plots (Flag key appendix 8.4).

CTD	RP	Flag	Reason for Flag or Action
17	6	133	Result Bad. Operator error. Cause: incomplete titration, not all the titrant was added to the sample. Not reported in final data.
43	4	0	Result Good. Large difference between bottle and CTD value. CTD DO outlier in vertical profile plot (below). Cause unknown. CTD data flagged suspect



Deployment 43

6.4 CTD Dissolved Oxygen - Bottle Dissolved Oxygen Plot

The CTD dissolved oxygen values in this plot are unprocessed raw data.

Bottle samples are mostly less than 20 uM greater than the raw CTD data. The highest point in the plot is deployment 43 RP 4; suspect CTD data.

The CTD dissolved oxygen values are not reported in the hydrology data set. Please contact the <u>DataLibrariansOAMNF@csiro.au</u> for this data.

Note: dots = bottle samples, circles = CTD instrument (unprocessed)

UNITS: µmol L⁻¹



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

The normality of the thiosulphate titrant (0.2N) varied less than 0.001N for all standardisations. Red lines indicate \pm 0.0005 N either side of the mean titrant (thiosulfate) normality. The normality should not vary more than 0.0005 N for consecutive standardisations.

The blank correction range was less than 0.0006 mL with a voyage mean of 0.0005 mL and standard deviation of 0.0001 mL (n=32).

For reference, titre volumes for dissolved oxygen bottle samples lay in the range 0.2 to 0.7 mL.

0.0001 mL of titrant is equivalent to 0.04uM dissolved oxygen.





7 Nutrient Data Processing

7.1 Nutrient Assay Parameter Summary

Details							
CSIRO Software	HyPro 5.7	HyPro 5.7					
Instrument	Seal AA3HR						
Instrument Software	Seal AACE 7.0)					
CSIRO Hydrochem. Method, sampling	WI_Nut_001						
CSIRO Hydrochem. Method, nutrient	SOP001	SOP002	SOP003	SOP003	SOP004		
Nutrient	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonium		
Upper Concentration	112 μΜ 3.0 μΜ 42 μΜ 1.4 μΜ 2.0 μΝ						
Method Detection Limit (MDL)	0.2 μM	0.02 μM	0.06 µM	0.02 μM	0.02 μM		
Matrix Corrections	none	none	none	none	none		
Analysts	Peter Hughes	s, Julie Janssen	IS				
Lab Temperature (±1°C)	Variable, 21–	- 23°C					
Reference Material	KANSO, RMNS lot CB and CJ						
Sampling Container type	CTD and UWY: 50ml HDPE with screw cap lids.						
Sample Storage	< 2 hrs at room temperature or ≤ 12 hrs @ 4°C						
Pre-processing of Samples	None.						
Comments	None						

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/Iom3.10294

Nutrient samples are assayed on a Seal AA3HR segmented flow auto-analyser fitted with 1cm flowcells 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-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 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 Missing or Suspect Nutrient Data.

The table below identifies all flagged data. Data flagged 63, below detection limit, are not included in the table below. Flag Key in Appendix 8.4.

Data is flagged as Good, Suspect or Bad in HyPro based on notes from CTD sampling log sheet, observations during analysis, and examination of depth profile and waterfall plots (Flag key appendix 8.4).

CTD	RP	Nutrient	Flag	Reason for Flag or Action		
51	1	NO _x	69	Data suspect. Flagged by HyPro. Duplicate samples differ by more than 0.06 μ mol I ⁻¹ (MDL). Cause		
				unknown. Results: 37.09, 37.25		

7.4 HyPro Processing Parameters

All instrument parameters, 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	6	6	6	
Forced through zero?	Ν	N	Ν	Ν	N	
Matrix correction	Ν	Ν	Ν	Ν	N	
Blank correction	Ν	Ν	Ν	Ν	Ν	
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.	Ν	Ν	Ν	Ν	Ν	
Medium of Standards	Low nutrient seawater (LNSW, bulk on deck of Investigator) collected on 28/9/2016. 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	dium of Baseline 18.2 Ω water. Dispensed from Milli Q					
Proportion of samples in duplicate.	<10%. Duplicate samples collected from surface and greatest depth of deployment. Single samples collected for remaining depths.					
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in appendix 8.2.					

7.5 HyPro Data Processing Summary

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.

With suspect calibration points, their contribution to the curve is given less weighting dependent on their distance from the final 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 WOCE¹).
- Within 0.02uM for phosphate, nitrite and ammonium.

HyPro classifies the quality of data as good, suspect or bad and flags accordingly. Refer Appendix 8.4 for flag key.

Missing or suspect nutrient data is tabulated in section 7.3.

¹ World Ocean Circulation Experiment

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

Japanese KANSO certified RMNS lot CJ or CB was assayed in quadruplicate in each run to monitor accuracy. The certified values are in table 1.

For in2019_v03, the majority of RMNS results are within 1% of their certified mean and within $0.02\mu M$ for nitrite. Plots of RMNS values for all runs are below.

The assayed RMNS values per CTD deployments are listed in appendix 8.2

The GO-SHIP criteria (Hyde *et al.*, 2010), appendix 8.6, specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy.

RMNS	NO₃ (µmol L⁻¹)	NO₂ (μmol L ⁻¹)	NO ₃ + NO ₂ (NO _x , μmol L ⁻¹)	PO₄ (µmol L⁻¹)	SiO₄ (µmol L ⁻¹)
Lot CB	36.649 ± 0.276	0.119 ± 0.006	36.768 ± 0.282	2.580 ± 0.022	111.821 ± 0.635
Lot CJ	16.588 ± 0.205	0.032 ± 0.007	16.620 ± 0.212	1.219 ± 0.020	39.424 ± 0.410

Table 1: RMNS concentrations with expanded uncertainty at 21°C

KANSO publishes the RMNS nutrient values in μ mol kg⁻¹. These are converted to μ mol L⁻¹ at 21°C. The RMNS is not certified for ammonium. NO_x is derived by adding the NO₃ and NO₂ values.

Plot key. The green pink and red lines are the 1%, 2% and 3% contours from the RMNS certified mean value. Exception: nitrite, the contours are at 0.02 μ M increments from the certified value. The blue line is the expanded uncertainty of the certified value (KANSO).



7.6.1 Silicate RMNS Plot (µmol L⁻¹)





7.6.2 Phosphate RMNS Plot (µmol L⁻¹)

1.2

1.19

1.18

10

20

30

Run no.

40

50



7.6.3 Nitrate + Nitrite (NOx) RMNS Plot (µmol L⁻¹)



7.6.4 Nitrite RMNS Plot (µmol L⁻¹)



7.7 Analytical Precision

7.7.1 Nutrient 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).

Calculated Meas	urement Uncertai	nty @ 1 μmol L ⁻¹		
Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
±0.017	±0.024	±0.019	±0.14	$\pm 0.30^{4}$

*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.

7.7.2 Nutrient Method Detection Limit

For in2019_v03, the measured detection limits (MDL) for all runs are lower than the nominal detection limits, indicating high analytical precision at lower concentrations. The MDL is 3 times the standard deviation of four measurements of LNSW assayed in each nutrient run. See appendix 8.3 for the measured MDL per CTD deployments.

MDL	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Nominal MDL (μmol L ⁻¹)	0.20	0.02	0.06	0.02	0.02
Standard Dev. Min	0.0	0.0	0.0	0.0	0.0
Standard Dev. Max	0.06	0.006	0.006	0.019	0.006
Standard Dev. Mean	0.02	0.002	0.002	0.001	0.0
Standard Dev. Median	0.0	0.0	0.0	0.001	0.0
Precision of MDL (stdev)	0.02	0.002	0.002	0.001	0.0

7.7.3 Reference Material for Nutrients in Seawater

Precision values are calculated from intra-analysis measurements, multiple measurements are taken at a time, typically 3-4.

Precision achieved is better than the RMNS expanded uncertainty.

Units: µmole L⁻¹

RMNS	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Certified RMNS CB w/std deviation	111.821 ± 0.635	2.580 ± 0.022	36.768 ± 0.282	0.119 ± 0.006	-
Minimum	111.5	2.58	36.68	0.139	1.36
Maximum	112.2	2.61	36.98	0.147	1.38
Mean	111.8	2.59	36.79	0.143	1.37
Median	111.7	2.59	36.75	0.142	1.3
Precision (Stdev)	0.1	0.01	0.03	0.001	0.0

RMNS	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Certified RMNS CJ w/std deviation	39.424 ± 0.410	1.219 ± 0.020	16.620 ± 0.212	0.032 ± 0.007	-
Minimum	38.4	1.21	16.31	0.038	0.88
Maximum	40.3	1.24	16.92	0.062	1.16
Mean	39.3	1.23	16.62	0.045	0.99
Median	39.3	1.23	16.62	0.045	1.00
Precision (Stdev)	0.1	0.00	0.02	0.001	0.02

7.8 Sampling Precision

Sampling precision is monitored by assaying duplicate samples collected from the greatest depth for each CTD deployment. The sampling precision is good if the difference between the duplicate concentrations is less than the MDL value. The exception is nitrate+nitrite, which uses 0.06 μ M as the MDL boundary.

Plots of the difference between the duplicate and their mean for the CTD deployments are below. The red line is the boundary below which sampling precision is deemed good.

For in2019_v03, the sampling precision is good.

7.8.1 Silicate Duplicates Plot



7.8.2 Phosphate Duplicates Plot





7.8.3 Nitrate + Nitrite (NOx) Duplicates Plot

7.8.4 Nitrite Duplicates Plot





7.8.5 Ammonia Duplicates Plot





7.9 Redfield Ratio Plot (14.0) for CTD Deployments.

Plots consist of phosphate versus NOx for all CTD deployments. Best fit ratio = 14.18

7.10 Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry lab and within the AA3HR instrument where measured and logged in the following locations:

(1) Above the AA3HR instrument, temperature only. Average 21.2°C, stdev 0.8.

(2) Above the AA3HR instrument on the other side, ship's instrument (Grafana). Data on request.

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

Refer to "in2019_v03_hyd_voyagereport.docx" for room temperature graphs.

The laboratory temperature was measured and recorded on the nutrient run sheets at the start each analysis run. The temperature remained within 19 to 23°C over the course of the voyage.

The ambient conditions during nutrient analysis are within normal laboratory parameters.

8 Appendix

8.1 Salinity: Reference Material Used

OSIL IAPSO Standard Seawater			
Batch	P161	P162	
Use by date	3 rd May 2020	16 th April 2021	
K ₁₅	0.99987	0.99983	
PSU	35.995	35.994	

8.2 Nutrients: RMNS results for each CTD Deployment.

CTD Deployment	Silicate (Si)	Phosphate (PO ₄)	Nitrate + Nitrite (NO _x)	Nitrite (NO ₂)
	39.4	1.219	16.65	0.0320
1,2	39.2	1.219	16.57	0.042
3	39.3	1.234	16.55	0.046
4	39.2	1.239	16.48	0.045
5	39.1	1.228	16.58	0.047
6	39.2	1.224	16.34	0.042
7	39.2	1.229	16.64	0.043
8	39.4	1.231	16.57	0.042
9	39.3	1.223	16.48	0.049
10	39.4	1.223	16.58	0.046
11	39.4	1.228	16.34	0.046
12	39.4	1.227	16.64	0.044
13	39.4	1.224	16.69	0.042
14	39.5	1.228	16.69	0.043
15	39.4	1.222	16.67	0.048
16	39.5	1.222	16.64	0.048
17	39.4	1.229	16.60	0.045
18	39.3	1.219	16.52	0.043
19	39.6	1.230	16.75	0.053
20	39.2	1.216	16.51	0.057
21	39.3	1.228	16.59	0.046
22	39.4	1.233	16.59	0.050
23	39.6	1.222	16.68	0.047
24	39.6	1.222	16.64	0.048
25	39.3	1.225	16.59	0.047
26	39.2	1.222	16.64	0.041
27	39.4	1.226	16.61	0.048
30	39.1	1.230	16.62	0.044
31	39.2	1.223	16.63	0.041

8.2.1 RMNS Lot CJ Results (µmol L⁻¹)

32	39.4	1.224	16.65	0.044
33	39.4	1.224	16.91	0.045
34	39.4	1.227	16.87	0.039
35	39.2	1.227	16.59	0.041
36	39.3	1.223	16.58	0.042
37	39.2	1.232	16.52	0.042
38	39.4	1.231	16.50	0.042
43	39.4	1.230	16.57	0.043
45	39.5	1.230	16.60	0.042
47	39.1	1.231	16.61	0.048
48	39.2	1.233	16.58	0.045
49	39.2	1.232	16.62	0.048
50	39.4	1.230	16.64	0.047
51	39.4	1.220	16.57	0.049
52	39.3	1.222	16.55	0.048
53	39.1	1.221	16.48	0.047
54	39.3	1.218	16.59	0.051

8.2.2 RMNS lot CB Results (µmol L⁻¹)

CTD Deployment	Silicate (Si)	Phosphate (PO ₄)	Nitrate + Nitrite (NO _x)	Nitrite (NO ₂)
	111.8	2.580	36.77	0.119
13	111.8	2.582	36.75	0.142
14	111.9	2.591	36.72	0.141
28	111.7	2.597	36.71	0.147
29	111.6	2.597	36.96	0.142

Nutrient results do <u>NOT</u> 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

8.3 Nutrients: Measured Detection Limit for each CTD Deployment.

Units: (umol L⁻¹)

CTD Deployment	Silicate (Si)	Phosphate (PO ₄)	Nitrate + Nitrite (NO _x)	Nitrite (NO ₂)	Ammonium (NH₄)
1,2	0.1	0.01	0.01	0.002	0.003
3	0.0	0.01	0.01	0.002	0.002
4	0.0	0.01	0.01	0.003	0.004
5	0.1	0.01	0.02	0.004	0.002
6	0.2	0.00	0.01	0.006	0.005
7	0.0	0.00	0.01	0.002	0.003
8	0.1	0.01	0.01	0.004	0.003
9	0.0	0.01	0.01	0.002	0.002
10	0.0	0.00	0.00	0.002	0.003
11	0.0	0.00	0.00	0.002	0.002
12	0.0	0.01	0.01	0.004	0.004
13	0.0	0.01	0.01	0.004	0.003
14	0.0	0.01	0.01	0.003	0.003
15	0.0	0.00	0.01	0.004	0.002
16	0.1	0.01	0.00	0.001	0.002
17	0.0	0.01	0.00	0.003	0.002
18	0.0	0.01	0.01	0.002	0.003
19	0.2	0.01	0.01	0.005	0.002
20	0.1	0.00	0.01	0.003	0.003
21	0.1	0.01	0.00	0.002	0.002
22	0.1	0.00	0.00	0.006	0.004
23	0.1	0.00	0.00	0.001	0.003
24	0.1	0.01	0.01	0.003	0.003
25	0.0	0.01	0.01	0.002	0.003
26	0.1	0.01	0.01	0.001	0.003
27	0.1	0.01	0.00	0.002	0.003
28	0.0	0.00	0.00	0.001	0.003
29	0.1	0.00	0.01	0.003	0.003

30	0.0	0.00	0.01	0.003	0.004
31	0.0	0.00	0.01	0.002	0.003
32	0.0	0.00	0.00	0.001	0.002
33	0.1	0.00	0.01	0.001	0.002
34	0.1	0.00	0.01	0.004	0.001
35	0.0	0.00	0.01	0.004	0.001
36	0.0	0.01	0.01	0.001	0.002
37	0.1	0.00	0.01	0.001	0.001
38	0.2	0.00	0.01	0.002	0.002
43	0.0	0.00	0.01	0.001	0.003
45	0.1	0.01	0.01	0.002	0.002
47	0.0	0.00	0.01	0.001	0.002
48	0.1	0.00	0.01	0.002	0.001
49	0.1	0.00	0.01	0.002	0.002
50	0.0	0.00	0.01	0.002	0.002
51	0.1	0.00	0.01	0.003	0.003
52	0.1	0.00	0.01	0.002	0.002
53	0.1	0.00	0.01	0.002	0.003
54	0.1	0.01	0.01	0.003	0.003

8.4 Flag Key for Hydrology Data Set

Flag	Description
0	Data is GOOD.
63	Below nominal detection limit.
69	Data is SUSPECT. Flagged by operator or software.
133	Data is BAD. Flagged by operator.
141	Missing data, no result for sample ID. Used in netcdf file. Not used in csv file.

Please refer to the following sections within this document for details of flagged sample data:

Salinity: section 5.3

Dissolved Oxygen: section 6.3

Nutrients: section 7.3

8.5 GO-SHIP Specifications

8.5.1 Salinity

Accuracy of 0.001 is possible with AutosalTM 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°C is very important and should be recorded².

8.5.2 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.

8.5.3 SiO2

Approximately 1-3% accuracy¹, 0.2% precision³, full scale.

8.5.4 PO4

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

8.5.5 NO3

Approximately 1% accuracy¹, 0.2% precision³, full scale.

8.5.6 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.

9 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)
- Kérouel, 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: p. 23-31.