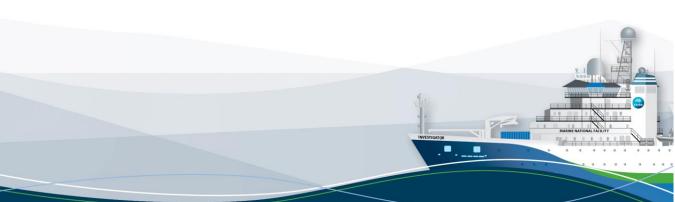


# RV *INVESTIGATOR*HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	in2022_v08			
Chief Scientist	Tim O'Hara			
Principal Investigator	Alan Williams, Shane Ahyong, Nerida Wilson, Lisa Kirkendale, Andrew Hosie			
Voyage title:	Biodiversity Assessment of Australia's Indian Ocean Territories			
Report compiled by:	Julie Janssens and Pavie Nanthasurasak			





## Contents

1	Ex	xecuti	ve Summary	4
	1.1	Ob.	jectives	4
	1.2	Ge	neral Hydrochemistry Information	5
2	lt	inerar	у	6
3	K	ey per	sonnel list	7
4	Sı	umma	ry	8
	4.1	Sar	nple Type and Number Assayed	8
	4.	.1.1	CTD Samples (Conductivity, Temperature, Density)	8
	4.	.1.2	TSG Samples (Thermosalinograph)	8
	4.2	Dat	ta Processing Overview	9
5	Sa	alinity		10
	5.1	Sal	inity Measurement Parameters	10
	5.2	Sal	inity Method	10
	5.3	СТІ	O Salinity vs Bottle Salinity Plot	10
6	D	issolv	ed Oxygen	12
	6.1	Dis	solved Oxygen Measurement Parameters	12
	6.2	Dis	solved Oxygen Method	12
	6.3	СТІ	D Dissolved Oxygen vs Bottle Dissolved Oxygen Plot	12
	6.4	Dis	solved Oxygen Instrument titrant: thiosulphate normality and blank correction	14
7	N	lutrien	ts	15
	7.1	Nu	trient Measurement Parameters	15
	7.2	Nu	trient Methods	15
	7.3	Hyl	Pro Processing Summary for Nutrients	16
	7.4	Acc	curacy - Reference Material for Nutrient in Seawater (RMNS)	17
	7.5	Nu	trient plots of RMNS	18
	7.	.5.1	Figure 6: Silicate RMNS Plot (μmol L <sup>-1</sup> )	19
	7.	.5.2	Figure 7: Phosphate RMNS Plot (μmol L <sup>-1</sup> )	20
	7.	.5.3	Figure 8: Nitrite RMNS Plot (μmol L <sup>-1</sup> )	21
	7.	.5.4	Figure 9: Nitrate + Nitrite (NOx) RMNS Plot (μmol L <sup>-1</sup> )	22
	7.6	Me	asurement Uncertainty	23
	7.7	Sar	npling Precision	23
	7.8	Red	dfield Ratio Plot (14.0) for CTD Deployments	24
	7.9	Ter	nperature & Humidity Change over Nutrient Analyses	26

8	App	end	ix	.27
	8.1	Salii	nity: Reference Material Used	. 27
	8.2	Nut	rients: Reference Material Used	. 27
	8.3	Nut	rients: RMNS lot CP results for each CTD Deployment	. 28
	8.4	Mis	sing or Suspect Salinity Data	. 30
	8.5	Mis	sing or Suspect Dissolved Oxygen Data	. 30
	8.6	Mis	sing or Suspect Nutrient Data	. 30
	8.7	Data	a Quality Flag Key	. 30
	8.8	GO-	SHIP Specifications	. 32
	8.8.	1	Salinity	. 32
	8.8.	2	Dissolved Oxygen	. 32
	8.8.	3	Si(OH) <sub>4</sub>	. 32
	8.8.	4	PO <sub>4</sub>	. 32
	8.8.	5	NO <sub>3</sub>	. 32
	8.8.	6	Notes	. 32
9	Ref	eren	ces	.33

# **1 Executive Summary**

Overall data collected was of very high quality. No significant sample collection, analysis, or data processing issues were encountered.

Concentration of ammonia were extremely low in most water samples measured. Lots of concentrations measured were within 3x of method detection limit. Some results are negative due to the concentration being lower than the instrument Milli-Q wash water. These negative values should be treated as  $0 \mu mol/L$ .

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

This voyage was the follow up voyage after in2021\_v04 was suspended in July 2021. Science operations resumed at Balthazar seamount where we left in 2021. The scientific aims of this voyage were to:

- 1. Characterise benthic biodiversity from seamounts (100-3500 m) across the Exclusive Economic Zone (EEZ) in Australia's Indian Ocean Territories (IOT) for the first time.
- 2. To provide specimen and tissue samples to taxonomists for species identification and descriptions.
- Test whether the proposed bioregionalisation for the territories (Brewer et al 2009, derived from environmental data) is an adequate surrogate for patterns of seafloor diversity across a series of depth strata.
- 4. Assess the conservation significance of these seamount communities, particularly the presence of Vulnerable Marine Ecosystems (VMEs) including cold water coral and sponge communities.
- 5. Document spatial and bathymetric patterns of oceanographic characteristics and plankton distribution.
- 6. Substantially contribute to the AusSeabed project by maximising new Multibeam coverage.
- 7. Understand the biogeographical relationships of the fauna through community and evolutionary (DNA) comparisons with other Australian, west Pacific and Indian Ocean faunas.

In parallel to this, we also engaged with Australians schools from the vessels through facilities set up by the BushBlitz program. The program also communicated with the local island communities through live video links with schools and community centre on Cocos Islands.

#### 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. See Appendix 8.3 for the CTD deployment versus measured RMNS values.

Missing and suspect hydrology samples 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: <a href="mailto:NCMI\_DataLibrarians@csiro.au">NCMI\_DataLibrarians@csiro.au</a>

# 2 Itinerary

**Table 1:** Voyage itinerary

	Depart	Arrive
Port	Darwin	Fremantle
Date	30/09/2022	03/11/2022
Time	1100	1700

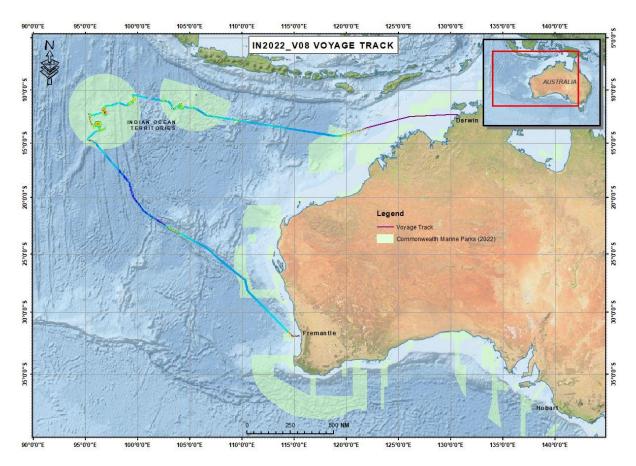


Figure 1: Voyage track

# 3 Key personnel list

Table 2: Key Personnel list

Name	Role	Organisation
Tim O'Hara	Chief Scientist	Museums Victoria
Margot Hind	Voyage Manager	CSIRO
Julie Janssens	Hydrochemist	CSIRO
Pavie Nanthasurasak	Hydrochemist	CSIRO

# 4 Summary

#### 4.1 Sample Type and Number Assayed

Table 3: Sample Type and Number Assayed

Analysis	Samples Assayed	Туре
Salinity	376 33	CTD TSG
Dissolved Oxygen	374	CTD
Nutrients	604	CTD

#### 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 44 CTD deployments were sampled by
  - Hydrochemistry: Julie Janssens and Pavie Nanthasurasak
  - Science party: Tiffany Sih, Melanie Mckenzie, Caroline Farrelly, Camille Moreau, Angelina Eichsteller, Ana Hara, Claire Rowe, Ingo Burghardt, Beth Flaxman, Jeremy Horowitz, Penny Berents, and Bruce Deagle

#### 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) at the completion of the voyage.

Refer to voyage EVERLog for TSG sample information.

#### 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).

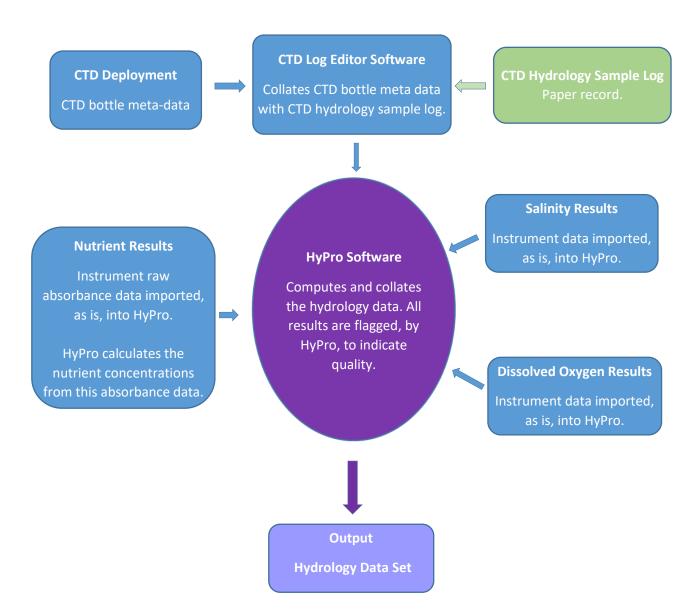


Figure 2: Hydrology Data Processing Flow Diagram.

# 5 Salinity

#### **5.1 Salinity Measurement Parameters**

**Table 4: Salinity Measurement Parameters** 

Details	
HyPro Version	5.7
Instruments	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71611 and SN 72088. Bath temperature 24.0°C
Software	Ocean Scientific International Ltd (OSIL) Data Logger version 1.2
Hydrochemistry Methods.	Sampling: WI_Sal_002 Measurement: SOP006
Accuracy	± 0.001 practical salinity units
Reference Material	OSIL IAPSO – Batch P164, use by 23/03/2023, $K_{15} = 0.99985$
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 22.5°C SD 0.78°C
Analysts	Julie Janssens and Pavie Nanthasurasak
Comments	See DAP report for CTD calibration details.

#### 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.

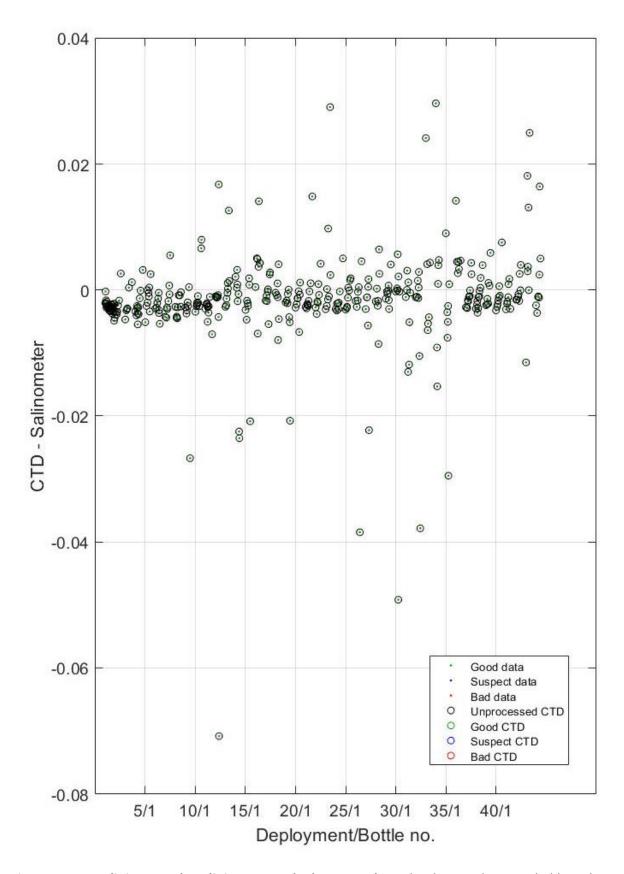
Before each lot of sample measurements, the Autosal is calibrated with standard seawater (OSIL, IAPSO) of known  $K_{15}$  ratio. A new bottle of OSIL standard is used for each calibration. The frequency of calibration is at least one per run.

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<sup>3</sup>. 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 metadata.

#### 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.



**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 5: 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 <sup>-1</sup>
Analysts	Julie Janssens and Pavie Nanthasurasak
Lab Temperature (±1°C)	Mean 19.4°C SD 0.4°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.

#### 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 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.

#### 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 15  $\mu$ mol L<sup>-1</sup>. The larger differences are for shallow samples across the sudden changes in the dissolved oxygen profile.

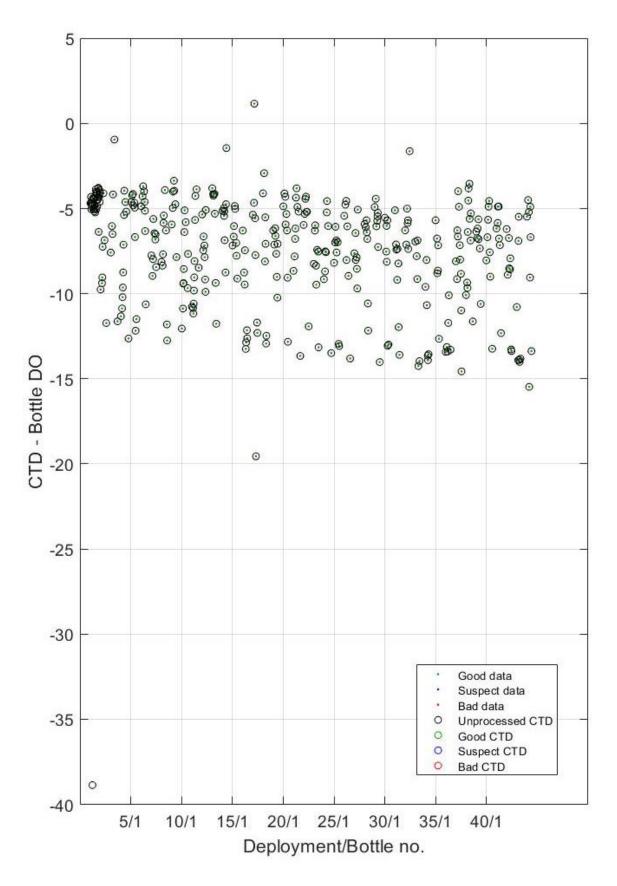


Figure 4. CTD Dissolved Oxygen - Bottle Dissolved Oxygen vs Deployment Plot. The data quality is coded by colour and delineated by a dot for the bottle DO and a circle for the CTD DO. Green = GOOD. Blue = SUSPECT. Black = UNPROCESSED. Units:  $\mu$ mol L<sup>-1</sup>

# 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. One batch of thiosulphate reagent was used during the voyage. The mean normality as follows:

CTD Deployment 1 to 44: Mean: 0.198425 N

SD: 0.000129 (n=26)

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<sub>3</sub> aliquot before the titration.

The red lines in figure 5 indicate  $\pm$  0.0005 N either side of the mean titrant (thiosulfate) concentration and the blank concentration. The titrant should not vary more than 0.0005 N between analyses.

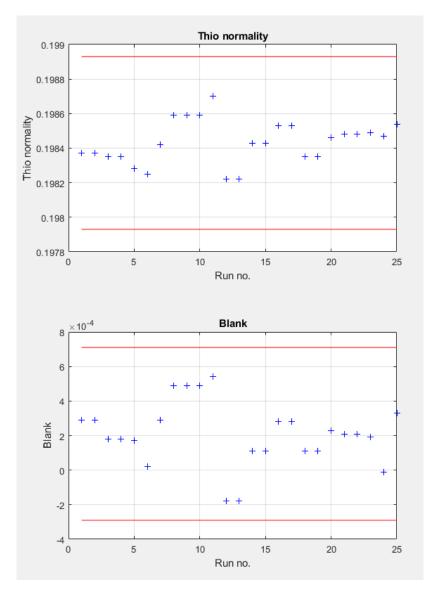


Figure 5. Thiosulphate standardisation and blank correction plots.

#### 7 Nutrients

#### 7.1 Nutrient Measurement Parameters

**Table 6: Nutrient measurement parameters.** All instrument parameters, reagent batches and instrument events are logged for each analysis run. This information is available on request.

	<del>.</del>					
Details						
Processing Software	CSIRO HyPro	CSIRO HyPro 5.7				
Instrument	Seal AA3HR	segmented flo	w analyser.			
Operating Software	AACE 7.10					
Hydrochemistry. Methods	Sampling: W	'I_DO_001				
	Assay:					
	SOP001	SOP002	SOP003	SOP004	SOP005	
	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonia	
Top concentration						
(μmol L <sup>-1</sup> )	140	3.0	42	1.4	2.0	
Method detection limit						
(μmol L <sup>-1</sup> )	0.20	0.02	0.02	0.02	0.02	
Reference Material	KANSO RMNS lot CP					
Sample Container	50 mL HDPE HCl	with screw ca	p lids. Reused	after acid wa	sh with 1M	
Sample Storage	< 4 hrs at ro	om temperatu	ire or < 12 hrs	@ 4°C		
Sample preparation	Assayed as r	eat. No filtrat	ion.			
Lab Temperature (°C)	Mean 19.4°(	C SD 0.4°C				
Analysts	Julie Janssens and Pavie Nanthasurasak					
Comments	N/A					

#### 7.2 Nutrient Methods

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-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-phthaldialdehyde 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.

#### 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 WOCE¹).
- 0.02umol<sup>-1</sup> 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 is tabulated in section 8.6

**Table 7: 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 <sup>-1</sup>	μmol L <sup>-1</sup>	μmol L <sup>-1</sup>	μmol L <sup>-1</sup>	μmol L <sup>-1</sup>
Calibration Curve degree	Linear	Linear	Linear	Quadratic	Quadratic
# of points in Calibration	7	6	7	6	6

<sup>&</sup>lt;sup>1</sup> Royal Netherlands Institute for Sea Research – Study Group on Nutrient Standards.

<sup>&</sup>lt;sup>1</sup> World Ocean Circulation Experiment

Result Details	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
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)	Υ	Υ	Y	Υ	Y
Baseline drift correction (HyPro)	Υ	Υ	Υ	Υ	Y
Sensitivity drift correction (HyPro)	Υ	Υ	Y	Υ	Υ
Data Adj for RMNS variance.	N	N	N	N	N
Medium of Standards	Low nutrient seawater (LNSW, bulk on deck of Investigator) collected in June 2021. 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 $\Omega$ water. Dispensed from the Milli Q IQ 7010.				
Duplicate samples.	CTD: Niskin 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 8.3.				

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

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

Japanese KANSO certified RMNS lot assayed in triplicate in each run to monitor accuracy. The certified values are in Table 8. Internal bulk quality control (BQC) was also analysed in each run in duplicates.

For in2022\_v08, the certified reference material results for NOx and silicate are within 1%, phosphate is within 3% and nitrite within 0.02  $\mu$ mol L<sup>-1</sup> 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 8: RMNS CP certified concentrations ± expanded uncertainty (U) at 21°C. Units: μmol L-1

RMNS	Silicate	Phosphate	Nitrite	NO <sub>3</sub> + NO <sub>2</sub>
	(Si(OH)₄)	(PO <sub>4</sub> )	(NO₂)	(NO <sub>X</sub> )
Lot CP	62.5687 ± 0.307	1.7951 ± 0.018	0.3175 ± 0.316	25.7136 ± 0.379

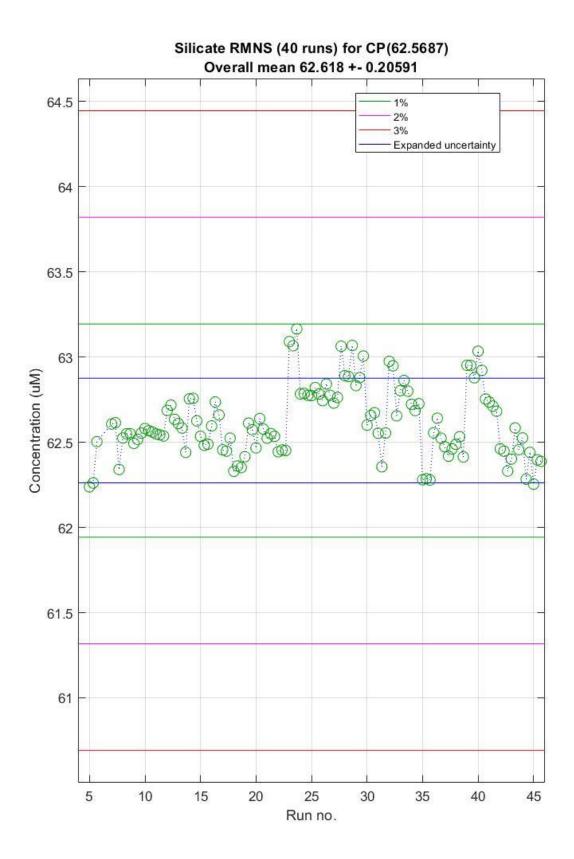
KANSO publishes the RMNS nutrient values in  $\mu$ mol kg<sup>-1</sup>. These are converted to  $\mu$ mol L<sup>-1</sup> at 21°C. The RMNS is not certified for ammonium. NO<sub>x</sub> is derived by summing the NO<sub>3</sub> and NO<sub>2</sub> values.

Table 9: RMNS CP statistics for of this voyage. Units: μmol L-1

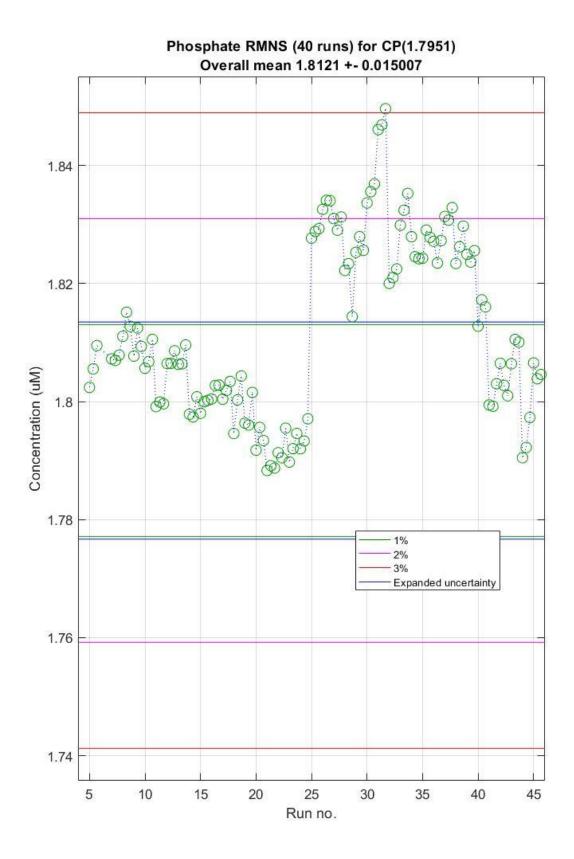
RMNS CP	Silicate (Si(OH)₄)	Phosphate (PO <sub>4</sub> )	Nitrite (NO <sub>2</sub> )	NO <sub>3</sub> + NO <sub>2</sub> (NO <sub>X</sub> )
Minimum	62.2	1.79	0.306	25.42
Maximum	63.2	1.85	0.327	25.87
Mean	62.6	1.81	0.316	25.60
Median	62.6	1.81	0.316	25.58
Repeatability	0.2	0.02	0.004	0.08

#### **7.5** Nutrient plots of RMNS

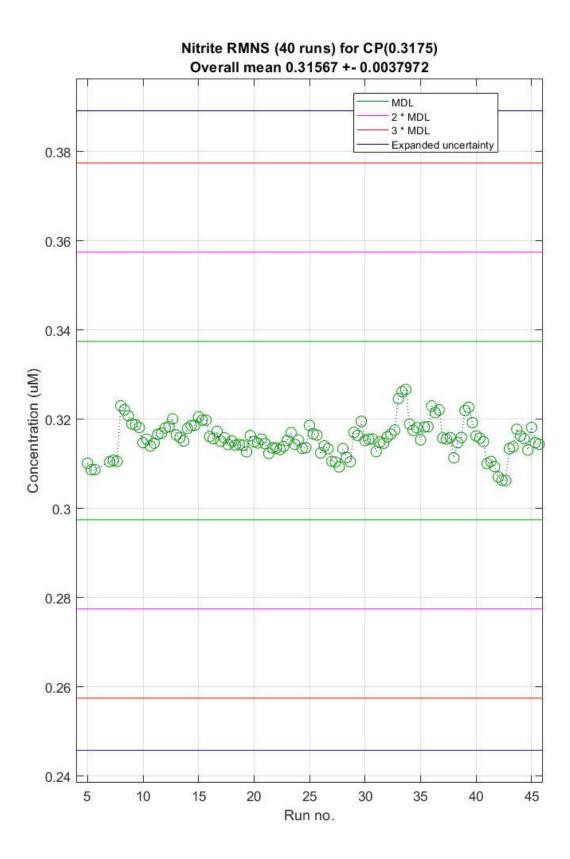
The green, pink, and red contours are at 1%, 2% and 3% from the RMNS certified mean value respectively. Exception: nitrite, the contours are at  $0.02~\mu mol~L^{-1}$  increments from the certified value. The blue line is the certified value's expanded uncertainty. Plots are RMNS value versus instrument run number.



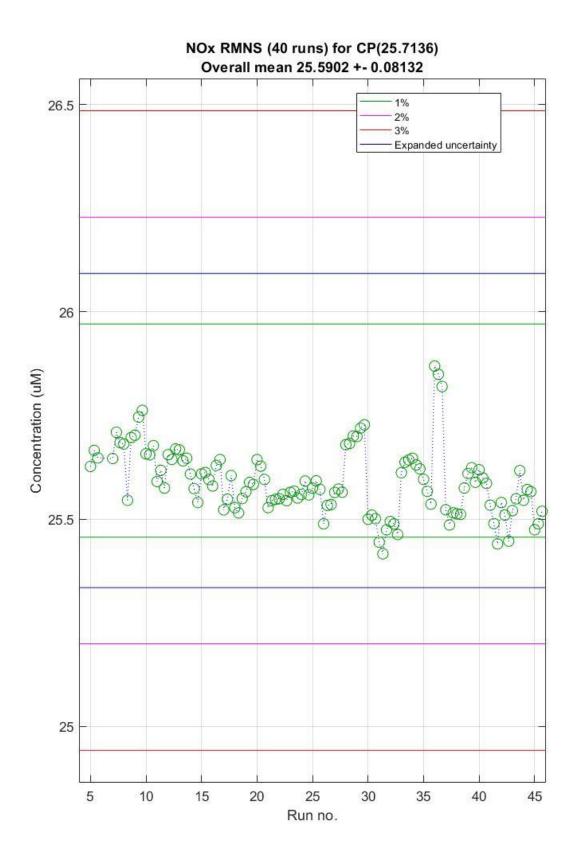
#### **7.5.1 Figure 6:** Silicate RMNS Plot (μmol L<sup>-1</sup>)



**7.5.2 Figure 7:** Phosphate RMNS Plot (μmol L<sup>-1</sup>)



#### **7.5.3 Figure 8:** Nitrite RMNS Plot (μmol L<sup>-1</sup>)



7.5.4 Figure 9: Nitrate + Nitrite (NOx) RMNS Plot (µmol L<sup>-1</sup>)

#### 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<sup>-1</sup>

Calculated Measurement Uncertainty @ 1 μmol L <sup>-1</sup>						
Silicate	Phosphate	Nitrite	Nitrate + Nitrite (NOx)	Ammonia		
±0.017	±0.024	±0.14	±0.019	±0.30 <sup>¥</sup>		

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

#### 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). 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 (table 6). The exception: NOx (nitrate+nitrite) which uses the limit  $0.06~\mu mol~L^{-1}$ 

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 44 Units: μmol L<sup>-1</sup>

	Silicate (Si(OH) <sub>4</sub> )	Phosphate (PO <sub>4</sub> )	Nitrite (NO <sub>2</sub> )	NO <sub>3</sub> + NO <sub>2</sub> (NO <sub>X</sub> )	Ammonia (NH₄)
Minimum	0.002	0.000	0.000	0.001	0.000
Maximum	0.131	0.003	0.002	0.045	0.016
Mean	0.050	0.001	0.001	0.014	0.001
Variance	0.035	0.001	0.000	0.012	0.003

<sup>\*</sup>The ammonia MU precision does not include data for the RMNS.

Table 12: CTD deployment 1. 35 bottles at 1000 dbar.

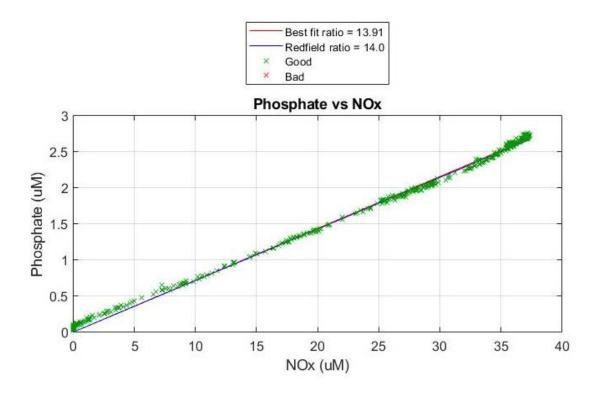
	Salinity (PSU)	Dissolved Oxygen µmol L <sup>-1</sup>	Silicate (Si(OH) <sub>4</sub> ) µmol L <sup>-1</sup>	Phosphate (PO <sub>4</sub> ) μmol L <sup>-1</sup>	Nitrite (NO <sub>2</sub> ) μmol L <sup>-1</sup>	NO <sub>3</sub> + NO <sub>2</sub> (NO <sub>X</sub> ) μmol L <sup>-1</sup>
Minimum	34.619	91.38	99.70	2.67	0.00	36.74
Maximum	34.624	93.97	100.60	2.69	0.01	36.92
Mean	34.622	92.11	100.22	2.68	0.01	36.82
SD	0.001	0.52*	0.22	0.00	0.00	0.05

<sup>\*</sup>Samples that were compromised during sampling process were excluded from this calculation.

# 7.8 Redfield Ratio Plot (14.0) for CTD Deployments.

The Redfield ratio for this voyage: 13.91

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.



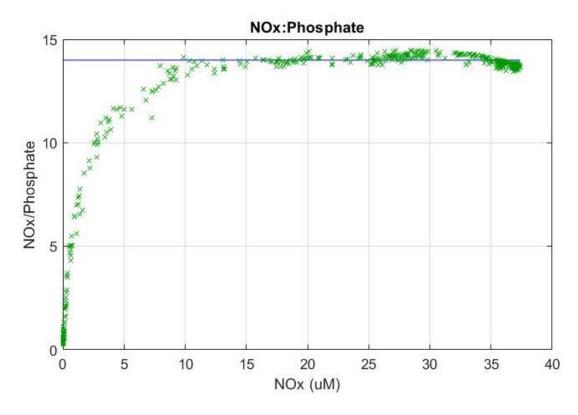


Figure 10. Redfield ratio plots.

#### 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 19.4°C SD 0.4°C
- (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:	P164	
Use by date:	23/03/2023	
K <sub>15</sub> :	0.99985	
PSU:	34.994	

## 8.2 Nutrients: Reference Material Used

RMNS	Silicate	Phosphate	Nitrite	NO <sub>3</sub> + NO <sub>2</sub>
	(Si(OH)₄)	(PO <sub>4</sub> )	(NO <sub>2</sub> )	(NO <sub>X</sub> )
Lot CP	62.5687 ± 0.307	1.7951 ± 0.018	0.3175 ± 0.316	25.7136 ± 0.379

# 8.3 Nutrients: RMNS lot CP results for each CTD Deployment.

Run analysis #	CTD Deployment #	Silicate (Si(OH) <sub>4</sub> ) (µmol L <sup>-1</sup> )	Phosphate (PO <sub>4</sub> ) (μmol L <sup>-1</sup> )	NOx (NO <sub>2</sub> + NO <sub>3</sub> ) (μmol L <sup>-1</sup> )	Nitrite (NO₂) (μmol L⁻¹)
5	1	62.334	1.806	25.647	0.309
7	2,3	62.521	1.807	25.680	0.311
8	4	62.542	1.813	25.641	0.322
9	5	62.521	1.810	25.737	0.319
10	6	62.569	1.808	25.663	0.315
11	7	62.543	1.800	25.594	0.316
12	8	62.680	1.807	25.656	0.319
13	9	62.545	1.807	25.651	0.316
14	10	62.713	1.799	25.574	0.318
15	11	62.502	1.799	25.606	0.320
16	12	62.664	1.802	25.618	0.316
17	13	62.476	1.802	25.558	0.315
18	14	62.347	1.800	25.531	0.315
19	15	62.534	1.798	25.579	0.314
20	16,17	62.561	1.794	25.622	0.315
21	18	62.537	1.789	25.540	0.314
22	19	62.450	1.792	25.551	0.314
23	20	63.107	1.792	25.561	0.316
24	21	62.782	1.794	25.570	0.314
25	22	62.793	1.829	25.580	0.317
26	23	62.787	1.834	25.519	0.313
27	24	62.852	1.830	25.567	0.310
28	25	62.948	1.820	25.687	0.312
29	26	62.906	1.826	25.715	0.318
30	27	62.644	1.835	25.503	0.315
31	28,29	62.488	1.848	25.445	0.314
32	30	62.860	1.821	25.482	0.317
33	31	62.822	1.833	25.631	0.326
34	32	62.712	1.826	25.633	0.318
35	33,34	62.281	1.827	25.566	0.317
36	35,36	62.574	1.826	25.846	0.322
37	37	62.451	1.832	25.508	0.316
38	38	62.478	1.826	25.533	0.314
39	39	62.927	1.825	25.608	0.321
40	40	62.903	1.815	25.601	0.316
41	41	62.710	1.801	25.488	0.310
42	42	62.413	1.803	25.499	0.307
43	43	62.481	1.809	25.562	0.315
44	44	62.416	1.793	25.561	0.315

#### 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

#### 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
NA	NA	NA	NA

#### 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
1	8	133	DO lid was put upside down before shaking resulting in small loss of sample volume and air gap under the stopper.  Data was marked as BAD by operator

#### 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
2	1	Ammonia	69	Duplicate of this bottle did not match and outside of set limit. The data was marked as SUSPECT by the HyPro
11	1	Ammonia	133	Abnormal vertical profile plot. Operator suspected sampling contamination. The data was marked as SUSPECT by operator
11	8	Ammonia	133	Abnormal vertical profile plot. Operator suspected sampling contamination. The data was marked as SUSPECT by operator
12	1	Ammonia	69 133	Duplicate of this bottle did not match. Abnormal vertical profile plot. Operator suspected sampling contamination. The data was marked as SUSPECT by operator
26	1	Ammonia	69 133	Duplicate of this bottle did not match and outside of set limit. Abnormal vertical profile plot. Operator suspected sampling contamination. The data was marked as SUSPECT by operator

#### 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<sup>TM</sup> 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 recorded<sup>2</sup>.

#### 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)<sub>4</sub>

Approximately 1-3% accuracy<sup>1</sup>, 0.2% precision<sup>3</sup>, full scale.

#### 8.8.4 PO<sub>4</sub>

Approximately 1-2% accuracy<sup>1</sup>, 0.4% precision<sup>3</sup>, full scale.

#### 8.8.5 NO<sub>3</sub>

Approximately 1% accuracy<sup>1</sup>, 0.2% precision<sup>3</sup>, full scale.

#### 8.8.6 Notes

- <sup>1</sup> If no absolute standards are available then accuracy should be taken to mean the reproducibility presently obtainable in the better laboratories.
- <sup>2</sup> 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.
- <sup>3</sup> 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 intercomparability 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: pp. 23-31.