

RV INVESTIGATOR

HYDROCHEMISTRY DATA PROCESS REPORT

Voyage:IN2016_v06Chief Scientist:Bernadette SloyanVoyage title:Sustained monitoring of the East Australian Current:
Mass, heat and freshwater transportsReport compiled by:Peter Hughes and Stephen Tibben



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1 Itinerary

Depart Leg	Date	Local Time
Brisbane	29 October 2016	0800
Arrive	Date	Time
Brisbane	13 November 2016	1300

2 Key personnel list

Name	Role	Organisation	
Bernadette Sloyan	Chief Scientist	CSIRO	
Tegan Sime	Voyage Manager	CSIRO	
Peter Hughes	Hydrochemist	CSIRO	
Stephen Tibben	Hydrochemist	CSIRO	

3 Summary

All finalized data can be obtained from the CSIRO data centre.

3.1 Hydrochemistry

Analysis parameter	Total	Processing Status at voyage end
Nutrients (Seal AA3)	263 CTD	Completed
Salinity (Guildline salinometer)	263 CTD	Completed
Dissolved Oxygen	262 CTD	Completed

Note: CTD-samples collected from NISKIN bottles on CTD rosette.

3.2 Rosette and CTD

- 12 CTD stations were sampled with a 24 bottle rosette (12 L).
- See in2016_v06HydrochemistryReport.pdf (voyage report) for more details on sample collection.

3.3 Procedure Summary

The procedure for data processing is outline in Figure 1.



Figure 1: The process above shows the data trail procedure from the initial data generated to output via HyPro for reporting.

4 Salinity Data Processing

4.1 Salinity Parameter Summary

Details						
HyPro Version	4.15					
Instrument	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71611					
Software	OSIL Data Logger					
Methods	Hydrochemistry Operations Manual + Quick Reference Manual					
Accuracy	± 0.001 salinity units					
Analyst(s)	Stephen Tibben and Peter Hughes					
Lab Temperature (±0.5°C)	20.0 -23.5°C during analysis.					
Bath Temperature	23.997°C					
Reference Material	Osil IAPSO - Batch P158					
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 held in Salt Room at least 8 hrs to reach 22°C before analysis					
Comments	Instrument 71611 running fine.					



4.2 CTD vs Hydro Salinities Plot

4.3 Missing or Suspect Salinity Data and Actions taken

CTD	RP	Assay run	Flag	Reason for Flag or Action
6	22	Sal004	69	Possible sampling error. This has the same salinity result as deployment 6 RP23 and does not follow the vertical profile trend.

5 Dissolved Oxygen Data Processing

5.1 Dissolved Oxygen Parameter Summary

Details					
HyPro Version	4.15				
Instrument	Automated Photometric Oxygen system				
Software	SCRIPPS				
Methods	SCRIPPS				
Accuracy	0.01 ml/L + 0.5%				
Analyst(s)	Stephen Tibben				
Lab Temperature (±1°C)	Variable, 21.0 - 24.0°C				
Sample Container type	Pre-numbered glass 140 mL glass vial w/stopper, sorted into 18 per box and boxes labelled A to S.				
Sample Storage	Samples were stored within Hydrochemistry lab under the forward starboard side bench until analysis. All samples were analysed within ~48 hrs				
Comments	All data recorded; no issues encountered during voyage.				



5.2 CTD vs Hydro DO Plot

5.3 Dissolved Oxygen thiosulphate normality across voyage





5.4 Dissolved Oxygen blank concentration across voyage

5.5 Missing or Suspect Dissolved Oxygen Data and Actions taken

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.

CTD	RP	Assay Run	Flag	Reason for Flag or Action
3	4	Oxy003	141	Acid not added during assay as required for a successful titration. No result obtained.

6 Nutrient Data Processing

6.1 Nutrient Parameter Summary

Details	Details							
HyPro Version	4.15							
Instrument	Seal AA3 HR							
Software	Seal AACE 6.10							
Methods	AA3 Analysis Metho	ods internal manual						
Nutrients analysed	Silicate	Phosphate	Nitrate + Nitrite	Nitrite				
Concentration range	140 µmol l ⁻¹	3 µmol l ⁻¹	36.4 µmol l⁻¹	1.4 μmol l ⁻¹				
Method Detection Limit* (MDL)	0.2 μmol l ⁻¹	0.02 μmol l ⁻¹	0.02 µmol l ⁻¹	0.02 μmol l ⁻¹				
Matrix Corrections	Ν	Ν	Ν	Ν				
Analyst(s)	Peter Hughes and Stephen Tibben							
Lab Temperature (±1°C)	Variable, 20.0 – 23.5°C							
Reference Material	Kanso RMNS lot CA and CC							
Sampling Container type	50 ml HDPE bottles							
Sample Storage	< 2 hrs at room temperature or \leq 12 hrs @ 4°C							
Pre-processing of Samples	None							
Comments	All data recorded; r	no issues encountered	d during voyage.					

6.2 Nutrient calibration and data parameter summary

During the course of the voyage all run parameters were recorded - LNSW batch, new cadmium column, new stock standard, daily standard information, fresh reagent information, AA3 instrument settings, pump tube changes and pump tube hours. This information is available upon request.

The raw data from each analysis run on the Seal AA3HR is imported into HyPro for peak determination, constructing the calibration curve, deriving the sample results and applying drift and carry-over corrections. The calibration curve is fitted to the standards by performing several passes over each standard point and weighting its contribution to the curve's fit depending on the magnitude of the difference between its measured and calculated value. The larger the difference the less weighting of the standard's contribution towards the curve's construction. The cut-off limits for good calibration data are

- ±0.5% the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE).
- 0.02uM for phosphate and nitrite.

Following standard procedures, the operator may choose to manually not include bad calibration points (see section 6.6 for edited data). Below are the corrections and settings that Hypro applied to the raw data.

Result Details	Silicate	Phosphate	Nitrate + Nitrite	Nitrite		
Data Reported as	µmol l⁻¹	µmol l ⁻¹	µmol l ⁻¹	µmol l ⁻¹		
Calibration Curve degree	Linear	Linear	Quadratic	Quadratic		
Forced through zero?	N	N	Ν	N		
# of points in Calibration	6 or 7	6	6	6		
Matrix Correction	Ν	Ν	Ν	Ν		
Blank Correction	Ν	Ν	Ν	Ν		
Carryover Correction (Hypro)	Y	Y	Y	Y		
Baseline Correction (Hypro)	Y	Y	Y	Y		
Drift Correction (Hypro)	Y	Y	Y	Y		
Data Adj for RMNS	Ν	Ν	Ν	Ν		
Peak Window Defined	HyPro	HyPro	HyPro	HyPro		
Medium of Standards	LNSW (bulk on deck of Investigator) collected on 28/9/2016. Sub-lot passed through a 10 micron filter and stored in 15 L carboys in the hydrochemistry laboratory at 21°C.					
Medium of Baseline	18.2 Ω MQ					
Proportion of samples in duplicate?	1 duplicate for each CTD deployment from NISKIN bottle 1.					
Comments	Calibration and QC data that was edited or removed is located in the table in section 6.6. The reported data is not corrected to the RMNS. RMNS data per CTD deployment can be found in Appendix 7.4.					

6.3 Accuracy - Reference Material for Nutrient in Seawater (RMNS) Plots

Japanese KANSO certified reference materials (CRM) for silicate, phosphate, nitrate and nitrite in seawater was used in each nutrient analysis run to ensure the accuracy of results. The RMNS was assayed 4 times after the calibration standards. A new RMNS was used in each run.

RMNS Lots CA and CC were used. Their stated values in μ mol/kg were converted to μ mol l⁻¹ at 21°C and are listed below.

Table	1:	RMNS	CA	and	CC	concentrations	(µM)	at 21°C
-------	----	------	----	-----	----	----------------	------	---------

RMNS	NO ₃	NOx	NO ₂	PO ₄	SiO ₄
СА	20.13	20.20	0.065	1.441	37.46
СС	31.62	31.74	0.119	2.130	88.23

The submitted nutrient results do NOT have RMNS corrections applied.

The following plots show RMNS values within 1% (green lines), 2% (pink lines) and 3% (red lines) of the published RMNS value except for nitrite. The nitrite limit is set to $\pm 0.020 \,\mu$ M (MDL) as 1% is below the method MDL. The GO-SHIP criteria (Hyde *et al.*, 2010), reference section 7.3, specifies

using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy. The calculated RMNS values per CTD deployment are reported in the table in section 7.4.





6.3.2 Phosphate RMNS Plot



6.3.3 Nitrate + Nitrite (NOx) RMNS Plot

6.3.4 Nitrite RMNS Plot



6.4 Analytical Precision

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

	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite
Calculated MU* @ 1 µmol l⁻¹	±0.017	±0.020	±0.017	±0.108

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

Method detection limits (MDL) achieved during the voyage were lower than the nominal detection limits, indicating high analytical precision at lower concentrations. Results are μ mol l⁻¹. The precision of the RMNS was also determined.

MDL	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite
Nominal MDL*	0.20	0.02	0.02	0.02
Min	0.008	0.005	0.004	0.001
Max	0.052	0.017	0.015	0.010
Mean	0.030	0.009	0.008	0.003
Median	0.023	0.008	0.008	0.002
Precision of MDL (stdev)	0.028	0.004	0.004	0.003

*MDL is based on 3 times the standard deviation of Low Nutrient Seawater (LNSW) analysed in each nutrient run.

Published RMNS CA	37.46	1.441	20.20	0.065
w/uncertainty	± 0.22	± 0.014	± 0.16	± 0.010
RMNS Min	36.77	1.437	20.17	0.067
RMNS Max	37.88	1.458	20.28	0.077
RMNS Mean	37.40	1.446	20.24	0.072
RMNS Median	37.44	1.445	20.26	0.073
RMNS Std Dev	0.36	0.007	0.33	0.003
Published RMNS CC	88.23	2.130	31.98	0.119
uncertainty	± 0.49	± 0.019	± 0.24	± 0.005
RMNS Min	88.28	2.111	31.86	0.115
RMNS Max	88.83	2.142	32.26	0.132
RMNS Mean	88.36	2.130	31.98	0.124
RMNS Median	88.29	2.131	31.97	0.123
RMNS Std Dev	0.25	0.008	0.09	0.006

6.5 Sampling Precision

Duplicates samples were collected from NISKIN bottle rosette position 1 to measure the precision of nutrient sampling (this is not a measurement of analytical precision). The duplicate measurements are reported in the data as an average when the duplicates are flagged GOOD. The sampling precision is deemed good if difference between duplicate concentrations is below the MDL for silicate, phosphate and nitrite and within 0.05 µM for nitrate.

6.5.1 Silicate Duplicate Plot





6.5.2 Phosphate Duplicate Plot



6.5.3 Nitrate + Nitrite (NOx) Duplicate Plot

6.5.4 Nitrite Duplicate Plot



6.5.5 Redfield Ratio Plot (14.0)

Plots consists of phosphate versus NOx, best fit ratio = 14.43



All calibration data was left as is for Hypro to evaluate. All calibrations where good, no outliers.

CTD	Assay Run	Peak	Analysis	Flag	Reason for Flag or Action
3	Nut004	Drift	Nitrite	133	Drift peaks off scale, no drift correction applied to run. Drift correction is typically <2% which is insignificant when applied to typical surface concentrations of 0.3uM

6.7 Investigation of Missing or Flagged Nutrient Data and Actions taken.

The table below identifies all flagged data and data that was repeated. Data that falls below the detection limit, Flag 63, is not captured in this table. All GOOD data is flagged 0 in the .csv and .netcdf files. Refer to Appendix 7.2 for flag explanations.

CTD	RP	Analysis	Flag	Reason for Flag or Action
11	4	All	69	Same value as RP05. Does not follow vertical profile trend. Possible sampling error – collected from RP05.
11	6	All	69	Same value as RP07. Does not follow vertical profile trend. Possible sampling error – collected from RP07.

6.8 Temperature & Humidity Change over Nutrient Analyses

The temperature and humidity within the AA3 chemistry module was logged using a temperature/humidity logger QP6013 (Jaycar) placed on the deck of the chemistry module.

The temperature spikes coincide with instrument operation whilst assaying samples.

Humidity data was not downloaded.

AA3 Temperature plot.



Refer to "in2016_v06_HydrochemistryReport.pdf" for room temperature graphs. Nutrient samples were placed on XY3 auto sampler at the average room temperature of 22°C.

7 Appendix

7.1 Salinity Reference Material

Reference	Batch	Use by Date	K ₁₅
Osil IAPSO Standard Seawater	P158	25/03/2018	0.99940

7.2 Hypro Flag Key for CSV & NetCDF file

Flag	Meaning
0	Data is GOOD – nothing detected.
192	Data not processed.
63	Below nominal detection limit.
69	Data flagged suspect by operator. Set suspect by software if Calibration or Duplicate data is outside of set limits but not so far out as to be flagged bad.
65	Peak shape suspect.
133	Error flagged by operator. Data is bad – operator identified by # in SLK file or by clicking on point.
129	Peak exceeds maximum A/D value. Data is bad.
134	Error flagged by software. Peak shape is bad - Median Absolute Deviation (MAD) analysis used. Standards, MDL's and Duplicates deviate from the median, Calibration data falls outside set limits.
141	Missing data, no result for sample ID. Used in the netcdf file. Not used in the csv file.
79	Method Detection Limit (MDL) during run was equal to or greater than nominal MDL. Data flagged as suspect.

7.3 GO-SHIP Specifications

- Salinity Accuracy of 0.001 is possible with Autosal[™] salinometers and concomitant attention to methodology, e.g., monitoring Standard Sea Water. 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. High precision of approximately 0.0002 PSS-78 is possible following the methods of Kawano (this manual) with great care and experience. Air temperature stability of ± 1°C is very important and should be recorded.¹
- O₂ 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.
- SiO₂ Approximately 1-3% accuracy[†], 2 and 0.2% precision, full-scale.
- PO₄ Approximately 1-2% accuracy[†], 2 and 0.4% precision, full scale.
- NO₃ Approximately 1% accuracy⁺, 2 and 0.2% precision, full scale.
- Notes: + If no absolute standards are available for a measurement then *accuracy* should be taken to mean the *reproducibility* presently obtainable in the better laboratories.

1 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 batches is recommended. The bottles should also be used in an interleaving fashion as a consistency check within a batch and between batches.

2 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.

CTD	SiO ₄	SiO ₄	PO ₄	PO ₄	NO ₂	NO ₂	NOx	NOx
	measured	expected	measured	expected	measured	expected	measured	expected
1	37.33	37.46	1.441	1.441	0.073	0.065	20.24	20.20
2	88.23	88.23	2.118	2.130	0.129	0.119	32.00	31.74
3	37.54	37.46	1.443	1.441	0.069	0.065	20.20	20.20
4	88.10	88.23	2.137	2.130	0.130	0.119	32.13	31.74
5	88.35	88.23	2.127	2.130	0.123	0.119	31.98	31.74
6	88.81	88.23	2.125	2.130	0.129	0.119	31.95	31.74
7, 8	37.84	37.46	1.457	1.441	0.075	0.065	20.26	20.20
9	88.20	88.23	2.138	2.130	0.118	0.119	31.95	31.74
10	88.23	88.23	2.132	2.130	0.117	0.119	31.98	31.74
11, 12	88.61	88.23	2.134	2.130	0.121	0.119	31.88	31.74

7.4 RMNS Values for each CTD Deployment

7.5 Nutrient Methods

CSIRO Oceans and Atmosphere Hydrochemistry nutrient analysis is performed with a segmented flow auto-analyser – Seal AA3HR – to measure silicate, phosphate, nitrite and nitrate plus nitrite.

Details							
Instrument	AA3						
Software	Seal AACE 6.10						
Methods	AA3 Analysis Methods internal manual						
Nutrient	Silicate	Phosphate	Nitrate + Nitrite	Nitrite			
Concentration range	140 µmol l ⁻¹	3 µmol l ⁻¹	42 μmol l ⁻¹	1.4 μmol l ⁻¹			
Method Detection Limit (MDL)	0.2 μmol l ⁻¹	0.02 μmol l ⁻¹	0.02 µmol l ⁻¹	0.02 µmol l ⁻¹			

Table 2: Calibration range and detection limits of nutrient analysis

Silicate analysis is based on a modified Armstrong et al. (1967) method. Silicate in seawater reacts with acidified ammonium molybdate to produce silicomolybdic acid. This solution will also react with phosphate producing a phosphomolybdic acid. Tartaric acid is introduced to remove this interference. Finally, Stannous Chloride (Tin II Chloride) is added to reduce silicomolybdic acid to the blue compound silicomolybdous acid which can be detected at 660 nm or 820 nm.

Phosphate measurement is based on the original Murphy and Riley (1962) method with some modifications developed at the NIOZ-SGNOS Practical Workshop 2012 optimizing antimony catalyst/phosphate ratio and reduction of silicate interferences by pH. Phosphate in seawater forms a phosphomolybdenum blue complex with acidified ammonium molybdate reduced by ascorbic acid which can be detected at 880 nm.

Nitrate is determined by first reducing to nitrite via a basic buffered copperized cadmium column before the colour reaction (Wood et al., 1967). Nitrite in seawater will react with sulphanilamide under acidic conditions to form a diazo compound. This compound couples with 1-N-naphthly-ethylenediamine di-hydrochloride to produce a reddish purple azo complex which can be detected at 520 nm.

Detailed SOPs can be obtained from the CSIRO Oceans and Atmosphere Hydrochemistry Group on request.

8 References

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