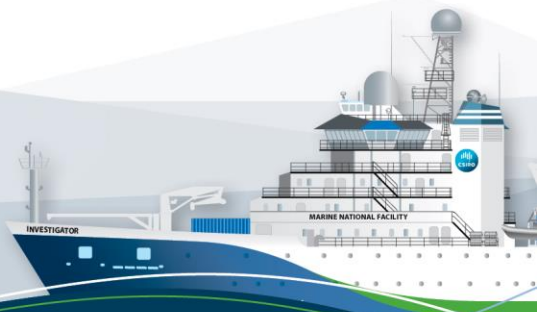
RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESSING REPORT:

AA100 Underway Nutrients

Voyage:	in2018_v04
Chief Scientist:	Michael Ellwood
Voyage title:	Constraining external iron inputs and cycling in the southern extension of the East Australian Current.
Report compiled by:	Kendall Sherrin, Christine Rees & Stephen Tibben



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1 Executive Summary

The primary objective of the voyage was to characterize the sources and biogeochemical cycling of iron and associated nutrients and their impact on productivity from east of mainland Australia to south of Tasmania. Hydrochemistry analysed salinity, oxygen and nutrient samples from the CTD, TMR and experimental samples.

This data processing report will pertain to everything AA100 related, which was the instrument used to analyse seawater nutrients underway. This was the first voyage that the AA100 was used on, it worked exceptionally well and did not have many issues with running underway. Nitrate as NO_x (NO_3 + NO_2) and Phosphate were analysed on the instrument.

There is no data from the very first analytical run, this analysis had a catastrophic failure during analysis, rendering all of the data unusable. After this analytical run the instrument inlet setup was modified in order to prevent a similar issue occurring again.

All of the data included in the final dataset is to be considered as good data. The dataset has had a reasonable level of quality control applied to ensure there are no apparent erroneous data points. However due to the size and sheer amount of data points there is a small chance there could still be some values that may be incorrect.

A large geographic gap (on the east – west transect) in data was due to a flood occurring in the underway seawater laboratory, which also meant the AA100 was covered in seawater. This instrument was removed from the laboratory and taken apart for cleaning. During this time the ship continued transiting, accounting for the missing data on the large east to west transect south of Tasmania.

The matched latitude and longitude coordinates supplied in the dataset were obtained from the ships underway computing systems. Nutrient analysis data points were matched using UTC time stamps, no time correction was applied to account for the residence time in the ships piping or in the instrument. Approximate timings are outlined in the report if this needs to be corrected for.

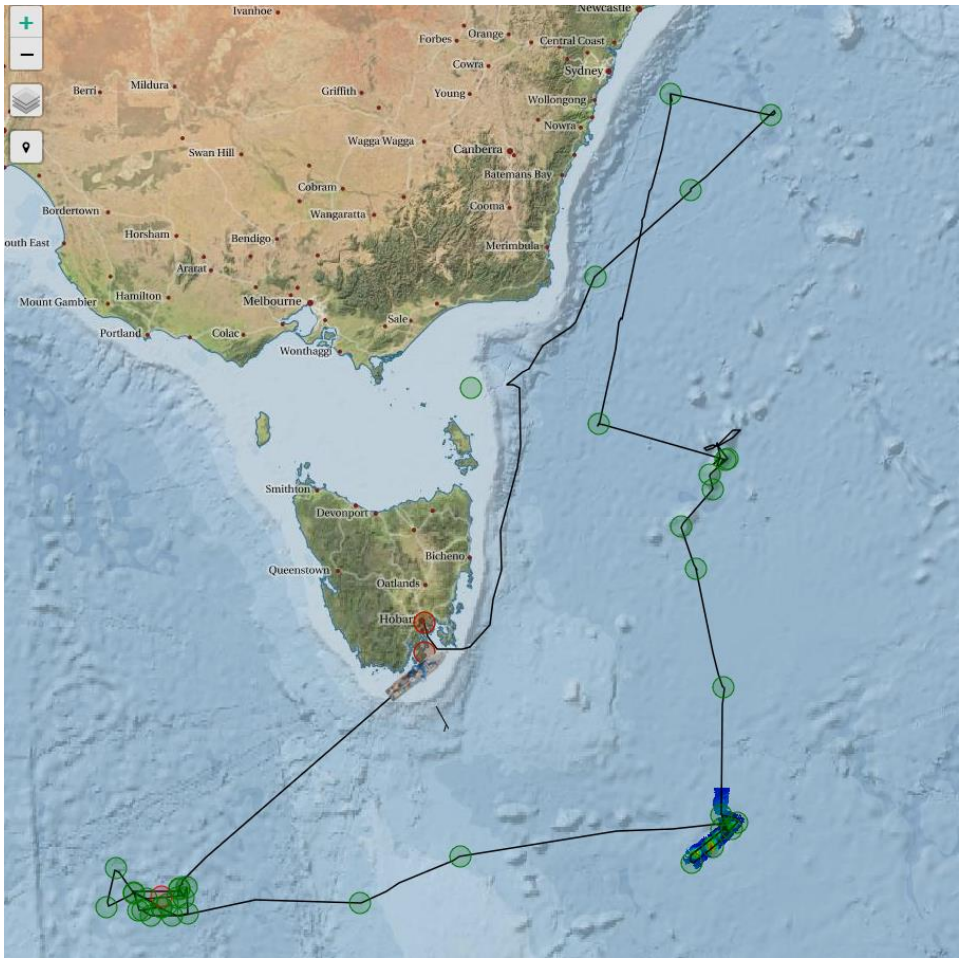
The final dataset includes latitude, longitude, UTC time stamps, NO_x (nitrate + nitrite in µM), phosphate (PO_4 in µM) and analysis file. The analysis file for each data point is included if correcting to the reference material used for each analysis is required. The measurements for the reference material are provided in appendix 6.2. Time stamps in the .csv data file are given in a written format set to UTC. In the NetCDF file, the time stamps are provided as seconds since the start of 2018, eg 01/01/2018 00:00:00.

For further enquiries about this dataset please contact: DataLibrariansOAMNF@csiro.au

2 Itinerary

Hobart to Hobart, September 11th - October 8th, 2018.

Voyage Track:



3 Key personnel list

Name	Role	Organisation
Michael Ellwood	Chief Scientist	ANU
Max McGuire	Voyage Manager	CSIRO
Christine Rees	Hydrochemist	CSIRO
Stephen Tibben	Hydrochemist	CSIRO
Kendall Sherrin	Hydrochemist (AA100)	CSIRO

4 Summary

4.1 Sample Type and Number Assayed

Analysis (instrument)	Number of Samples	Processing Status at voyage end
Underway Nutrients (Seal AA100)	6890 UWY (data points)	Completed

4.2 Analysis and Data Processing Overview

The following flowchart provides an overview of how the AA100 was setup to analyse the underway seawater on the ship. Also outlined is the process of how the data is automatically matched with the ship underway data to provide latitude and longitude for the data points.

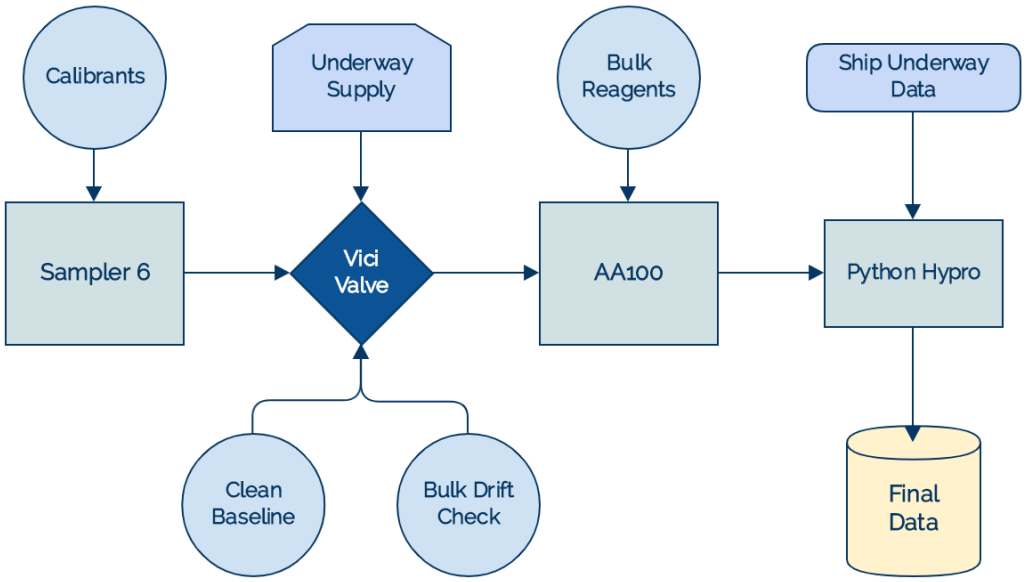


Figure 1: Underway Nutrient Analysis and Data Processing Flow Diagram.

5 Underway Nutrients Analysis & Data Processing

5.1 Nutrient Assay Parameter Summary

Details		
CSIRO Software	HyPro	
Instrument	Seal AA100	
Instrument Software	Seal AACE 7.10	
CSIRO Hydrochem. Method, sampling	WI_Nut_001	
CSIRO Hydrochem. Method, nutrient	AA100_SOP01	AA100_SOP02
Nutrient	Phosphate	Nitrate + Nitrite
Concentration range	0 - 3.0 µM	0 – 14 µM
Method Detection Limit (MDL)	0.02 µM	0.02 µM
Matrix Corrections	None	none
Analysts	Kendall Sherrin	
Lab Temperature (±1°C)	Variable, 18– 25°C	
Reference Material	KANSO, RMNS lot CD	
Sampling Container type	12ml PP tubes with screw cap lids.	
Sample Storage	≤ 12 hrs @ 4°C	
Pre-processing of Samples		
Comments		

5.2 Nutrient Methods

Nutrient samples are assayed on a Seal AA100 segmented flow auto-analyser fitted with 1.5cm debubbled flow-cells for colorimetric measurements.

Phosphate (AA100:SOP01): colourimetric, molybdenum blue method. Based on Murphy and Riley (1962) with modifications from the NIOZ-SGNOS 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 (AA100:SOP02): 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.

SOP methods can be obtained from the CSIRO Oceans and Atmosphere Hydrochemistry Group.

5.3 Nutrient Analysis Overview

Despite being the very first voyage the AA100 was implemented on, the data produced was of very high quality. The instrument chemistry method is the same as on the main hydrochemistry nutrient analyser, the AA3. This method results in high quality absorbance peaks that are very reproducible.

The detection limit for Phosphate appears to be rather similar to the AA3, however for Nitrate it is perhaps a little bit higher. With the concentrations seen on this voyage, the slightly higher detection limit isn't critical.

The highest calibration standard used for Nitrate analysis was 14µM, on the voyage there was surprisingly water masses that contained higher concentrations. Although these data points were above the highest standard, the data was salvageable as the calibration curve is still linear above this range. The only reason for capping the calibration at 14µM was to have higher resolution within this range.

Underway water was fed into the AA100 via a cup that was continually overflowing, allowing the AA100 to draw an unpressurised sample. The cup only held a volume of approximately 20mL, with the seawater flowrate between 3.5-4.0L/min. With such a small dead volume and high flowrate the sample could be as true as possible.

The residence time of the seawater through the underway piping would have contributed to a significant amount of lag in measurements. Testing and cleaning of the underway system while the ship was in port leads us to believe there is about a 2 minute delay between water entering the drop keel to when it reaches the underway seawater laboratory.

Time required for the chemistry and measurement to take place is approximately 6 minutes for Nitrate and 7 minutes for Phosphate. The time stamps provided in the dataset are produced by the AA100 software when a peak has been first measured, this means the time stamps were produced for the Nitrate channel. If a correction was to be made for the time required for analysis, 6 minutes would be subtracted from the time stamps in the dataset.

5.4 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	Phosphate	Nitrate + Nitrite (NOx)
Data Reported as	µmol l^{-1}	µmol l^{-1}
Calibration Curve degree	Linear	Linear
# of points in Calibration	6	6
Forced through zero?	N	N
Matrix correction	N	N
Blank correction	N	N

Result Details	Phosphate	Nitrate + Nitrite (NOx)
Peak window defined by	HyPro	HyPro
Carryover correction (HyPro)	Y	Y
Baseline drift correction (HyPro)	Y	Y
Sensitivity drift correction (HyPro)	Y	Y
Data Adj for RMNS variance.	N	N
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 20 L carboys in the clean dry laboratory at 22°C.	
Medium of Baseline	18.2 Ω water. Dispensed from Milli Q	
Comment	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in appendix 8.3.	

5.5 HyPro Data Processing Summary

After a run, the raw absorbance 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 nitrate+nitrite (as per WOCE).
- Within 0.02uM for phosphate

HyPro was used to automatically assign latitude and longitude values to the data points. The latitude and longitude coordinates were extracted from the ships underway file by matching the UTC time stamps.

There are no flags provided with the final dataset as it is assumed all points within the dataset are good. HyPro automatically removes points that are bad based on criteria specified above. The dataset has also had a manual pass over to check the quality of the data. There is the small chance that there is bad data in the dataset, however this should be very unlikely.

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

Japanese KANSO certified RMNS lot CD was assayed in triplicate in each run to monitor accuracy. The certified values are in table 1.

For in2018_v04, the majority of RMNS results are within 1% for Nitrate and 2% for Phosphate of their certified mean. Plots of RMNS values for all runs are below.

The assayed RMNS values per Analysis run are listed in appendix 6.1.

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.

Table 1: RMNS concentrations with expanded uncertainty (µmol L^{-1}) at 21°C

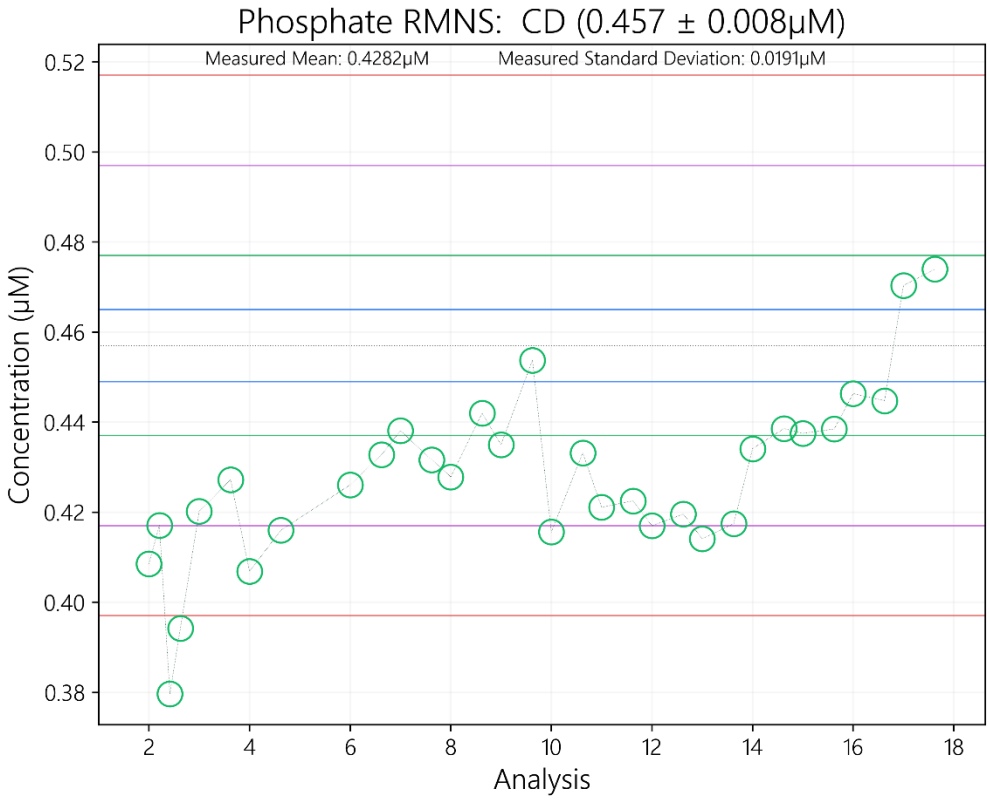
RMNS	NO_3	NO_2	NO_3+ NO_2 (NO_X)	PO_4	SiO_4
Lot CD	5.630 ± 0.051	0.018 ± 0.005	5.648 ± 0.056	0.457 ± 0.008	14.264 ± 0.10

KANSO publishes the RMNS nutrient values in µ mol kg^{-1}. These are converted to µ mol l^{-1} at 21°C. Lot CD is not certified for ammonium. NO_x is derived by adding the NO_3 and NO_2 values.

RMNS 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 µM increments from the certified value. The blue line is the expanded uncertainty of the certified value.

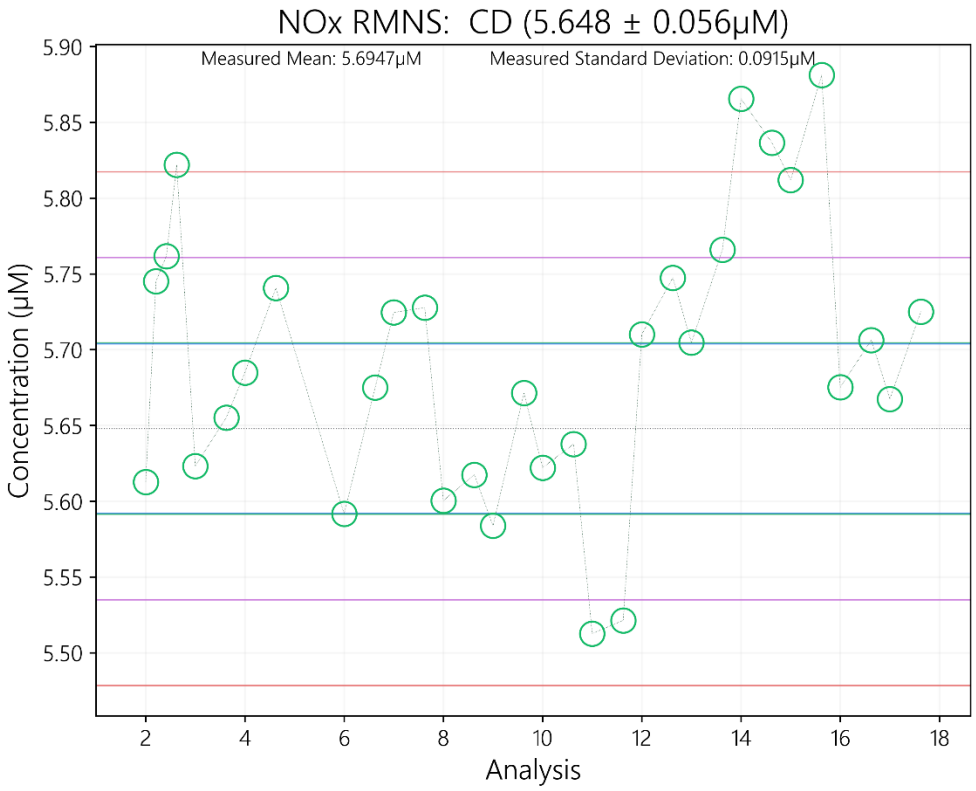
5.6.1 Phosphate RMNS Plot

Phosphate RMNS (19runs) for BW (1.578) | measured mean: 0.428µM



5.6.2 Nitrate + Nitrite (NOx) RMNS Plot

NOx RMNS (18 runs) for lot CD (5.648) | measured mean: 5.694µM



5.7 Analytical Precision

5.7.1 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 Measurement Uncertainty @ 1 µmol L^{-1}				
Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
±0.017	±0.024	±0.019	±0.14	±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.

5.7.2 Nutrient Method Detection Limit

For in2018_v04, the measured detection limits for each run are much lower than the nominal detection limits, indicating high analytical precision at lower concentrations.

MDL	Phosphate	Nitrate + Nitrite (NOx)
Nominal MDL*	0.02	0.02
Standard Dev. Min	0.006	0.006

Standard Dev. Max	0.024	0.08
Standard Dev. Mean	**0.019**	**0.06**
Standard Dev. Median	0.022	0.07
Precision of MDL (stdev)	0.023	0.07

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

5.7.3 Reference Material for Nutrients in Seawater

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

RMNS CD	Phosphate	Nitrate + Nitrite (NOx)
Published RMNS CD (µmol l^{-1}) w/std deviation	0.46 ± 0.001	5.65 ± 0.004
Minimum	0.38	5.51
Maximum	0.47	5.88
Mean	0.43	5.69
Median	0.43	5.68
Precision (Stdev)	0.01	0.08

5.8 Temperature & Humidity Change over Nutrient Analyses

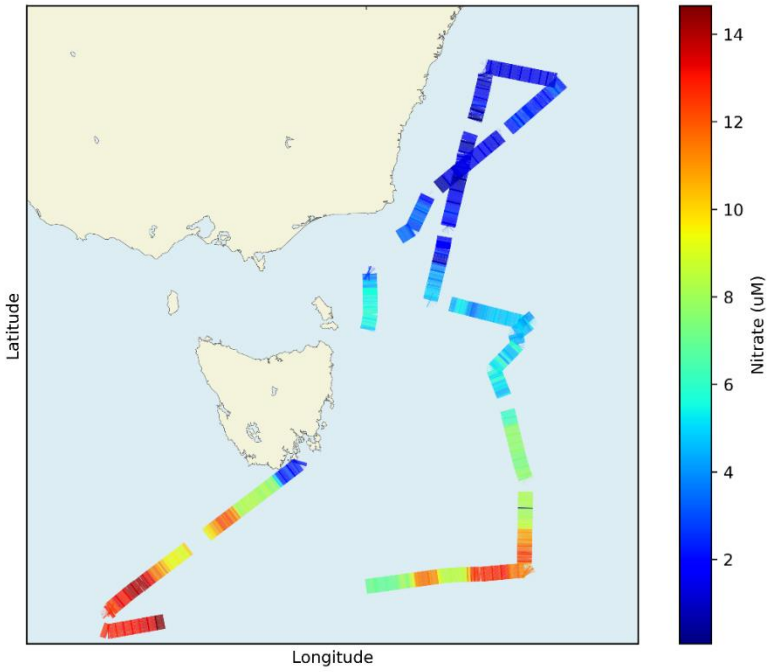
The temperature and humidity within the AA3 chemistry module was logged using a temperature/humidity logger, Lascar Electronics Easylog, placed on the deck of the chemistry module.

Refer to “in2018_v04_UWYNUTS_voyagereport.docx” for room temperature graphs.

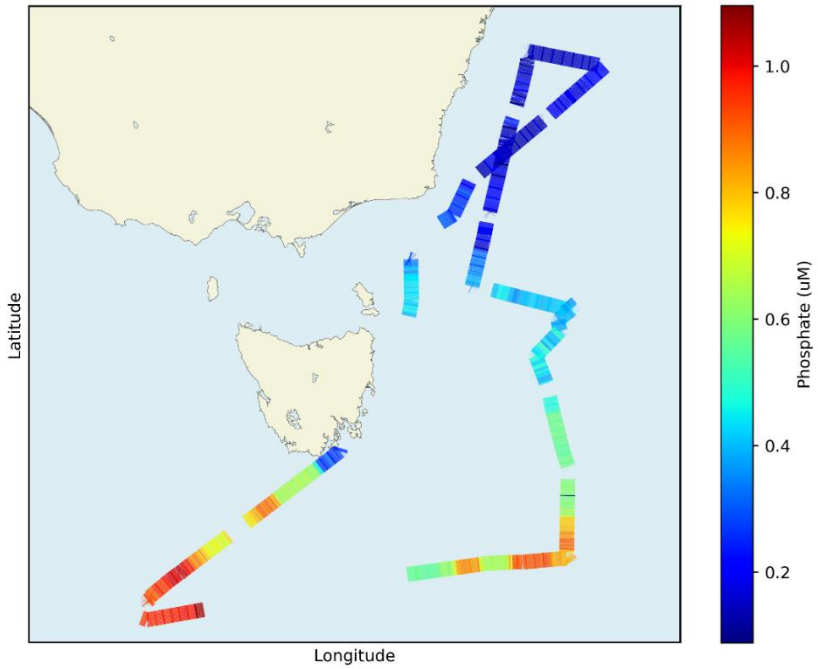
6 Appendix

6.1 Nutrient Data Plotted to Map

6.1.1 Nitrate



6.1.2 Phosphate



6.2 Nutrients: RMNS results for each Analysis Run & CTD Deployment.

6.2.1 RMNS Lot CD Results

Analysis Run	Phosphate	NO_x (NO2 + NO3)
CD reported	*0.46*	*5.65*
1	-	-
2	0.399	5.73
3	0.407	5.70
4	0.408	5.70
5	0.416	5.69
6	0.418	5.68
7	0.420	5.69
8	0.422	5.68
9	0.425	5.67
10	0.424	5.67
11	0.424	5.66
12	0.424	5.66
13	0.423	5.66
14	0.424	5.68
15	0.425	5.69
16	0.426	5.69
17	0.429	5.69

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

6.3 GO-SHIP Specifications

6.3.1 PO4

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

6.3.2 NO3

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

6.3.3 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.

7 References

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