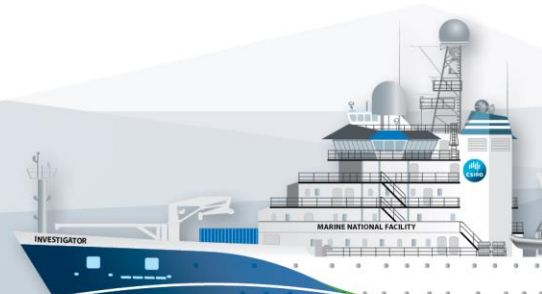


RV INVESTIGATOR

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	in2021_t01
Chief Scientist:	Viena Puigcorbé Lacueva
Voyage title:	Transit Brisbane to Darwin
Report compiled by:	Peter Hughes & Stephen Tibben



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1 Executive Summary

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Limnol. Oceanogr: Methods, 17(1): pp. 25-41.

doi:10.1002/lom3.10294

If publishing ammonium data, please cite the following:

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Frontiers in Marine Science, 8.

doi:10.3389/fmars.2021.581901

1.1 Objectives

Multiple projects undertaken focussing on micro-plastics, biological carbon pump flux, planktonic assemblage classification and cosmic ray measurements.

1.2 General Hydrochemistry Information

Water samples collected from CTD deployments, 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. The results for the reference material for nutrients in seawater (RMNS lot CJ), that was used, were within 2% of its certified values. The RMNS results for each CTD deployment are listed in table 8.3.

Missing and suspect hydrology samples are listed in Appendix 8

CTD Deployments 3, 5 & 7 were not sampled for hydrochemistry.

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

For Data, contact: NCMI_DataLibrarians@csiro.au

2 Itinerary

Brisbane to Darwin, 11th June 2021 – 23rd June 2021.

Figure 1: Voyage track



3 Key personnel list

Table 1: Key Personnel list

Name	Role	Organisation
Viena Puigcorb� Lacueva	Chief Scientist	Edith Cowan University
Rod Palmer	Voyage Manager	CSIRO
Claire Grubb	Deputy Voyage Manager	CSIRO MNF
Peter Hughes	Hydrochemist	CSIRO
Stephen Tibben	Hydrochemist	CSIRO

4 General Summary

4.1 Sample Type and Number Assayed

Table 2: Sample Type and Number Assayed

Analysis	Samples Assayed	Type
Salinity	75	CTD
	18	TSG
Dissolved Oxygen	75	CTD
Nutrients	75	CTD

4.1.1 CTD Samples (Conductivity, Temperature, Density)

- CTD samples were collected from the 12 L Ocean Test Equipment bottles on the CTD rosette that is deployed at depth for water collection.
- A total of 5 CTD deployments were sampled by hydrochemistry (Peter Hughes, Stephen Tibben) with help from the science party (Rachele Bernasconi, Hannah McCleary, Breeanna Muggleton, Kate Kiefer, George Cresswell).

4.1.2 TSG Samples (Thermosalinograph)

- TSG samples were collected from the underway instrument clean seawater line supplying the pCO₂ instrument in the underway laboratory.
- TSG results emailed to Vito Dirita (CSIRO) during the transit back to port.

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).

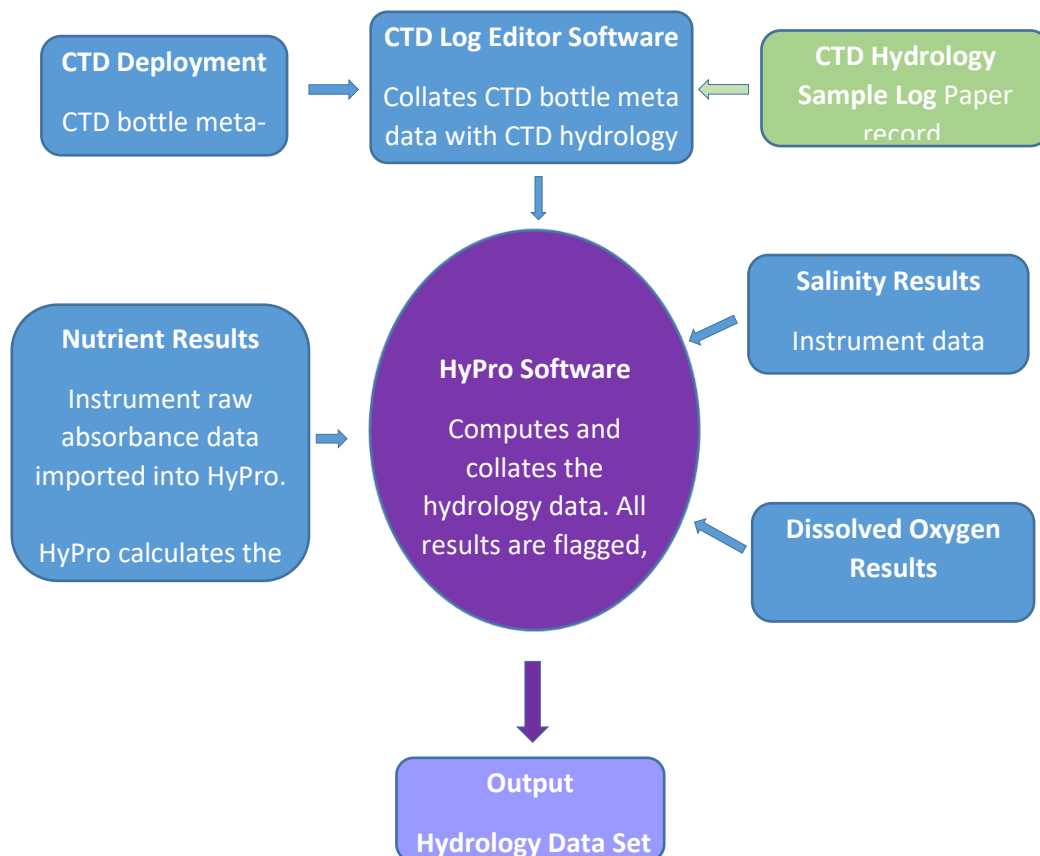


Figure 2: Hydrology Data Processing Flow Diagram.

5 Salinity Analysis

5.1 Salinity Measurement Parameters

Table 3: Salinity Measurement Parameters

Details	
HyPro Version	5.7
Instruments	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71611. Bath temperature 24.0°C
Software	Ocean Scientific International Ltd (OSIL) Data Logger ver 1.2
Hydrochemistry Methods.	Sampling: WI_Sal_002 Measurement: SOP006
Accuracy	± 0.001 practical salinity units
Reference Material	OSIL IAPSO - Batch P163, use by 10/04/2022, $K_{15} = 0.99985$
Sample Container	200 ml volume OSIL bottles made of type II glass (clear) with disposable plastic insert and plastic screw cap.
Sample Storage	Stored in salinometer lab > 8 hrs before measurement.
Lab Temperature	Mean 20.6°C SD 1.6 °C
Analysts	Stephen Tibben
Comments	Good agreement between bottle salinity and unprocessed CTD salinity results. Discrepancies >0.01 PSU occur at shallower depths across the greatest change in the thermohaline profile. 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 (one run consists of samples from up to two CTD deployments).

Method: The salinity sample is collected in a 200ml OSIL bottle. The bottle is rinsed then filled from the bottom, via a polytetrafluoroethylene (PTFE) straw, till overflowing. The bottle is removed from the straw and the sample is decanted to allow a headspace of approximately 25cm³. A dry plastic insert is fitted, the bottle inverted and rinsed with water then capped and stored cap-down until measured. To measure, the Autosal cell is flushed three times with the sample and then measured after the fourth and fifth flush. The OSIL data logger software captures the conductivity ratio and calculates the practical salinity.

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

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.

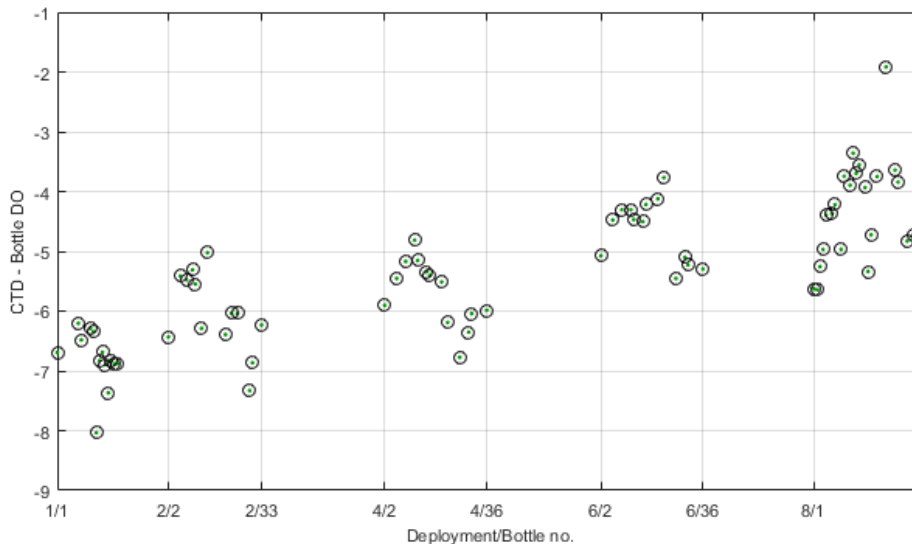
The thiosulphate solution is standardised with a 10ml aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two titres of consecutive additions of 1 mL aliquots of potassium iodate to the same blank sample. The standardisation is done at least once per 12-hour shift, when samples are being assayed.

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

6.3 CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

For this voyage, the difference between the unprocessed CTD value and the measured bottle value is generally less than $9 \mu\text{mol L}^{-1}$.

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: $\mu\text{mol L}^{-1}$



7 Nutrients Analysis

7.1 Nutrient Measurement Parameters

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

Details					
Processing Software	CSIRO HyPro 5.7				
Instrument	Seal AA3HR segmented flow analyser.				
Operating Software	AACE 7.10				
Hydrochemistry. Methods	Sampling: WI_DO_001				
	Assay:				
	SOP001	SOP002	SOP003	SOP004	SOP005
	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonia
Top concentration ($\mu\text{mol L}^{-1}$)	120	3.0	42	1.4	2.0
Method detection limit ($\mu\text{mol L}^{-1}$)	0.2	0.02	0.02	0.02	0.02
Medium of Standards	Low nutrient seawater (LNSW, bulk on deck of Investigator) collected on in2019_v05. Sub-lot passed through a 10-micron filter and stored in 20 L carboys in the clean dry laboratory at 21°C.				
Medium of Baseline	18.2 Ω water. Dispensed from the Milli Q Integral 10 unit.				
Reference Material	KANSO RMNS lot CJ				
Duplicate samples.	CTD: Niskin fired at the greatest depth sampled in duplicate. Single samples collected for remaining depths.				
Sample Container	50 mL HDPE with screw cap lids. Reused after acid wash with 1M HCl				
Sample Storage	< 4 hrs at room temperature or < 12 hrs @ 4°C				
Sample preparation	Assayed as neat. No filtration.				
Lab Temperature (°C)	Mean 20.1°C SD 0.6 °C				
Analysts	Peter Hughes, Stephen Tibben				
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in appendix 8.3 Nitrite wavelength changed from 520 nm to 540 nm for AA3 runs 5 and 6 (CTD deployments 7 & 8). 540 nm is more sensitive.				

7.2 Nutrient Methods

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Frontiers in Marine Science, 8.

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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 K rouel and Aminot (1997). Ammonium reacted with ortho-phthaldialdehyde and sulphite at a pH of 9.0-9.5 to produce an intensely fluorescent product. Its emission is measured at 460nm after excitation at 370nm.

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

¹ Royal Netherlands Institute for Sea Research – Study Group on Nutrient Standards.

7.3 HyPro Processing Summary for Nutrients

After a run, the raw absorbance/ fluorescence data is exported from the instrument and processed by HyPro. For each analyte, HyPro re-creates the peak traces, defines the region on the peak's plateau

(peak window) used to determine the peak heights, constructs the calibration curve, applies corrections for carry-over, baseline and sensitive drifts then, derives the nutrient concentrations for each sample. The corrections are quantified using dedicated solutions included in every run.

HyPro uses criteria to identify suspect calibration points, noisy peaks, method detection limits that are above the nominal limit and, duplicate sample results that do not match.

Suspect calibration points are weighted less when fitting the calibration curve. The cut-off limits for good calibration data are:

- $\pm 0.5\%$ of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE¹).
- $0.02\mu\text{mol}^{-1}$ for phosphate, nitrite and ammonium.

HyPro classifies the quality of data as good, suspect or bad and flags accordingly. The Flag key is in Appendix 8.7. Missing or suspect nutrient data is tabulated in section 8.6,

¹ World Ocean Circulation Experiment

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

Japanese KANSO certified RMNS lot CJ was assayed in triplicate in each run to monitor accuracy. The certified values are in appendix 8.2.

The RMNS lot CJ results are within 1.5% for NO_x and silicate, within 2% for phosphate and within 0.06 $\mu\text{mol L}^{-1}$ for nitrite of their certified mean concentrations.

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

7.5 Temperature & Humidity Change over Nutrient Analyses

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

- (1) Above the AA3HR instrument, temperature only. Mean 20.1°C SD 0.6°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

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

8.2 Nutrients: Reference Material

KANSO RMNS	Silicate (Si(OH) ₄)	Phosphate (PO ₄)	Nitrite (NO ₂)	Nitrate (NO ₃)	NO ₃ + NO ₂ (NO _x)
Lot CJ	39.42 ± 0.41	1.219 ± 0.020	0.032 ± 0.007	16.588 ± 0.205	16.62 ± 0.21

8.3 Nutrients: RMNS lot CJ results for each CTD Deployment.

CTD Deployment	Silicate (Si(OH) ₄) (μmol L ⁻¹)	Phosphate (PO ₄) (μmol L ⁻¹)	NO _x (NO ₂ + NO ₃) (μmol L ⁻¹)	Nitrite (NO ₂) (μmol L ⁻¹)
1	39.7	1.24	16.80	0.056
2	39.8	1.24	16.85	0.072
4	39.4	1.24	16.71	0.070
6	39.4	1.24	16.72	0.053
8	39.4	1.22	16.68	0.050

The reported 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
8	29	0	Maximum salinity difference of all deployments (0.073 PSU). Unprocessed CTD 34.863 vs bottle salinity 34.936. Coincides with the largest change in the salinity temperature profile of the water column.

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

NO missing or suspect dissolved oxygen data.

8.6 Missing or Suspect Nutrient Data.

Not included, Data flagged 63 (below detection limit). Flag key: appendix 8.7

CTD	RP	Flag	Reason for Flag
2	6	69	Slight outlier on depth profile plot. Cause unknown. Flagged 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.

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