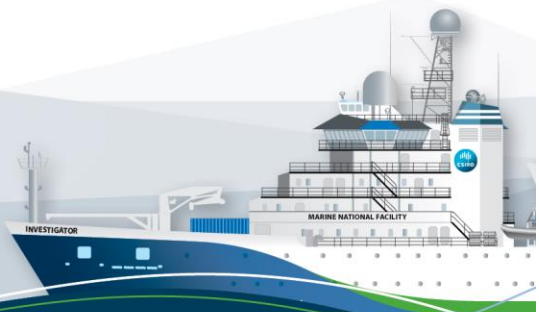
RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage:	in2022_v07
Chief Scientist:	Jody Webster
Voyage title:	Halimeda bioherm origins, function and fate in the northern Great Barrier Reef.
Report compiled by:	Peter Hughes



Contents

1 Executive Summary

Water samples collected from CTD deployments/ box corer and multi corer deployments/ nutrient incubation experiments and underway seawater, were assayed in the ship's hydrochemistry laboratory

Data Quality (accuracy and precision) of the Nutrient, Salinity and Dissolved Oxygen sample results are GOOD. Missing and suspect data tabulated in Appendix 8.

Nutrients: Reference material RMNS lot CP results per sample data tabulated in Appendix 8

Salinity: Slightly larger offset than average between CTD salinity (raw) and bottle salinity results for CTD deployments 17, 18, 19. Offset ranges from 0.006 to 0.019 PSU.

Final hydrology data, analytical methods, related log sheets and processing notes can be obtained from the CSIRO data centre. Please contact NCMI_DataLibrarians@csiro.au

Please cite the following manuscripts when reporting or publishing nutrient data:

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

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

Objectives

(1) define the spatial distribution and morphological variation of the *Halimeda* bioherms;

(2) explore the relationship of the bioherms to the deeper undersea landscape (channels, passages, slope, submarine canyons and basin) and key oceanographic processes;

(3) develop new 3D models explaining their origin and development, generate Holocene paleo-climate data, including novel archives of upwelling, paleo-flooding and water quality;

(4) quantify their total volume/area as a regional geological carbon sink within the context of the global carbon budget; and

(5) assess the importance of the bioherms as modern, inter-reef benthic habitats

2 Itinerary

Brisbane to Cairns, 14th August 2021 – 7th September 2021.

Figure 1: Voyage track

3 Key personnel list

Table 1: Key Personnel list

Name	Role	Organisation
Jody Webster	Chief Scientist	Sydney University
David Flynn	Voyage Manager	CSIRO
Peter Hughes	Hydrochemist	CSIRO

4 General Summary

Sample Type and Number Assayed

Table 2: **Sample Type and Number Assayed**

Analysis	Samples Assayed	Type
Salinity	156	CTD
	18	TSG
	26	EXP
Dissolved Oxygen	155	CTD
	26	EXP
Nutrients	175	CTD
	401	EXP

CTD Samples (Conductivity, Temperature, Density)

- CTD seawater samples were collected from the 12 L Ocean Test Equipment bottles on the CTD rosette that is deployed at depth.
- A total of 21 CTD deployments were sampled by the science party ().

TSG Samples (Thermosalinograph)

- TSG seawater 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 15/09/2023)

EXP Samples

- EXP samples were collected from three sources. (1) 5L Niskin on the box and multi-corer, (2) underway clean instrument seawater supply, (3) incubation tanks.
- EXP results given to Helen Bostock (UQU) at end of voyage.

Data Processing Overview

The sample meta-data, measured bottle salinities, dissolved oxygen assays, and 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).

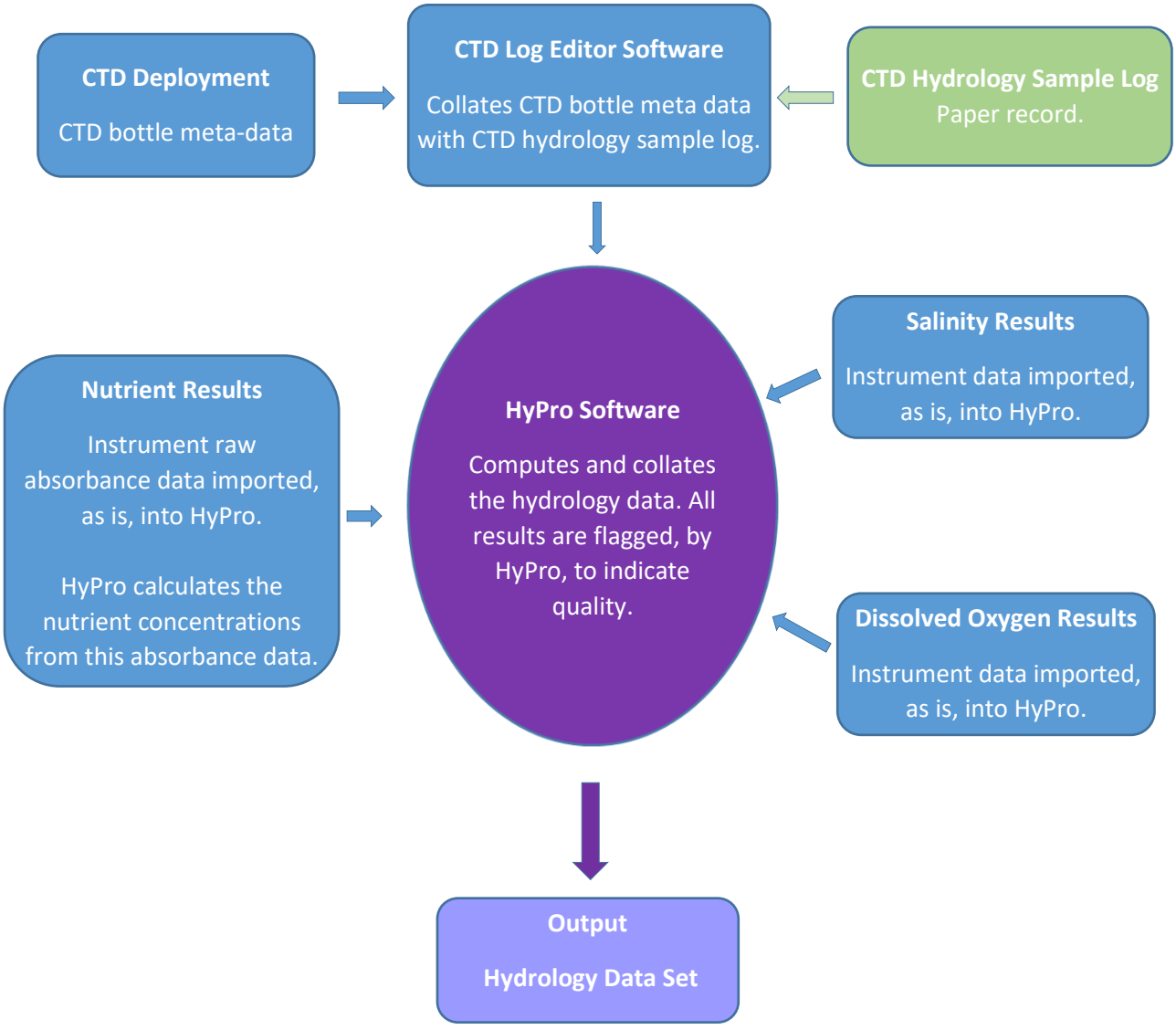


Figure 2: Hydrology Data Processing Flow Diagram.

5 Salinity Analysis

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 P164, use by 23/04/2023, 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.
Analysts	Peter Hughes
Comments	Good agreement between bottle salinity and unprocessed CTD salinity results for deployments 1 to 16, 20, 21. Some discrepancies occur for CTD deployments 17 to 19, up to 0.019 PSU difference. Cause unknown. See DAP report for CTD calibration details.

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).

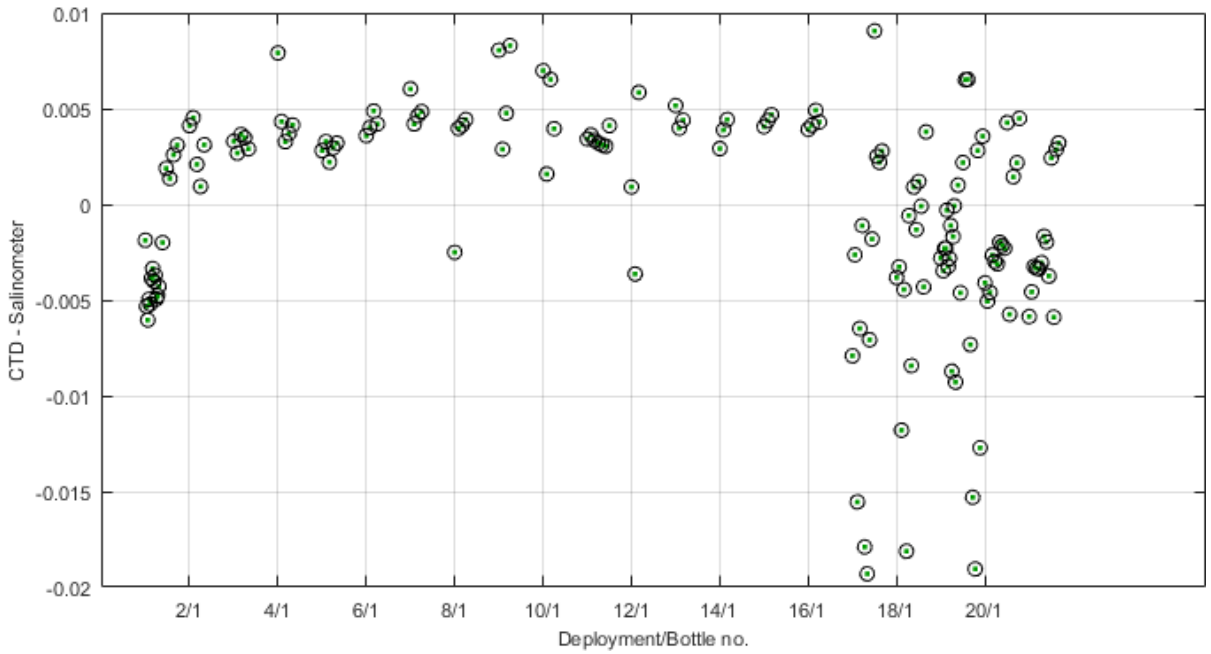
Method: The salinity sample is collected in a 200ml OSIL bottle. The bottle is rinsed then filled from the bottom, via a polytetrafluoroethylene (PTFE) straw, till overflowing. The bottle is removed from the straw and the sample is decanted to allow a headspace of approximately 25cm^3. A dry plastic 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.

CTD Salinity vs Bottle Salinity Plot

For this voyage, the difference between the unprocessed (raw) CTD value and the measured bottle value were less than 0.006 PSU for shallow deployments 2 to 16 (< 100m depth).

Figure 3: Salinity CTD - Bottle vs CTD deployment plot. CTD salinity is raw. Units: PSU (dimensionless).



6 Dissolved Oxygen Analysis

Dissolved Oxygen Measurement Parameters

Table 4: 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^{-1}
Analysts	Peter Hughes
Sample Container type	140 mL glass iodine determination flasks with glass stopper.
Sample Storage	Samples stored in the hydrochemistry lab until analysis.
Comments	Good agreement between bottle and CTD results with the difference <12 µmol L^{-1}. See DAP report for CTD calibration details.

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.

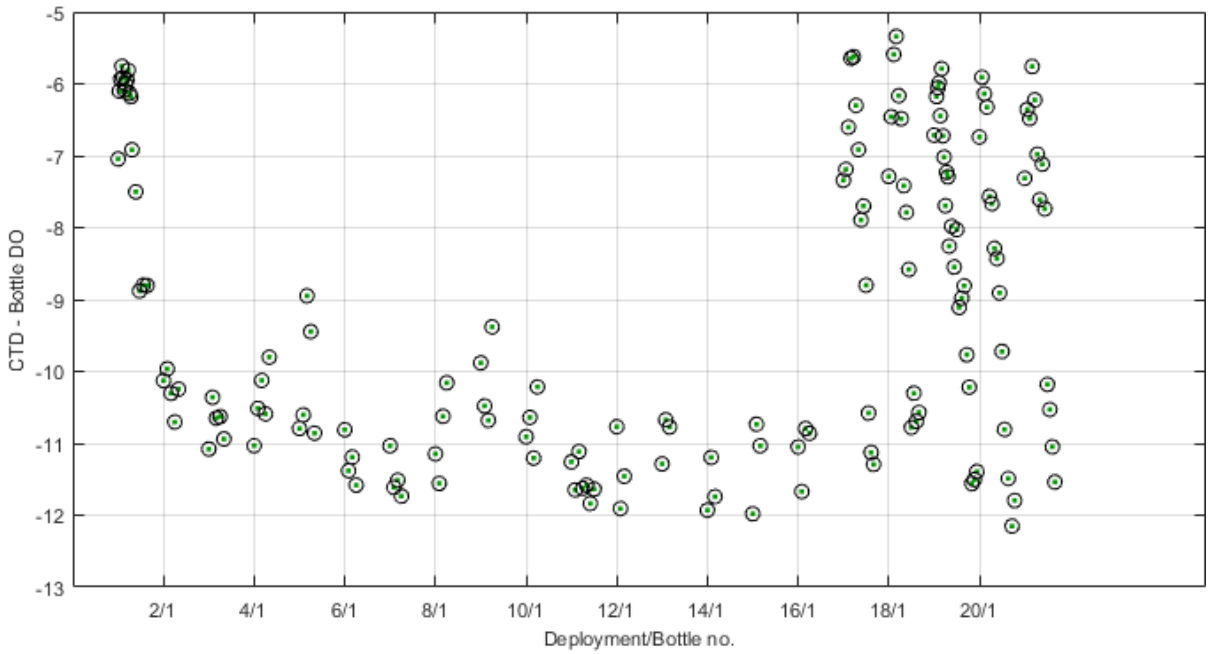
The thiosulphate solution is standardised with a 10ml aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two titres of consecutive additions of 1 mL aliquots of potassium iodate to the same blank sample. The standardisation is done at least 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.

CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

For this voyage, the difference between the unprocessed (raw) CTD value and the measured bottle value is less than 12 µmol L^{-1}. Deployments 2 to 16 are shallow, < 100m depth.

Figure 4. Dissolved Oxygen, CTD - Bottle vs Deployment Plot. Units: µmol L^{-1}



7 Nutrients Analysis

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				
	Analysis				
	SOP001	SOP002	SOP003	SOP003	SOP004
	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonia
Top concentration (µmol L^{-1})	84 112 (CTD 1,17-21)	3.0	36.5	1.4	2.0
Method detection limit (µmol 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 June 2021. Filtered (10 micron) July 2022. Stored in 20 L carboys in the clean dry laboratory at 21°C.				
Medium of Baseline	18.2 Ω water. Dispensed from a Milli Q IQ7015.				
Reference Material	KANSO RMNS lot CP				
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	CTD Assayed as neat. No filtration EXP. Assayed as received. Some dilutions required for incubation samples.				
Analysts	Peter Hughes				
Comments	The reported data is not corrected to the RMNS. Results per RMNS data and missing and suspect data tabulated in Appendix 8				

Nutrient Methods

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

Nutrient samples are assayed on a Seal AA3HR segmented flow auto-analyser fitted with 1cm flow-cells for colorimetric measurements and a JASCO FP2020 instrument for fluorescent measurement (ammonium).

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-SGNOS[1] 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 540 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.

HyPro Processing Summary for Nutrients

Hypro uses the raw absorbance/ fluorescence data to measure the peak heights, construct the calibration curves, apply corrections for carry-over, baseline and sensitivity drifts and derive the sample nutrient concentrations.

HyPro classifies the data as good, suspect or bad and flags accordingly. The Flag key: See Appendix 8.

Missing or suspect nutrient data is tabulated in Appendix 8.

Accuracy - Reference Material for Nutrient in Seawater (RMNS)

The accuracy for this voyage is GOOD

Japanese KANSO certified RMNS lot CP was assayed in triplicate in each run to monitor accuracy.

The RMNS lot CP results are within 1.2% for NOx, silicate, phosphate and within 0.07 µmol L^{-1} for nitrite of the certified mean concentrations.

The measured RMNS values and associated nutrient samples are tabulated in Appendix 8.

Table 6: RMNS certified concentrations ± expanded uncertainty (U) at 21°C. Units: µmol L-1

RMNS	Silicate (Si(OH)4)	Phosphate (PO4)	Nitrite (NO2)	NO3+ NO2 (NOX)
Lot CP	62.57 ± 0.31	1.795 ± 0.018	0.32 ± 0.07	25.71 ± 0.38

Table 7: RMNS CP statistics for of this voyage. Units: µmol L-1

RMNS Lot CP	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	61.7	1.78	0.312	25.50
Maximum	63.4	1.81	0.327	25.99
Mean	62.75	1.80	0.320	25.74
Median	62.3	1.79	0.320	25.74
Repeatability	0.14	0.003	0.002	0.002

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 at the same depth, then sampled for hydrochemistry (Results: Tables 9, 10). Duplicate samples are also collected from the greatest depth of subsequent CTD deployments (Results: Table 8).

For nutrients, the sampling precision is good if the difference from the mean of duplicate measurements is less than the nominal method detection limit. The exception: NOx (nitrate+nitrite) which uses the limit 0.06 µmol L^{-1}. Duplicate samples that exceed this limit are flagged 69 (suspect).

Table 8: Difference between duplicate samples. CTD 8 to CTD 21 (n= 26) Units: µmol L-1

	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)	Ammonia (NH_4)
Minimum	0.0	0.00	0.000	0.00	0.00
Maximum	0.1	0.01	0.005	0.07	0.02
Mean	0.0	0.00	0.002	0.01	0.00
SD	0.0	0.00	0.001	0.02	0.00

Table 9: CTD deployment 1. 10 bottles at 1000 dbar. Units: µmol L-1

	Salinity (PSU)	Dissolved Oxygen	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	34.447	185.6	45.3	2.15	0.037	31.28
Maximum	34.450	185.8	45.6	2.16	0.048	31.40
Mean	34.448	185.73	45.4	2.15	0.043	31.35
SD	0.0007	0.09	0.08	0.003	0.004	0.03

Table 10: CTD deployment 1. 10 bottles at 10 dbar. Units: µmol L-1

	Silicate ($Si(OH)_4$)	Phosphate (PO_4)	Nitrite (NO_2)	NO_3+ NO_2 (NO_X)
Minimum	0.7	0.10	0.038	0.15
Maximum	0.8	0.11	0.047	0.16
Mean	0.8	0.105	0.043	0.16
SD	0.04	0.005	0.004	0.003

Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry laboratory were measured and logged. Data on request.

(1) Above the AA3HR instrument, temperature only. Range: 19.5 to 21.5°C

(2) On the outboard laboratory bulkhead, temperature, humidity, pressure.

8 Appendix

8.1 Salinity: Reference Material

OSIL IAPSO Standard Seawater	
Batch:	P164
Use by date:	23/04/2023
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 CP	62.6 ± 0.3	1.795 ± 0.018	0.32 ± 0.07	25.4 ± 0.3	25.72 ± 0.37

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

CTD Deployment	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)
1	62.6	1.79	25.8	0.320
2, 3, 4, 5	62.8	1.79	25.6	0.328
6, 7	62.2	1.80	25.8	0.344
8, 9	62.9	1.80	25.9	0.333
10, 11, 12	62.6	1.81	25.8	0.330
13	63.0	1.80	25.8	0.342
14, 15, 16	62.8	1.80	25.8	0.339
17	62.4	1.78	25.7	0.341
18	63.1	1.78	25.7	0.332
19	62.7	1.79	25.8	0.340
20	62.4	1.79	25.5	0.328
21	62.3	1.79	25.7	0.322

8.4 Nutrients: RMNS lot CP results for each EXP sample.

uwy	Corers 5L Niskin	Incubation Experiment	Silicate ($Si(OH)_4$) ($\mu mol\ L^{-1}$)	Phosphate (PO_4) ($\mu mol\ L^{-1}$)	NOx ($NO_2 + NO_3$) ($\mu mol\ L^{-1}$)	Nitrite (NO_2) ($\mu mol\ L^{-1}$)
1-16			63.0	1.80	25.8	0.322
17-29			63.1	1.80	25.8	0.323
30-48			62.9	1.80	25.5	0.325
		1 & 2 (t = 0)	62.8	1.79	25.6	0.328
49-56	1-10		62.5	1.80	25.6	0.342
		1 & 2	62.6	1.80	25.7	0.332
		3 & 4	62.7	1.80	25.7	0.344
57-68	11-13		62.6	1.79	25.8	0.344
	14-16	5 & 6 (t = 0)	62.9	1.79	25.9	0.333
		5 & 6	62.6	1.81	26.0	0.341
69-73	17		62.6	1.81	25.8	0.330
		7 & 8	63.1	1.80	25.8	0.311
	18-22		63.0	1.80	25.8	0.342
74-89			63.1	1.80	25.7	0.322
	23-24		62.8	1.80	25.8	0.339
		9 &10	62.5	1.80	25.7	0.318
	25-26		62.4	1.78	25.7	0.341

The reported nutrient results DO NOT have RMNS corrections applied.

8.5 Missing or Suspect Salinity Data

CTD	RP	Flag	Reason for Flag
1	3	0	Bottle salinity result offset from CTD (raw) -0.006 PSU. Cause unknown. Not an outlier on depth profle.
17	1,5,7,11,15,19	0	Bottle salinity result offset from CTD (raw) ranging from 0.006 to 0.019 PSU. Cause unknown.
18	5,9,13	0	Bottle salinity result offset from CTD (raw) ranging from 0.008 to 0.018 PSU. Cause unknown.
19	10,13,21,23,25,27,33	0	Bottle salinity result offset from CTD (raw) ranging from 0.006 to 0.019 PSU. Cause unknown.

8.6 Missing or Suspect Dissolved Oxygen Data

NO missing or suspect dissolved oxygen data.

8.7 Missing or Suspect Nutrient Data.

Data flagged 63 (below detection limit) not included.

CTD	RP	Analyte	Flag	Reason for Flag
1	29	NH4	69	Outlier on depth profile. Cause: contamination during sampling. Flagged suspect by operator.
19	1	NOx	69	Duplicate sample results do not match. Results 36.39, 36.46 µmol L^{-1} Flagged suspect by HyPro

8.8 Data Quality Flag Key

Flag	Classification	Description
0	Data is GOOD	
63	Data below detection.	Nutrients only. Data below nominal detection limit.
65	Data is SUSPECT.	Nutrients only: absorbance peak shape 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).
79	Data is SUSPECT.	Nutrients only. Measured Method Detection Limit (MDL) 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.	Flagged by operator.
141	NO Data.	Netcdf data file only.

9 References

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