

# ***RV INVESTIGATOR***

## **HYDROCHEMISTRY DATA PROCESSING REPORT**

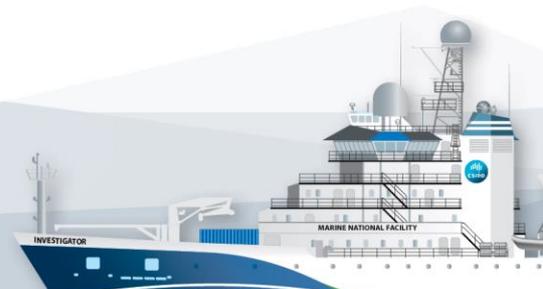
**Voyage:** in2019\_v05

**Chief Scientist** Bernadette Sloyan

**Principal Investigator** Iain Suthers

**Voyage title:** Integrated Marine Observing System: monitoring of East Australian Current property transports at 27°S.  
Dynamics of larval fish diversity for ocean observing off North Stradbroke Island.

**Report compiled by:** Christine Rees, Merinda McMahon and Kendall Sherrin



## Contents

1	Executive Summary.....	3
2	Itinerary and Personnel .....	4
3	Sample Summary .....	5
3.1	CTD (Conductivity, Temperature, Density) .....	5
3.2	TSG (Thermosalinograph) .....	5
4	Data Processing Overview .....	6
5	Salinity Data Processing .....	7
5.1	Salinity Method.....	7
5.2	CTD Salinity vs Bottle Salinity Plot .....	9
5.4	OSIL Salinity Standard PSU across the Voyage.....	10
5.5	Salinity Measurement Uncertainty .....	10
6	Dissolved Oxygen Data Processing .....	11
6.1	Dissolved Oxygen Method .....	11
6.2	CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot .....	12
6.3	Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.....	13
6.3.1	Dissolved Measurement Uncertainty .....	13
7	Nutrient Data Processing .....	14
7.1	Nutrient Methods .....	14
7.2	HyPro Data Processing Summary for Nutrients.....	16
7.3	Accuracy and Precision - Reference Material for Nutrient in Seawater (RMNS).....	17
7.4	Nutrient plots of RMNS.....	19
7.4.1	Silicate RMNS Plot ( $\mu\text{M}$ ) .....	19
7.4.2	Phosphate RMNS Plot ( $\mu\text{M}$ ) .....	20
7.4.3	Nitrate + Nitrite ( $\text{NO}_x$ ) RMNS Plot ( $\mu\text{M}$ ) .....	20
7.4.4	Nitrite RMNS Plot ( $\mu\text{M}$ ) .....	21
7.5	Measurement Uncertainty.....	21
7.6	Method Detection Limit for Nutrients .....	22
7.7	Sampling Precision .....	22
7.8	Redfield Ratio.....	23
7.9	Temperature & Humidity Change over Nutrient Analyses .....	23
8	GO-SHIP Specifications .....	24
9	Appendix .....	25
10	References .....	30

## 1 Executive Summary

Please cite the following manuscript when reporting or publishing data for silicate, phosphate, nitrate+nitrite (NO<sub>x</sub>) 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**

The main objective of the voyage was to recover and re-deploy an array of six full-depth current meter and property (temperature, salinity and pressure) moorings from the continental slope to the abyssal waters off Brisbane (27°S). The East Australian Current (EAC) mooring array is a component of Integrated Marine Observing System (IMOS) and is used to capture the mean and time varying flow of the EAC. The supplementary project object was to investigate the dynamics of larval fish diversity for ocean observing off North Stradbroke Island. Both projects required Conductivity, Temperature, Depth (CTD) measurements to characterise the sea water at each location.

Hydrochemistry oversaw the collection of salinity, oxygen and nutrient samples (silicate, phosphate, nitrate + nitrite (NO<sub>x</sub>), nitrite, and ammonium) from the CTD. All samples were analysed in the ship's hydrochemistry laboratory.

The AA100 segmented flow analyser was connected to the underway water supply to map the concentrations of nitrate and phosphate in the surface waters. A separate report for the AA100 data is available.

High quality data were produced for the three measured parameters. Certified reference materials for nutrients in seawater were within the specified limits of the certified value for silicate, nitrate+nitrite (NO<sub>x</sub>), and nitrite. However, phosphate was slightly lower than usual, a correction has been applied to the phosphate data using the certified reference material.

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

Contact: [DataLibrariansOAMNF@csiro.au](mailto:DataLibrariansOAMNF@csiro.au)

## 2 Itinerary and Personnel

Brisbane to Brisbane, September 9<sup>th</sup> – 29<sup>th</sup>, 2019.

Voyage Track:



**Table 1:** Key personnel list

Name	Role	Organisation
Bernadette Sloyan	Chief Scientist	CSIRO
Iain Suthers	Principal Investigator	UNSW
Linda Gaskell	Voyage Manager	CSIRO
Christine Rees	Hydrochemist	CSIRO
Merinda McMahon	Hydrochemist	CSIRO
Kendall Sherrin	Hydrochemist	CSIRO

### 3 Sample Summary

*Table 2: Sample summary and number assayed.*

Analysis (instrument)	Number of Samples
Salinity (Guildline Salinometer)	388 CTD 21 TSG
Dissolved Oxygen (SIO automated titration)	385 CTD
Nutrients (Seal AA3HR segmented flow)	385 CTD 7 UWY

#### 3.1 CTD (Conductivity, Temperature, Density)

- Sampling point, 24 bottle rosette with 12 L Ocean Test Equipment bottles (Niskin) deployed at depth for water collection.
- 27 CTD deployments in total. Deployments were sampled by Bo Lui, Clare Cao, Amelia Caley, Jonathan Lawley, Aaron Puckeridge, Madelaine Langley, Violaine Pellichero, Oceane Richet, Amanda Black and Chris Chapman.

#### 3.2 TSG (Thermosalinograph)

- Samples collected by data acquisition and processing (DAP) staff or hydrochemistry staff from underway lab for calibration of thermosalinograph.

For underway (UWY) and TSG sample information refer to the eLogs from the voyage.

## 4 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 in figure 1.

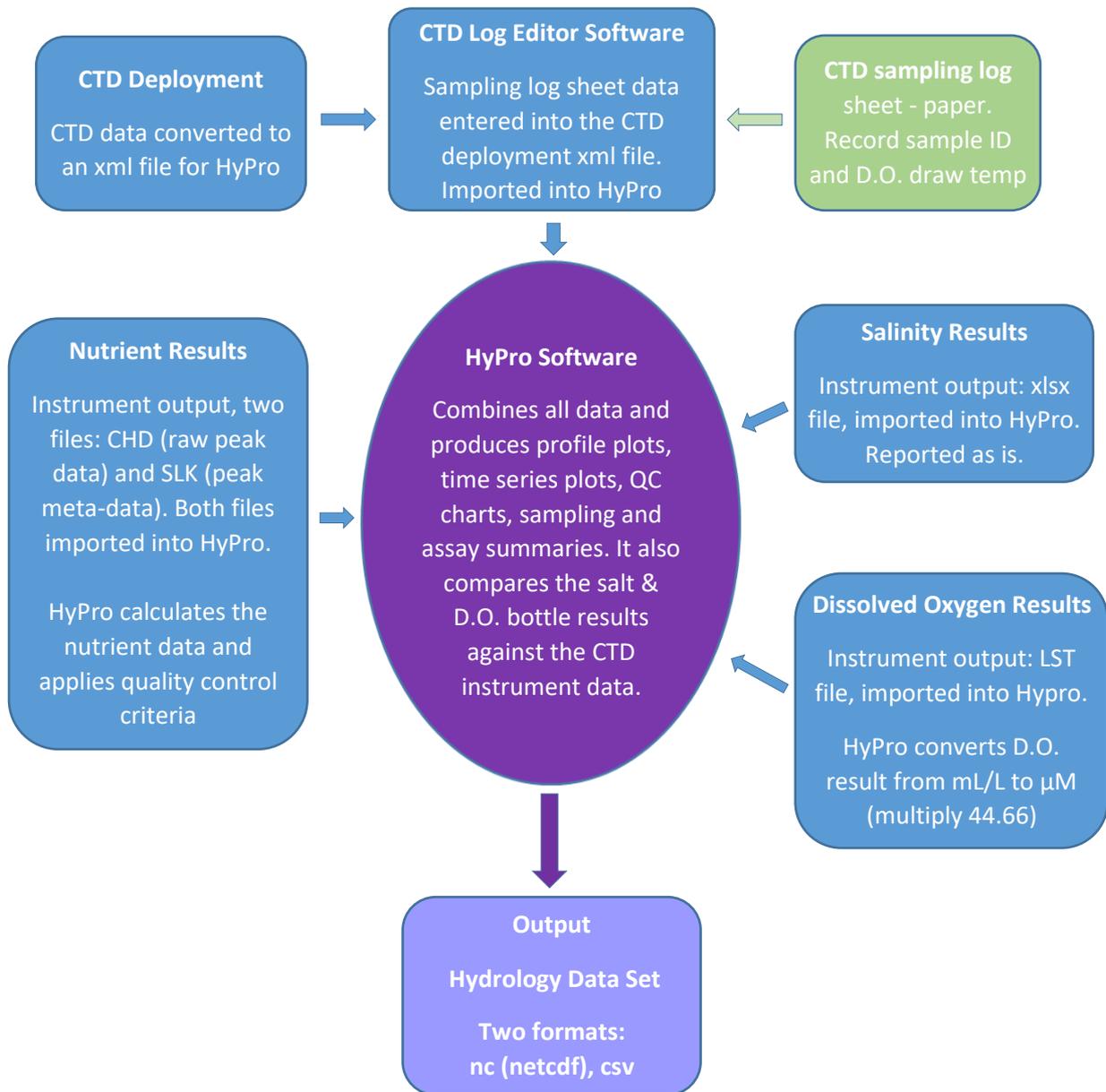


Figure 1: Hydrology Data Processing Flow Diagram.

## 5 Salinity Data Processing

### 5.1 Salinity Method

Salinity samples are measured on a high precision laboratory salinometer (Guildline Autosol 8400B) which is operated in accordance with its technical manual.

Practical salinity ( $S$ ) is defined in terms of the ratio ( $K_{15}$ ) of the electrical conductivity measured at 15°C 1 atm of seawater to that of a potassium chloride (KCl) solution of mass fraction  $32.4356 \times 10^{-3}$ .

Before each batch of sample measurements, the Autosol is calibrated with the International Association for the Physical Sciences of the Oceans (IAPSO) standard seawater of known  $K_{15}$  ratio. A new bottle of standard solution is used for each calibration. The frequency of calibration is one per set of samples per CTD deployment or if not all Niskin bottles are sampled when there is enough samples equivalent to 24 or 36. During the voyage the analysis run consisted of either one CTD deployment or 2 CTD deployment worth of samples.

Method synopsis: Salinity samples are collected into 200 ml Ocean Scientific International Ltd (OSIL) bottles, filled from the bottom, via a polytetrafluoroethylene (PTFE) straw connected to the Niskin spigot with silicon tubing. The bottles are rinsed 3 times before filling and overflowing for 3 seconds. The straw is removed from the bottle and the sample is decanted to allow a headspace of approximately 25 cm<sup>3</sup>. A plastic insert is fitted, the bottle inverted and rinsed with water and dried then capped and stored cap-down until measured. To measure, the Autosol cell is flushed three times with the sample and then measured after the fourth and fifth fill of the cell. The OSIL data logger software captures the conductivity ratio and calculates the practical salinity.

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

Table 3: Salinity parameters summary.

Details	
HyPro Version	5.7
Instrument	Guildline Autosal Laboratory Salinometer 8400(B) – SN 72088
Software	Ocean Scientific International Ltd (OSIL) Data Logger ver 1.2
CSIRO Hydrochem Method.	Sampling: WI_Sal_002 Measurement: SOP006
Accuracy	± 0.001 practical salinity units (PSU)
Analysts	Merinda McMahon and Christine Rees
Lab Temperature	20.7-24.5°C during analysis.
Bath Temperature	24.02°C
Reference Material	OSIL IAPSO <sup>1</sup> - Batch P162, use by 16/04/2021, K <sub>15</sub> = 0.99983
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 stored in the Salt lab for a minimum of 8 hrs before measurement.
Comments	TSG samples were analysed on SN 72089.

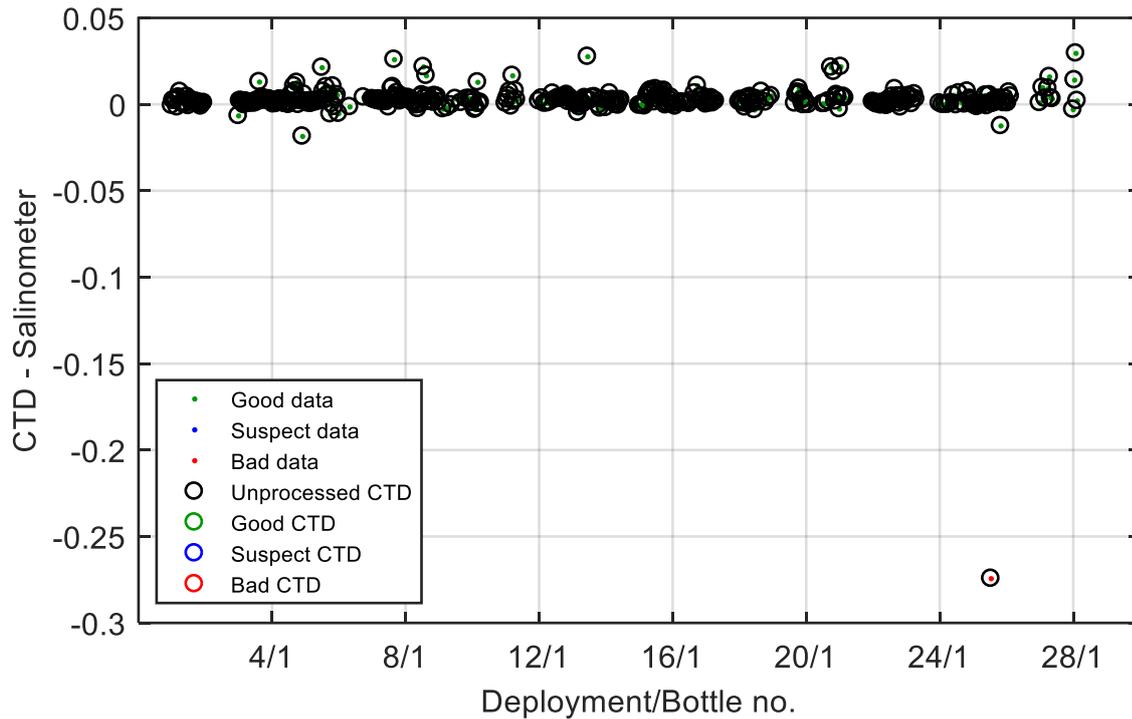
<sup>1</sup> International Association for the Physical Sciences of the Oceans

## 5.2 CTD Salinity vs Bottle Salinity Plot

The CTD values in this plot are unprocessed raw data. The difference between the unprocessed (uncorrected) CTD values and the measured bottle salinities is generally less than 0.03 PSU.

The unprocessed CTD values are adjusted (corrected) by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the [DataLibrariansOAMNF@csiro.au](mailto:DataLibrariansOAMNF@csiro.au) for corrected CTD data.

Note: dots = bottle samples, circles = CTD instrument (unprocessed)



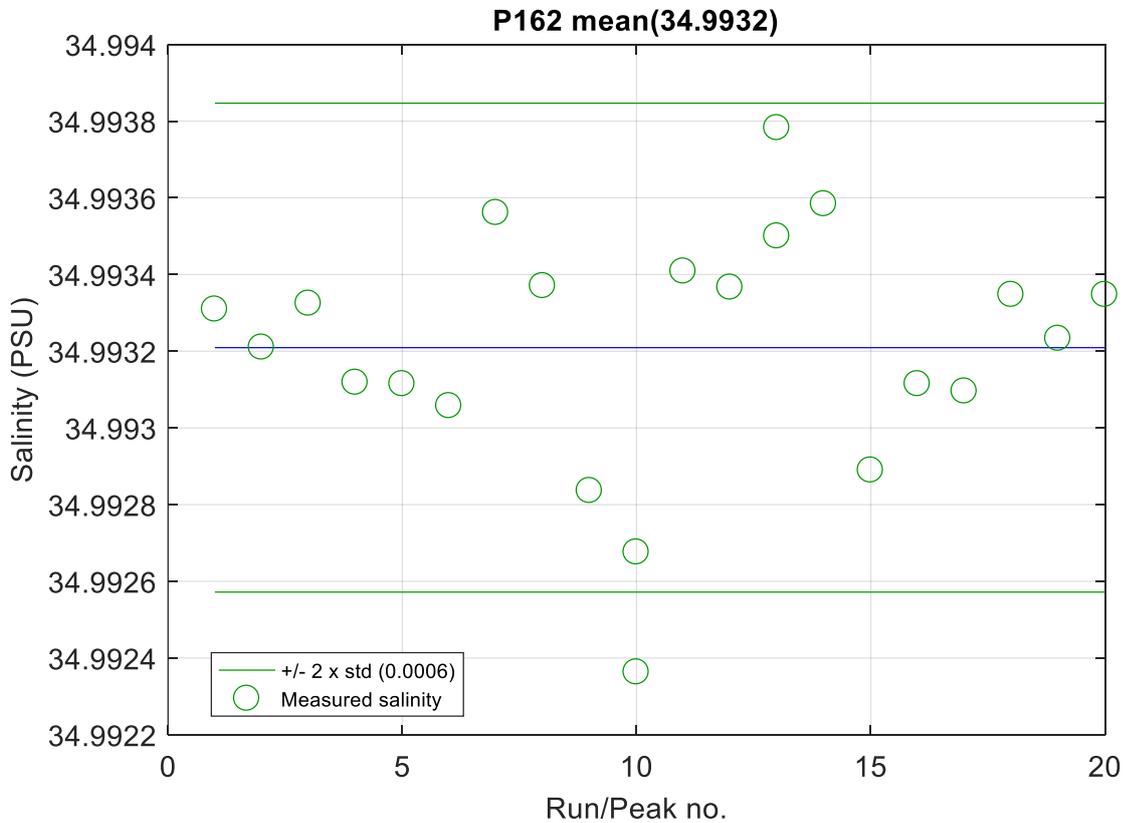
**Figure 2:** Plot of the measured bottle salinity results against the unprocessed CTD salinity values.

**Table 4:** Missing or suspect salinity data. 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 (Flagging key in Table 19 in the Appendix).

CTD	RP	Run	Flag	Reason for Flag or Action
25	14	sal019	133	Flagged Bad, outlier in profile. Niskin misfire.

## 5.4 OSIL Salinity Standard PSU across the Voyage

The instrument is calibrated with IAPSO standard seawater lot P162 (PSU = 34.993). The graph below shows the IAPSO lot P162 measured results after the same bottle was used for the calibration. The blue line represents the mean of all standards measured for standardisation.



**Figure 3:** The measured P162 IAPSO standard salinity (PSU) for each analysis during the voyage.

## 5.5 Salinity Measurement Uncertainty

The measurement uncertainty (MU) was calculated for this voyage using the precision obtained from deployment 1, where all Niskin bottles were fired at a depth of 3412 m. The reported uncertainty (U) is an expanded uncertainty using a coverage factor of 2 giving a 95% level of confidence. The U for this voyage was 0.00004 PSU or expressed as a percentage 0.004% PSU.

## 6 Dissolved Oxygen Data Processing

### 6.1 Dissolved Oxygen Method

The method used to measure the dissolved oxygen concentration is the Scripps Institution of Oceanography (SIO) method. This method is based on the Winkler titration with the whole-bottle modification from Carpenter (1965) and modifications by Culberson *et al* (1991).

Method synopsis: The sample is collected in an iodine determination flask of known volume. 1 mL 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 20 times. The dissolved oxygen oxidizes an equivalent amount of Mn (II) to Mn (IV) which precipitates. Just before titration, the sample is acidified, reducing the Mn (IV) back to the divalent state liberating iodine twice the original dissolved oxygen content of the sample. The tri-iodine is auto-titrated with a standardised thiosulphate solution using a Metrohm 665 Dosimat fitted with a 1 mL burette. The endpoint is determined by measuring changes in the UV absorption of the tri-iodide ion at 365 nm. The point at which there is no change in absorbance is the endpoint.

Before each batch of sample assays, the thiosulphate solution is standardised by using it to titrate a 10 mL aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two consecutive titres for 1 mL aliquots of the same potassium iodate solution.

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

**Table 5:** Dissolved oxygen parameters summary

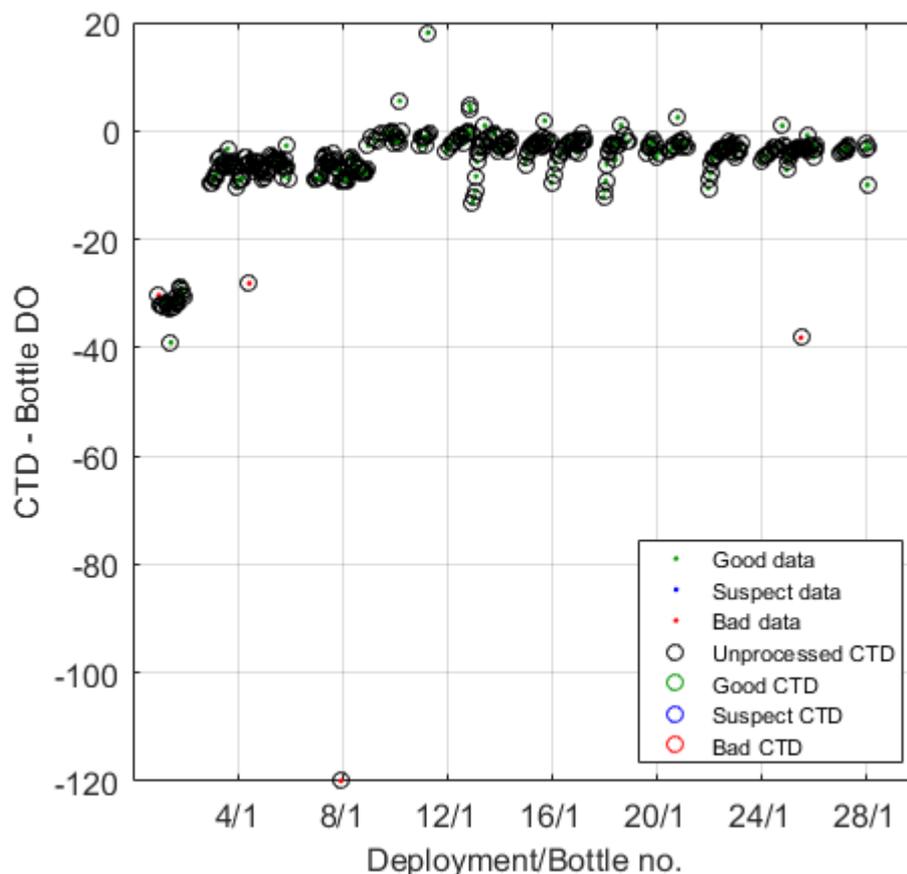
Details	
HyPro Version	5.7
Instrument	Automated Photometric Oxygen System
Software	Scripps Institution of Oceanography (SIO)
CSIRO Hydrochem. Method	Sampling: WI_DO_001 Assay: SOP005
Accuracy	± 0.5 µM
Analyst(s)	Merinda McMahon, Christine Rees and Kendall Sherrin
Lab Temperature	Variable, 19.6 - 22.0°C
Sample Container type	Pre-numbered 140 mL glass iodine determination flasks with glass stopper. 24 or 18 flasks per light-proof container.
Sample Storage	Samples stored in the hydrochemistry lab until analysis. All samples were analysed within ~48 hrs
Comments	None.

## 6.2 CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

The CTD values in this plot are unprocessed raw data. Measurements from the conductivity sensor on the CTD were incorrect for the first deployment.

The unprocessed CTD values are adjusted (corrected) by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the [DataLibrariansOAMNF@csiro.au](mailto:DataLibrariansOAMNF@csiro.au) for corrected CTD data.

Note: dots = bottle samples, circles = CTD instrument (unprocessed)



**Figure 4:** Plot of the measured dissolved oxygen bottle results against the unprocessed CTD dissolved oxygen values.

**Table 6:** Missing or suspect dissolved oxygen data. 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 (Flagging key in Table 19 in the Appendix).

CTD	RP	Run	Flag	Reason for Flag or Action
1	01	oxy001	133	Bad result. Operator error. Incorrect flask number entered.
7	24	oxy004	133	Bad result, higher than all other samples for entire voyage. Does not match CTD oxygen.
4	12	oxy003	133	Bad result. Bubbles in the sample.
22	07	oxy016	141	Missing data – sample temperature not recorded
25	14	oxy018	133	Bad result, outlier in depth profile, Niskin misfired.

### 6.3 Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.

The normality of the thiosulphate titrant (0.2 N) varied less than 0.0001 N for all dissolved oxygen sample titrations. The blank correction is less than 0.0009 mL with a voyage mean of 0.0007 mL and standard deviation of 0.0002 mL (n=13).

Red lines 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.

For reference, titre volumes for dissolved oxygen bottle samples lay in the range 0.38 to 0.62 mL.

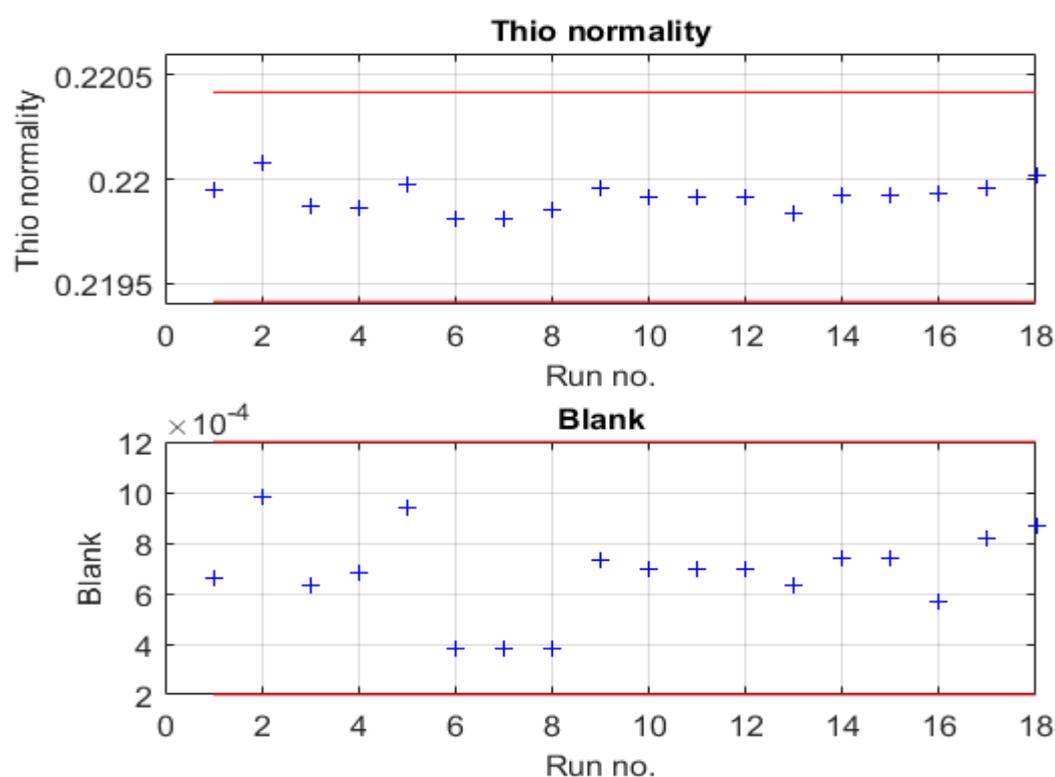


Figure 5: The measured thiosulphate normality for each analysis during the voyage.

Figure 6: The measured blank normality for each analysis during the voyage.

#### 6.3.1 Dissolved Measurement Uncertainty

The measurement uncertainty (MU) was calculated for this voyage using the precision obtained from deployment 1, where all Niskin bottles were fired at a depth of 3412 m. The reported uncertainty (U) is an expanded uncertainty using a coverage factor of 2 giving a 95% level of confidence. The U for this voyage was 0.02  $\mu$ M or expressed as a percentage 2%.

## 7 Nutrient Data Processing

### 7.1 Nutrient Methods

When using silicate, phosphate, nitrate+nitrite (NO<sub>x</sub>) and nitrite data set for publication, please cite the paper:

**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 were assayed on a Seal AA3HR segmented flow auto-analyser fitted with 1 cm flow-cells for colorimetric measurements and a JASCO FP2020 fluorescence instrument as the ammonium detector. A synopsis of the methods is given below:

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 660 nm.

Phosphate (SOP002): colourimetric, molybdenum blue method. Based on Murphy and Riley (1962) with modifications from the NIOZ-SGNOS<sup>1</sup> 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 880 nm.

Nitrate (SOP003): colourimetric, Copper (Cu)-Cadmium (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 –Cu-Cd 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 Cu-Cd reduction column and buffer.

Ammonium (SOP004): fluorescence, ortho-phtaldialdehyde method. Based on K erouel and Aminot (1997). Ammonium in seawater reacts with ortho-phtaldialdehyde and sulphite at a pH of 9.0-9.5 to produce an intensely fluorescent product. Its emission is measured at 460 nm after excitation at 370 nm.

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

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

**Table 7: Nutrient assay parameters summary.**

Details					
CSIRO Software	HyPro 5.7				
Instrument	Seal AA3HR				
Instrument Software	Seal AACE 7.09				
CSIRO Hydrochem. Method, sampling	WI_Nut_001				
CSIRO Hydrochem. Method, nutrient	SOP001	SOP002	SOP003	SOP003	SOP004
Nutrient	Silicate	Phosphate	Nitrate + Nitrite	Nitrite	Ammonium
Concentration range	140 µM	3.0 µM	42 µM	1.4 µM	2.0 µM
Method Detection Limit (MDL)	0.2 µM	0.02 µM	0.02 µM	0.02 µM	0.02 µM
Matrix Corrections	none	none	none	none	none
Analysts	Merinda McMahon and Christine Rees				
Lab Temperature (±1°C)	Variable, 19.6 - 22.0°C				
Reference Material	KANSO, RMNS lot CG in every analytical run.				
Sampling Container type	CTD: 50 mL HDPE with screw cap lids. UWY: 12 mL PP tubes with screw cap lids.				
Sample Storage	< 2 hrs at room temperature or ≤ 12 hrs @ 4°C				
Pre-processing of Samples	CTD and UWY: None.				
Comments	From Run 15 the concentration range for ammonium was 1.0 µM.				

**Table 8:** Missing or suspect nutrient data. All flagged data and any samples that had repeated analyses performed to obtain good data are reported here. 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 (Flagging key in Table 19 in the Appendix). Data flagged 63, below detection limit, are not included in the table below. Data flagged BAD (133) are not included in the .csv results (File: in2019\_v05HydroDep.csv).

CTD	RP	Run	Nut	Flag	Reason for Flag or Action
1	10	2	NH4	133	Flagged Bad as very different to all other samples which were collected at same depth.
3	2	3	NH4, PO4, SiO4, NOx	133	Flagged bad as air spike occurred from sample tubing becoming disconnected.
18	01	16	SiO4	133	One of the duplicate results flagged bad as the result was different to the first time the duplicates were analysed in previous run (nut015B).
19	18	16	All	133	Bad result looks like an outlier compared to all other depth profiles for the entire voyage, incorrect bottle sampled for nutrients.
22	01	19	NOx	133	One of the duplicate results was flagged bas as the result was different to all previous NOx measurements made at this depth.
25	14	21	All	133	Outlier in depth profile, temperature was also incorrect for Dissolved Oxygen.

## 7.2 HyPro Data 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.

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:

- $\pm 0.5\%$  of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE<sup>1</sup>).
- Within 0.02  $\mu\text{M}$  for phosphate, nitrite and ammonium.

HyPro classifies the quality of data as good, suspect or bad and flags accordingly. The flagged nutrient calibration data is in the Appendix.

Missing or suspect nutrient data is tabulated in table 8, the flags are also in the final hydrology data set. The Flagging key is in table 19 in the Appendix.

<sup>1</sup> World Ocean Circulation Experiment

**Table 9:** 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 (NO <sub>x</sub> )	Nitrite	Ammonia
Data Reported as	µM	µM	µM	µM	µM
Calibration Curve degree	Linear	Linear	Quadratic	Quadratic	Quadratic
# of points in Calibration	7	6	7	6	6
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)	Y	Y	Y	Y	Y
Baseline drift correction (HyPro)	Y	Y	Y	Y	Y
Sensitivity drift correction (HyPro)	Y	Y	Y	Y	Y
Data Adj for RMNS variance.	N	N	N	N	N
Medium of Standards	Low nutrient seawater (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				
Proportion of samples in duplicate.	<10%. CTD: Niskin fired at the greatest depth sampled in duplicate. Single samples collected for remaining depths.				
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in the Appendix.				

### 7.3 Accuracy and Precision - Reference Material for Nutrient in Seawater (RMNS)

Descriptive statistics were used to ascertain the accuracy and precision of the analysis from the repetitive measurement of the RMNS for silicate, phosphate, NO<sub>x</sub>, and nitrite in seawater.

Japanese KANSO certified RMNS lot CG was assayed in triplicate in each run to monitor accuracy. The certified values are in table 10. Lots CG, CJ and CB were analysed at the beginning of the voyage to confirm the accuracy of the calibration curves.

For in2019\_v05, the certified reference material results for NO<sub>x</sub>, and silicate were within 1%, phosphate was within 2% and nitrite within 0.02 µM of the certified concentration. However, it came to the attention that the phosphate stock standard was slightly higher in concentration than the previous phosphate stock standard which caused the RMNS not to be within 1%. The phosphate data has been corrected using the RMNS Lot CG with the following calculation.

$\text{Ratio} = \frac{\text{Certified RMNS Concentration}}{\text{Measured RMNS Concentration in each run}}$ $\text{Corrected Concentration} = \text{Ratio} \times \text{Measured Nutrient Concentration}$
---

The GO-SHIP criteria (Hyde *et al.*, 2010), section 8, specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy.

The assayed RMNS values per analysis run and CTD deployments are listed in the Appendix.

**Table 10:** RMNS certified concentrations ± expanded uncertainty (U) at 21°C.

RMNS	SiO <sub>4</sub> (µM)	PO <sub>4</sub> (µM)	NO <sub>3</sub> + NO <sub>2</sub> (NO <sub>x</sub> , µM)	NO <sub>3</sub> (µM)	NO <sub>2</sub> (µM)
Lot CD	14.264 ± 0.10	0.457 ± 0.008	5.647 ± 0.055	5.629 ± 0.051	0.018 ± 0.004
Lot CG	57.75 ± 0.51	1.74 ± 0.02	24.330 ± 0.24	24.27 ± 0.205	0.061 ± 0.03
Lot CJ	39.424 ± 0.410	1.219 ± 0.020	16.620 ± 0.212	16.588 ± 0.205	0.032 ± 0.007
Lot CB	111.821 ± 0.635	2.580 ± 0.022	36.768 ± 0.282	36.649 ± 0.276	0.119 ± 0.006

KANSO publishes the RMNS nutrient values in µmol kg<sup>-1</sup>. These are converted to µmol 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.

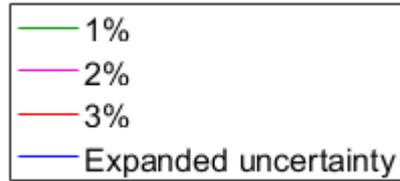
**Table 11:** Statistical results of RMNS for the voyage. The minimum, maximum, mean, median, and reproducibility (standard deviation) are of all the analytical measurements performed during the voyage.

RMNS CG	Silicate (µM)	Phosphate (µM)	Nitrate + Nitrite (NO <sub>x</sub> , µM)	Nitrite (µM)	Ammonia (µM)
Minimum	56.95	1.71	24.26	0.058	0.73
Maximum	58.10	1.73	24.53	0.078	0.92
Mean	57.67	1.72	24.42	0.068	0.81
Median	57.67	1.72	24.44	0.070	0.81
Reproducibility	0.24	0.01	0.06	0.005	0.06

RMNS CJ	Silicate (µM)	Phosphate (µM)	Nitrate + Nitrite (NO <sub>x</sub> , µM)	Nitrite (µM)	Ammonia (µM)
Minimum	39.085	1.203	16.465	0.039	0.904
Maximum	39.477	1.212	16.650	0.052	1.188
Mean	39.285	1.208	16.603	0.045	1.050
Median	39.302	1.208	16.627	0.045	1.078
Reproducibility	0.168	0.003	0.070	0.004	0.112

## 7.4 Nutrient plots of RMNS

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  $\mu\text{M}$  increments from the certified value. The blue line is the expanded uncertainty of the certified value.



### 7.4.1 Silicate RMNS Plot ( $\mu\text{M}$ )

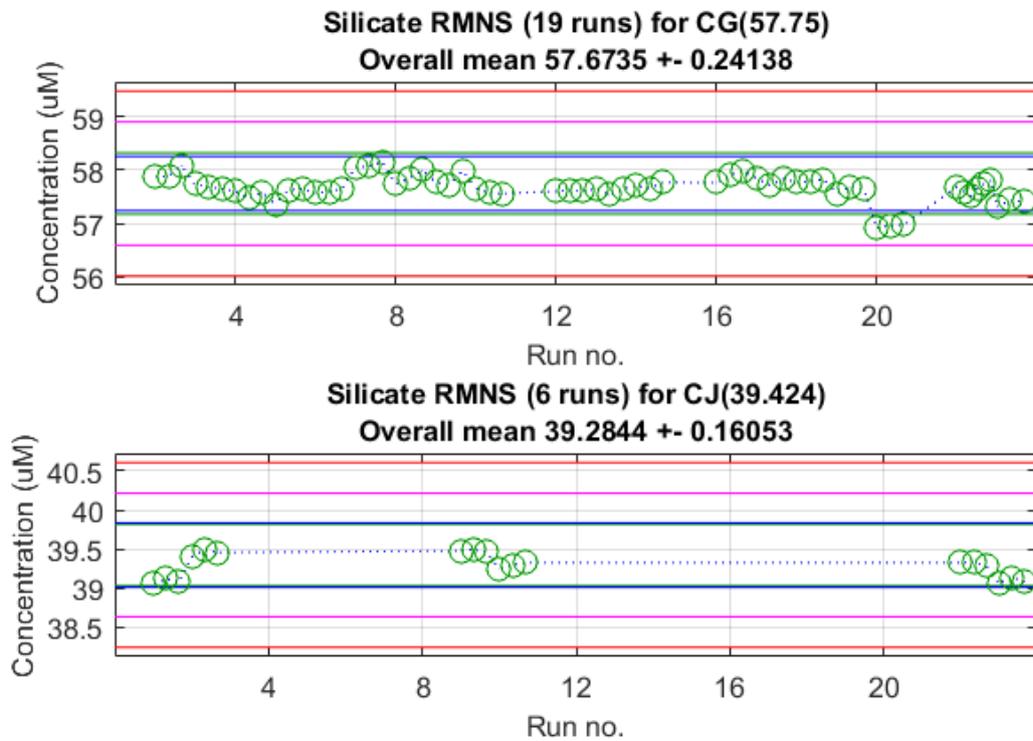


Figure 7: Silicate RMNS plots.

### 7.4.2 Phosphate RMNS Plot ( $\mu\text{M}$ )

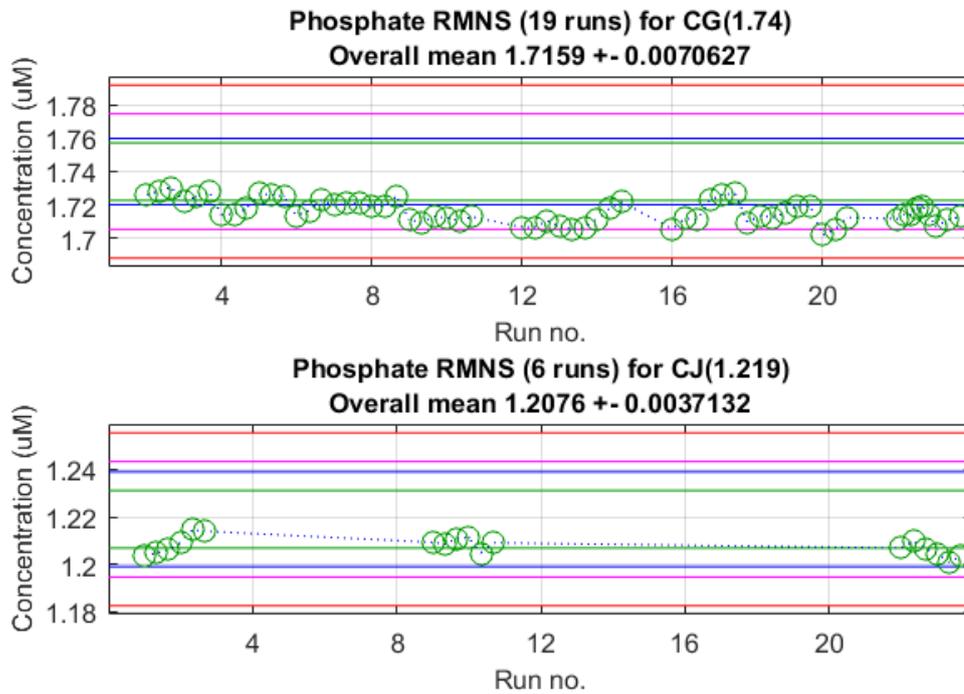


Figure 8: Phosphate RMNS plots.

### 7.4.3 Nitrate + Nitrite ( $\text{NO}_x$ ) RMNS Plot ( $\mu\text{M}$ )

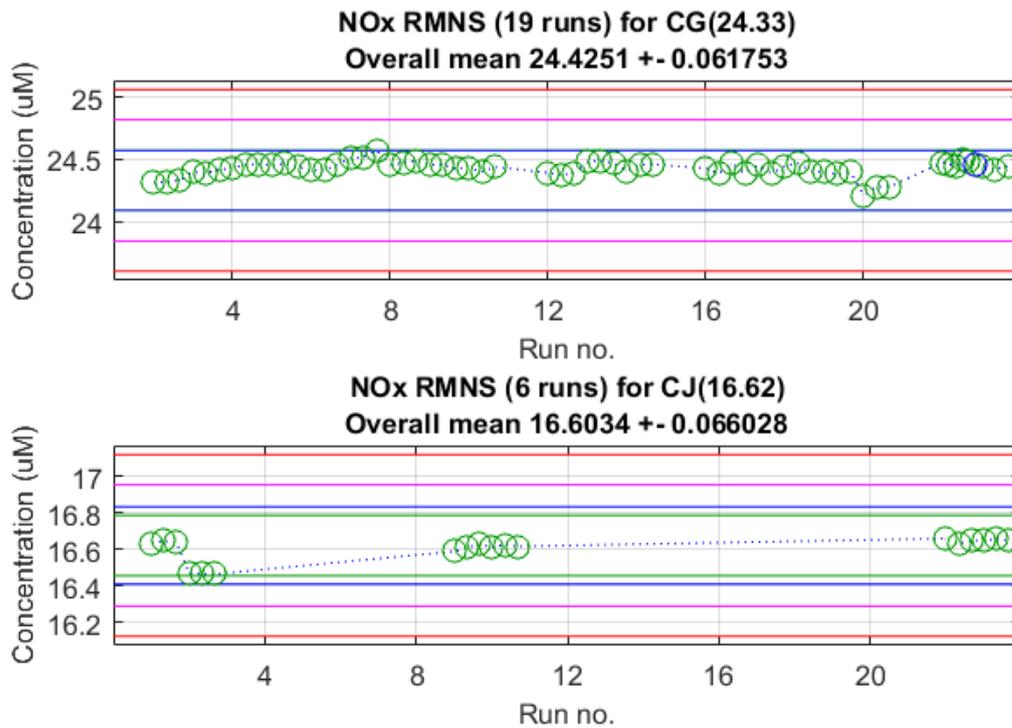


Figure 9: Nitrate + Nitrite ( $\text{NO}_x$ ) RMNS plots.

#### 7.4.4 Nitrite RMNS Plot ( $\mu\text{M}$ )

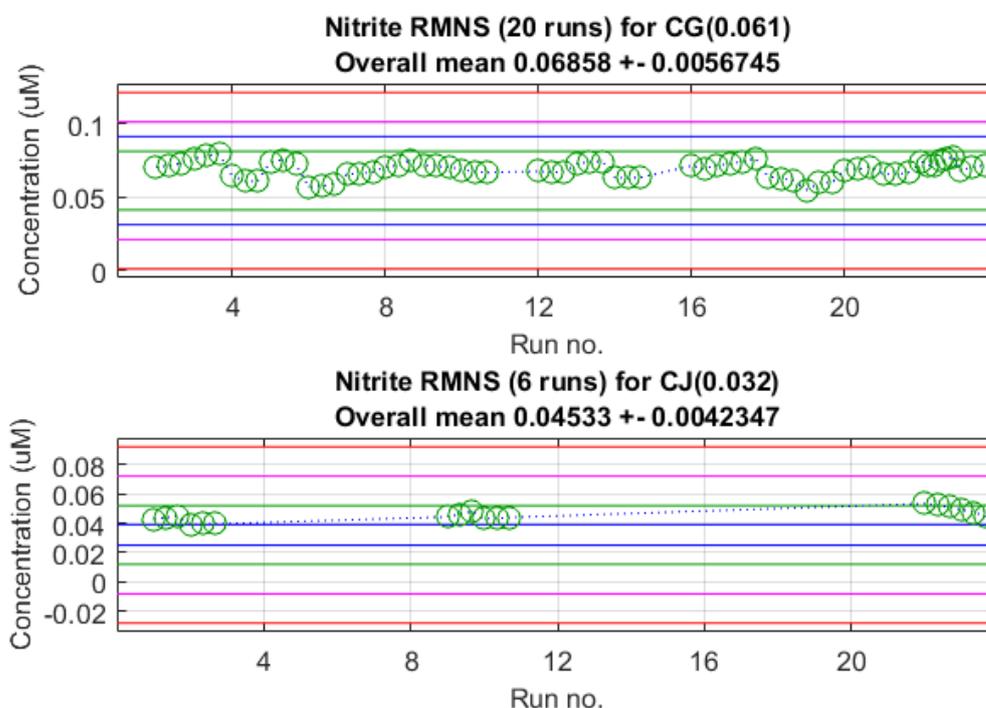


Figure 10: Nitrite RMNS plots

### 7.5 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 12: Nutrient measurement uncertainty.

Calculated Measurement Uncertainty @ 1 $\mu\text{M}$				
Silicate ( $\mu\text{M}$ )	Phosphate ( $\mu\text{M}$ )	Nitrate + Nitrite (NO <sub>x</sub> ) ( $\mu\text{M}$ )	Nitrite ( $\mu\text{M}$ )	Ammonia ( $\mu\text{M}$ )
±0.017	±0.024	±0.019	±0.14	±0.30 <sup>‡</sup>

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

‡The ammonia MU precision does not include data for the RMNS.

**Table 13:** Calculated measurement uncertainty during in2019\_v05. The expanded uncertainty (U) was calculated for this voyage using the precision and bias. The precision was obtained from deployment 1 where all Niskin bottles were fired at a depth of 3412 m and from the repeated measurement of RMNS over the voyage. The bias was calculated from the accuracy of the RMNS measurement over the voyage. The expanded uncertainty is also expressed as a percentage U%.

	Silicate ( $\mu\text{M}$ )	Phosphate ( $\mu\text{M}$ )	Nitrate + Nitrite (NOx) ( $\mu\text{M}$ )
<b>U*</b>	0.0149	0.0187	0.0153
<b>U%</b>	1	2	2

\*The depth at which Niskin bottles were fired does not contain nitrite or ammonium, therefore no precision measurements were available.

## 7.6 Method Detection Limit for Nutrients

The method detection limit (MDL) samples consisting of low nutrient seawater (LNSW) were analysed in every run. Nominal MDL was determined previously by measuring nutrients in LNSW 10 times. The MDL was set to three times the standard deviation of the results (National Association of Testing Authorities 2013). The nominal detection limits are reported in the table. During the voyage, LNSW was analysed 3 times within each analytical run and the standard deviation was calculated and multiplied by three to compare against the nominal MDL. The resultant MDL was used to assess the analysis precision at low concentrations. The MDL for each run are much lower than the nominal detection limits, indicating high analytical precision at lower concentrations. See the Appendix for the measured MDL per CTD deployments.

**Table 14:** Statistical results of MDL for the voyage. The minimum, maximum, mean, median, and reproducibility (standard deviation) of all the analytical measurements performed during the voyage.

MDL	Silicate ( $\mu\text{M}$ )	Phosphate ( $\mu\text{M}$ )	Nitrate + Nitrite (NOx) ( $\mu\text{M}$ )	Nitrite ( $\mu\text{M}$ )	Ammonia ( $\mu\text{M}$ )
<b>Nominal MDL</b>	0.20	0.02	0.02	0.02	0.02
<b>Min</b>	0.012	0.001	0.002	0.002	0.001
<b>Max</b>	0.144	0.013	0.032	0.043	0.004
<b>Mean</b>	0.059	0.005	0.010	0.006	0.002
<b>Median</b>	0.049	0.003	0.010	0.004	0.002
<b>Reproducibility</b>	0.037	0.004	0.008	0.008	0.001

## 7.7 Sampling Precision

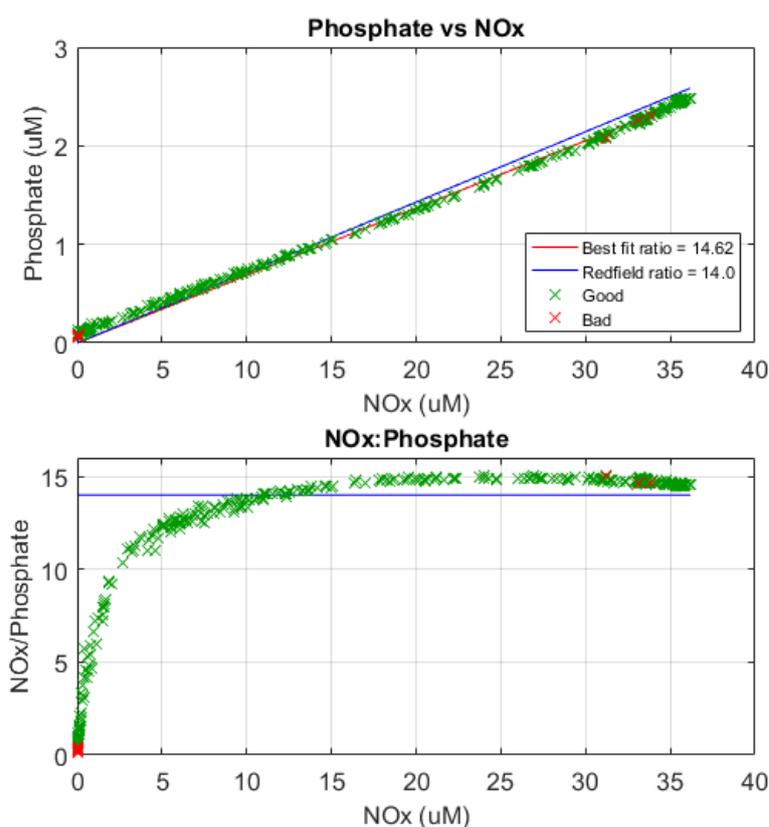
Sampling precision was monitored by assaying duplicate samples collected from the greatest depth for each CTD deployment. The sampling precision is deemed “good” if the difference between the

duplicate concentrations is less than the MDL value. The exception is nitrate+nitrite, which uses 0.06  $\mu\text{M}$  as the MDL boundary.

For in2019\_v05, the sampling precision was within the acceptable criteria.

## 7.8 Redfield Ratio

Calculating and plotting 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. The ratio is very consistent in the deep ocean with phosphate to nitrate equalling 14. The ratio for this voyage was 14.62



**Figure 11:** Plots consist of the phosphate concentration against the NOx concentration for all CTD deployments. Green crosses are good data and red crosses are bad data.

## 7.9 Temperature & Humidity Change over Nutrient Analyses

The ambient conditions in the hydrochemistry lab and within the AA3HR instrument were measured and logged in the following locations:

- (1) Above the AA3HR instrument, temperature only. Average 21.15°C, Standard Deviation 0.39°C.
- (2) Above the AA3HR instrument on the other side, ship's instrument (Grafana).
- (3) On the deck of the nitrate & nitrite AA3HR chemistry module, temperature and humidity. Data on request.

Refer to “in2019\_v05\_hyd\_voyagereport.docx” for room temperature graphs, nutrient samples were placed on XY3 auto sampler at the average room temperature of **21.15°C**. **This is the temperature that should be used to convert the results from  $\mu\text{M}$  to  $\mu\text{mol kg}^{-1}$ .**

The laboratory temperature was measured and recorded on the nutrient run sheets at the start each analysis run. The temperature varied between 19.57 and 22.00 °C over the course of the voyage.

## 8 GO-SHIP Specifications

### Salinity

Accuracy of 0.001 is possible with Autosol™ 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. Autosol 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  $\pm 1^\circ\text{C}$  is very important and should be recorded<sup>2</sup>.

### 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.

### Silicate

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

### Phosphate

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

### Nitrate

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

### 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 Appendix

**Table 15: Salinity Reference Material**

OSIL IAPSO Standard Seawater	
Batch	P162
Use by date	16/04/2021
K <sub>15</sub>	0.99983
PSU	34.993

**Table 16: Nutrient flagged calibration and quality control data.** HyPro classifies the quality of data as good, suspect or bad and flags accordingly. Points flagged as suspect have less weighting in the calibration curve, points flagged as bad are not used within the calibration curve.

CTD	Peak	Run	Analysis	Reason for Flag or Action
3	Cal 2 & 4	Nut003	NH4	Cal 2 both points bad. Cal 4 first point suspect and second bad.
5	Cal 1, 2 & 4	Nut005	NH4	<70% of calibration peaks are within calibration limits. Both cal 1 points bad, 1 <sup>st</sup> cal 2 point suspect and 2 <sup>nd</sup> cal 4 point suspect.
5	Cal 5	Nut005	SiO4	Cal 5 second point bad.
8	Cal 1	Nut007	NH4	Both cal 1 points bad.
12	Cal 4	Nut009	NH4	Both cal 4 points bad.
14&15	Cal 4	Nut012	NH4	Both cal 4 points suspect.
16	Cal 2 & 3	Nut013	NH4	Both cal 2 points suspect, 1 <sup>st</sup> cal 3 point suspect.
17	Cal 4	Nut014	NH4	Both cal 4 points suspect.
18&19	Cal 3 & 4	Nut016	NH4	Both cal 3 points suspect, 1 <sup>st</sup> cal 4 points suspect.
20	Cal 4	Nut017	PO4	1 <sup>st</sup> cal 4 point suspect.
21	Cal 5	Nut018	SiO4	1 <sup>st</sup> cal 5 point suspect.
22&23	Cal 6	Nut019	SiO4	2 <sup>nd</sup> cal 6 point suspect.
24	Cal 6	Nut020	SiO4	2 <sup>nd</sup> cal 6 point suspect.

**Table 17:** Nutrient results for RMNS Lot CG for each analytical run and CTD deployment.

Analysis Run	CTD #	Silicate (Si, $\mu\text{M}$ )	Phosphate ( $\text{PO}_4$ , $\mu\text{M}$ )	Nitrite ( $\text{NO}_2$ , $\mu\text{M}$ )	NOx ( $\text{NO}_2 + \text{NO}_3$ , $\mu\text{M}$ )
Lot CG certified value	-	57.75	1.74	0.061	24.33
2	1	57.95	1.73	0.071	24.32
3	3	57.69	1.73	0.078	24.40
4	4	57.56	1.72	0.062	24.45
5	5	57.55	1.73	0.074	24.45
6	7	57.61	1.72	0.058	24.42
7	8	58.10	1.72	0.066	24.53
8	9, 10 & 11	57.86	1.72	0.072	24.47
9	12	57.83	1.71	0.071	24.45
10	13	57.60	1.71	0.067	24.42
11	Aborted				
12	14 & 15	57.61	1.71	0.067	24.38
13	16	57.61	1.71	0.073	24.48
14	17	57.71	1.72	0.063	24.44
15	18			0.071	
16	19 & repeat 18	57.89	1.71	0.074	24.43
17	20	57.80	1.73	0.063	24.41
18	21	57.80	1.71	0.058	24.44
19	22 & 23	57.63	1.72	0.069	24.39
20	24	56.95	1.71	0.071	24.26
21	25			0.066	
22	26, 27 & repeat 25	57.67	1.72	0.074	24.47
23	28	57.39	1.71	0.070	24.44

Note: CTD 18 was repeated as the  $\text{NO}_2$  baseline was greater than 2%. The repeated results for all other channels were similar to the original run therefore it was decided to use the repeat as the final results. CTD 25 was repeated due to poor peak shapes for  $\text{PO}_4$ ,  $\text{SiO}_4$  and  $\text{NO}_x$ . The peak shapes for  $\text{NO}_2$  and  $\text{NH}_4$  were acceptable therefore the results from the original run were used for these nutrients.

**The submitted nutrient results do NOT have RMNS corrections applied, except for the phosphate data refer to section 7.3 for details.**

**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

**Table 18:** Nutrient measured detection limit (MDL) for each analytical run and CTD deployment.

Measured Detection Limit						
Analysis Run	CTD #	Silicate (Si, $\mu\text{M}$ )	Phosphate ( $\text{PO}_4$ , $\mu\text{M}$ )	Nitrite ( $\text{NO}_2$ , $\mu\text{M}$ )	NOx ( $\text{NO}_2 + \text{NO}_3$ , $\mu\text{M}$ )	Ammonia ( $\text{NH}_4^+$ , $\mu\text{M}$ )
2	1	0.093	0.013	0.004	0.005	0.002
3	3	0.044	0.003	0.004	0.01	0.002
4	4	0.076	0.003	0.003	0.032	0.004
5	5	0.046	0.005	0.004	0.022	0.003
6	7	0.144	0.004	0.043	0.005	0.002
7	8	0.023	0.005	0.009	0.005	0.003
8	9, 10 & 11	0.012	0.003	0.002	0.018	0.003
9	12	0.021	0.005	0.005	0.003	0.002
10	13	0.059	0.001	0.004	0.009	0.003
11	Aborted					
12	14 & 15	0.024	0.002	0.005	0.003	0.002
13	16	0.103	0.002	0.002	0.002	0.003
14	17	0.035	0.009	0.005	0.002	0.002
15	18					0.001
16	19 & repeat 18	0.03	0.011	0.004	0.014	0.001
17	20	0.052	0.001	0.002	0.01	0.002
18	21	0.069	0.01	0.003	0.012	0.003
19	22 & 23	0.039	0.001	0.002	0.014	0.001
20	24	0.077	0.002	0.006	0.007	0.001
21	25			0.009		0.002
22	26, 27 & repeat 25	0.072	0.012	0.003	0.002	0.002
23	28	0.022	0.003	0.005	0.014	0.002

Note: CTD 18 was repeated as the  $\text{NO}_2$  baseline was greater than 2%. The repeated results for all other channels were similar to the original run therefore it was decided to use the repeat as the final results. CTD was 25 was repeated due to poor peak shapes for  $\text{PO}_4$ ,  $\text{SiO}_4$  and  $\text{NO}_x$ . The peak shapes for  $\text{NO}_2$  and  $\text{NH}_4$  were acceptable therefore the results from the original run were used for these nutrients.

**Table 19:** Hydrology data flagging key.

Flag	Description
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 is 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 netcdf file as an array compiles results. Not used in csv file.
79	Method Detection Limit (MDL) during run was equal to or greater than nominal MDL. Data flagged as suspect.

## 10 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 inter-comparability 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)
- K erouel, 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.