

RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESSING REPORT

Voyage: in2018_v06

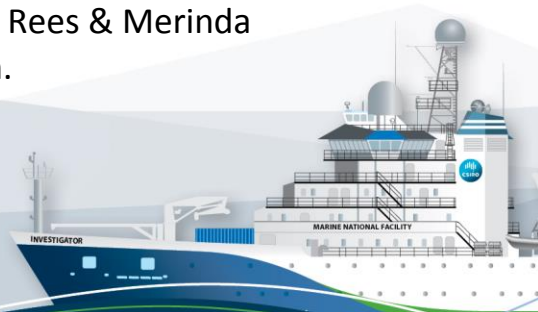
Chief Scientist: Alan Williams

Principal Investigators: Dr Nic Bax, Dr Malcom Clark & Dr Thomas Schlacher

Voyage title: Status and Recovery of deep-sea coral communities on seamounts in iconic Australian marine reserves.

Supplementary Project: Spatial and temporal variability in the distribution and abundance of seabirds.

Report compiled by: Stephen Tibben, Christine Rees & Merinda McMahon.



Contents

| | | |
|-------|---|----|
| 1 | Executive Summary | 4 |
| | Itinerary..... | 5 |
| 2 | Key personnel list | 5 |
| 3 | Summary..... | 6 |
| 3.1 | Sample Type and Number Assayed..... | 6 |
| 3.1.1 | CTD (Conductivity, Temperature, Density) | 6 |
| 3.1.2 | TSG (Thermosalinograph) | 6 |
| 3.2 | Data Processing Overview | 7 |
| 4 | Salinity Data Processing..... | 8 |
| 4.1 | Salinity Parameter Summary | 8 |
| 4.2 | Salinity Method..... | 8 |
| 4.3 | CTD Salinity vs Bottle Salinity Plot | 9 |
| 5.4 | OSIL Salinity Standard PSU across the Voyage..... | 9 |
| 5.5 | Missing or Suspect Salinity Data | 10 |
| 5 | Dissolved Oxygen Data Processing | 11 |
| 5.1 | Dissolved Oxygen Parameter Summary..... | 11 |
| 5.2 | Dissolved Oxygen Method | 11 |
| 5.3 | CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot | 12 |
| 5.4 | Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction..... | 13 |
| 5.5 | Missing or Suspect Dissolved Oxygen Data. | 13 |
| 6 | Nutrient Data Processing..... | 14 |
| 6.1 | Nutrient Assay Parameter Summary | 14 |
| 6.2 | Nutrient Methods | 14 |
| 6.3 | HyPro Processing Parameters..... | 16 |
| 6.4 | HyPro Data Processing Summary..... | 16 |
| 6.5 | Accuracy - Reference Material for Nutrient in Seawater (RMNS) | 17 |
| 6.5.1 | Silicate RMNS Plot..... | 18 |
| 6.5.2 | Phosphate RMNS Plot | 18 |
| 6.5.3 | Nitrate + Nitrite (NO _x) RMNS Plot | 19 |
| 6.5.4 | Nitrite RMNS Plot..... | 19 |
| 6.6 | Analytical Precision | 19 |
| 6.6.1 | Nutrient Measurement Uncertainty..... | 19 |
| 6.6.2 | Nutrient Method Detection Limit | 20 |
| 6.6.3 | Reference Material for Nutrients in Seawater..... | 20 |

| | | |
|-------|--|----|
| 6.7 | Sampling Precision | 21 |
| 6.7.1 | Silicate Duplicates Plot | 22 |
| 6.7.2 | Phosphate Duplicates Plot | 22 |
| 6.7.3 | Nitrate + Nitrite (NO _x) Duplicates Plot | 23 |
| 6.7.4 | Nitrite Duplicates Plot | 23 |
| 6.7.5 | Ammonia Duplicates Plot | 24 |
| 6.8 | Redfield Ratio Plot (14.0) for CTD Deployments | 25 |
| 6.9 | Missing or Suspect Nutrient Data. | 26 |
| 6.10 | Temperature & Humidity Change over Nutrient Analyses | 26 |
| 7 | Appendix | 27 |
| 7.1 | Salinity: Reference Material Used | 27 |
| 7.2 | Nutrients: Flagged Calibration and Quality Control Data | 27 |
| 7.3 | Nutrients: RMNS results for each Analysis Run & CTD Deployment. | 28 |
| 7.3.1 | RMNS Lot CJ Results | 28 |
| 7.4 | Flag Key for Hydrology Data Set | 29 |
| 7.5 | GO-SHIP Specifications | 30 |
| 7.5.1 | Salinity | 30 |
| 7.5.2 | Dissolved Oxygen | 30 |
| 7.5.3 | SiO ₂ | 30 |
| 7.5.4 | PO ₄ | 30 |
| 7.5.5 | NO ₃ | 30 |
| 7.5.6 | Notes | 30 |
| 8 | References | 31 |

1 Executive Summary

The main objectives of the voyage were to determine the extent, ecological characteristics, and conservation status of deep-sea coral reefs on Tasmanian seamounts inside and outside of existing reserves. Measure recovery trajectories and dynamics of deep-sea coral communities (multi-species and successional changes) following cessation of bottom trawling. Provide the first set of empirical data on conservation status, resilience and recovery potential to enhance management and conservation of deep-sea coral habitats nationally. Also to collect and analyse salinity, oxygen and nutrient samples from the CTD at each seamount.

Water samples collected during the voyage were assayed in the ship's hydrochemistry laboratory for nutrients, dissolved oxygen, and their salinity measured. The samples came from deployments of the CTD rosette.

High quality data was produced for the three measured parameters. Five nutrients were analysed; silicate, phosphate, nitrate + nitrite, nitrite and ammonium. Certified reference materials for nutrients in seawater were within the specified limits of the certified value.

Underway thermosalinograph (TSG) samples were collected and analysed during the voyage.

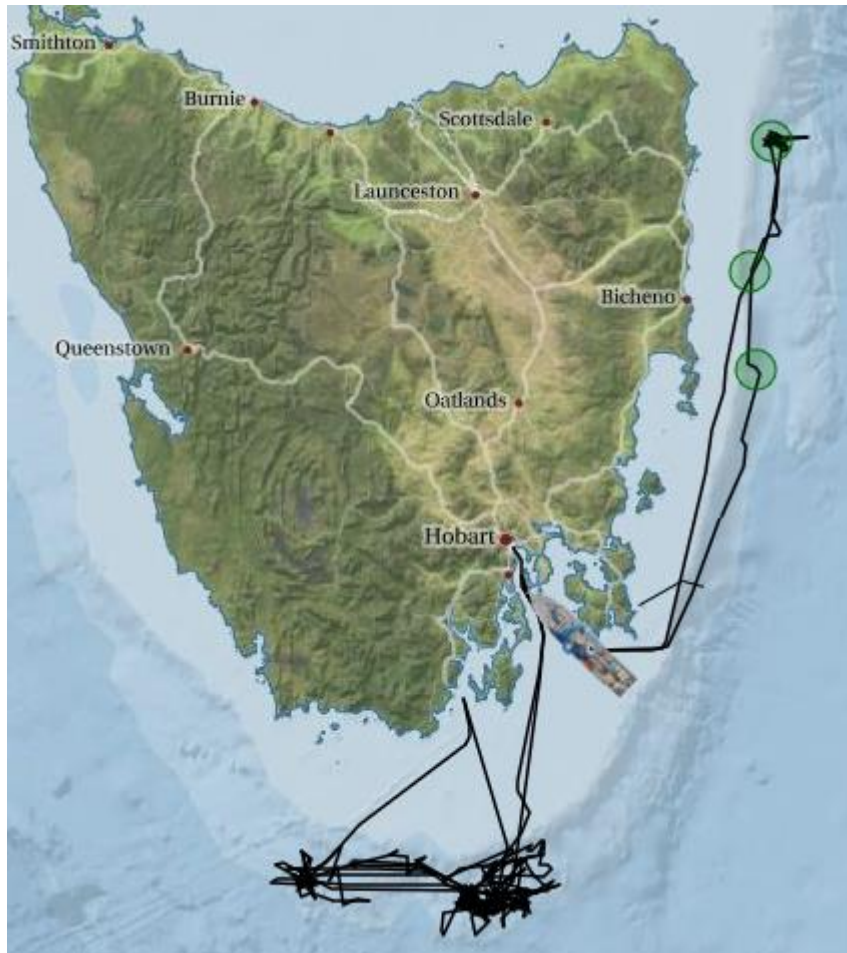
Final hydrology data, analytical methods, and related log sheets and processing notes can be obtained from the CSIRO data centre.

Contact: DataLibrariansOAMNF@csiro.au

Itinerary

Hobart to Hobart, November 23rd – December 19th, 2018.

Voyage Track:



2 Key personnel list

| Name | Role | Organisation |
|-----------------|-----------------|--------------|
| Alan Williams | Chief Scientist | CSIRO |
| Max McGuire | Voyage Manager | CSIRO |
| Stephen Tibben | Hydrochemist | CSIRO |
| Christine Rees | Hydrochemist | CSIRO |
| Merinda McMahon | Hydrochemist | CSIRO |

3 Summary

3.1 Sample Type and Number Assayed

| Analysis (instrument) | Number of Samples |
|--|-------------------|
| Salinity (Guildline Salinometer) | 156 CTD 24 TSG |
| Dissolved Oxygen (automated titration) | 156 CTD |
| Nutrients (Seal AA3HR) | 157 CTD |

3.1.1 CTD (Conductivity, Temperature, Density)

- Sampling point, 36 bottle rosette with 12L Ocean Test Equipment bottles (Niskin) deployed at depth for water collection.
- 12 CTD deployments in total. Deployments 1 through 12 sampled by Stephen Tibben, Christine Rees, Tiffany Sih, Cath Sampson, Alexandra Weber, Kylie Maguire, Candice Untiedt, Ricky-Lee Ericson, Cassie Layton and Merinda McMahon.

3.1.2 TSG (Thermosalinograph)

- 24 Samples collected by hydrochemistry from underway lab for calibration of thermosalinograph.

For TSG sample information refer to the eLog's from the voyage.

3.2 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 in figure 1.

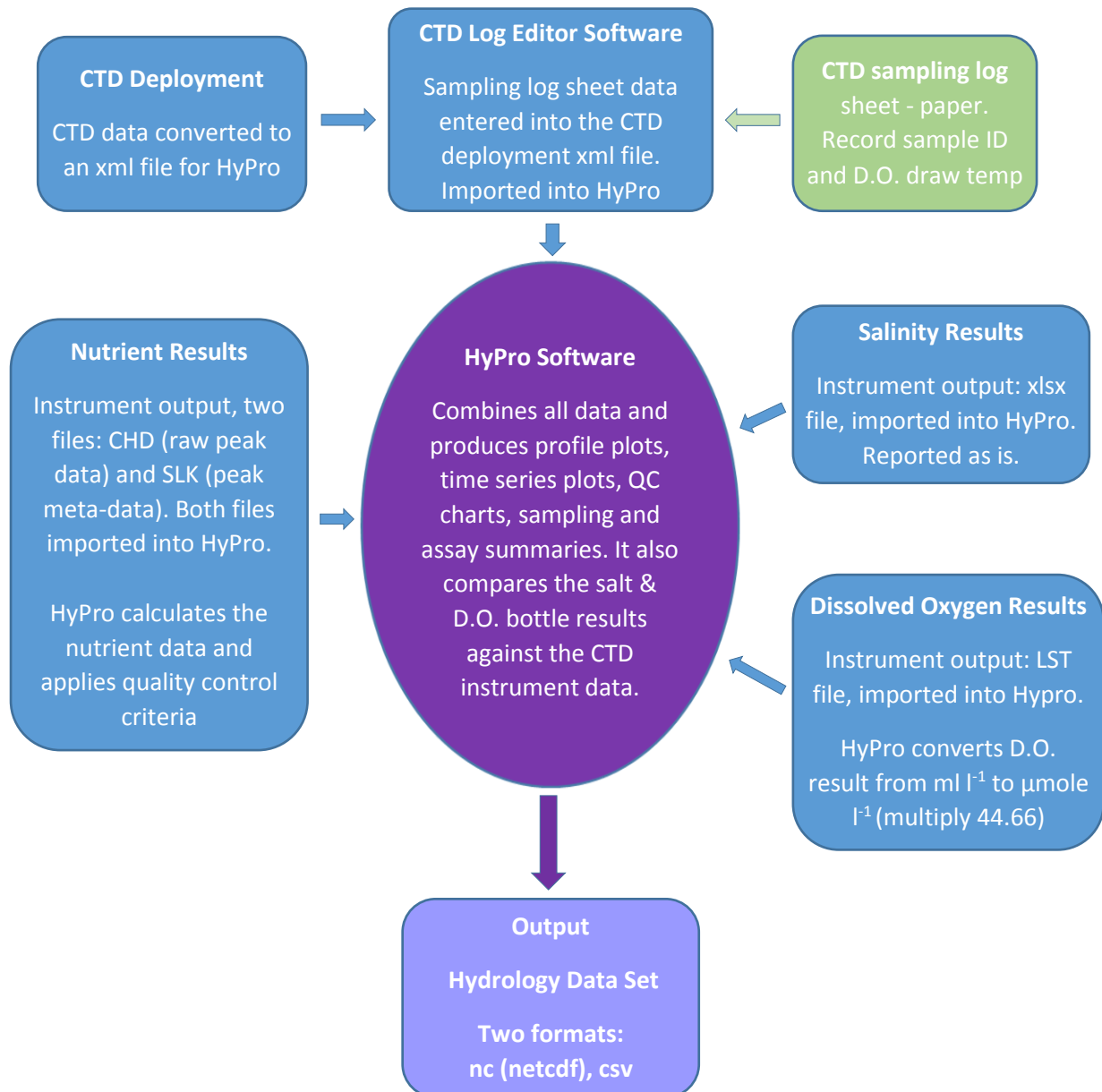


Figure 1: Hydrology Data Processing Flow Diagram.

4 Salinity Data Processing

4.1 Salinity Parameter Summary

| Details | |
|--------------------------|--|
| HyPro Version | 5.7 |
| Instrument | Guildline Autosal Laboratory Salinometer 8400(B) – SN 72151 |
| Software | OSIL Data Logger ver 1.2 |
| CSIRO Hydrochem Method. | Sampling: WI_Sal_002 Measurement: SOP006 |
| Accuracy | ± 0.001 practical salinity units |
| Analysts | Christine Rees & Merinda McMahon |
| Lab Temperature (±0.5°C) | 20.5 -23.7°C during analysis. |
| Bath Temperature | 24.01°C |
| Reference Material | Osil IAPSO - Batch P161, use by 03/05/2020, $K_{15} = 0.99987$ |
| 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 | None. |

4.2 Salinity Method

Salinity samples are measured on a high precision laboratory salinometer (Guildline Autosal 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 1atm of seawater to that of a potassium chloride (KCl) solution of mass fraction 32.4356×10^{-3} .

Before each batch of sample measurements, the Autosal is calibrated with standard seawater (OSIL, IAPSO) of known K_{15} ratio. A new bottle of OSIL solution is used for each calibration.

Method synopsis: Salinity samples are collected into 200ml OSIL bottles, filled from the bottom, via a 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 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. Further flush-measurement cycles are done where the initial values are more than 3 digits different. The Osil Data logger software captures the conductivity ratio and calculates the practical salinity.

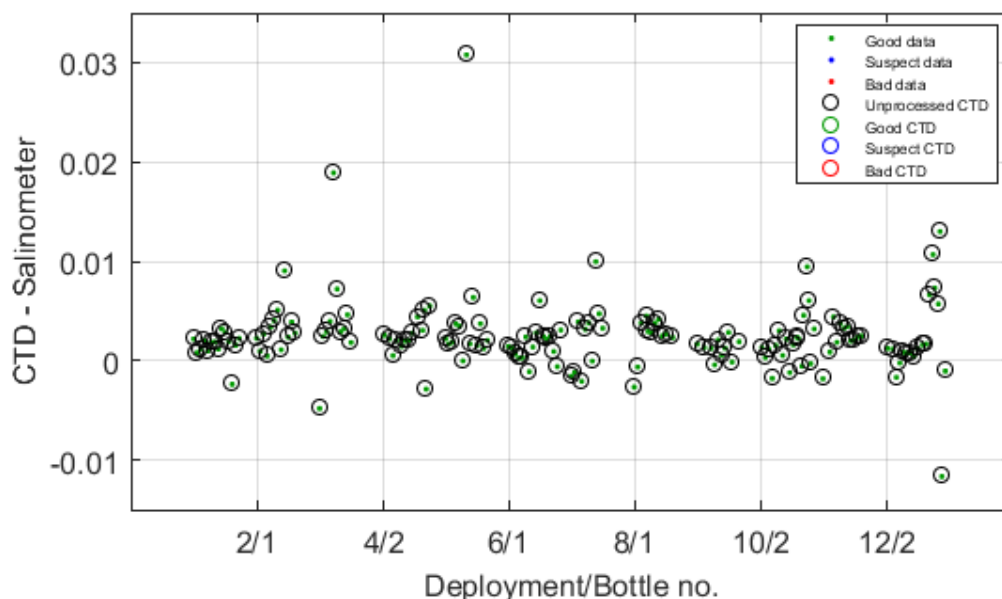
The output from the data logger software is imported into HyPro and collated with the CTD deployment meta-data.

4.3 CTD Salinity vs Bottle Salinity Plot

The difference between the unprocessed (uncorrected) CTD values and the measured bottle salinities is generally less than 0.01 PSU.

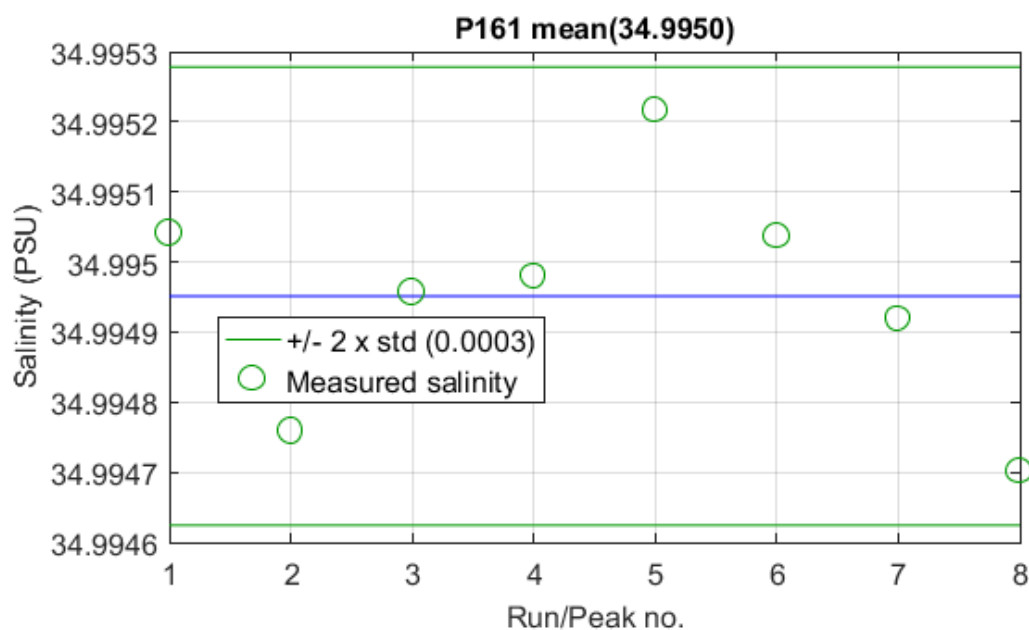
The unprocessed CTD values are adjusted (corrected) by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the DataLibrarians@csiro.au for corrected CTD data.

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



5.4 OSIL Salinity Standard PSU across the Voyage

Practical salinity (PSU) of P161 is 34.995, the blue line is the mean of all standards measured which were used to standardise the salinometers.



5.5 Missing or Suspect Salinity Data

Data is flagged based on notes from CTD sampling log sheet, observations during analysis, and examination of depth profile and waterfall plots.

HyPro classified the quality of data as good, no suspect or bad data to report.

5 Dissolved Oxygen Data Processing

5.1 Dissolved Oxygen Parameter Summary

| Details | |
|-------------------------|--|
| HyPro Version | 5.7 |
| Instrument | Automated Photometric Oxygen system (SIO) |
| Software | SCRIPPS |
| CSIRO Hydrochem. Method | Sampling: WI_DO_001 Assay: SOP005 |
| Accuracy | ± 0.5 µM |
| Analyst(s) | Stephen Tibben & Merinda McMahon |
| Lab Temperature (±1°C) | Variable, 20.19– 23.18°C, Average 21.35°C |
| Sample Container type | Pre-numbered 140 mL glass iodine determination flasks with glass stopper. 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. |

5.2 Dissolved Oxygen Method

SCRIPPS method used. The method is based on the whole-bottle modified Winkler titration of Carpenter (1965) plus modifications by Culberson *et al* (1991).

Method synopsis: The sample is collected in an iodine determination flask of known volume. 1mL of manganese (II) chloride solution followed by 1mL of alkaline iodide solution is added to the sample, the flask stoppered and inverted a minimum ten times. The dissolved oxygen oxidizes an equivalent amount of Mn (II) to Mn (IV) which precipitates. Just before titration, the sample is acidified, oxidizing 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 1ml 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 10ml aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two consecutive titres for 1ml aliquots of the same potassium iodate solution.

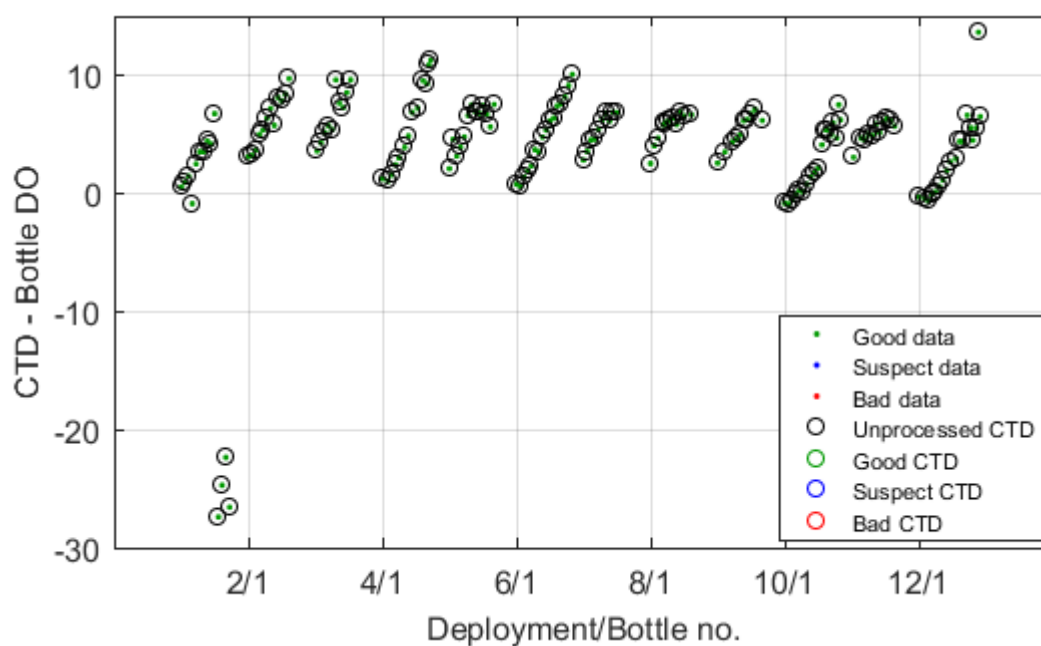
The output from the SIO instrument software is imported into HyPro and collated with the CTD deployment meta-data.

5.3 CTD Dissolved Oxygen vs Bottle Dissolved Oxygen Plot

The CTD values in this plot are unprocessed raw data.

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 DataLibrarians@csiro.au for corrected CTD data.

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



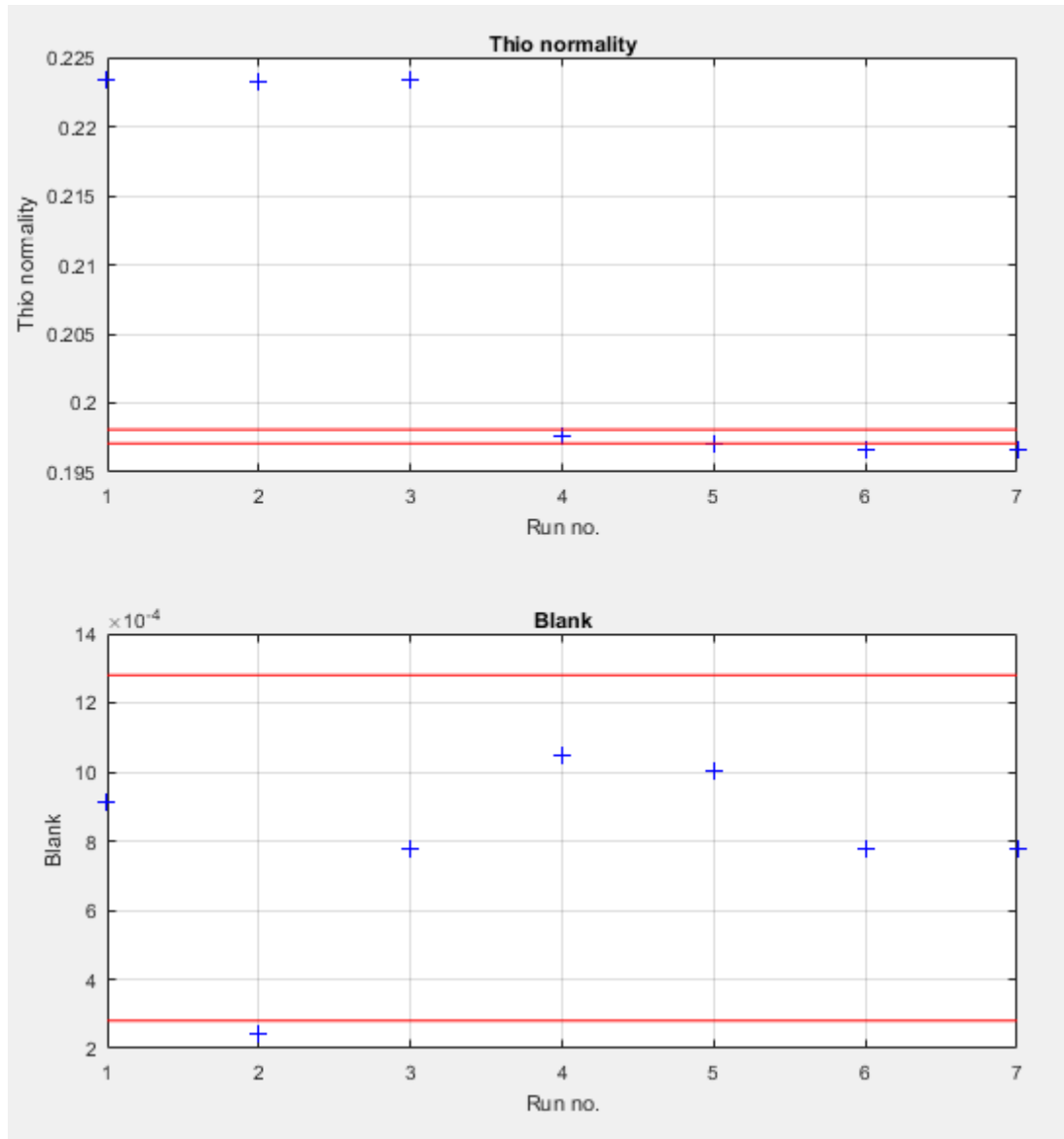
Also note that the large offsets in deployment 1 were due to sensor failure, not bad bottle samples.

5.4 Dissolved Oxygen Instrument titrant: thiosulphate normality and blank correction.

The normality of the thiosulphate titrant (0.2N) varied less than 0.0005 N for all dissolved oxygen sample titrations. The blank correction is less than 0.00012 ml.

Red lines indicate ± 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. The large difference between Run 3 and Run 4 corresponds to a change in thiosulfate.

For reference, titre volumes for dissolved oxygen bottle samples lay in the range 0.46 to 0.80 ml.



5.5 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.

There was no missing or suspect data for dissolved oxygen.

6 Nutrient Data Processing

6.1 Nutrient Assay Parameter Summary

| Details | | | | | |
|-----------------------------------|---|-----------|-------------------|---------|----------|
| CSIRO Software | HyPro 5.7 | | | | |
| Instrument | Seal AA3HR | | | | |
| Instrument Software | Seal AACE 6.10 | | | | |
| 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 ¹ | 0.02 µM | 0.02 µM | 0.02 µM | 0.02 µM |
| Matrix Corrections | none | none | none | none | none |
| Analysts | Christine Rees, Stephen Tibben & Merinda McMahon | | | | |
| Lab Temperature (±1°C) | Variable, 20.19– 23.18°C, Average 21.35°C | | | | |
| Reference Material | KANSO, RMNS lot CJ | | | | |
| Sampling Container type | CTD: 50ml HDPE with screw cap lids. | | | | |
| Sample Storage | < 2 hrs at room temperature | | | | |
| Pre-processing of Samples | CTD None. | | | | |
| Comments | RMNS lots CD, CC, & CB were also analysed sporadically throughout the voyage. | | | | |

6.2 Nutrient Methods

Please cite the following paper when using Hydrochemistry data for silicate, phosphate, nitrate+nitrite (NO_x) and nitrite analysis:

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 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 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-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 (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-naphthyl-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-phthalaldehyde method. Based on Roger K  rouel and Alain Aminot, IFREMER (1997 Mar.Chem.57). Ammonium reacted with ortho-phthalaldehyde 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.

6.3 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 _x) | Nitrite | Ammonia |
|--------------------------------------|---|----------------------|--------------------------------------|----------------------|----------------------|
| Data Reported as | μmol l ⁻¹ | μmol l ⁻¹ | μmol l ⁻¹ | μmol l ⁻¹ | μmol l ⁻¹ |
| 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 | LNSW (bulk on deck of Investigator) collected on 28/9/2016. Sub-lot passed through a 10 micron filter on the 15/10/2018 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 appendix 7.3. | | | | |

6.4 HyPro Data Processing Summary

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).
- Within 0.02 μM 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 appendix 7.2.

Missing or suspect nutrient data is tabulated in section 6.9, the flags are also in the final hydrology data set. The Flag key is in Appendix 7.4.

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

Japanese KANSO certified RMNS lot CJ was assayed in triplicate in each run to monitor accuracy. Lots CC, CD & CB were also analysed sporadically throughout the voyage. The certified values are in table 1.

For in2018_v06, the majority of RMNS results are within 1% of their certified mean and within 0.02 μM for nitrite. Plots of RMNS values for all runs are below.

The assayed RMNS values per Analysis run and CTD deployments are listed in appendix 7.3

The GO-SHIP criteria (Hyde *et al.*, 2010), appendix 7.5 specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy.

Table 1: RMNS concentrations with expanded uncertainty ($\mu\text{mol L}^{-1}$) at 21°C

| RMNS | NO_3 | NO_2 | $\text{NO}_3 + \text{NO}_2$ (NO_x) | PO_4 | SiO_4 |
|---------------|--------------------|-------------------|--|-------------------|---------------------|
| Lot CD | 5.629 ± 0.051 | 0.018 ± 0.004 | 5.647 ± 0.055 | 0.457 ± 0.008 | 14.264 ± 0.10 |
| Lot CC | 31.621 ± 0.246 | 0.119 ± 0.006 | 31.740 ± 0.252 | 2.130 ± 0.019 | 88.228 ± 0.492 |
| Lot CB | 36.649 ± 0.276 | 0.119 ± 0.006 | 36.768 ± 0.282 | 2.580 ± 0.022 | 111.821 ± 0.635 |
| Lot CJ | 16.588 ± 0.205 | 0.032 ± 0.007 | 16.620 ± 0.212 | 1.219 ± 0.020 | 39.424 ± 0.410 |

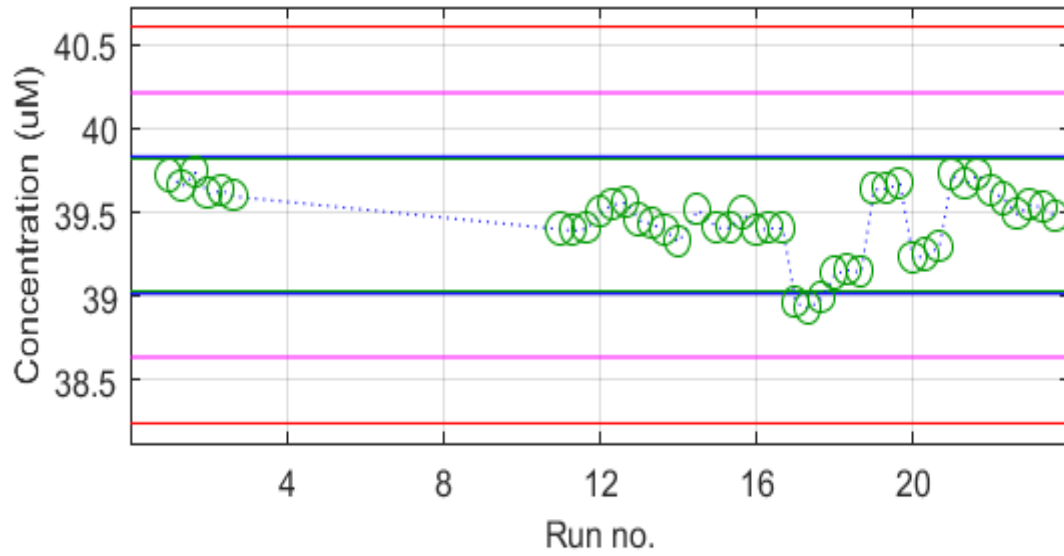
KANSO publishes the RMNS nutrient values in $\mu\text{mol kg}^{-1}$. These are converted to $\mu\text{mol l}^{-1}$ at 21°C. RMNS does not have a certified value for ammonium (NH_4). NO_x is derived by adding the NO_3 and NO_2 values.

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.

Please note: there is only RMNS values for phosphate runs 4 to 10 as these were experiments conducted only for phosphate and not part of the voyage samples.

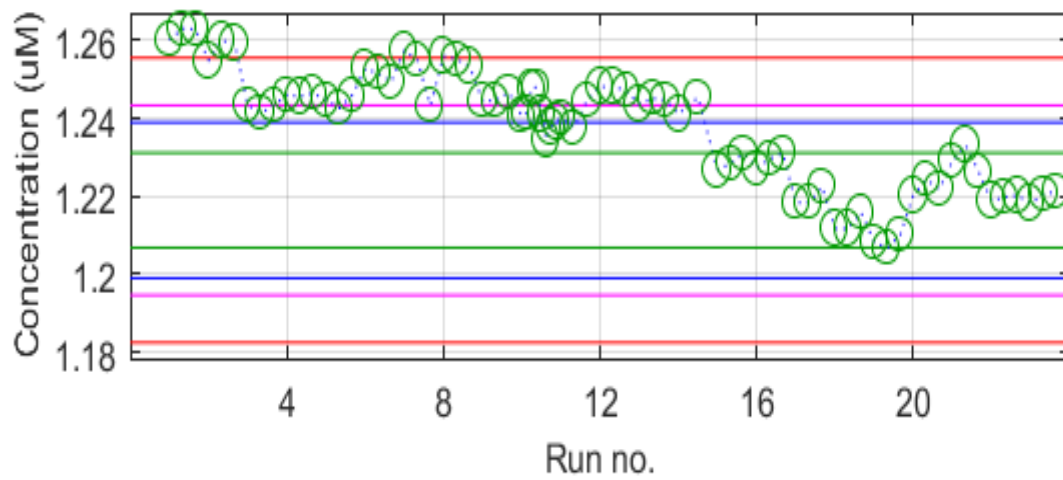
6.5.1 Silicate RMNS Plot

Silicate RMNS (15 runs) for CJ (39.42)
Overall mean 39.45 ± 0.21



6.5.2 Phosphate RMNS Plot

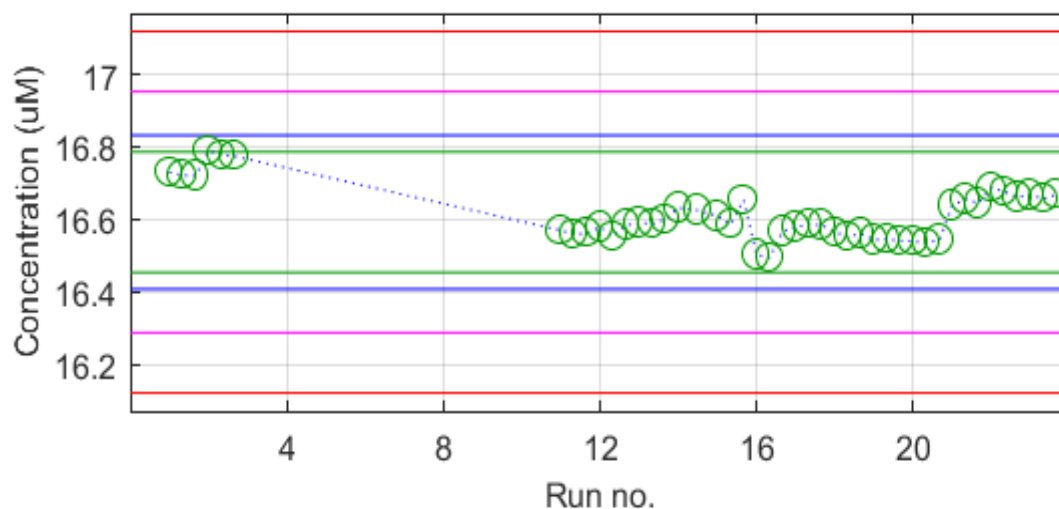
Phosphate RMNS (23 runs) for CJ (1.219)
Overall mean 1.238 ± 0.014



6.5.3 Nitrate + Nitrite (NO_x) RMNS Plot

NO_x RMNS (15 runs) for CJ (16.62)

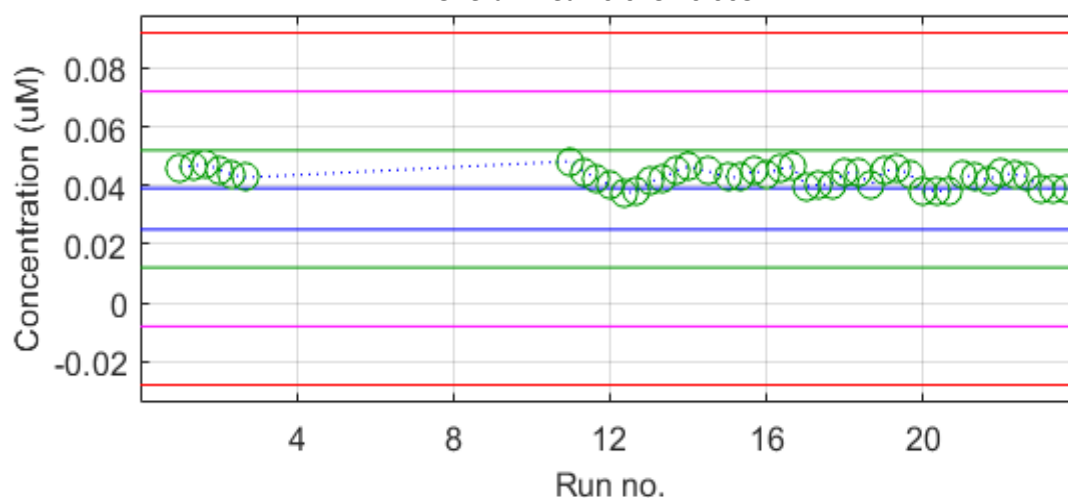
Overall mean 16.61 ± 0.07



6.5.4 Nitrite RMNS Plot

Nitrite RMNS (15 runs) for CJ (0.032)

Overall mean 0.043 ± 0.003



6.6 Analytical Precision

6.6.1 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 (NO _x) | Nitrite | Ammonia |
| ± 0.017 | ± 0.024 | ± 0.019 | ± 0.14 | $\pm 0.30^{\text{§}}$ |

*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.

6.6.2 Nutrient Method Detection Limit

For in2018_v06, the measured detection limits for each run are much lower than the nominal detection limits, indicating high analytical precision at lower concentrations.

| MDL | Silicate | Phosphate | Nitrate + Nitrite (NO _x) | Nitrite | Ammonia |
|---------------------------------|-------------|--------------|--------------------------------------|--------------|--------------|
| Nominal MDL* | 0.20 | 0.02 | 0.02 | 0.02 | 0.02 |
| Standard Dev. Min | 0.01 | 0.001 | 0.004 | 0.00 | 0.002 |
| Standard Dev. Max | 0.22 | 0.042 | 0.019 | 0.005 | 0.004 |
| Standard Dev. Mean | 0.06 | 0.006 | 0.009 | 0.003 | 0.003 |
| Standard Dev. Median | 0.04 | 0.004 | 0.007 | 0.003 | 0.003 |
| Precision of MDL (stdev) | 0.05 | 0.008 | 0.004 | 0.001 | 0.001 |

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

6.6.3 Reference Material for Nutrients in Seawater

Precision values are calculated from intra-analysis measurements, multiple measurements are taken at a time, typically 3-4.

| RMNS CJ | Silicate | Phosphate | Nitrate + Nitrite (NO _x) | Nitrite | Ammonia |
|--|----------------|----------------|--------------------------------------|-----------------|---------|
| Published RMNS CJ (μmol l⁻¹) w/std deviation | 39.42 ±0.41 | 1.219 ±0.21 | 16.62 ±0.21 | 0.032 ±0.007 | - - |
| Minimum | 38.96 | 1.208 | 16.52 | 0.038 | 0.80 |
| Maximum | 39.71 | 1.262 | 16.78 | 0.047 | 1.17 |
| Mean | 39.45 | 1.238 | 16.61 | 0.043 | 0.92 |
| Median | 39.44 | 1.243 | 16.59 | 0.043 | 0.94 |
| Precision (Stdev) | 0.21 | 0.014 | 0.07 | 0.003 | 0.09 |

| RMNS CD | Silicate | Phosphate | Nitrate + Nitrite (NO _x) | Nitrite | Ammonia |
|--|------------------|-----------------|---|------------------|---------|
| Published RMNS CD (μmol l⁻¹) w/std deviation | 14.26 ± 0.009 | 0.46 ± 0.001 | 5.65 ± 0.004 | 0.018 ± 0.001 | - - |
| Minimum | 14.27 | 0.445 | 5.51 | 0.031 | 1.55 |
| Maximum | 14.63 | 0.483 | 5.64 | 0.037 | 2.11 |
| Mean | 14.42 | 0.466 | 5.52 | 0.034 | 1.79 |
| Median | 14.39 | 0.467 | 5.51 | 0.033 | 1.76 |
| Precision (Stdev) | 0.16 | 0.013 | 0.07 | 0.003 | 0.24 |

| RMNS CC | Silicate | Phosphate | Nitrate + Nitrite (NO _x) | Nitrite | Ammonia |
|--|----------------|-----------------|---|-----------------|---------|
| Published RMNS CC (μmol l⁻¹) w/std deviation | 88.23 ±0.49 | 2.130 ±0.019 | 31.74 ±0.25 | 0.119 ±0.006 | - - |
| Minimum | 87.85 | 2.105 | 31.81 | 0.134 | 1.69 |
| Maximum | 88.84 | 2.197 | 32.18 | 0.135 | 1.94 |
| Mean | 88.50 | 2.163 | 31.81 | 0.135 | 1.80 |
| Median | 88.82 | 2.186 | 32.00 | 0.135 | 1.78 |
| Precision (Stdev) | 0.56 | 0.050 | 0.22 | 0.001 | 0.13 |

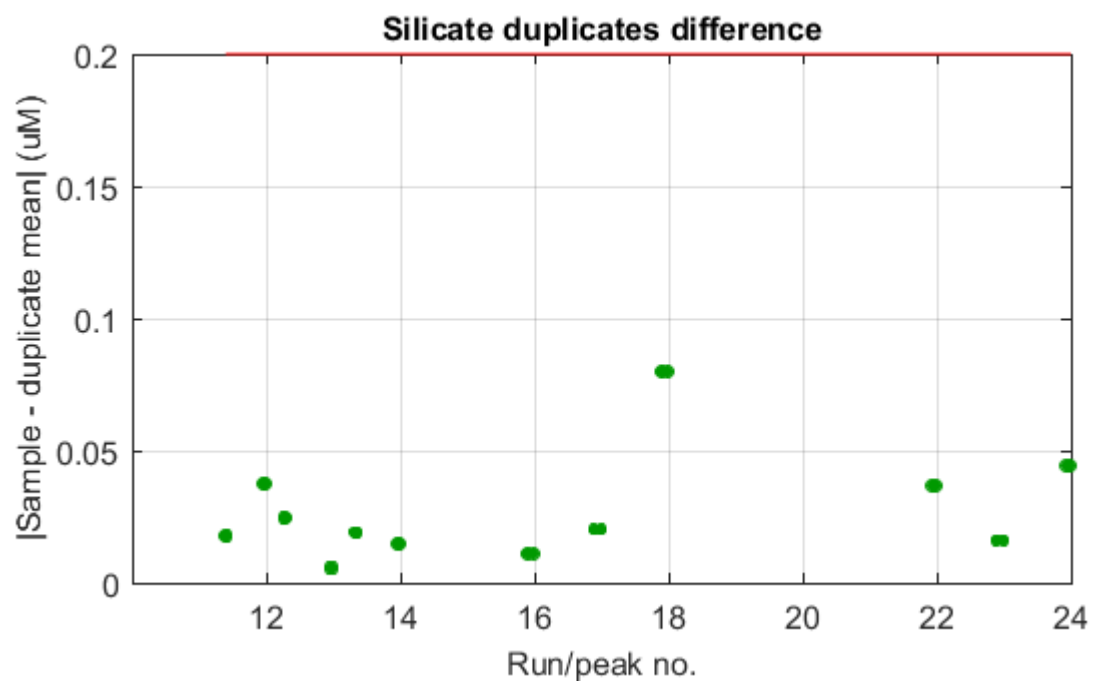
6.7 Sampling Precision

Sampling precision is monitored by assaying duplicate samples collected from the greatest depth for each CTD deployment. The sampling precision is good if the difference between the duplicate concentrations is less than the MDL value. The exception is nitrate+nitrite, which uses 0.06 μM as the MDL boundary.

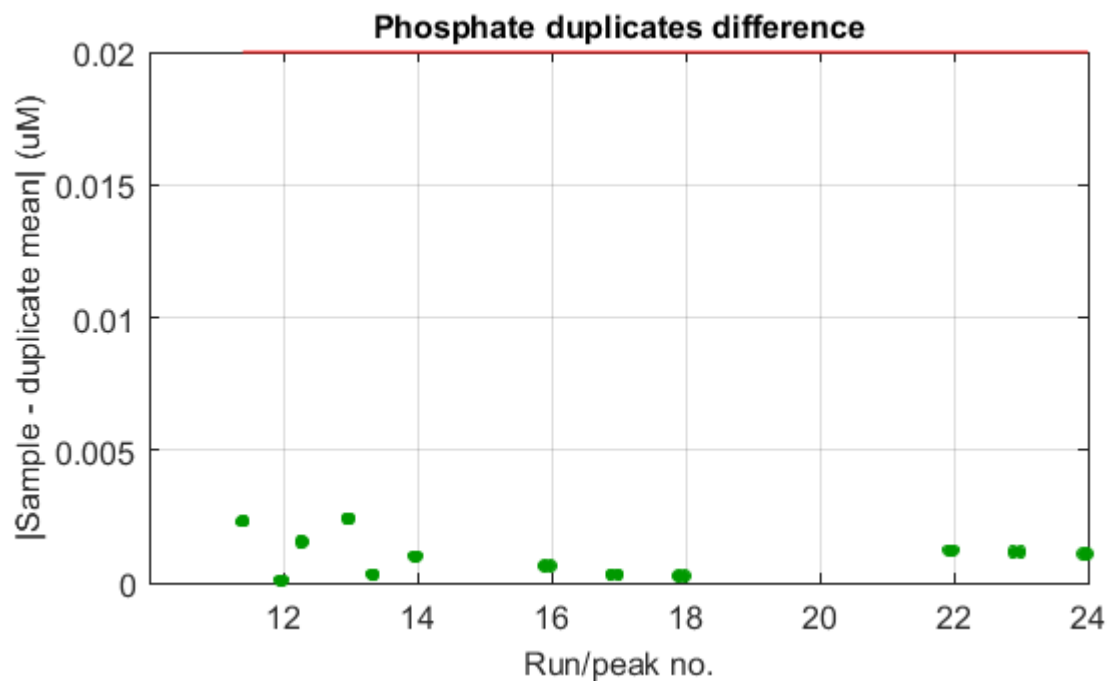
Plots of the difference between the duplicate and their mean for the CTD deployments are below. The red line is the boundary below which sampling precision is deemed good.

For in2018_v06, the sampling precision is good.

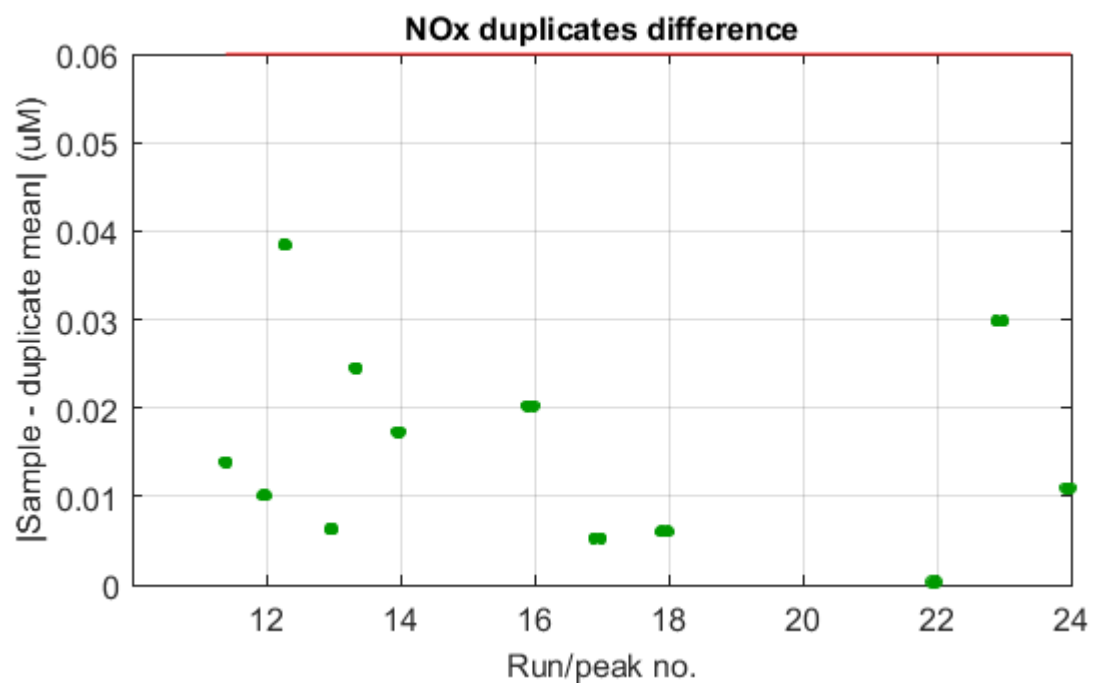
6.7.1 Silicate Duplicates Plot



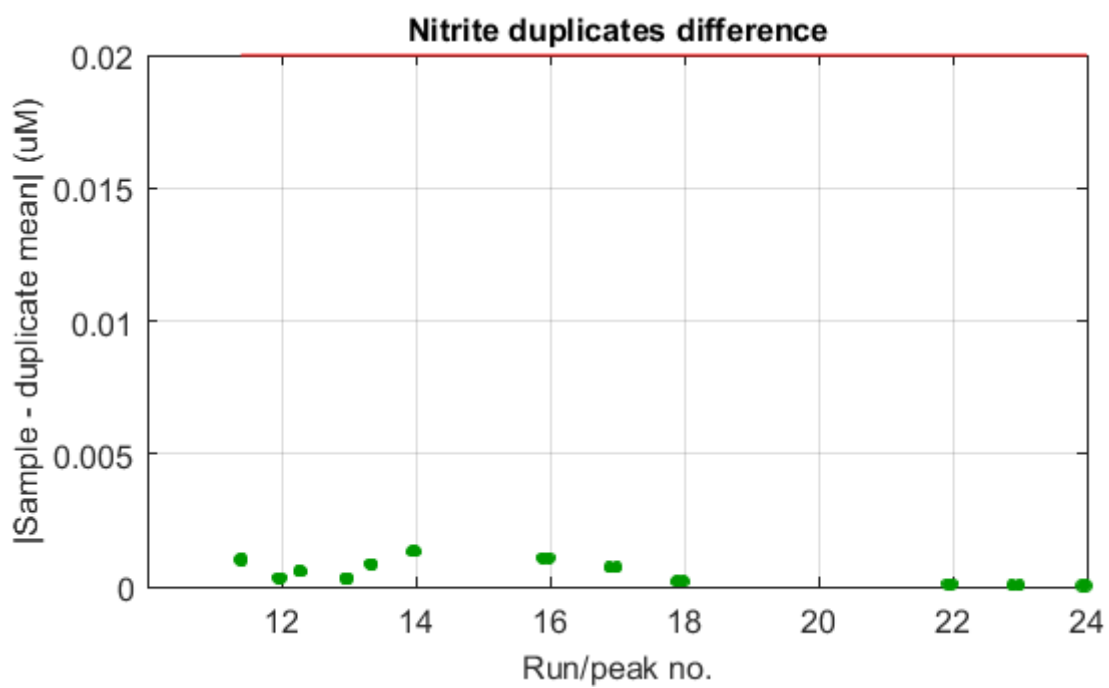
6.7.2 Phosphate Duplicates Plot



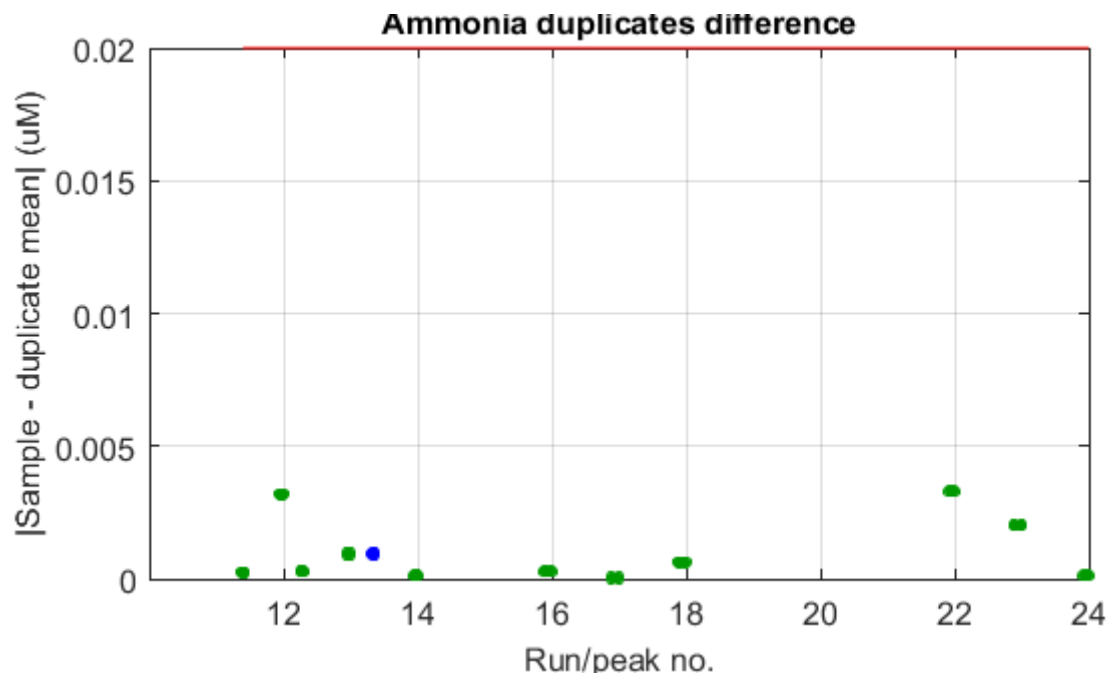
6.7.3 Nitrate + Nitrite (NO_x) Duplicates Plot



6.7.4 Nitrite Duplicates Plot

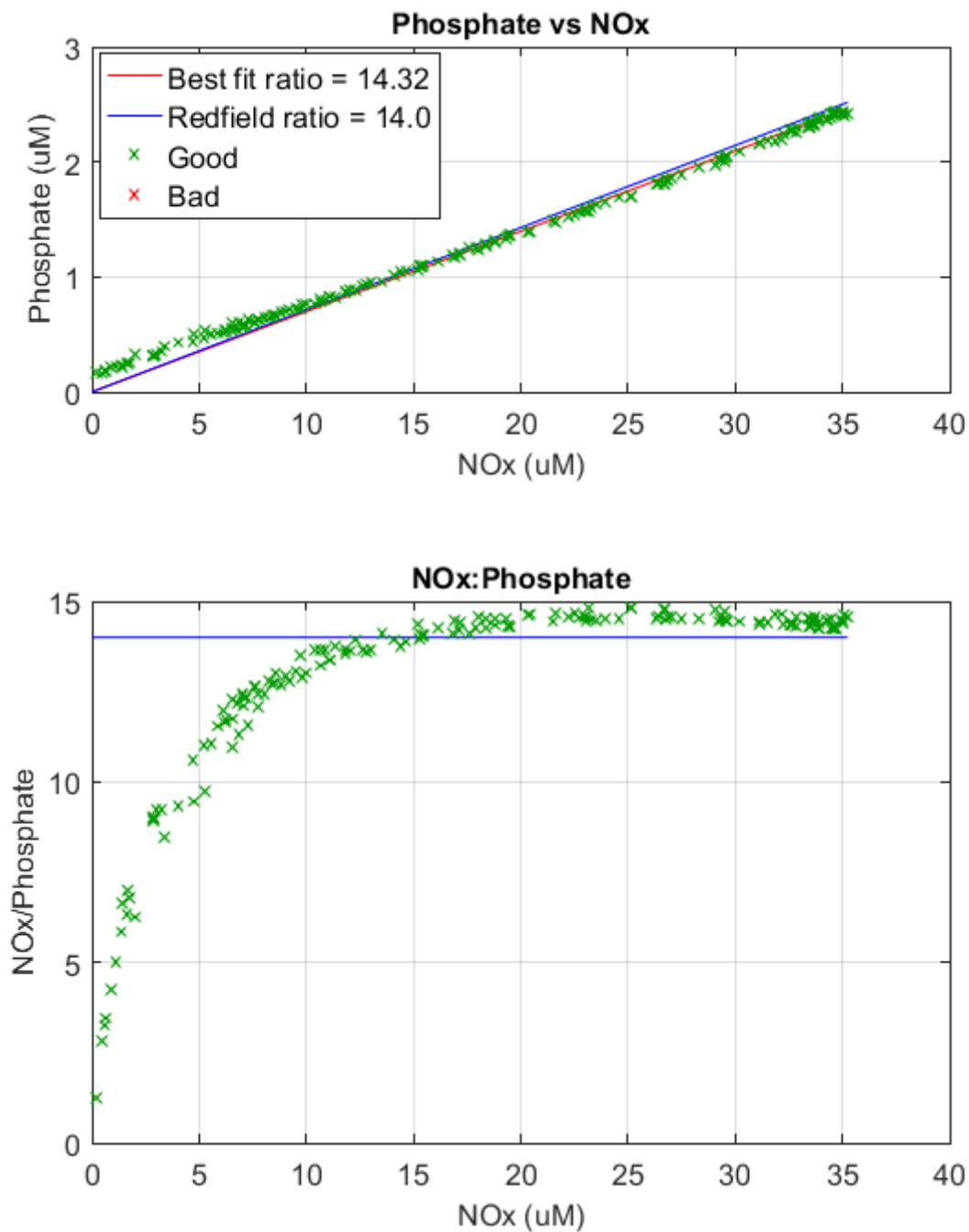


6.7.5 Ammonia Duplicates Plot



6.8 Redfield Ratio Plot (14.0) for CTD Deployments.

Plots consist of phosphate versus NOx for all CTD deployments. Best fit ratio = 14.32



6.9 Missing or Suspect Nutrient Data.

The table below identifies all flagged data and any samples that had repeated analyses performed to obtain good data. Data that falls below the detection limit, Flag 63, is not captured in this table. All GOOD data is flagged 0 in the .csv and .netcdf files. Data that is flagged BAD is not exported to the .csv files. Flag Key in Appendix 7.4.

| CTD | RP | Run | Flag | Reason for Flag or Action |
|-----|----------|-----|------|---|
| 5 | 1, 3, 11 | 13 | 133 | Uncharacteristic and high values for Ammonia samples, suggesting contamination. |
| 12 | 4, 8 | 23 | 133 | Uncharacteristic and high values for Ammonia samples, suggesting contamination. |

6.10 Temperature & Humidity Change over Nutrient Analyses

The temperature and humidity within the AA3 chemistry module was logged using a temperature/humidity logger QP6013 (Jaycar) placed on the deck of the chemistry module, refer to in2018_v06_hyd_voyagereport.docx for this data.

Nutrient samples were placed on the XY3 auto sampler at the average room temperature of 21.35°C. Refer to “in2018_v06_hyd_voyagereport.docx” for room temperature graphs.

The laboratory temperature was measured and recorded on the nutrient run sheets at the start each analysis run. The temperature varied between 20.19 and 23.18°C over the course of the voyage.

7 Appendix

7.1 Salinity: Reference Material Used

| Osil IAPSO Standard Seawater | |
|------------------------------|------------|
| Batch | P161 |
| Use by date | 03/05/2020 |
| K ₁₅ | 0.99987 |
| PSU | 35.995 |

7.2 Nutrients: Flagged Calibration and Quality Control Data

HyPro classifies the quality of data as good, suspect or bad and flags accordingly.

| CTD | Peak | Run | Analysis | Reason for Flag or Action |
|-------|-----------|--------|----------|---|
| 1 & 2 | Cal 1 | Nut011 | NH4 | Both points bad, not used in calibration curve. |
| 3 & 4 | Cal 4 | Nut012 | NH4 | Both points suspect, less weighting in calibration curve. <70% of calibration peaks are within calibration limits. Cal 2 & 3 suspect less weighting in calibration curve. |
| 5 & 6 | Cal 2 | Nut013 | PO4 | 1 st point suspect, less weighting in calibration curve. |
| 5 & 6 | Cal 3 | Nut013 | NH4 | Both points suspect, less weighting in calibration curve. |
| 8 | Cal 1 & 6 | Nut016 | NOx | Cal 1 1 & Cal 6 2 nd point suspect, less weighting in calibration curve. |
| 9 | Cal 1 & 4 | Nut017 | NH4 | Both points are suspect, less weighting in the calibration. |

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

7.3.1 RMNS Lot CJ Results

| Analysis Run | CTD # | Silicate | Phosphate | Nitrite | NOx (NO2 + NO3) |
|---------------------|-------|----------|-----------|---------|-----------------|
| <i>CJ certified</i> | - | 39.424 | 1.219 | 0.0320 | 16.621 |
| 1 | - | 39.703 | 1.262 | 0.047 | 16.724 |
| 2 | - | 39.610 | 1.258 | 0.044 | 16.780 |
| 3 | - | - | 1.243 | - | - |
| 4 | - | - | 1.246 | - | - |
| 5 | - | - | 1.244 | - | - |
| 6 | - | - | 1.251 | - | - |
| 7 | - | - | 1.252 | - | - |
| 8 | - | - | 1.255 | - | - |
| 9 | - | - | 1.245 | - | - |
| 10 | - | - | 1.241 | - | - |
| 11 | 1 & 2 | 39.396 | 1.241 | 0.045 | 16.564 |
| 12 | 3 & 4 | 39.533 | 1.248 | 0.038 | 16.572 |
| 13 | 5 & 6 | 39.424 | 1.244 | 0.043 | 16.593 |
| 14 | - | 39.417 | 1.243 | 0.046 | 16.629 |
| 15 | 7 | 39.435 | 1.229 | 0.043 | 16.616 |
| 16 | 8 | 39.399 | 1.229 | 0.045 | 16.521 |
| 17 | 9 | 38.958 | 1.220 | 0.040 | 16.583 |
| 18 | - | 39.142 | 1.213 | 0.043 | 16.559 |
| 19 | - | 39.648 | 1.208 | 0.045 | 16.544 |
| 20 | - | 39.253 | 1.222 | 0.038 | 16.540 |
| 21 | 10 | 39.704 | 1.229 | 0.043 | 16.646 |
| 22 | 11 | 39.563 | 1.220 | 0.044 | 16.675 |
| 23 | 12 | 39.514 | 1.220 | 0.039 | 16.665 |

The submitted 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

7.4 Flag Key for Hydrology Data Set

| 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. |

7.5 GO-SHIP Specifications

7.5.1 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 recorded².

7.5.2 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.

7.5.3 SiO₂

Approximately 1-3% accuracy¹, 0.2% precision³, full scale.

7.5.4 PO₄

Approximately 1-2% accuracy¹, 0.4% precision³, full scale.

7.5.5 NO₃

Approximately 1% accuracy¹, 0.2% precision³, full scale.

7.5.6 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.

8 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 Research*, 14, pp.381-389.
- 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.
- K  rouel, Roger and Alain Aminot. (1997) Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. *Journal of Marine Chemistry* 57 pp. 265-275.
- Murphy, J. And Riley, J.P. (1967) A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Anal.Chim.Acta*, 27, p.30, (1962)
- 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* Doi:10.1002/lom3.10294
- Wood, E.D., F.A.J. Armstrong, and F.A. Richards. (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.