RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESS REPORT

Voyage:	IN2017_C01
Chief Scientist:	Asra Talukder
Voyage title:	GAB Deep Water Geological and Benthic Ecology Program
Report compiled by:	Cassie Schwanger

Contents

1   Itinerary

Depart Leg 1	Date	Time
Hobart	11/4/2017	0800
Arrive	Date	Time
Hobart	28/4/2017	0800

2   Key personnel list

Name	Role	Organisation
Asra Talukder	Chief Scientist	CSIRO
Tegan Sime	Voyage Manager	CSIRO
Cassie Schwanger	Hydrochemist	CSIRO

3   Summary

All finalized data can be obtained from the CSIRO data centre Contact: DataLibrariansOAMNF@csiro.au.

3.1   Hydrochemistry

Analysis	Sampled
Salinity (Guildline Salinometer)	30 CTD
Dissolved Oxygen (automated titration)	27 CTD
Nutrients (AA3)	30 CTD

Note:

- CTD; samples collected from NISKIN bottles on CTD rosette.

3.2   Rosette and CTD

- 6 CTD stations were sampled with a 36 bottle rosette (12 L) – not all bottles were fired.
- Deployments 3-5 & 7 were aborted

- See in2017_c01_HYD_VoyageReport.pdf (voyage report) for more details on sample collection.

3.3 Data Procedure Summary

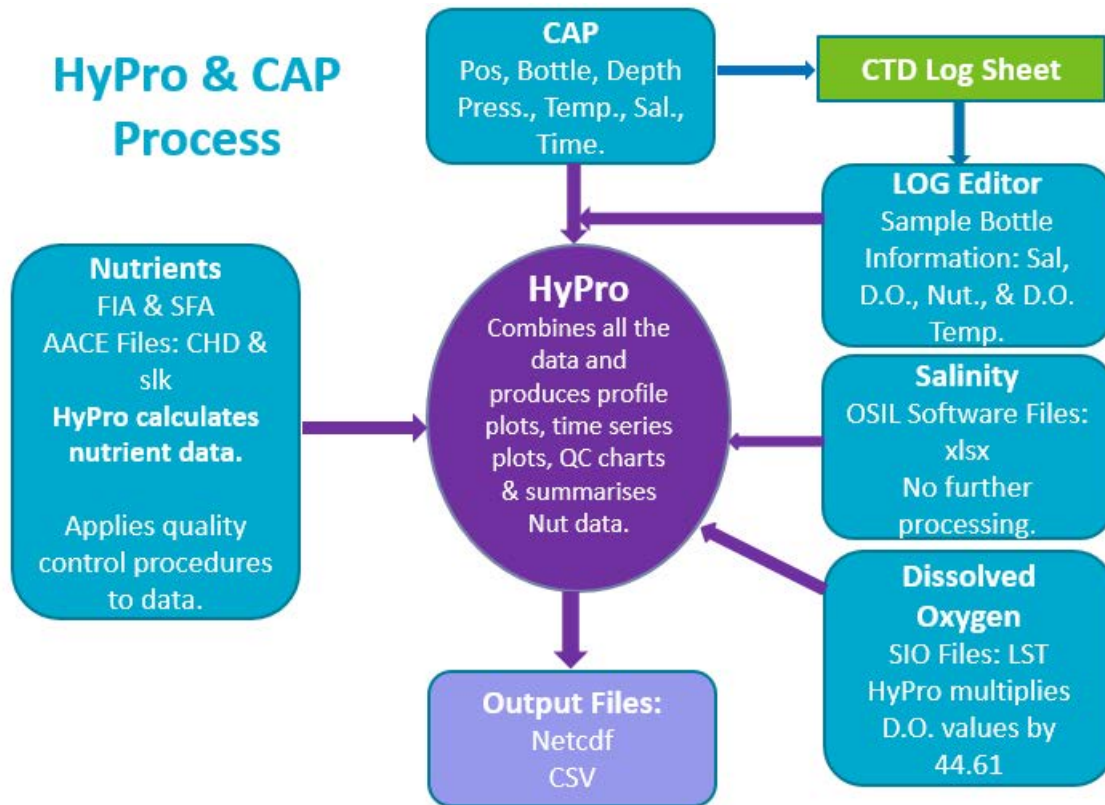The procedure for data processing is outlined in Figure 1.

Figure 1: The processing steps for hydrology data following sample assay.

4 Salinity Data Processing

4.1 Salinity Parameter Summary

| Details	
HyPro Version	4.17
Instrument	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71611
Software	OSIL Data Logger ver 1.2
Methods	Hydrochemistry Operations Manual + Quick Reference Manual
Accuracy	± 0.001 practical salinity units
Analyst(s)	Cassie Schwanger
Lab Temperature (±0.5°C)	21.0 -22.5.0°C during analysis.
Bath Temperature	23.996°C
Reference Material	Osil IAPSO - Batch P158 (see appendix 7.1)
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 held in Salt Room for 24 hrs to reach 22°C before analysis
Comments	All bottles were washed in Miele dishwasher and stored dry with clean caps at the end of the voyage to prevent biofouling/algal growth.

4.2 Salinity Method

The method uses 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 x 10^{-3}.

The Autosal is calibrated with standard seawater (OSIL, IAPSO) of known conductivity ratio against which the samples are measured. The Autosal is calibrated before each batch run of samples.

Salinity samples are collected into 200ml OSIL bottles –from the bottom via a PTFE straw filled till overflowing. The sample is decanted to allow a headspace of approximately 25cm^3. A plastic insert is fitted, the bottle inverted and rinsed then capped and stored cap-down until measured. To measure, the salinometer 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 conductivity ratio data is captured by the Osil data logger v1.2 program which then calculates the practical salinity.

4.3 CTD vs Hydro Salinities Plot
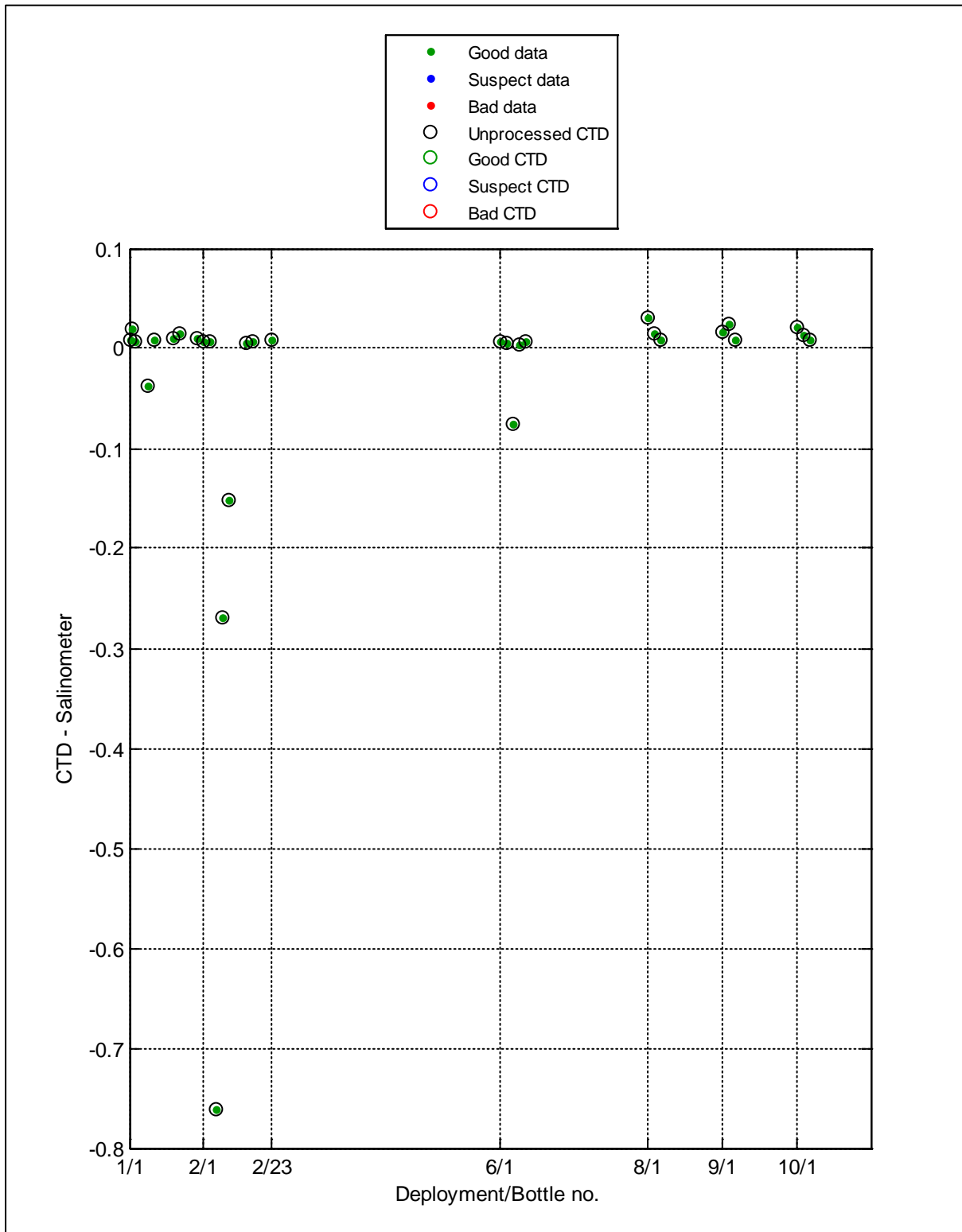
4.4 Missing or Suspect Salinity Data and Actions taken

Data is flagged based on notes from CTD sampling log sheet, observations during analysis, and examination of depth profile and waterfall plots. No salinity samples were marked suspect or missing.

5 Dissolved Oxygen Data Processing

5.1 Dissolved Oxygen Parameter Summary

Details	
HyPro Version	4.17
Instrument	Automated Photometric Oxygen system
Software	SCRIPPS
Methods	SCRIPPS
Accuracy	0.01 ml/L + 0.5%
Analyst(s)	Cassie Schwanger
Lab Temperature (±1°C)	Variable, 20.0 - 23.0°C
Sample Container type	Pre-numbered glass 140 mL glass vial w/stopper, sorted into 18 per box and boxes labelled A to S.
Sample Storage	Samples were stored within Hydrochemistry lab under the forward starboard side bench until analysis. All samples were analysed within ~48 hrs
Comments	Surface bottles were not collected for analysis

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).

Manganese chloride followed by alkaline iodide, is added to the sample, and the precipitated manganous hydroxide is distributed evenly throughout the bottle by shaking. At this stage, the dissolved oxygen oxidizes an equivalent amount of Mn(II) to Mn(IV). Just before titration, the sample is acidified, converting the Mn (IV) back to the divalent state liberating an amount of Iodine equivalent to the original dissolved oxygen content of the water. The Iodine is auto-titrated with a standardised thiosulphate solution using a Metrohm 665 Dosimat 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.

The thiosulphate solution is standardised by titrating a 10ml aliquot of potassium iodate primary standard. The blank correction is determined from the difference between two consecutive titres for 1ml aliquots of the same potassium iodate solution.

5.3   CTD vs Hydro DO Plot
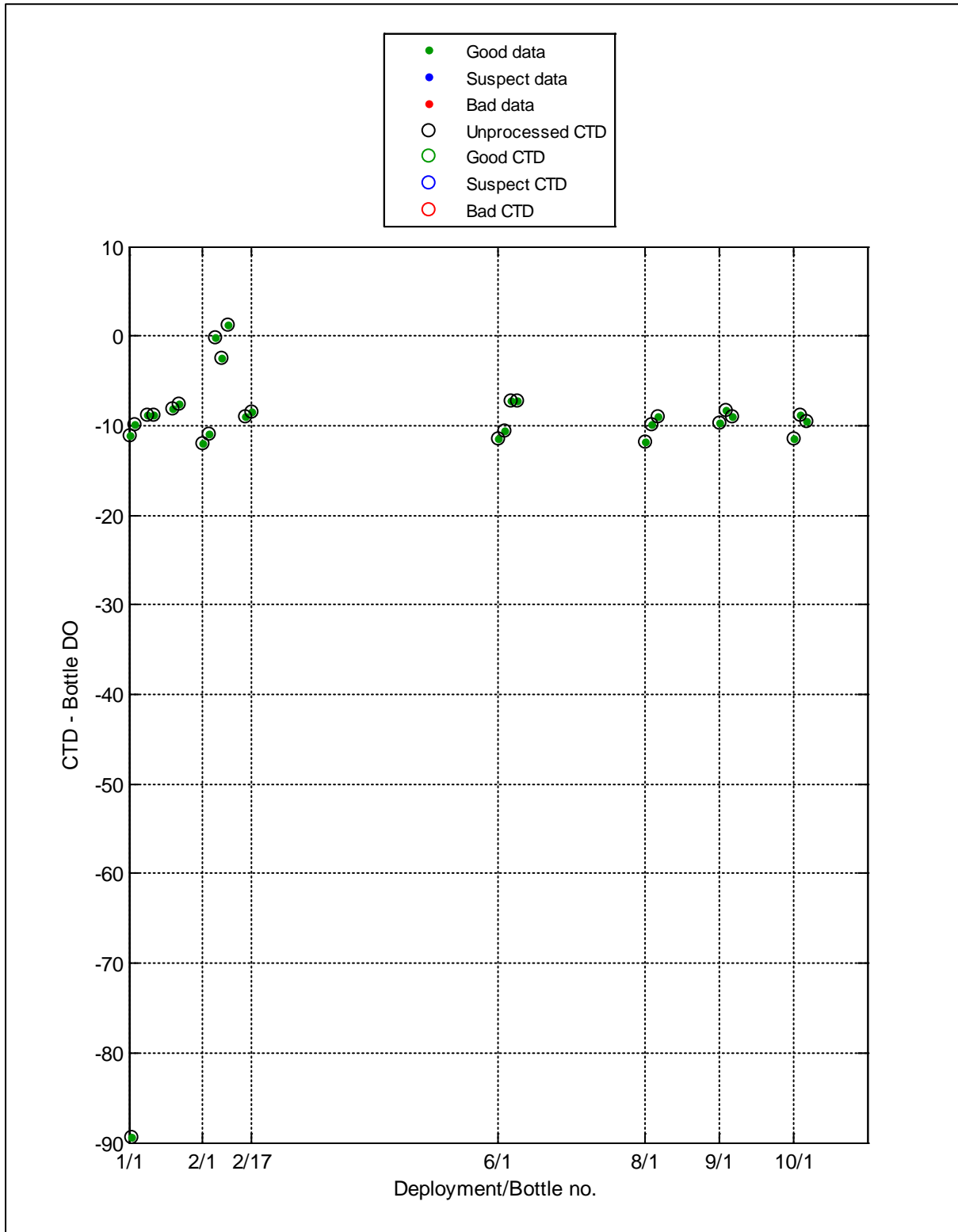
5.4   Dissolved Oxygen thiosulphate normality across voyage
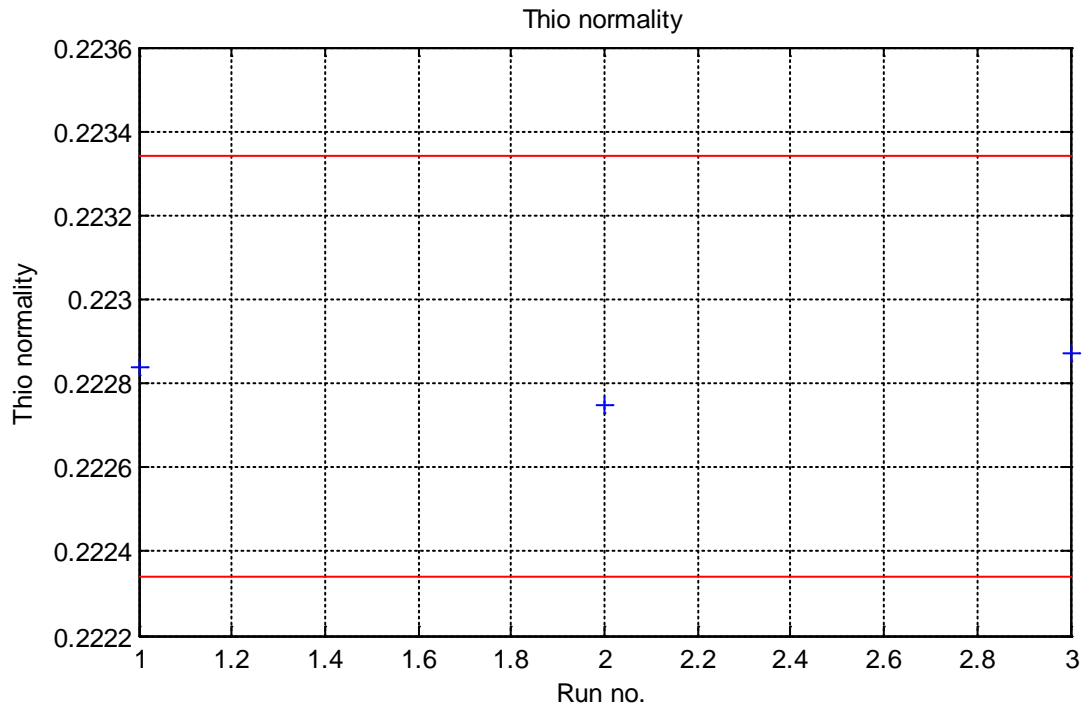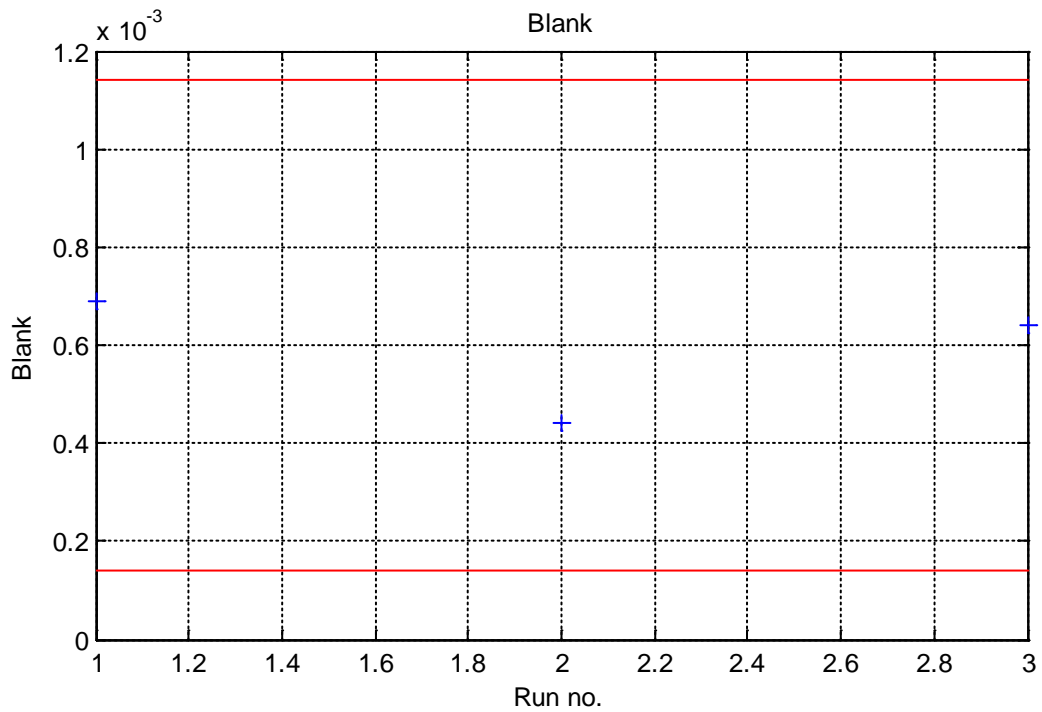

5.5   Dissolved Oxygen blank concentration across voyage

5.6   Missing or Suspect Dissolved Oxygen Data and Actions taken

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. No dissolved oxygen samples are missing or suspect.

6   Nutrient Data Processing

6.1   Nutrient Parameter Summary

Details					
HyPro Version	4.17				
Instrument	AA3				
Software	Seal AACE 6.10				
Methods	AA3 Analysis Methods internal manual				
Nutrients analysed	☒ Silicate	☒ Phosphate	☒ Nitrate + Nitrite	☒ Nitrite	☒ Ammonia
Concentration range	140 µmol l^{-1}	3 µmol l^{-1}	42.0 µmol l^{-1}	1.4 µmol l^{-1}	2.0 µmol l^{-1}
Method Detection Limit* (MDL)	0.2 µmol l^{-1}	0.02 µmol l^{-1}	0.02 µmol l^{-1}	0.02 µmol l^{-1}	0.02 µmol l^{-1}
Matrix Corrections	N	N	N	N	N
Analyst(s)	Cassie Schwanger				
Lab Temperature (±1°C)	Variable, 20.0 – 23.0°C				
Reference Material	RMNS – CC, BW, CD				
Sampling Container type	30 ml polypropylene sample tubes				
Sample Storage	< 2 hrs at room temperature or ≤ 18 hrs @ 4°C				
Pre-processing of Samples	None				
Comments	Frozen and refrigerated samples from in2017_v02 were analysed as experimental samples.				

6.2   Nutrient Methods

CSIRO Oceans and Atmosphere Hydrochemistry nutrient analysis is performed with a segmented flow auto-analyser – Seal AA3 HR – to measure silicate, phosphate, nitrite, nitrate plus nitrite (NOx), and ammonium

Silicate: 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: 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 molybate. It is then reduced by ascorbic acid and its absorbance is measured at 880nm.

Nitrate: colourimetric analysis, Cu-Cd reduction – Naphthylenediamine photometric method. Based on Wood et.al (1967). Nitrate is reduced to nitrite by first adding an ammonium chloride buffer then sending it through a copper - cadmium column. Sulphanilamide is added under acidic conditions to form a diazo compound. This compound is coupled with 1-N-naphthly-ethylenediamine di-hydrochloride to produce a reddish purple azo complex and its absorbance is measured at 520 nm.

Nitrite: colourimetric analysis, Naphthylenediamine photometric method. As per nitrate method without the copper cadmium reduction column and buffer.

Ammonium: fluorescence analysis, ortho-phtaldiadehyde method. Based on Roger Kérouel and Alain Aminot, IFREMER (1997 Mar.Chem.57). Ammonium reacted with ortho-phtaldialdehyde and sulfite at a pH of 9.0-9.5 to produce an intensely fluorescent product. Its emission is measured at 460nm after excitation at 370nm.

Detailed SOPs can be obtained from the CSIRO Oceans and Atmosphere Hydrochemistry Group on request.

6.3   Instrument Calibration and Data Parameter Summary

All instrument parameters and reagent batch compositions are logged for each analysis run. This information is available on request.

The raw data from each analysis run on the Seal AA3HR is imported into HyPro for peak height determination, constructing the calibration curve, deriving the sample results and applying drift and carry-over corrections. The calibration curve is fitted to the standards by performing several passes over each standard point and weighting its contribution to the curve depending on the magnitude of the difference between its measured and calculated value. The larger the difference, the less weighting is given to the standard's contribution towards the curve construction. The cut-off limits for good calibration data are

- ±0.5% of the concentration of the top standard for silicate and nitrate+nitrite (as per WOCE).

- 0.02uM for phosphate, nitrite and ammonium.

Following standard procedures, the operator may choose to not include bad calibration points (see section 6.7 for edited data). Below are the corrections and settings that HyPro applied to the raw data.

All runs have a corresponding "AA3_Run_Analysis_sheet" to record the following: sample details, LNSW batch, cadmium column, working standards, reagent information, instrumentation settings, and pump tube hours. The NUT### file numbers that correspond to each analytical run and the details of the samples analysed are in "Nut_AACE_LogLin2017_v02.doc". These files along with calibration summary data for each analysis run are in the voyage documentation and available upon request.

Result Details	Silicate	Phosphate	Nitrate + Nitrite	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
Forced through zero?	N	N	N	N	N
# of points in Calibration	6	6	6	6	6
Matrix Correction	N	N	N	N	N
Blank Correction	N	N	N	N	N
Carryover Correction (Hypro)	Y	Y	Y	Y	Y
Baseline Correction (Hypro)	Y	Y	Y	Y	Y
Drift Correction (Hypro)	Y	Y	Y	Y	Y
Data Adj for RMNS	N	N	N	N	N
Window Defined*	HyPro	HyPro	HyPro	HyPro	HyPro
Medium of Standards	LNSW (bulk on deck of Investigator) collected on 28/9/2016. Sub-lot passed through a 10 micron filter and stored in 20 L carboys in the hydrochemistry laboratory at 22°C.				
Medium of Baseline	18.2 Ω MQ				
Proportion of samples in duplicate?	The deepest sample was always collected in duplicate for error in sampling precision.				
Comments	Calibration and QC data that was edited or removed is located in the table in section 6.7. The reported data is not corrected to the RMNS. Per deployment RMNS data can be found in appendix 7.4.				

6.4 Accuracy - Reference Material for Nutrient in Seawater (RMNS) Plots

Japanese KANSO certified reference materials (RMNS) for silicate, phosphate, nitrate and nitrite in seawater was used in each nutrient analysis run to determine the accuracy. For each analysis run, a new RMNS bottle was opened and used. The RMNS was assayed in quadruplicate after the calibration standards.

RMNS lots BW, CC and CD were used. Their stated values in μ mol/kg are converted to μ mol l^{-1} at 21°C and are listed below. RMNS do not have certified ammonium values.

Table 1: RMNS BW, CC and CD concentrations with expanded uncertainty (µmol/L) at 21°C

RMNS	NO_3	NO_X	NO_2	PO_4	SiO_4
BW	25.18 ± 0.21	25.25 ± 0.22	0.069 ± 0.010	1.58 ± 0.01	61.45 ± 0.43

**The submitted nutrient results do <u>NOT</u> have RMNS corrections applied.**

RMNS Correction

% error = (RMNS measured – RMNS Published)/RMNS Published

Corrected Nutrient Concentration = Nutrient measured – (nutrient measured x error)

Note: NOx data should be corrected as NO_3 and NO_2.

The following plots show RMNS values within 1% (green lines), 2% (pink lines) and 3% (red lines) of the published RMNS value except for nitrite. The nitrite limit is set to ±0.020 µM (MDL) as 1% is below the method MDL. The GO-SHIP criteria (Hyde *et al.*, 2010), appendix 7.3, specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy. The assayed RMNS values per CTD deployment are reported in the table in appendix 7.4.
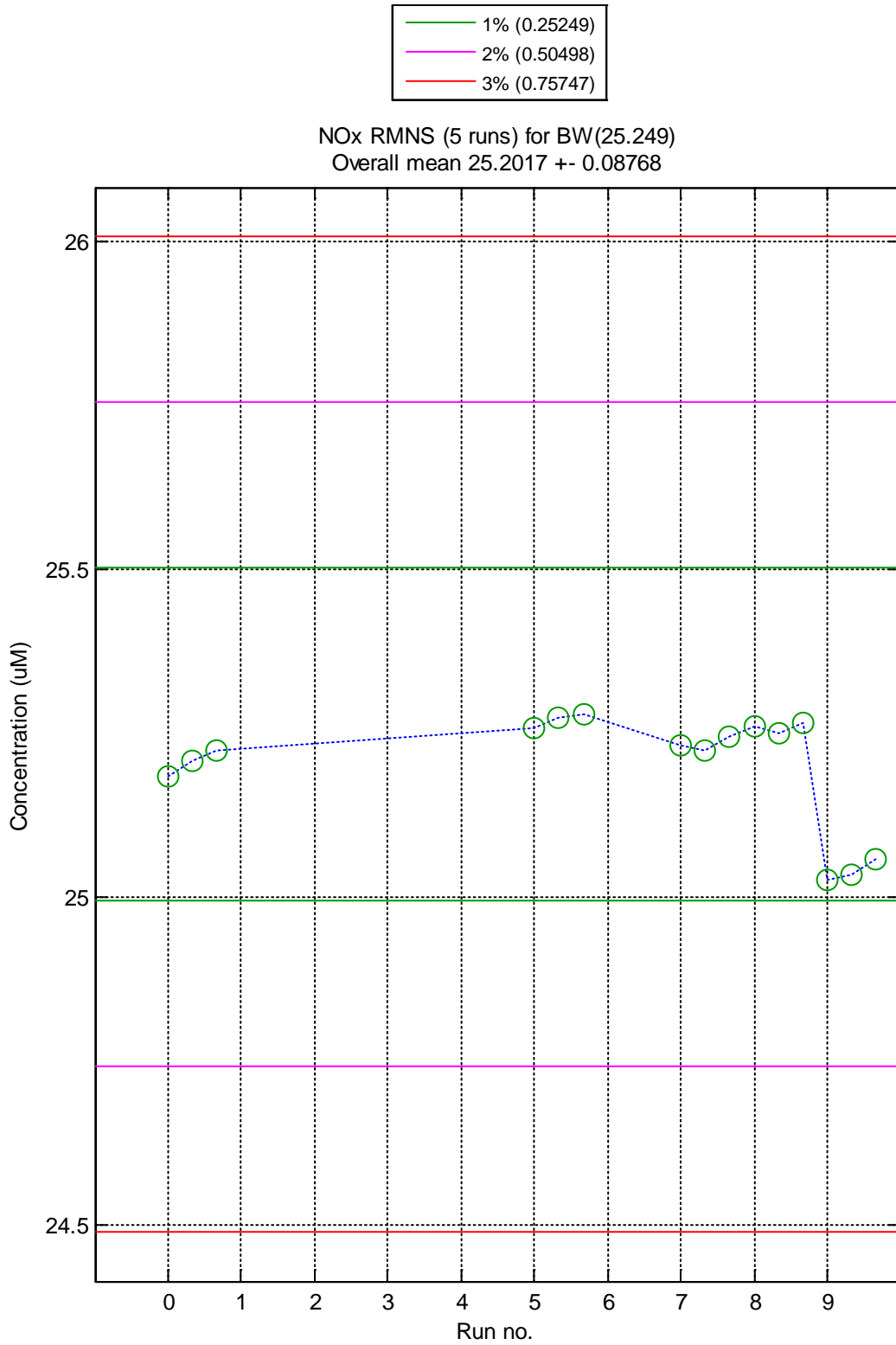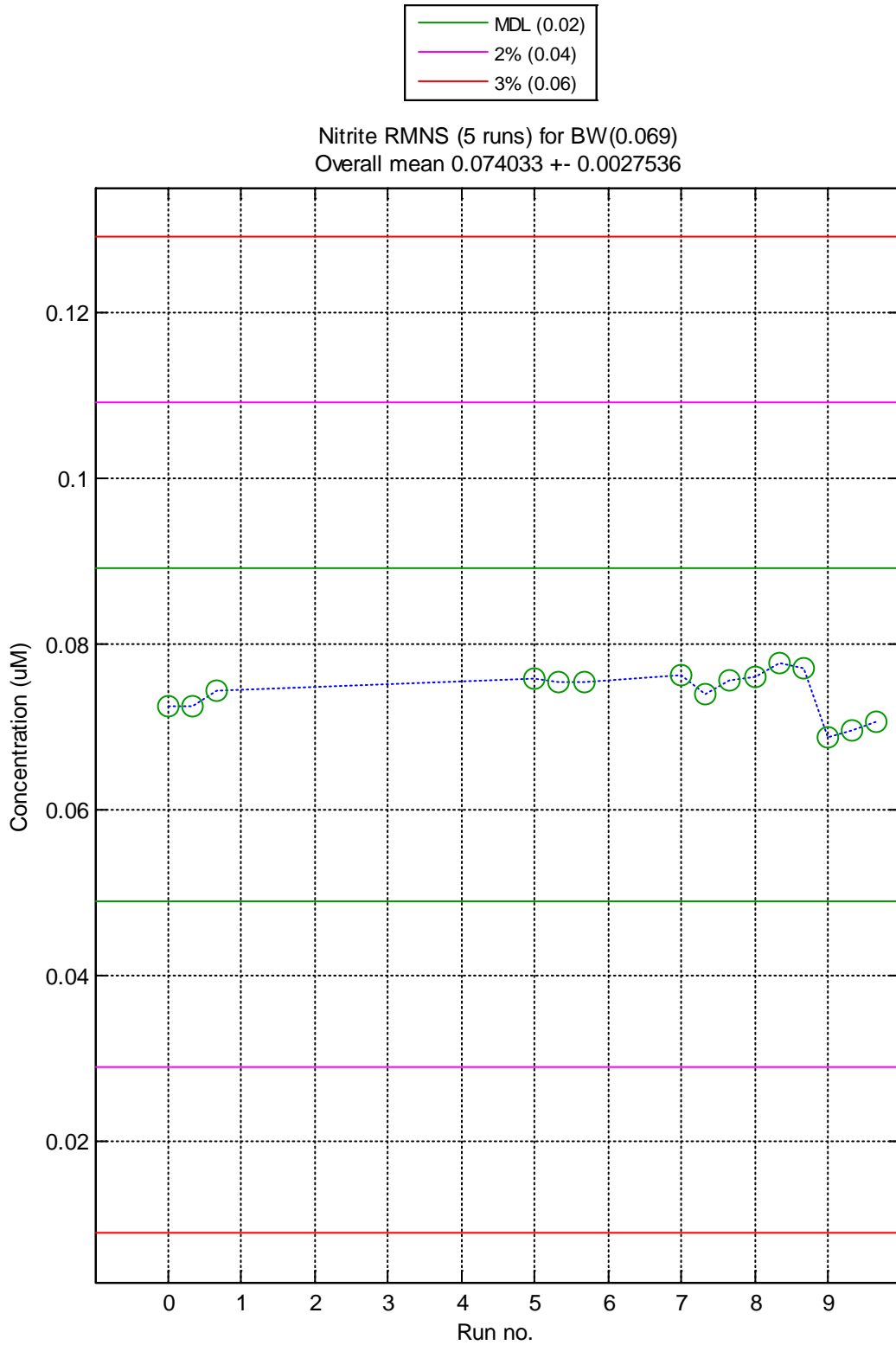
6.4.1   Silicate RMNS Plot
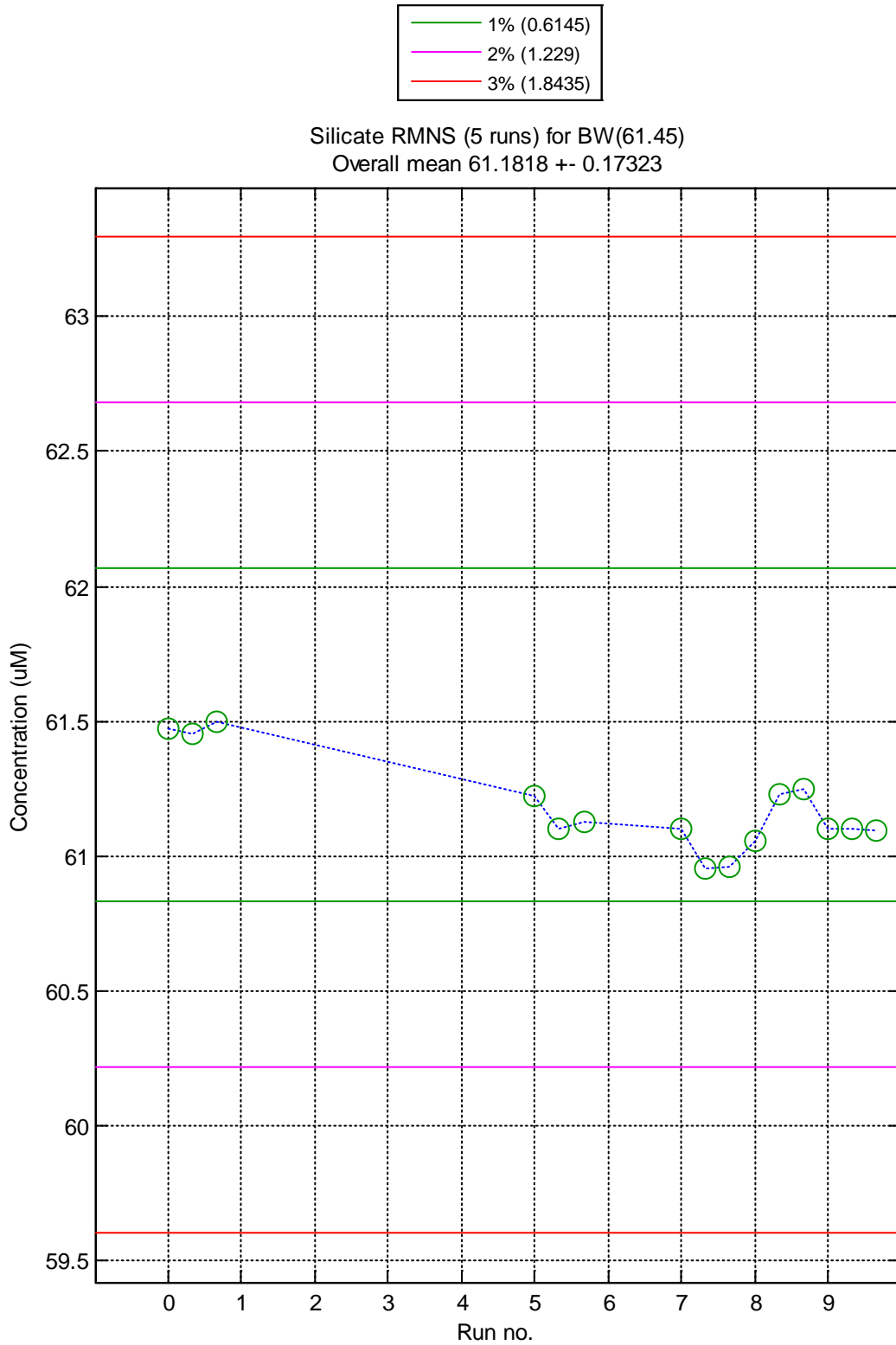
6.4.2 Phosphate RMNS Plot
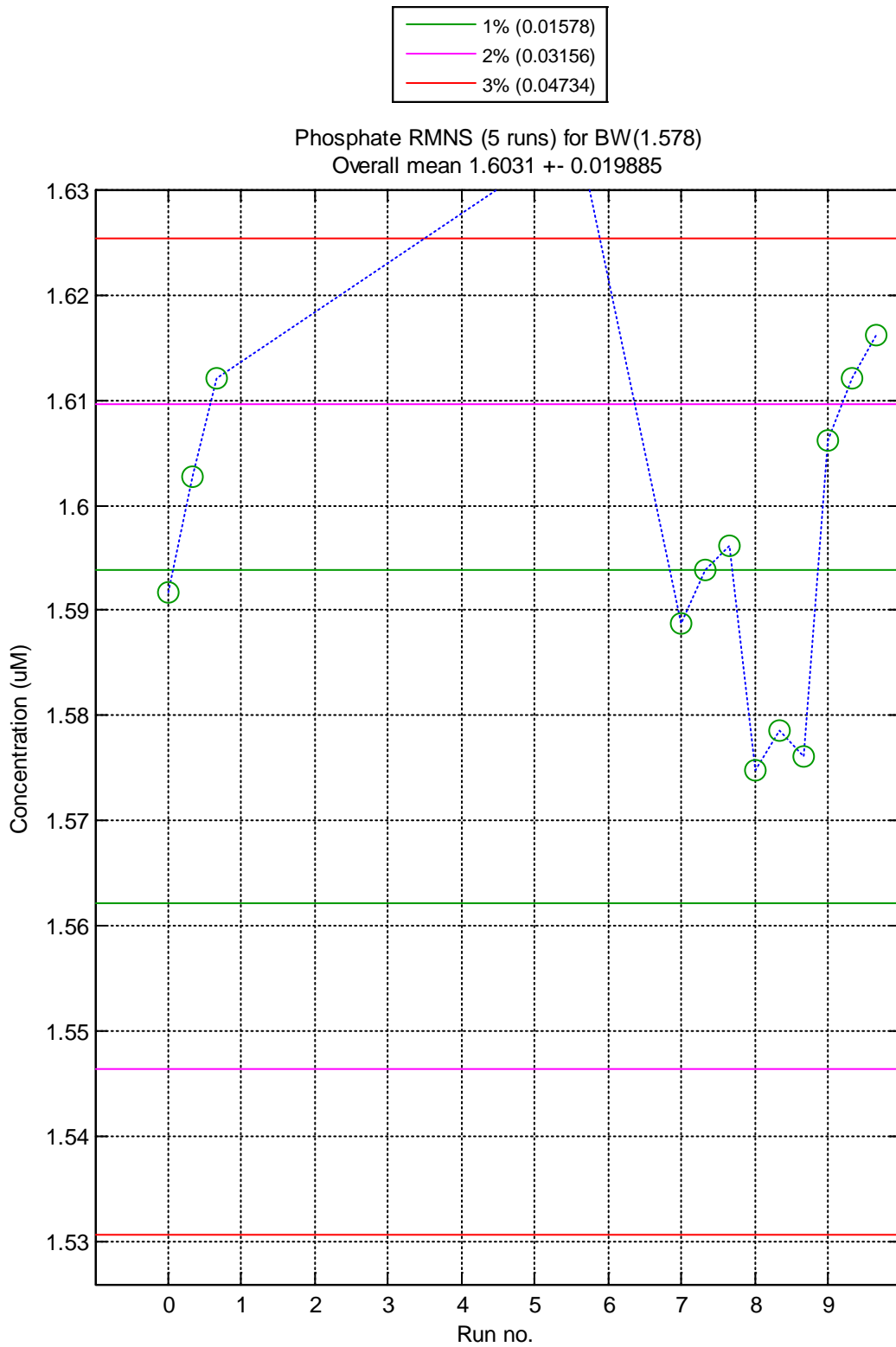
6.4.3   Nitrate + Nitrite (NOx) RMNS Plot

6.4.4 Nitrite RMNS Plot

6.5 Analytical Precision

The CSIRO Hydrochemistry method measurement uncertainty (MU) has been calculated for each nutrient based on variation in the calibration curve, calibration standards, pipette and glassware calibration, and precision of the RMNS over time (Armishaw 2003).

	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Calculated MU* @ 1 µmol l^{-1}	±0.017	±0.020	±0.017	±0.108	±0.066[¥]

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

[¥]The ammonia MU precision component does not include data on the RMNS.

Method detection limits (MDL) achieved during the voyage were much lower than the nominal detection limits, indicating high analytical precision at lower concentrations. RMNS and MDL precision data listed below. Results are µmol l^{-1}.

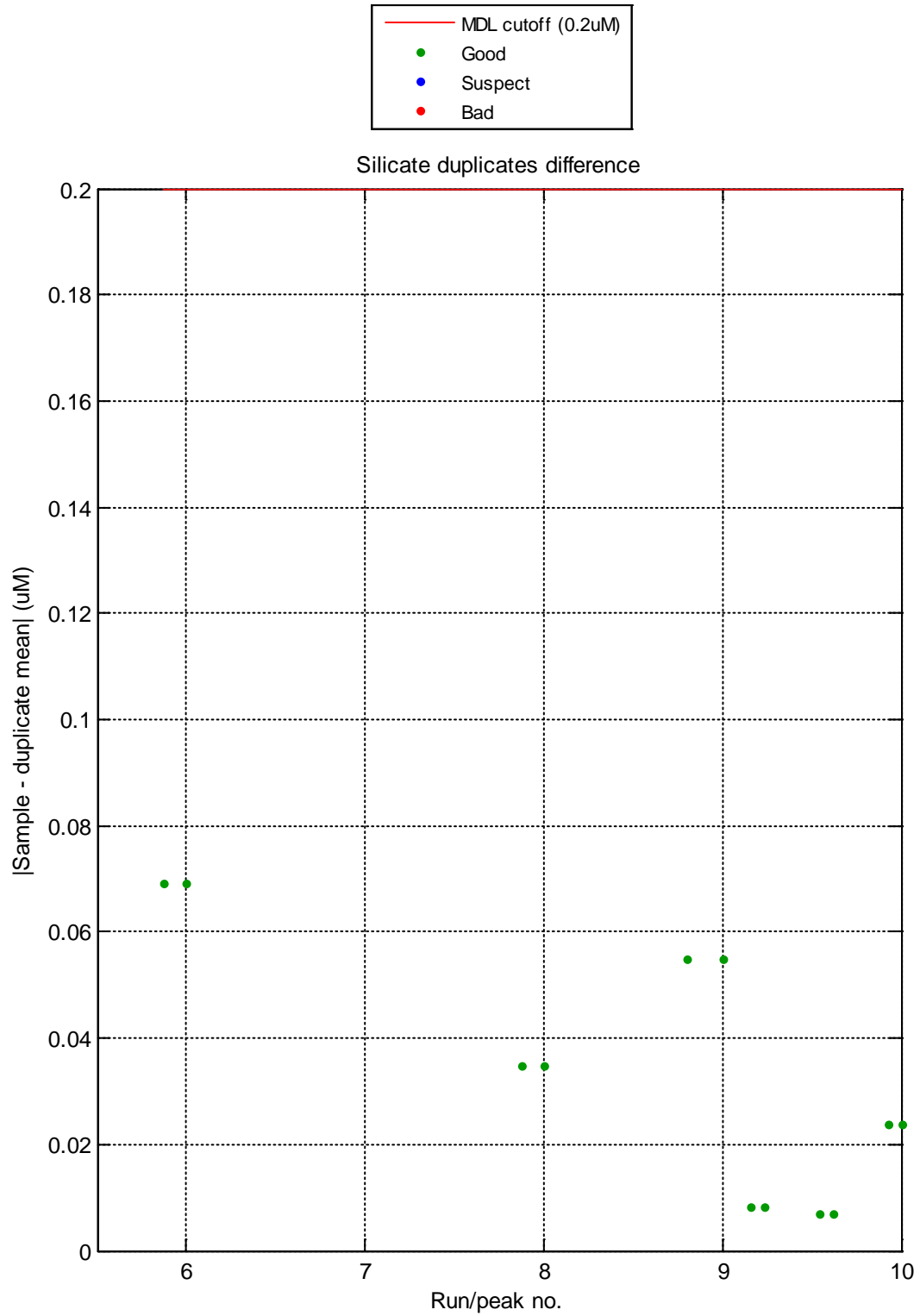| MDL	Silicate	Phosphate	Nitrate + Nitrite (NOx)	Nitrite	Ammonia
Nominal MDL*	0.20	0.02	0.02	0.02	0.02
Min	0.021	0.002	0.007	0.001	0.004
Max	0.102	0.021	0.038	0.007	0.007
Mean	**0.059**	**0.010**	**0.018**	**0.005**	**0.005**
Median	0.062	0.010	0.013	0.005	0.005
Precision of MDL (stdev)	0.026	0.005	0.011	0.002	0.001

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

|					
Published RMNS BW (µmol l^{-1})	61.45	1.58	25.25	0.069	-
w/uncertainty	± 0.43	± 0.01	± 0.22	± 0.010	-
RMNS Min	61.00	1.577	25.04	0.073	
RMNS Max	61.48	1.633	25.30	0.078	
RMNS Mean	61.18	1.603	25.20	0.074	1.18
RMNS Median	61.18	1.607	25.26	0.075	
RMNS Std Dev	0.17	0.020	0.09	0.003	0.07

6.6 Sampling Precision

Duplicates were collected from the deepest niskin for each cast. The multiple measurements are reported in the data as an average, when all measurements are flagged GOOD. The sampling precision is deemed good if the difference between the concentrations is below the MDL for silicate, phosphate and nitrite and within 0.06 µM for nitrate.
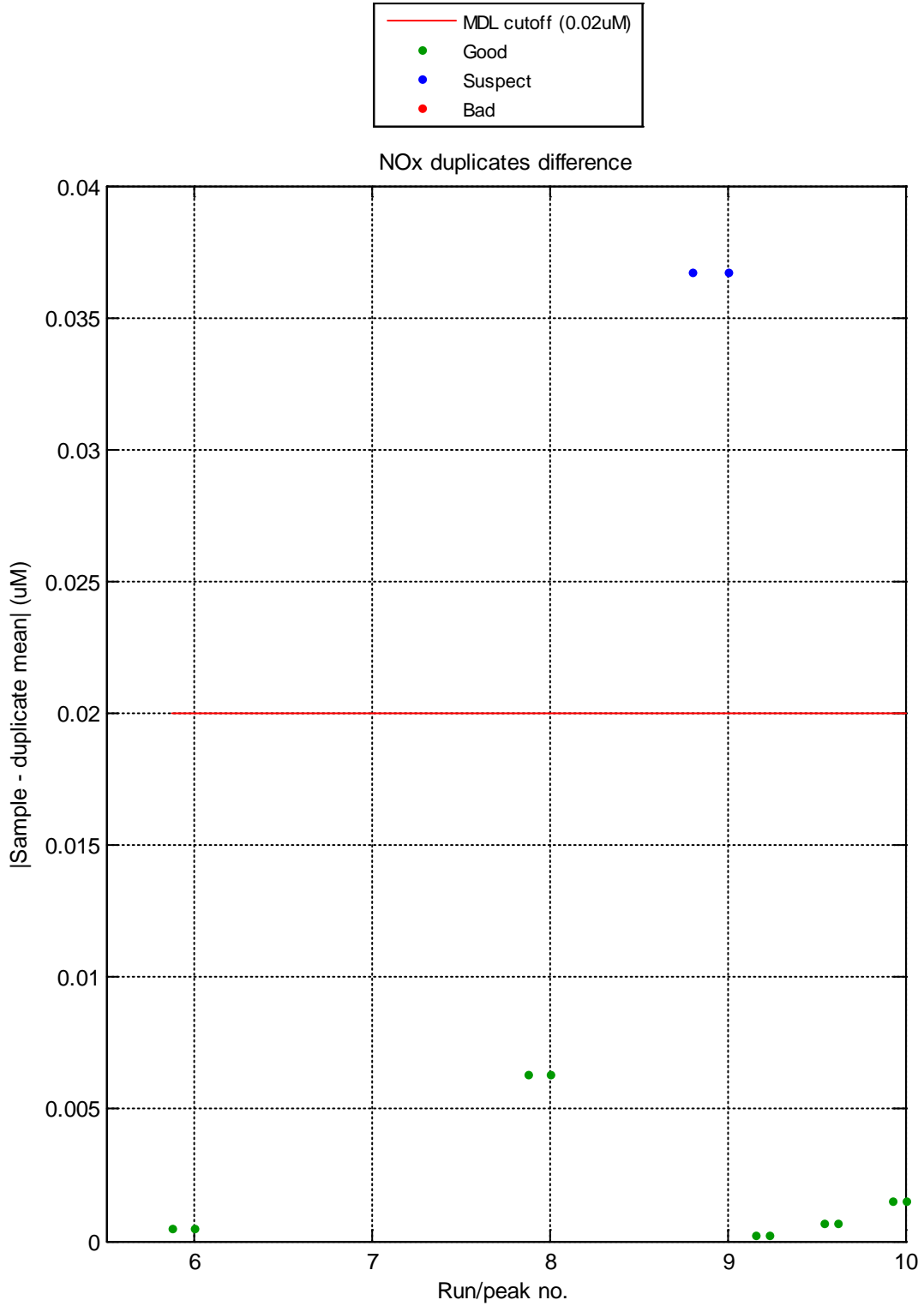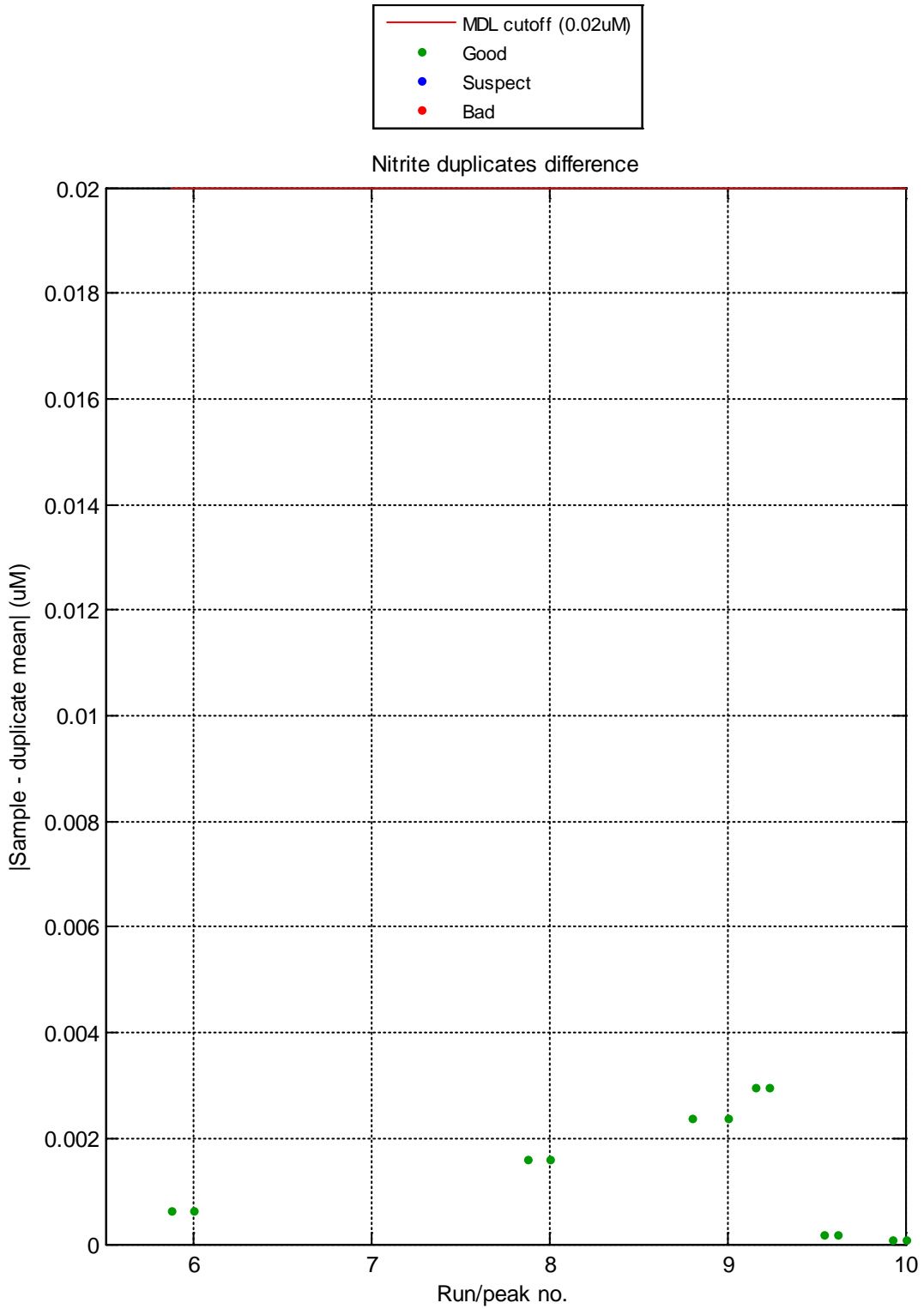
6.6.1   Silicate Duplicate/Replicates Plot

6.6.2   Phosphate Duplicate/Replicates Plot
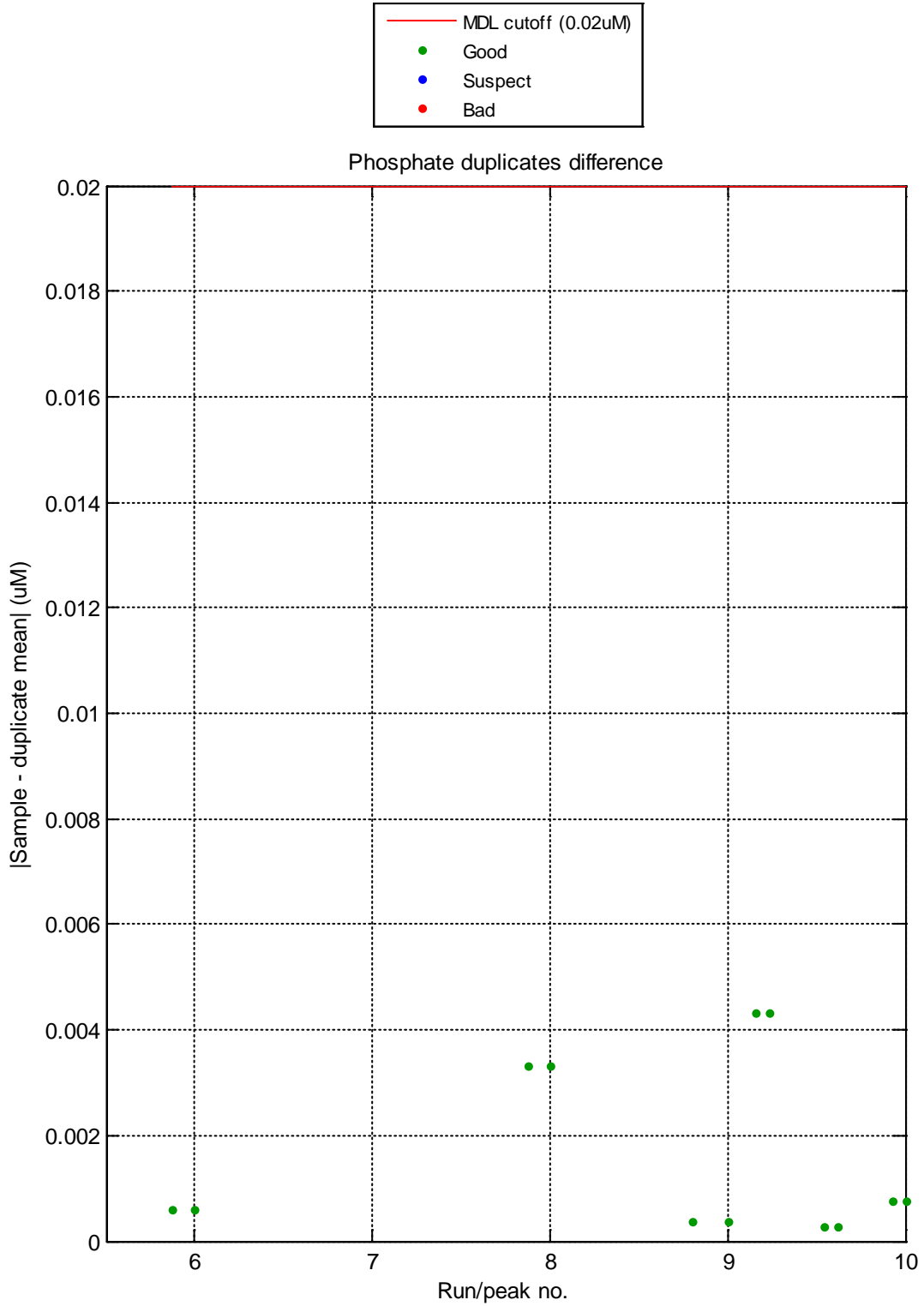
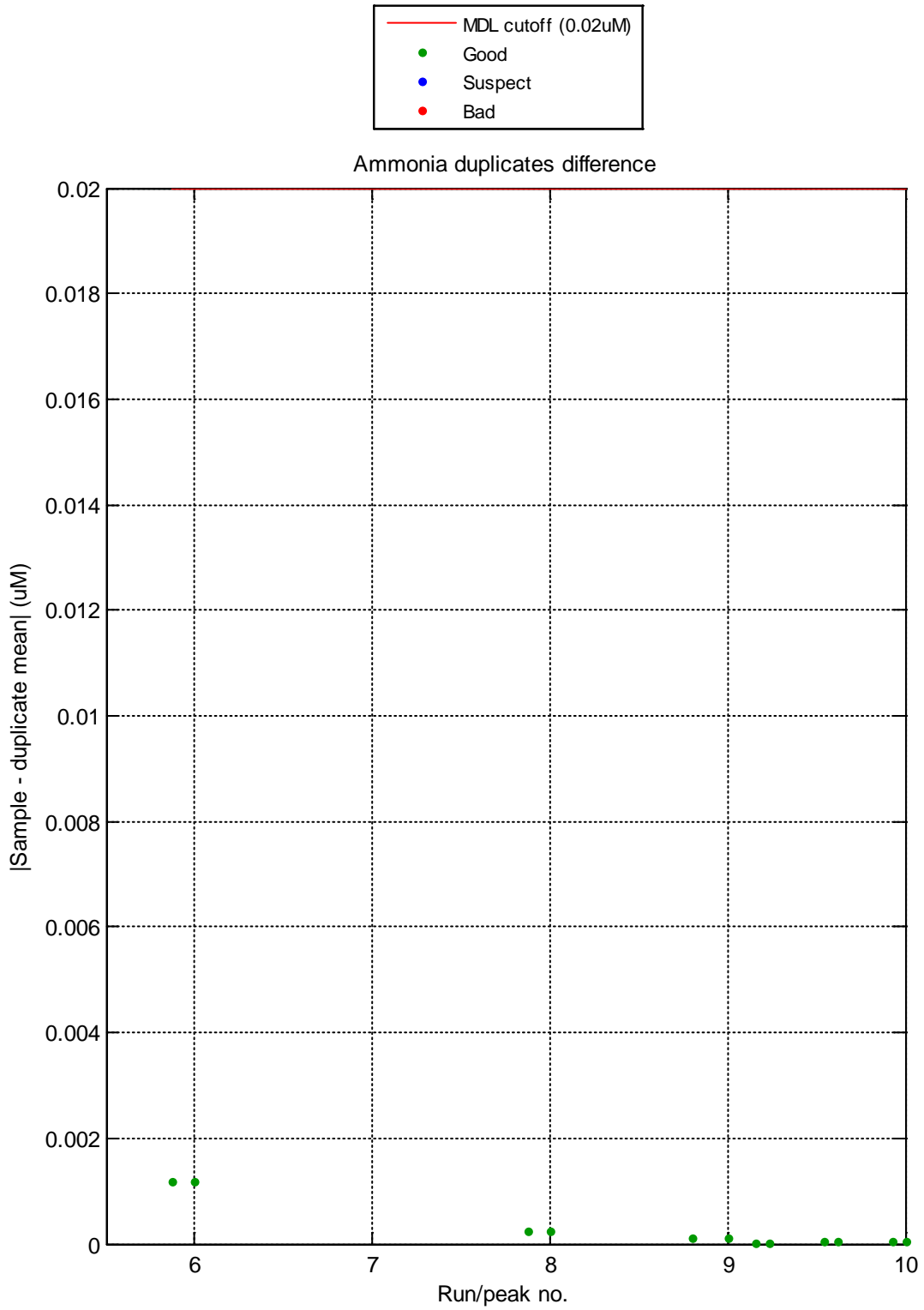### 6.6.3   Nitrate + Nitrite (NOx) Duplicate/Replicates Plot

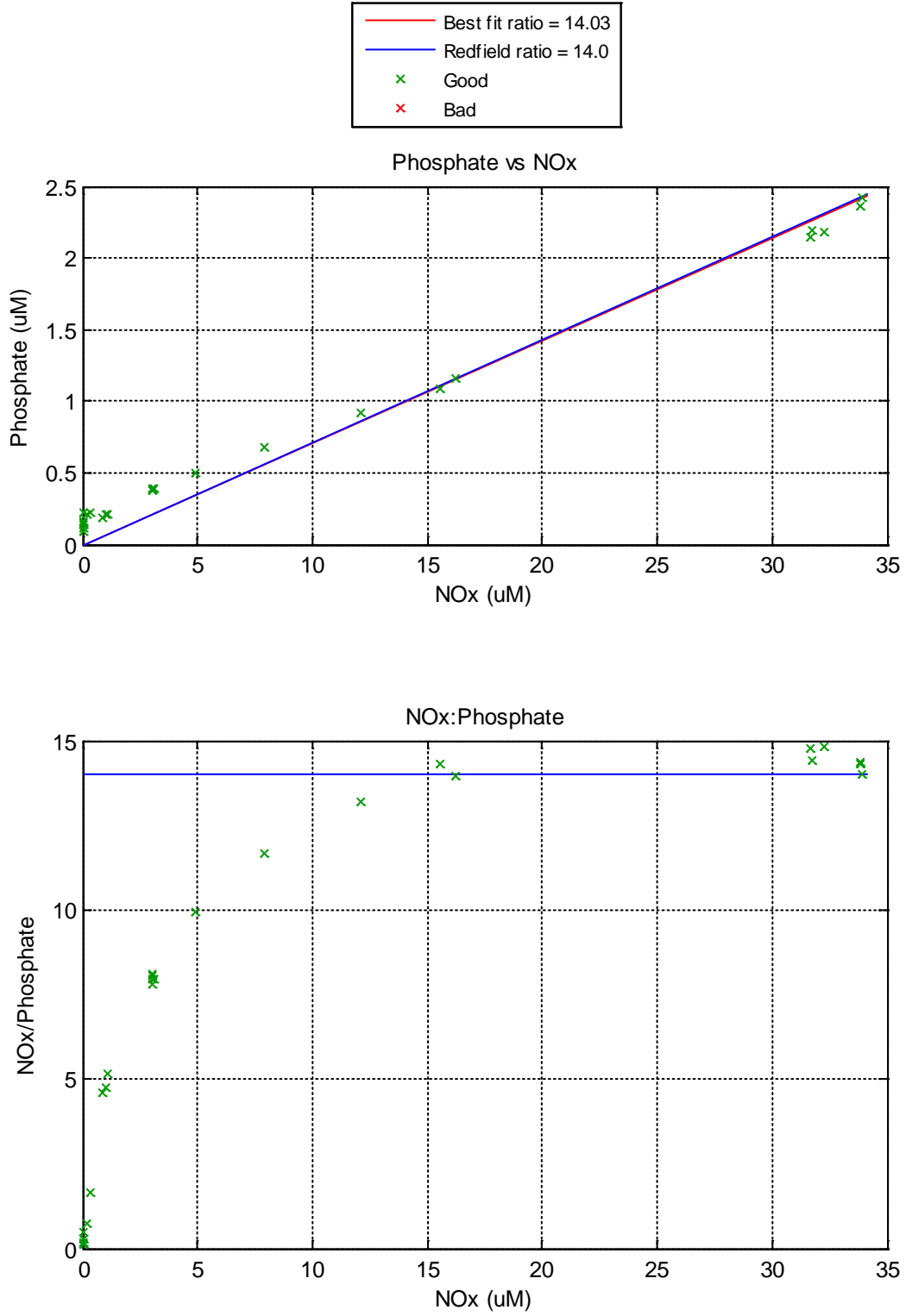## 6.6.4   Nitrite Duplicate/Replicates Plot

6.6.5   Ammonia Duplicate/Replicates Plot

6.6.6 Redfield Ratio Plot (14.0)

Plots consists of phosphate versus NOx, best fit ratio = 14.03.

6.7   Calibration and QC edited data

CTD	Peak	Run	Analysis	Reason for Flag or Action
1	Cal 2	Nut005	PO4	Suspect - greater than calibration error
6	Cal 4	Nut008	PO4	Suspect - greater than calibration error
6	Cal 1 & 3	Nut008	NH4	Suspect - greater than calibration error
8/9/10	Cal 2	Nut009	PO4	Suspect - greater than calibration error
8/9/10	Cal 1 & 3	Nut009	NH4	Bad – outside of calibration limits

6.8   Investigation of Missing or Flagged Nutrient Data and Actions taken.

The table below identifies all flagged data and any data that was repeated. 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. Refer to Appendix 7.2 for flag explanations.

CTD	RP	Run	Analysis	Flag	Reason for Flag or Action
6	1	Nut008	NOx	none	Data good, duplicates higher than set limit

6.9   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 "in2017_c01_hyd_voyagereport.docx" for room temperature graphs, nutrient samples were placed on XY3 auto sampler at the average room temperature of 21.5°C.
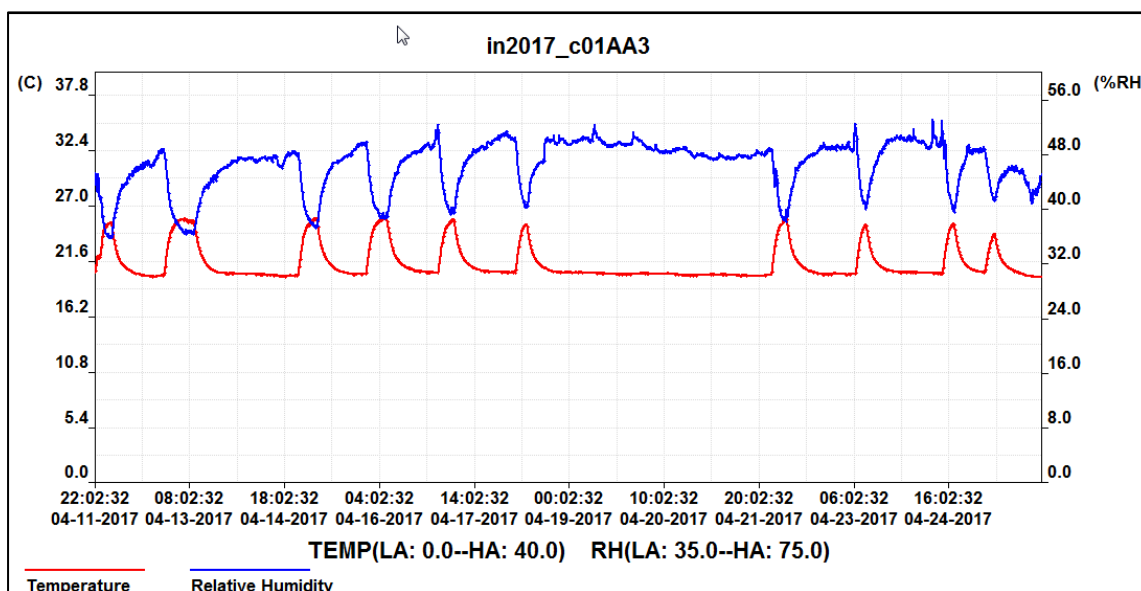
7 Appendix

7.1 Salinity Reference Material

Osil IAPSO Standard Seawater	
Batch	P158
Use by date	25/03/18
K_{15}	0.99940

7.2 HyPro Flag Key for CSV & NetCDF file

Flag	Meaning
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.

Flag	Meaning
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.

7.3 GO-SHIP Specifications

Salinity Accuracy of 0.001 is possible with Autosal™ salinometers and concomitant attention to methodology, e.g., monitoring Standard Sea Water. Accuracy with respect to one particular batch of Standard Sea Water can be achieved at better than 0.001 PSS-78. Autosal precision is better than 0.001 PSS-78. High precision of approximately 0.0002 PSS-78 is possible following the methods of Kawano (this manual) with great care and experience. Air temperature stability of ± 1°C is very important and should be recorded.[1]

O_2 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.

SiO_2 Approximately 1-3% accuracy†, 2 and 0.2% precision, full-scale.

PO_4 Approximately 1-2% accuracy†, 2 and 0.4% precision, full scale.

NO_3 Approximately 1% accuracy†, 2 and 0.2% precision, full scale.

Notes: † If no absolute standards are available for a measurement then *accuracy* should be taken to mean the *reproducibility* presently obtainable in the better laboratories.

1 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 batches is recommended. The bottles should also be used in an interleaving fashion as a consistency check within a batch and between batches.

2 Developments of reference materials for nutrients are underway that will enable improvements in the relative accuracy of measurements and clearer definition of the performance of laboratories when used appropriately and the results are reported with the appropriate meta data.

7.4   RMNS Values for each CTD Deployment

CTD	SiO_4 measured	PO_4 measured	NO_2 measured	NOx measured
BW reported	61.45	1.578	0.069	25.25
1	61.15	1.633	0.075	25.27
2	61.00	1.593	0.075	25.25
6	61.18	1.577	0.077	25.25
8, 9, 10	61.20	1.612	0.070	25.04

8   References

Armishaw, Paul, "Estimating measurement uncertainty in an afternoon. A case study in the practical application of measurement uncertainty." Accred Qual Assur, 8, pp. 218-224 (2003).

Armstrong, F.A.J., Stearns, C.A., and Strickland, J.D.H., *"The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment,"* Deep-Sea Research, 14, pp.381-389 (1967).

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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*

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Kérouel, Roger and Alain Aminot, *"Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis".* Journal of Marine Chemistry 57 (1997) pp. 265-275.

Murphy, J. And Riley, J.P.,"*A Modified Single Solution Method for the Determination of Phosphate in Natural Waters"*, Anal.Chim.Acta, 27, p.30, (1962)

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.