Marine National Facility

RV *INVESTIGATOR*

HYDROCHEMISTRY DATA PROCESS REPORT

Voyage:	IN2017_v05
Chief Scientist:	John Keesing
Voyage title:	Long term recovery of trawled marine communities.
Report compiled by:	Peter Hughes

CSIRO

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Contents

1 Executive Summary

Water samples collected from CTD deployments and the underway clean seawater supply where assayed for nutrients, dissolved oxygen and salinity in the hydrochemistry lab on the ship.

This report details the analytical methods and data processing steps involved in producing the hydrology data set.

Final data, analytical methods, log sheets and processing notes are available at the CSIRO data centre. Contact:

DataLibrariansOAMNF@csiro.au

Depart	**Date**	**Time**
Broome	11/10/2017	0900
Arrive	**Date**	**Time**
Perth (Henderson)	10/11/2017	0800

2 Key personnel list

Name	**Role**	**Organisation**
Peter Hughes	Chief Scientist	CSIRO
Max McGuire	Voyage Manager	CSIRO
Peter Hughes	Hydrochemist	CSIRO

3 Summary

3.1 Samples Collected

Analysis (instrument)	Number of Samples	
Salinity (Guildline Salinometer)	89	CTD
	19	UWY
Dissolved Oxygen (automated titration)	94	CTD
Nutrients (AA3HR)	274	CTD
	17	UWY

3.1.1 CTD

- Sampling point, 36 bottle Rosette with 12L Ocean Test Equipment bottles deployed for water collection.
- 72 deployments in total. 64 sampled and assayed by hydrochemistry.
- Deployments not sampled: 10, 19, 29, 11, 50, 57

3.1.2 UWY

- Sampling point, the underway clean seawater supply in the PCO_2 lab.
- 19 salinity samples for TSG calibration collected by Peter Hughes. Results and meta-data saved in the ship's v05 elog
- 17 nutrient samples collected by Morgane Perron (UTAS) and assayed by hydrochemistry. Results issued to Morgane during the voyage.

3.2 Data Procedure Summary

The procedure for data processing is illustrated in figure 1.

The CSIRO program HyPro collates and processes the CTD deployment met-data with the sample assay data to produce the final hydrology data set. The final data set is issued as a single csv file plus nc files for each deployment.

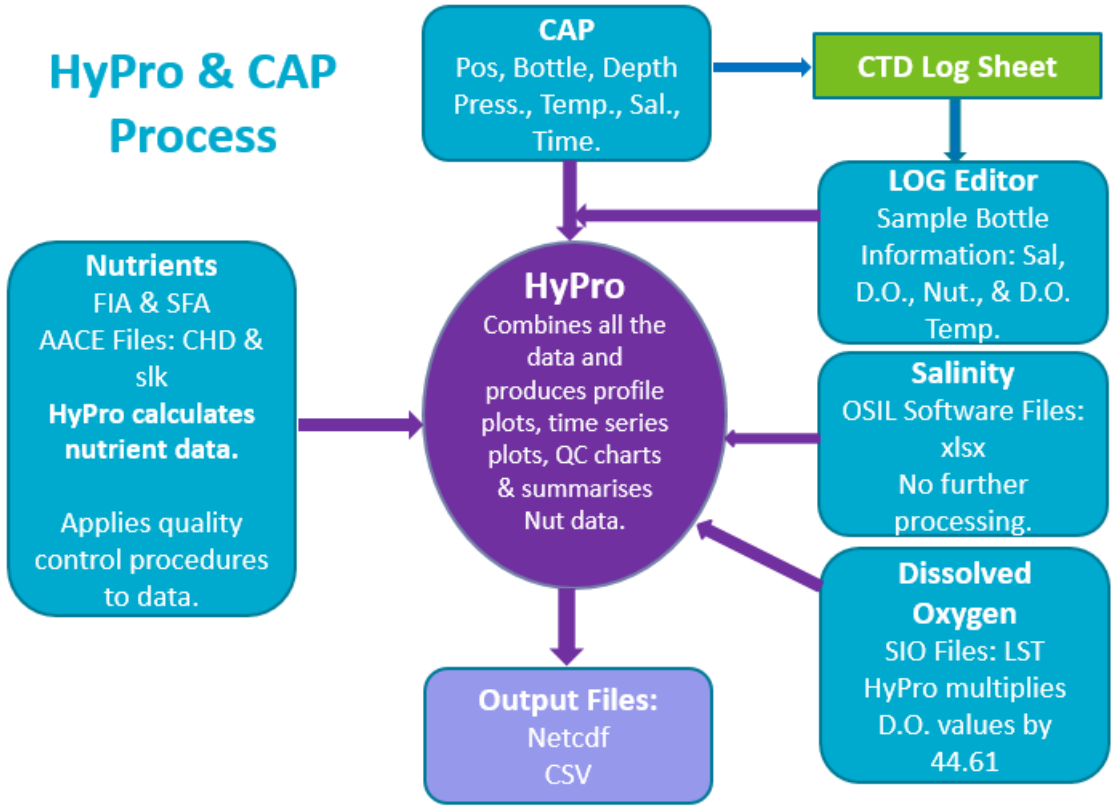


Figure 1: The processing steps for hydrology data following sample assay.

4 Salinity Data Processing

4.1 Salinity Parameter Summary

Details	
HyPro Version	5.3
Instrument	Guildline Autosal Laboratory Salinometer 8400(B) – SN 71613
Software	OSIL Data Logger v1.2
Methods – In house, Hydrochemistry	Sampling: WI_Sal_002 Measurement: SOP006
Accuracy	± 0.001 practical salinity units
Analyst	Peter Hughes
Lab Temperature (±0.5°C)	21.0 - 22.5 °C
Bath Temperature	24.013°C
Reference Material	Osil IAPSO - Batch P158, use by 25/03/2018, K_{15} = 0.99940
Sampling Container type	200 ml OSIL bottles made of type II glass (clear) with disposable plastic insert and plastic screw cap.
Sample Storage	Samples stored in salt room until analysis. All samples measured within 120 hours of collection.
Comments	One or two depths sampled per deployment. Deployments less than 100m deep.

4.2 Salinity Method

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}. 35 PSU has a K_{15} of one.

The electrical conductivity is measured with a salinometer (Guildline Autosal 8400B). The Autosal is calibrated with standard seawater (OSIL, IAPSO) of known K_{15} ratio before each batch run of samples.

Synopsis: Salinity samples are collected into 200ml OSIL bottles, filled from the bottom, via a PTFE straw, 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 conductivity, 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 captures the conductivity ratio and calculates the practical salinity.

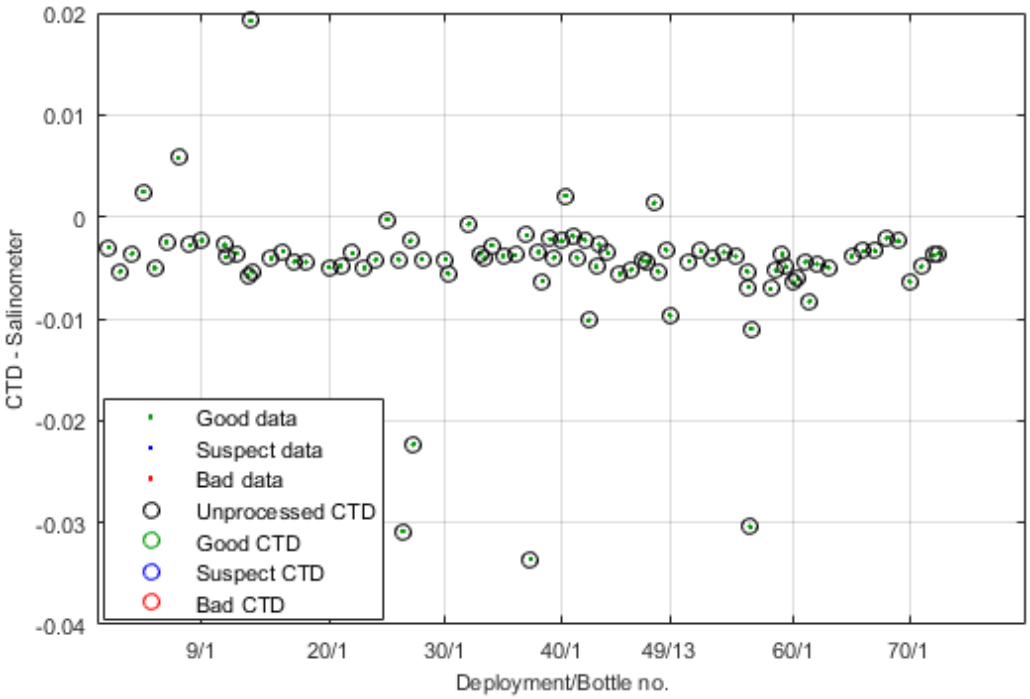
The conductivity data and the calculated practical salinity results are imported into HyPro as is.

4.3 CTD salinity vs Bottle Salinity Plot

The difference between the uncorrected CTD instrument values and the measured bottle salinities is less than 0.04 PSU.

The CTD instrument values are 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



4.4 Missing or Suspect Salinity Data

None.

5 Dissolved Oxygen Data Processing

5.1 Dissolved Oxygen Parameter Summary

Details	
HyPro Version	5.3
Instrument	Automated Photometric Oxygen system (SCRIPPS)
Software	SCRIPPS
Methods – In house, hydrochemistry	Sampling: WI_DO_001 Assay: SOP005
Accuracy	± 0.5 µM
Analyst	Peter Hughes
Lab Temperature (±1°C)	Variable, 20.0 - 23.0°C
Sample Container type	140 mL iodine determination flask with glass stopper. Each flask uniquely numbered and its stoppered volume known to the nearest 0.01mL.
Sample Storage	Samples stored in the Hydrochemistry lab until analysis. All samples were assayed within 144 hours of collection.
Comments	One or two depths sampled per deployment. Deployments less than 100m deep.

5.2 Dissolved Oxygen Method

Samples are collected and assayed in accordance with CSIRO hydrochemistry procedures. Procedures are based on the whole-bottle modified Winkler titration of Carpenter (1965) plus modifications by Culberson *et al* (1991).

Synopsis: The sample is collected in an iodine determination flask of known volume. A 1mL aliquot each of manganese (II) chloride solution followed by alkaline iodide solution is added to the sample, the flask stoppered and shaken. 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 equivalent to the original dissolved oxygen content of the sample. The iodine is present as the tri-iodide in solution. The tri-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.

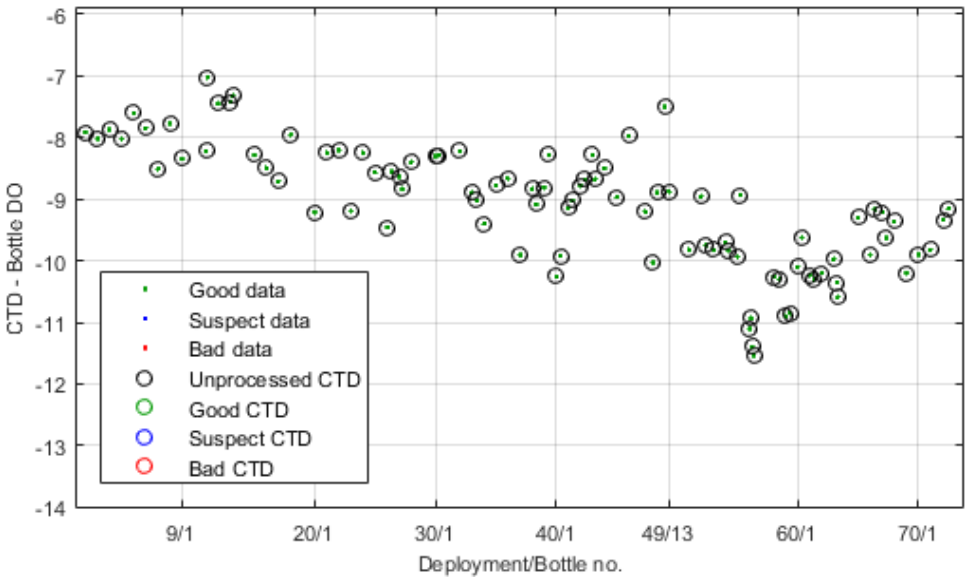
The thiosulphate solution is standardised by titrating a 10ml aliquot of potassium iodate primary standard. A blank correction is also determined from the difference between two consecutive titres for 1ml aliquots of the same potassium iodate solution.

5.3 CTD vs Hydro DO Plot

The difference between the uncorrected CTD instrument values and the measured bottle samples is less than 12 µ mole/l.

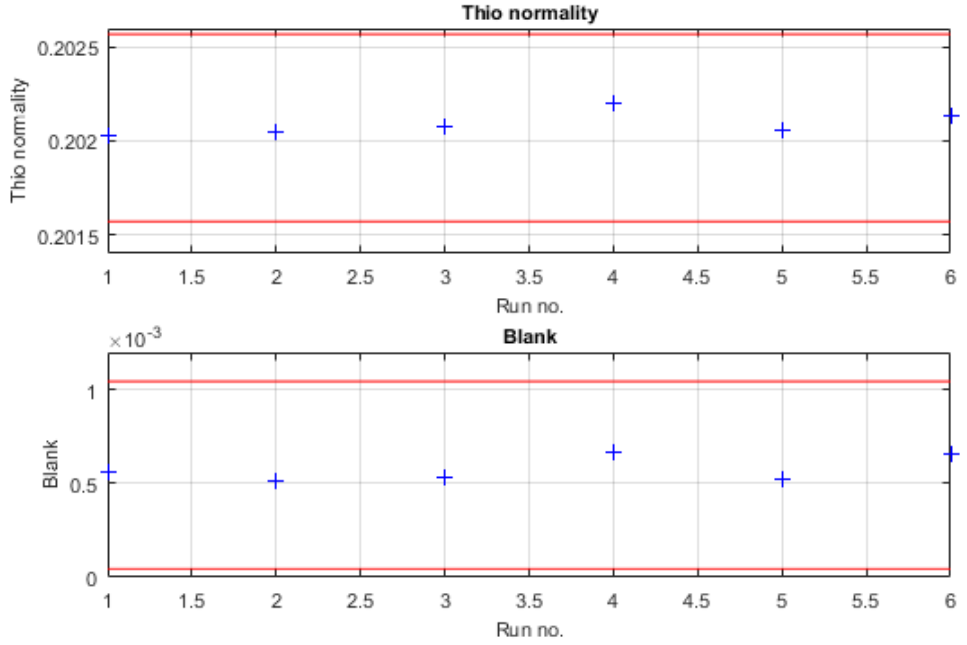
The CTD instrument values are corrected by DAP using the bottle results. The corrected values are not reported in the hydrology set. Please contact the DataLibrarians@csiro.au for corrected CTD data.

Note: dots = bottle samples, circles = CTD instrument



5.4 Thiosulphate normality and blank concentrations

The normality of the thiosulphate titrant varied less than 0.0002 for all dissolved oxygen sample titrations. Our procedure SOP005 sets the acceptable upper limit of change in thiosulphate normality at 0.0005 / day.



5.5 Missing or Suspect Dissolved Oxygen Data and Actions taken

CTD	RP	Analysis	Flag	Reason for Flag or Action
47	1	DO	141	No Result. Sample collected, assay aborted. Sample not acidified before titration titrated. No endpoint.

6 Nutrient Data Processing

6.1 Nutrient Parameter Summary

Details					
HyPro Version	5.3				
Instrument	Seal AA3HR				
AA3HR Software	Seal AACE 6.10				
Methods – In house, Hydrochemistry	Sampling: WI_Nut_001 Assay: SOP001 to SOP004				
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	Peter Hughes				
Lab Temp. (±1°C)	Variable, 20.0 – 23.0°C				
Reference Material	KANSO, RMNS lot CD				
Sample Container	50 ml polypropylene sample tubes				
Sample Storage	< 2 hrs at room temperature or ≤ 18 hrs @ 4°C				
Pre-processing of Samples	None				
Comments	Every depth sampled per deployment. Deepest in duplicate. Deployments less than 100m deep.				

6.2 Nutrient Methods

Nutrient samples where assayed on a Seal AA3HR segmented flow auto-analyser fitted with 1cm flow-cells and a JASCO FP2020 fluorescence 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 molybate. It is then reduced by ascorbic acid and its absorbance is measured at 880nm.

Nitrate (SOP003): 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 (SOP003): colourimetric analysis, Naphthylenediamine photometric method. As per nitrate method without the copper cadmium reduction column and buffer.

Ammonium (SOP004): fluorescence analysis, ortho-phtaldiadehyde method. Based on Roger Kérouel and Alain Aminot, IFREMER (1997 Mar.Chem.57). Ammonium reacted with ortho-phtaldialdehyde and sulphite at a pH of 9.0-9.5 to produce an intensely fluorescent product. Its emission is measured at 460nm after excitation at 370nm.

6.3 Instrument Calibration and Data Parameter Summary

The absorbance/ fluorescence raw data for each analysis run is processed in HyPro – a CSIRO program. HyPro identifies the peaks, determines their height, constructs the calibration curve, derives the sample result and applies corrections for instrument drift and peak carry-over. The calibration curve is a regression fit using two iterations with each point's contribution weighted inversely to its difference from the curve. 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.

Instrument parameters, reagent batch compositions, analysis run details and processing steps are documented.

Below are the main settings and corrections used for the instrument and HyPro program.

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	5	5	5	5	5
Corrections: baseline drift, sensitivity drift, peak carry-over (AA3HR instrument)	N	N	N	N	N
Corrections: baseline drift, sensitivity drift, peak carry-over (HyPro)	Y	Y	Y	Y	Y
Data Adj for RMNS	N	N	N	N	N
Peak Window Defined	HyPro	HyPro	HyPro	HyPro	HyPro
Medium of Standards: LNSW (low nutrient seawater)	1000L surface seawater collected 28/9/2016. Bulk stored outside on deck 2 of Investigator. Sub-lot passed through a 10 micron filter and stored in 20 L carboys in the hydrochemistry laboratory at 21°C.				
Medium of Baseline	18.2 Ω water (type1)				
Proportion of samples in duplicate?	One duplicate set per deployment. Deepest point used for duplicate.				
Comments	The reported data is not corrected to the RMNS. Per deployment RMNS data tabulated in appendix 7.1				

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

Japanese KANSO certified reference material Lot CD for silicate, phosphate, nitrate and nitrite in seawater was assayed in triplicate in each instrument run to determine accuracy. See www.kanso.co.jp for product detail.

The RMNS is packaged in 100ml bottles. To economise, one bottle of RMNS was spilt between two runs. Before a pair of runs, a new bottle of RMNS is opened and half the contents decanted into a cleaned/ dry/ dedicated bottle (ex-RMNS). This decanted split was assayed in the first run. The remaining solution, in the original bottle, was refrigerated until it was assayed in the second run.

KANSO issue RMNS values in µ mole/kg. The values in table 1 are converted at 21°C and a density of 1.024 g/kg. RMNS lot CD does not have a certified ammonium value.

Table 1: RMNS concentrations with expanded uncertainty (µmol/l) at 21°C

RMNS	NO_3	NO_X	NO_2	PO_4	SiO_4
CD	5.630 ± 0.051	5.648 ± 0.056	0.018 ± 0.005	0.457 ± 0.008	14.26 ± 0.10

The issued (final) nutrient results do NOT have RMNS corrections applied.

The RMNS values per deployment are reported in appendix 7.1

The following plots show the RMNS lot CD results. The 1%, 2% and 3% lines are from the certified concentration excluding the expanded uncertainty. The nitrite limit is set to ±0.02 µM (MDL) as 1% is below the method MDL.

The GO-SHIP criteria (Hyde *et al*., 2010), appendix 7.4, specifies using 1-3 % of full scale (depending on the nutrient) as acceptable limits of accuracy.

RMNS Plot Legend:

Blue boundary = expanded uncertainty from RMNS value

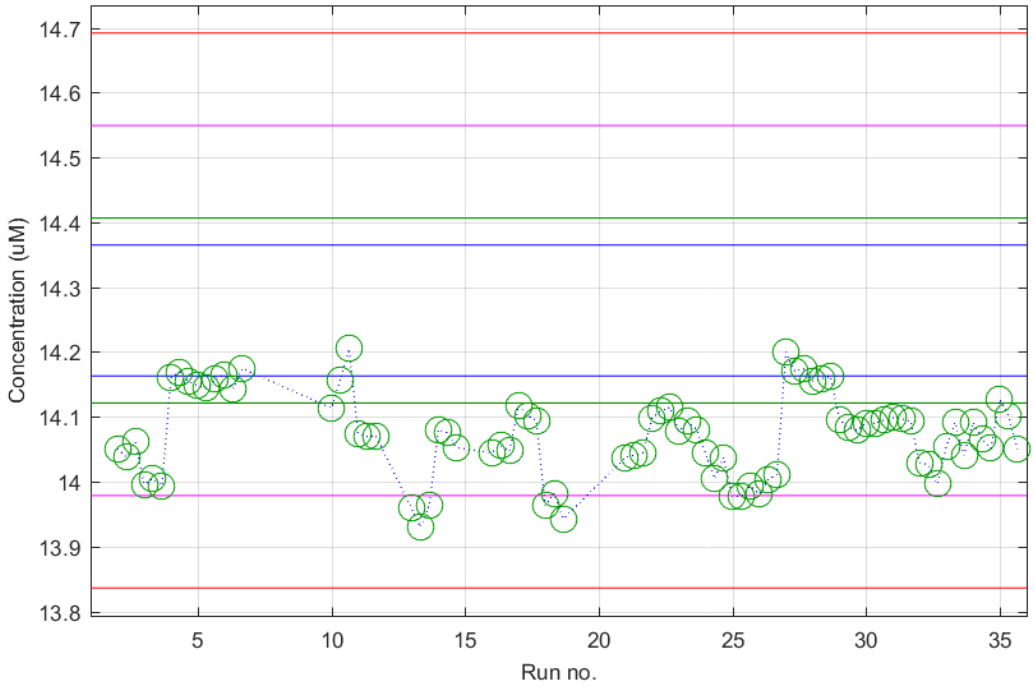
Green boundary = 1% from RMNS value

Pink boundary = 2% from RMNS value

Red boundary = 3% from RMNS value

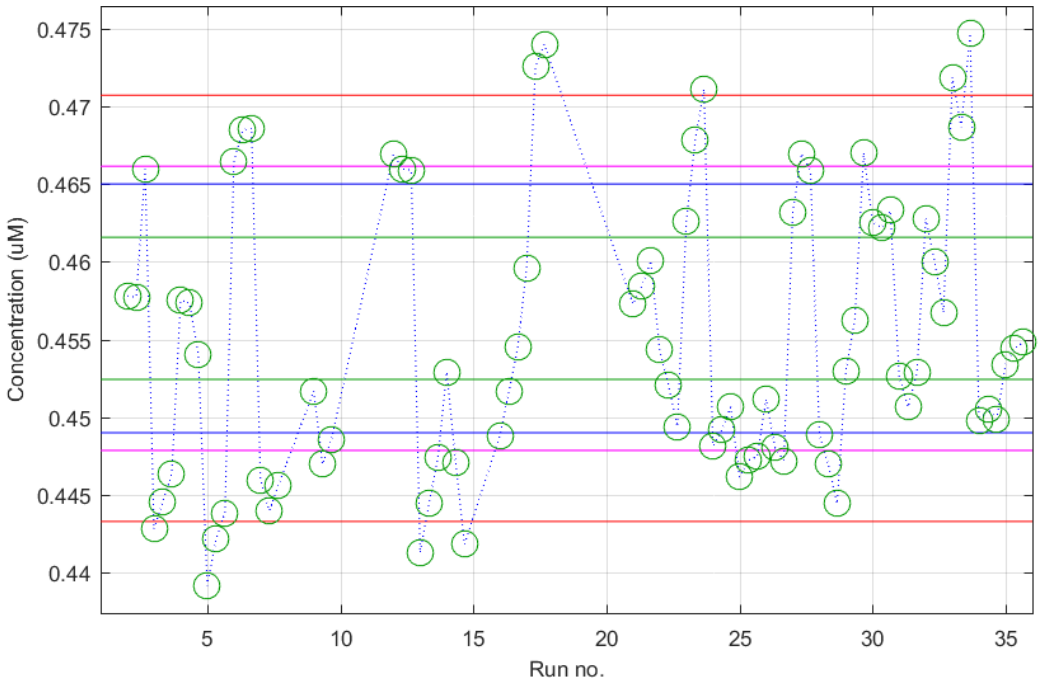
6.4.1 Silicate RMNS Plot

Lot CD (14.26 ± 0.10), 27 runs
Overall mean 14.07 ± 0.06



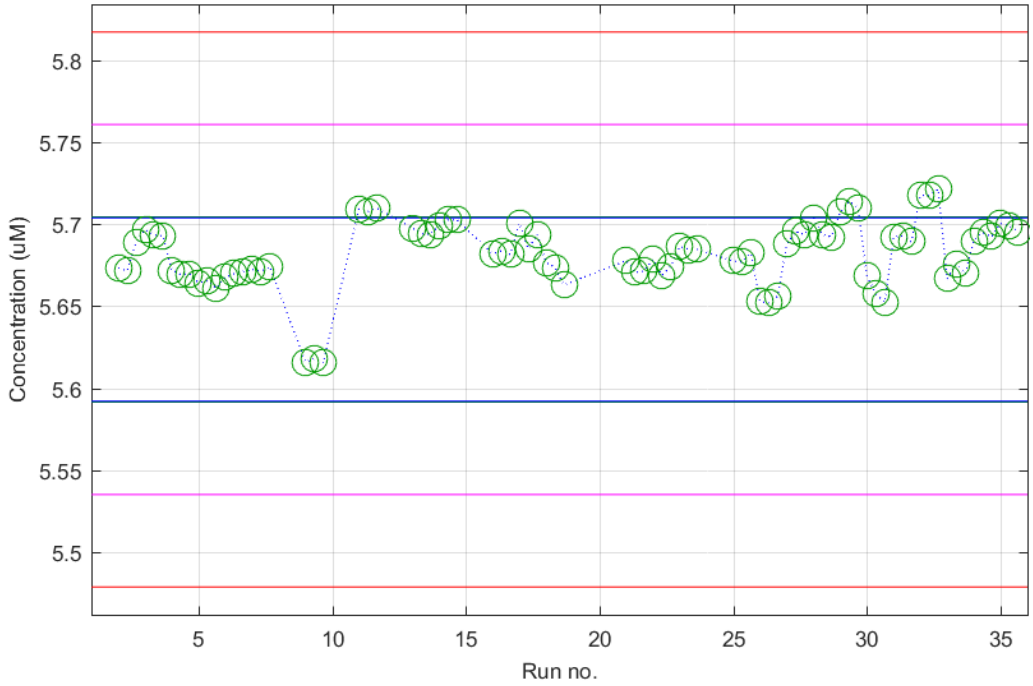
6.4.2 Phosphate RMNS Plot

Lot CD (0.457 ± 0.008), 27runs
Overall mean 0.455 ± 0.009



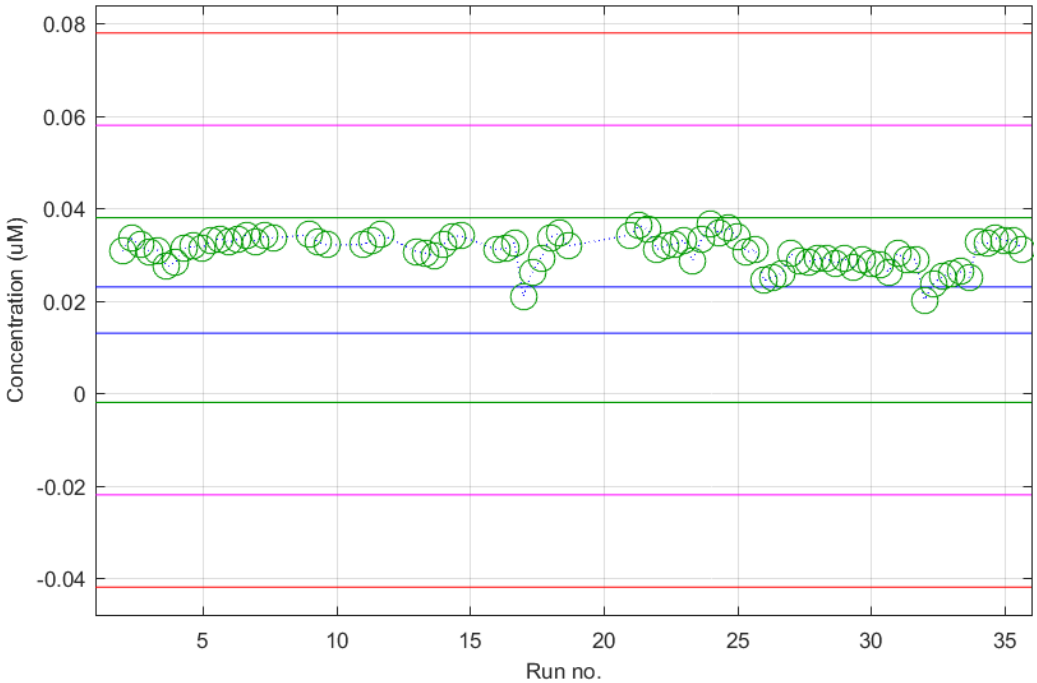
6.4.3 Nitrate + Nitrite (NOx) RMNS Plot

Lot CD (5.648 ± 0.056), 27 runs
Overall mean 5.68 ± 0.02



6.4.4 Nitrite RMNS Plot

Lot CD (0.018 ± 0.005), 28 runs
Overall mean 0.031 ± 0.003



6.5 Analytical Precision

The estimate of the measurement of uncertainty (MU) at 1 µmol l^{-1} for the CSIRO nutrient methods are tabulated below.

The reported uncertainty is an expanded uncertainty with coverage factor 2, 95% confidence level.

MU is calculated from the 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
MU @ 1 µmol l^{-1}	±0.016	±0.025	±0.019	±0.13	±0.29¥

¥The ammonia MU precision component does not include RMNS data.

Method detection limits (MDL) achieved during the voyage are 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	0.06	0.02	0.02
Min	0.007	0.002	0.001	0.001	0.003
Max	0.072	0.021	0.015	0.014	0.011
Mean	**0.037**	**0.011**	**0.004**	**0.003**	**0.006**
Median	0.039	0.011	0.003	0.003	0.005
Precision of MDL (stdev)	0.018	0.005	0.003	0.003	0.002

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

Published RMNS CD w/uncertainty	14.26 ± 0.10	0.457 ± 0.008	5.648 ± 0.056	0.018 ± 0.005	- -
RMNS Min	13.95	0.442	5.62	0.023	1.31
RMNS Max	14.18	0.472	5.72	0.035	1.75
RMNS Mean	14.07	0.455	5.68	0.031	1.49
RMNS Median	14.07	0.452	5.68	0.032	1.46
RMNS Std Dev	0.06	0.009	0.02	0.003	0.12

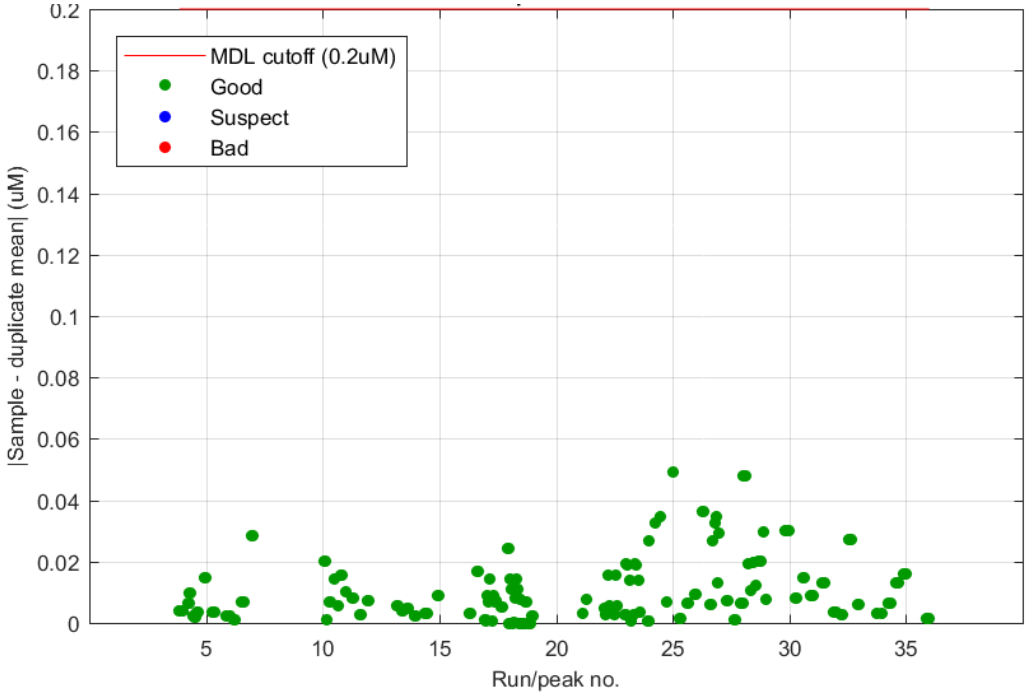
6.6 Sampling Precision

To monitor sampling precision, the deepest sample from each deployment is assayed in duplicate. Duplicate results whose difference from their mean is less than the MDL for silicate, phosphate, nitrite, ammonium and 0.06uM for nitrate pass the precision criteria.

The average of the duplicate is reported in the hydrology data set.

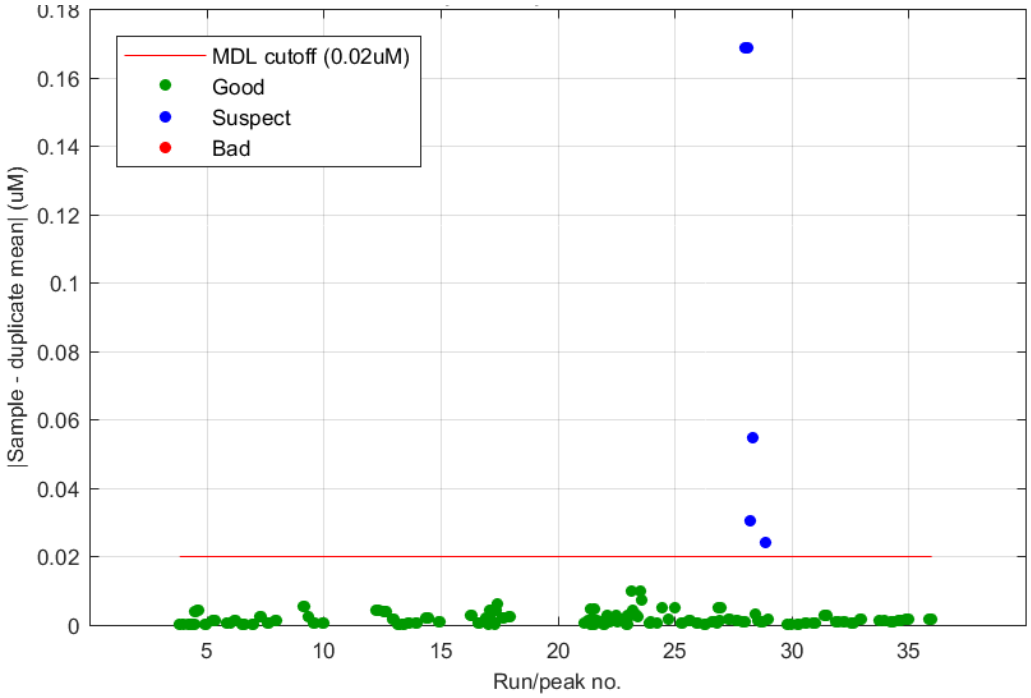
Below are the plots of the duplicate results for all deployments.

6.6.1 Silicate Duplicates Plot

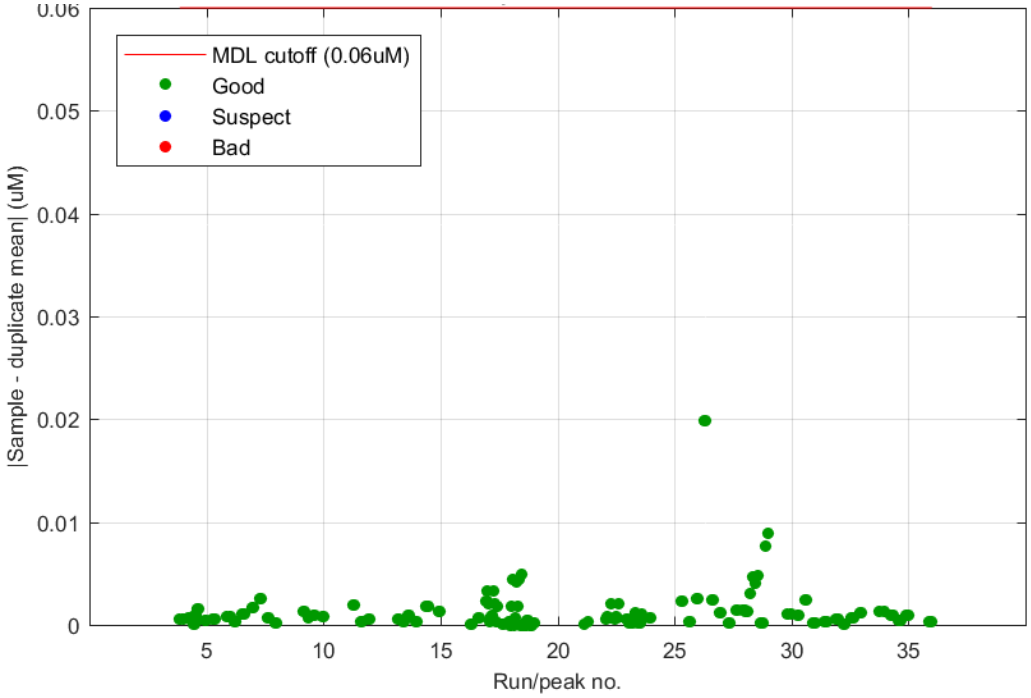


6.6.2 Phosphate Duplicates Plot

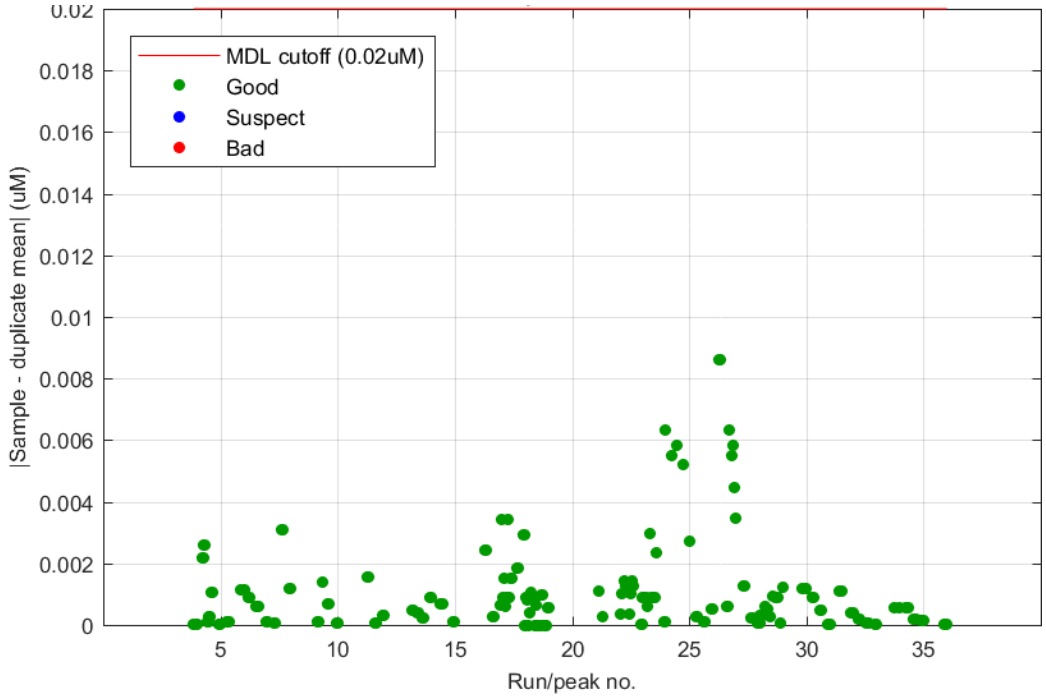
Suspect duplicate: deployment 56 in algal bloom, RP9, 13,



6.6.3 Nitrate + Nitrite (NOx) Duplicates Plot

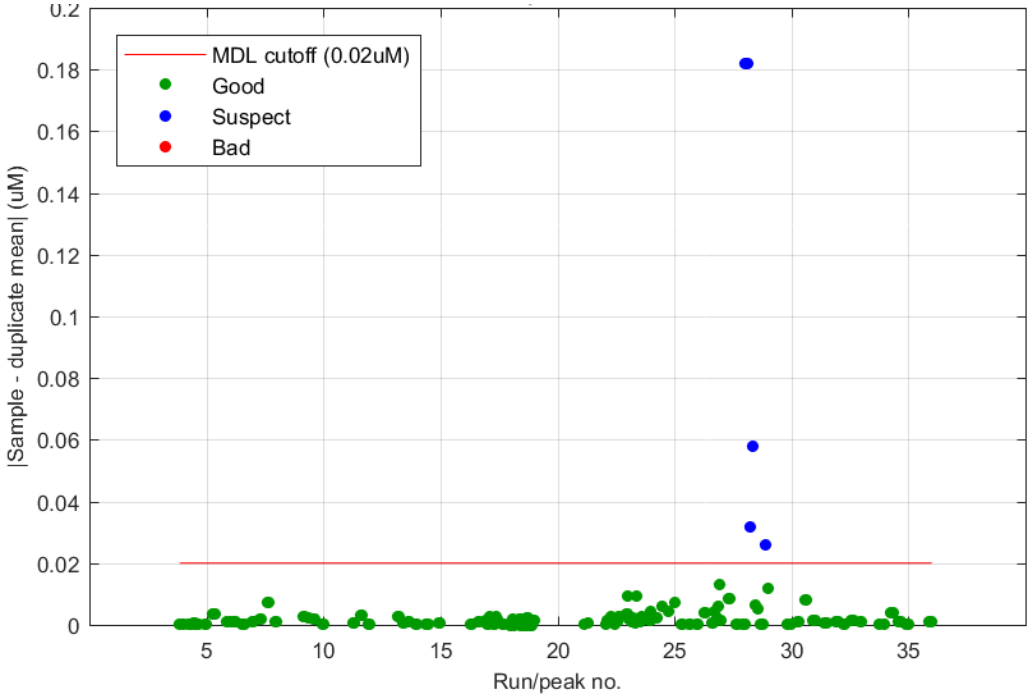


6.6.4 Nitrite Duplicates Plot



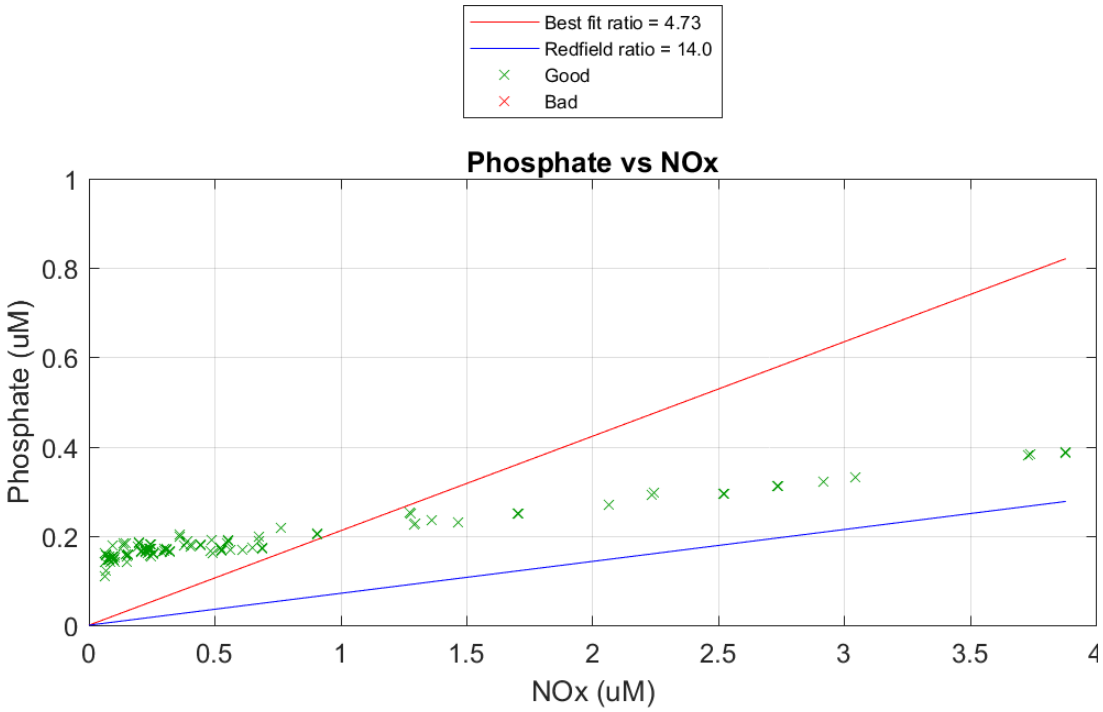
6.6.5 Ammonia Duplicates Plot

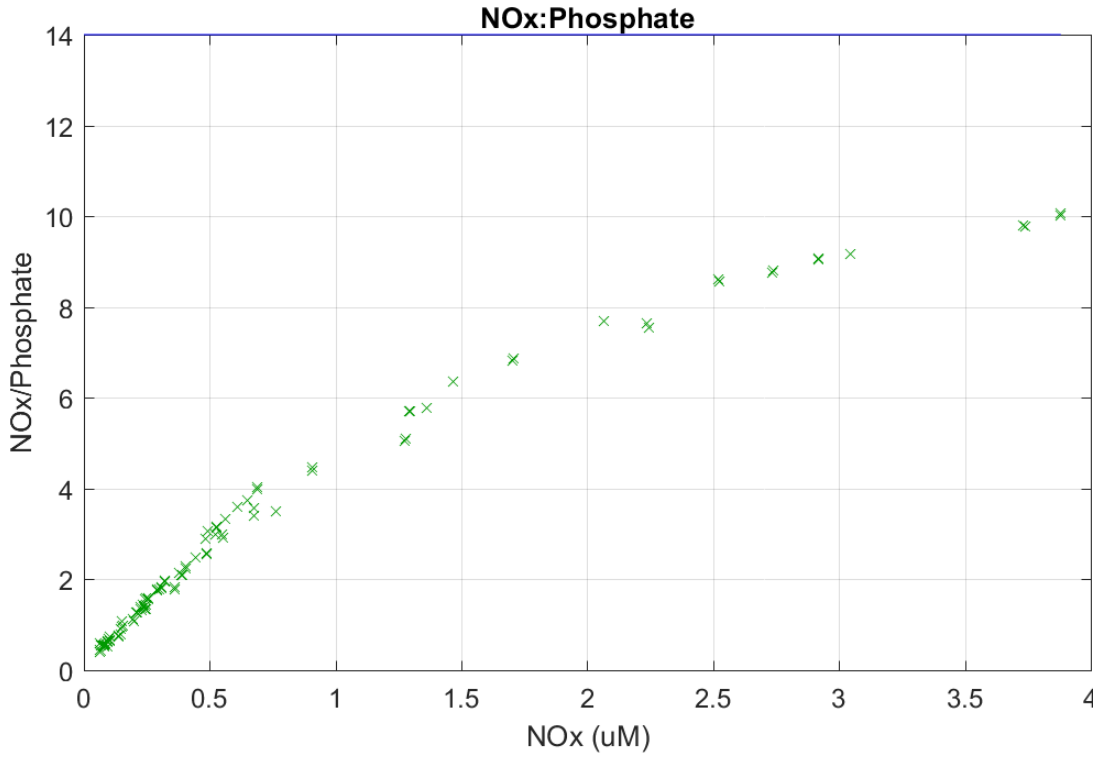
Suspect duplicates: deployment 56 in algal bloom, RP 9, 13



6.6.6 Redfield Ratio Plot (14.0)

Plots consists of phosphate versus NOx for all deployments.





6.7 Missing or Suspect Nutrient Data and Actions taken

CTD	RP	Analysis	Flag	Reason for Flag or Action
56	9,13	PO_4, NH_4	69	Deployment in algal bloom. Sample not filtered prior to assay. Duplicate / Triplicate results variable: All results below. Sample 5609: P (0.10, 0.18, 0.10), NH_4 (-0.01, 0.08, -0.01) Sample 5613: P (0.42, 0.09), NH_4 (0.46, 0.09)

6.8 Laboratory Temperature

The temperature in the hydrochemistry laboratory is measured at two locations. Above the AA3HR instrument (main laboratory) and in the room where salinity samples are measured (salt laboratory).

The laboratory temperature was stable for the voyage duration.

Location	Log Interval	Average	STD
Main Laboratory	3 minutes	21.4 °C	0.6
Salt Laboratory	1 minute	22.7 °C	0.4

7 Appendix

7.1 Nutrients: RMNS Lot CD results for each CTD Deployment

Units: µmole/litre

	SiO_4	PO_4	NO_2	NOx
Stated Value	14.26	0.457	0.018	5.65
Deployment	Result	Result	Result	result
1	14.0	0.45	0.030	5.69
2,3,4	14.2	0.46	0.031	5.71
5,6	14.2	0.44	0.033	5.70
7,8,9	14.2	0.47	0.033	5.67
11,12,13	14.2	0.44	0.034	5.67
15,16,17,18 uwy3,4,5	14.2	0.45	0.033	5.62
20,21,22, uwy6	14.1	0.47	0.033	5.71
23,24,25,26, uwy7	14.0	0.44	0.030	5.71
27,28	14.1	0.45	0.033	5.70
30,32,33 uwy8	14.0	0.45	0.032	5.68
34,35,36	14.1	0.47	0.025	5.69
37,38,39 uwy9	14.0	0.46	0.033	5.67
40,41, uwy10	14.0	0.46	0.035	5.67
42,43, uwy11	14.1	0.45	0.032	5.67
44,45,	14.1	0.47	0.032	5.68
46	14.0	0.45	0.036	5.65
47,48,49, uwy12	14.0	0.45	0.032	5.68
51,52	14.0	0.45	0.025	5.65
53,54,55, uwy13	14.2	0.46	0.029	5.69
56	14.2	0.45	0.029	5.70
58	14.1	0.46	0.029	5.71
59,60,61	14.1	0.46	0.027	5.66
62,63, uwy14	14.1	0.45	0.029	5.69
65,66,67	14.0	0.46	0.023	5.72
68, uwy15	14.1	0.47	0.026	5.67
69,70,71, uwy16	14.1	0.45	0.033	5.69
72, uwy17	14.1	0.45	0.032	5.70

7.2 All Flagged & Missing Data

CTD	RP	Analysis	Flag	Reason for Flag or Action
47	1	DO	141	Sample collected. No result. Sample not acidified for titration. No endpoint.
56	9,13	PO_4, NH_4	69	Deployment in algal bloom. Sample not filtered prior to assay. Triplicate results variable.

7.3 Flag Legend for CSV & NetCDF data

Flag	Meaning
0	Data is GOOD – nothing detected.
63	Nutrients: Below nominal detection limit.
69	Nutrients Only: flagged suspect by operator/ software. Set suspect by software when calibration or duplicate data lies outside of limits set for good data but less than that set for bad data.
79	Nutrients Only: Method Detection Limit (MDL) during run was equal to or greater than the nominal MDL. Data is suspect.
129	Nutrients Only: AA3HR instrument, peak exceeds maximum A/D value. Data is bad.
133	Flagged by operator/ software. Data is bad.
134	Nutrients Only: flagged by software. AA3HR analysis chart trace 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	nc file only: Missing data, no result for sample ID. Not flagged in csv file.
192	Data not processed. Raw data only.

7.4 GO-SHIP Specifications (paraphrased)

7.4.1 Salinity

Accuracy of 0.001 is possible with Autosal™ 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. Autosal 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 ± 1°C is very important and should be recorded.[2]

7.4.2 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.

7.4.3 SiO_2

Approximately 1-3% accuracy[1,3] 0.2% precision, full scale.

7.4.4 PO_4

Approximately 1-2% accuracy[1,3] 0.4% precision, full scale.

7.4.5 NO_3

Approximately 1% accuracy[1,3] 0.2% precision, full scale.

7.4.6 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

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