### Nutrients

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#### Sampling

Seawater samples for nutrient determination were collected at every station and depth into new 16 mL polystyrene tubes after the tube and cap had been rinsed three times with the sample water. A total of 221 unique samples were collected, all in duplicates. In addition, 19 samples were taken in quadruplet so a set of duplicates could be filtered for a filtration test. Those samples were collected at station UTBS-5, UTBC-2, UTBS-4 and UTBS-1 (Cast 14, 15, 17 and 18, respectively). All samples were immediately frozen after collection for storage at -20°C until analysis back onshore.

Nutrient samples were also collected by Jacqueline Grebmeier (UMCE). Are we reporting their data???

#### Standards, reference material samples and reagents

Primary stock standards of nitrate (nitrate + nitrite, NO3), phosphate (PO4) and silicate (SiO4) were prepared at IOS in October 2015 by Linda White, and were calibrated against Kanso certified reference materials, lot CA (NO3 = 20.1 µM, SiO4 = 36.96 µM, PO4 = 1.454 µM). The primary stock standards were prepared in Milli-Q water, using high purity grade dry chemicals (Fluka puriss. grade for sodium hexafluorosilicate, and Fluka ultra p.a. for potassium nitrate and Potassium phosphate monobasic), and grade “A” glass volumetric flasks, according to Barwell-Clarke and Whitney (1996).

A set of 5 working standards, were prepared daily from the primary standard solutions, using freshly prepared 3.4% sodium chloride, 0.02% sodium bicarbonate solution. Concentrations of the standards were selected to bracket the expected nutrient levels in the samples (NO3: 0 to 24.53 µM, SiO4 : 0 to 49.02 µM and PO4: 0 to 2.449 µM).

For quality assurance and quality control purposes, Kanso certified reference material (CRM), deep water reference (DWR), medium check (3rd lowest working standard) and drift cup samples for each nutrient were analyzed at the beginning of a day’s run, every ~ 25 samples and at the end of a day’s run. Wako reference material for nitrate (10 µg atom/L), and silicate (10 µg atom/L) were analyzed at the beginning and end of a day’s run.

The assigned values from CRM KANSO (CA series) were nitrate + nitrite, 20.10 ± 0.016 µmol/L; silicate, 36.96 ± 0.027 µmol/L; and phosphate, 1.454 ± 0.005 µmol/L. Onboard DWR samples were collected from sample # 1181, which was collected during the JOIS 2015-06 cruise in October 2015. Two other “deep water” references were collected during JOIS 2015-06 cruise and analysed with the SWL 2015-07 samples. These were not true deep water samples as they were collected from either 1016 m or a combination of 3055 m and 2545 m depths. Deep water reference samples were sub-sampled into new 16 polystyrene 16 mL tubes, frozen at -20°C, and thawed as required in tepid water.

Reagents were prepared as required, using ACS grade dry chemicals and water from ultrapure water system (Direct-Q5, Millipore) that produced 18.2 MΩ-cm resistance Type I reagent grade water. The system is supplied was supplied with the ship’s distilled water. Two pre-filters are installed before the Direct-Q5 system, a 1 µm sediment filter and a 5 µm carbon filter. The 34 g/L solution of sodium chloride, 0.2 g/L sodium bicarbonate (Sigma, BioXtra grade) was prepared, as needed, and was used as the wash solution between samples, to prepare the working standards and as the blank samples.

#### Sample analysis

Unfiltered nutrients (nitrate, silicate and phosphate) samples were analyzed 5 months after collection at the IOS nutrient laboratory by Sarah-Ann Quesnel on a three channel Seal Analytical nutrient Auto-Analyser 3 (AA3), following the methods described by the manufacturer. There was o exception; we removed the Brij-23 surfactant from the ammonium chloride reagent for nitrate analysis. We found that Brij-23 in this reagent was significantly decreasing the sensitivity of the analysis within a run (> 5% downward drift).

Samples were thawed for 2 hours at 25°C in a water bath prior to analysis. At the beginning of each day, the AA3 was allowed to equilibrate for at least 30-60 minutes, with reagents and wash solution hooked- up to the platen tubing. Nitrate, phosphate and silicate were analyzed simultaneously with the AA3. A typical sample run would consist of a drift cup, carryover cup, 5 point standard curve, a set of reference material, a set of cadmium column recovery samples, blanks, followed by a first set of replicates. A set of reference material (medium check, Kanso, DWR, drift cup and a blank) were analysed every ~ 25 samples. A complete set of reference material were analyzed at the end of a day’s run, along with a second set of cadmium column recovery samples. After each run, wash solutions were run roughly 20 minutes through the system for cleaning. The 2nd set of replicates were analysed on a different day to account for day-to-day variation. Data were logged using the AACE (version 7.02) software provided with the AA3 system, which calculated all standards, reference materials and sample concentrations, correcting for drift, carryover and baseline. When the nitrate level in surface samples was below detection limit, it was reported as zero.

A salt correction was applied to all measured NO3 and SiO4 concentration to adjust for the effect of salinity difference between the sample and the standards. The salt correction equations were as follows:

[NO3]corr = [NO3]meas \* [0.00006 \* (Ss – Sstd)^2 +0.0080 \* (Ss – Sstd) + 0.99909

R2= 0.8438

[SiO4]Scorr = [SiO4]meas \* [-0.0008 \* (Ss – Sstd)^2 +0.00236 \* (Ss – Sstd) + 0.99718

R2= 0.9684

Where:

* [NO3]corr and [SiO4]corr = corrected nitrate and silicate concentrations
* [NO3]meas and [SiO4]meas = measured nitrate and silicate concentrations
* Ss = salinity of the sample, from the bottle salinity
* Sstd = salinity of the standards (34 psu)

Another correction was applied to silicate measurements as the Kanso CRM was reading 2.6-4.2% lower than expected, and was consistent on a daily basis. So a correction factor was applied on a daily basis, as follows:

[SiO4]CAcorr = [SiO4]Scorr X [SiO4]CAexp / [SiO4]CA daily avge

Where:

* [SiO4]CAcorr = CA CRM corrected silicate concentration
* [SiO4]Scorr = salt effect corrected silicate concentration
* [SiO4]CAexp = CA CRM expected concentration = 36.96 µM
* [SiO4]CA daily avge = daily average of CA concentrations

#### Precision, Accuracy and L.o.D.

The precision was calculated as the pooled standard deviation (*sp*), with outliers rejected by the Chauvenet statistic, and the values for the 3 nutrients are given in Table 1 below.

Table 1. Water Sample Precision, L.o.D. and accuracy summary.

The outlier number that are in parentheses were removed “by eye”, meaning the difference between the 2 replicates were greater than 1 µM for nitrate and silicate and 0.2 µM for phosphate.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Chemistry Sample** | **Units** | **Minimum Range** | **Maximum Range** | **L.o.D** | **Precision (*s*p)** | **Number of Replicates (*n*)** | **Outliers**  | **Accuracy (% recovery)** |
| Nitrate (frozen) | µmol/L | 0 | 18.34 | 0.04 | 0.11 | 207 | 6 (6) | 98.02-99.77 |
| Silicate (frozen) | µmol/L | 1.77 | 50.12 | 0.01 | 0.09 | 212 | 2 (5) | 95.27-97.68 |
| Phosphate (frozen) | µmol/L | 0.329 | 2.308 | 0.012 | 0.046 | 215 | 2 (4) | 98.35-101.93 |

The accuracy of nutrient analysis was assured by daily analysis of Kanso Reference Material for Nutrients in Seawater (RMNS) (batch CA, NO3: 20.10 ± 0.016 μmol/L, SiO4: 36.96 ± 0.027 μmol/L; PO4: 1.454 ± 0.005 umol/L, salinity: 34.376 PSU). As mentioned earlier, a correction factor was applied to the samples when the daily % recovery of the analyte of the Kanso CRM was beyond 2% above or below the expected value.

The % recovery of the Kanso RMNS analytes ranged from 98.02 to 99.77% (n = 40) for NO3, 95.27 to 97.68% for SiO4 (n = 40) and 98.35 to 101.93% for PO4 (n = 40). The limit of detection (3 times the standard deviation of 10 blanks) were 0.04 μmol/L for NO3, 0.01 μmol/L for SiO4 and 0.012 μmol/L for PO4.

#### Sample Filtration test

##### Sampling

An extra set of 18 duplicates were collected at 4 stations (UTBS-5, -2, -4 and -1, cast 14, 15, 17 and 18, respectively) for the filtration test. The samples were collected in the barrel of 60 mL syringes that were rinsed 3 times with the sample water. A combusted GF/F filter, 25 mm housed in a swinnex filter holder was luer-locked to the syringe and 2-5 mL of sample water was filtered through before collecting the filtrate in 16 mL polystyrene tubes (also rinsed 3 times with filtrate before collection). The samples were immediately stored frozen at -20°C until analysis back onshore.

##### Results

Sample filtration increased the concentration (0.14 µM on average) and increased slightly variability between duplicates (Filtered - Unfiltered *sp* = 0.19 µM, all *sp* = 0.11 µM) for nitrate. This could either be attributed to contamination from filtration and/or release of dissolved nitrate as cells lyse during filtration. The later can possibly happen if too much pressure is applied on the barrel of the syringe. For silicate, we were expecting that filtration would contaminate the samples as the GF/F are made of borosilicate glass fibers. On the contrary, the results suggest that there was no contamination as the filtered samples were generally just slightly lower than the unfiltered ones (Figure 1). The results also show that the variability of the Filtered – Unfiltered (*sp* = 0.09 µM) was essentially the same as the variability for the whole data set (*sp* = 0.1 µM). For phosphate, the results suggest that filtering decreases the concentration compared to the whole data set (median Filtered – Unfiltered concentration difference = -0.061 µM and median all concentration difference = 0.039 µM, respectively, Figure 1), but with similar variability (Filtered – Unfiltered *sp* = 0.063 µM and .all *sp* = 0.046 µM, respectively).



Figure 1. Filtration test results.

The median of the difference in concentration between duplicates of the whole data set (All) and between the filtered and matching unfiltered samples (filt-unf) are plotted for nitrate (NO3), silicate (SiO4) and phosphate (PO4). The error bars represent the 25th percentile (lower error bars) and the 75th percentile (higher error bar).

#### Problems and Solutions

##### Analysis Issues

Nitrate:On 2 occasion (20 and 24 November 2015), the baseline and peaks were finely ragged for unknown reason. We applied a peak smoothing of 8, as we do for phosphate and the data looked good. We have never encountered this problem before since we acquired the AA3. The following days were back to normal. It is possible that electrical work being performed at IOS could of affected the colorimeter, although it was not seen for silicate and phosphate.

#### References