Use of reference materials for nutrients in seawater and comparability of nutrients in the world's oceans.

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Abstract:

Following issuance of the IPCC report in 2007, there has been renewed effort to improve the global comparability of nutrient measurements from seawater samples. The most recent inter-laboratory comparison studies were led by scientists at the Meteorological Research Institute (MRI), Japan, and have included many key laboratories around the world including the one at Scripps Institution of Oceanography/Shipboard Technical Support (SIO/STS). These comparison studies were made possible by the production of Reference Materials for Nutrients in Seawater (RMNS) by the General Environmental Technos Co., Japan (Aoyama et al 2006, Ota et al., 2010). Some of the participating laboratories have developed protocols for the use of RMNS to improve comparability between laboratories and cruises. SIO/STS participated in all of the recent comparison studies and helped develop protocols for use of the reference materials. The nutrient analysis section of the WOCE manual from the 1990s has also been updated and rewritten for GO-SHIP as part of these efforts. The original manual called for 1% accuracy or reproducibility for nitrate, 1-2% for phosphate, 1-3% for silicate (Joyce et al., 1991). The new GO-SHIP manual states, "These materials [RMNS] along with the use of best practice in using analysis equipment and improved internal standardization should make it commonly possible to achieve comparability of nutrient analyses to a level better than 1%." (Hydes et al.,2010).
The results from the comparison studies show that there is very good comparability and traceability between the participating core labs for nitrate, nitrite, and phosphate (Aoyama et al., 2006, 2007, 2008, and 2010); however, there are differences between some of the core labs in silicate. These inter-lab differences are on the order of 2% and are consistently present over the course of the comparison studies. The results suggest that the differences lie in standardization and lack of a primary silicate standard, thus indicating that the 2% silicate difference seen between some of the CLIVAR repeat cruises could be accounted for with the use of the reference materials. The chemistry lab at SIO/STS has been using the reference materials on cruises since 2009, specifically CLIVAR cruises P06, S04P, A20, A22, and P02, and the U.S. GEOTRACES cruises in 2011 and 2013. Comparison of the data from the recent CLIVAR cruises with the archived WHP data show very good agreement when SIO/STS was the analyzing lab for both expeditions.

Introduction:

Global comparability of nutrient data in the world's oceans has become increasingly important because nutrients play a significant role in the global carbon budget. Scientists are looking at data from different labs and cruises to determine long term changes in the carbon budgets as well as changes in the nutrient fields (Bindoff et al., 2007, Gouretski and Jancke 2001, Jutterstrom et al., 2008). The potential changes under examination are at or near the detection limits of most instruments. It is very important that differences in methodology or other inter-laboratory differences not contribute to the results.

There have been a number of comparison studies for nutrients over the past 30 years. The International Council for the Exploration of the Sea (ICES) first started comparison tests in the 1960's (UNESCO 1965, 1967; ICES 1967). After a lengthy break, there was another series of comparison experiments under ICES to support the WOCE program, summarized by Aminot 1995. Quality Assurance of Information for Marine Environmental Monitoring in Europe" - QUASIMEME - has developed into a program that allows individual laboratories to validate methods and procedures once or twice a year. (Topping, 1997). There have been other studies done more recently (Willie and Clancy, 2000; Clancy and Willie, 2003); however, when the IPCC report came out in 2007, global comparability and traceability of nutrients still had not been achieved.

The most recent nutrient comparison studies have been led by Dr. Michio Aoyama (MRI) in Japan(Aoyama, 2006, Aoyama et. al, 2007, 2008, and 2010). The production of RMNS used for the studies led by MRI is described by Ota (Ota et al., 2010). The production and use of these materials have allowed the participants of the comparison studies to form the IOC/ICES Study Group on Nutrient standards (SGONS). The ultimate goal of the study group is to establish comparability and traceability of nutrient data in the world's oceans through the development of the International Nutrients Scale System (INSS) (Aoyama, M., D. Hydes et al., 2010). To reach this goal the group has re-written and updated the WOCE nutrient analysis manual (GO-SHIP nutrients best practices), developed protocols for the use of the RMNS, encouraged collaboration between laboratories from different countries on global repeat cruises, and submitted a proposal for a IOC/SCOR Working Group on the "International Nutrients Scale System Panel." There have been meetings and a workshop on phosphate methods as part of these efforts.

RMNS have been used by Japanese scientists on the R/V Mirai as calibration standards on CLIVAR cruises since 2005. As a result they have established internal comparability of nutrient concentration values. Comparison of nutrient values at crossover points on R/V Mirai cruises since 2005 are also within the uncertainty of the measurements. (Sato, 2010). RMNS have been used on SIO/STS CLIVAR and other Global cruises starting in 2009. Analysis of the results from the RMNS and comparison of P06 data from 2003 (R/V Mirai cruise) and 2009/2010 were presented at the INSS workshop in Paris (Sato 2010). Since then, the SIO/STS chemistry laboratory has used RMNS on CLIVAR cruises in 2011 (S04P), 2012 (A20 and A22), 2013 (P02) in addition to an Atlantic GEOTRACES cruise in 2011 and 2013.

SIO/STS Nutrient methods and use of RMNS:

Nutrient analyses (phosphate, silicate, nitrate+nitrite, and nitrite) were performed on a Seal Analytical continuous-flow AutoAnalyzer 3 (AA3). The methods used are described by Gordon et al (1993) Hager et al. (1972), and Atlas et al. (1971). The details of modification of analytical methods used by SIO/STS are also compatible with the methods described in the nutrient section of the GO-SHIP repeat hydrography manual (Hydes et al., 2010)

Primary standards for silicate (Na2SiF6), nitrate (KNO3), nitrite (NaNO2), and phosphate (KH2PO4) were obtained from Johnson Matthey Chemical Co. and/or Fisher Scientific. The primary standards were dried and weighed out to 0.1 mg prior to the cruise. The exact weight was noted for future reference. When primary standards were made on the cruise, the flask volume at 20 °C, the weight of the powder, and the temperature of the solution were used to buoyancy-correct the weight, calculate the exact concentration of the solution, and determine how much of the primary was needed for the desired concentrations of secondary standard. Primary and secondary standards were made up every 7-10 days. The new standards were compared to the old before use.

A deep calibration "check" sample and a RMNS were run with each set of samples as a quality control check for the run. The water for deep check sample (10-20 liters) was collected during the test cast from a depth greater than 1500m. The water was poisoned with 1-2 mls of a saturated mercuric chloride solution and a subsample run with each set of samples. The RMNS preparation, verification, and suggested protocol for use of the material are described by (Aoyama et al., 2006, 2007, 2008) and Sato (2010). A new bottle of RMNS was opened every day. The values of the check sample and the RMNS were closely monitored to detect standardization or other analytical problems with the run. Adjustments to the data (if needed) were done on a run to run basis. The intra-cruise variability was determined from the standard deviation and percent variation of the repeat measurements of the deep check sample and analysis of same "batch" of the RMNS with each run.

Cruise Results:

CLIVAR P06:
This cruise provided a good opportunity to evaluate and compare data sets generated by different laboratories. These results were first presented at a SGONS workshop in Paris, France, 2010 (Becker et al., 2010) and were included in the results presented in Sato 2010. This CLIVAR line was occupied previously in 2003 by the Japanese scientists at JAMESTEC onboard the R/V Mirai. This was the first cruise on which RMNS were used at all stations. Dr. Michio Aoyama was able to provide 210 bottles of RMNS batch BE through a grant from the Japanese government for the 2009/2010 cruise on the R/V Melville. Dr Aoyama also provided the assigned values for the RMNS. A RMNS sample and a deep check sample were analyzed at every station as quality control checks. Cruise averages, standard deviations and coefficients of variation for each are tabulated below:

Table 1: P06 Deep check sample

Parameter	Conc (umol/L)	Stdev	%CV
P06 West			
NO3	32.92	0.28	0.84
PO4	2.29	0.02	1.09
SIL	119.5	1.05	0.88
P06 East			
NO3	33.25	0.22	0.67
PO4	2.33	0.02	0.67
SIL	121.7	0.6	0.49

Table 2: P06 RMNS Batch BE

Parameter	Conc (umol/kg)	Stdev	%CV	Assigned value	Difference
NO3	36.64	0.32	0.87	36.70	0.06
PO4	2.68	0.03	0.94	2.66	-0.02

SIL	101.8	0.71	0.68	99.2	-2.6

The average deep values (>2000m) in the water for the 2003 and 2009 cruises are summarized in table 3.

Table 3: Cruise Data

Parameter	2003 Conc (umol/kg)	2009 Conc (umol/kg)	difference
P06 West			
NO3	32.29	32.00	0.29
PO4	2.23	2.24	-0.01
SIL	116.4	119	-2.6
P06 East			
NO3	33.55	33.17	0.38
PO4	2.34	2.32	0.02
SIL	118.2	120.3	-2.1

The intra cruise variability for nitrate, phosphate and silicate were all on the order of 1%. The observed differences for deep water concentrations of nitrate and phosphate between the 2003 and 2009 data and the difference between the observed RMNS values and the assigned values are within, or very close, to the intra cruise variability. However, the difference between the observed vs. assigned RMNS values are greater than the intra cruise variability for silicate. The difference between the observed RMNS values and the assigned values for silicate are also outside the variability but very close to the differences seen in deep water concentrations between the 2003 and 2009 data sets.

CLIVAR S04P:

SIO/STS purchased vials of RMNS batch BE directly from the manufacturer (KANSO) for this cruise, and the manufacturer-assigned concentration values were supplied with the material. Again SIO/STS ran a deep check sample as well as the RMNS as quality control checks with each set of samples/station. Results are summarized in tables 4 and 5.

Table 4: S04P Deep check sample

Parameter	Conc (umol/L)	Stdev	%CV
NO3	32.60	0.13	0.40
PO4	2.26	0.01	0.44
SIL	104.5	0.56	0.54

Table 5: S04P RMNS Batch BE

Parameter	Conc (umol/kg)	Stdev	%CV	Assigned value	Difference
NO3	36.65	0.13	0.35	36.64	-0.01
PO4	2.66	0.01	0.37	2.67	0.01
SIL	100.0	0.57	0.57	101.2	1.2

Similarly to P06 the intra-cruise variability for nitrate, phosphate and silicate were all on the order of or less than one percent. Again the differences in the observed and assigned RMNS values for nitrate and phosphate fall within the intra cruise variability. The difference between the cruise determined RMNS values and assigned RMNS values for silicate are not as large for this cruise compared to P06, but are still not less than the intra cruise variability. This is due to a different assigned value from the manufacture as well as a different determined value for the RMNS.

CLIVAR A22 and A20:
Dr. Michio Aoyama was able to provide RMNS batches BS, BU, BT, and BD through a grant from the Japanese government. The RMNS were used as calibration standards on this cruise. Dr Aoyama also provided the values for the RMNS. The silicate values for the RMNS were adjusted based on past silicate results of SIO/STS analysis of RMNS for the comparison studies (Aoyama et al., 2006, 2007, and 2008), and P06 cruise results.

Since the RMNS were used as the calibration standards, the deep check sample was the only quality control sample run with each station. The results are consistent with past cruises, showing the intra cruise variability on the order of one percent.

Table 6: A22 and A20 deep check sample

Parameter	Conc (umol/L)	Stdev	%CV
A22			
NO3	17.20	0.04	0.24
PO4	1.17	0.009	0.78
SIL	18.57	0.15	0.79
A20			
NO3	18.22	0.07	0.39
PO4	1.22	0.01	0.83
SIL	18.8	0.20	1.04

Initial comparison of a few deep stations from A22 in 2003 and 2012 data sets show no differences in the deep values for nitrate, phosphate, or silicate.

CLIVAR P02:
RMNS were again provided by Dr. Aoyama through a grant from the Japanese government. Batch BX was used, with each bottle being analyzed once or twice before being discarded and a new one opened. Data for the deep check sample and RMNS BX are tabulated below

Table 7: P02 Deep check sample

Parameter	Conc (umol/L)	Stdev	%CV
P02 West			
NO3	41.70	0.21	0.50
PO4	2.94	0.01	0.34

SIL	162.1	0.58	0.36
P02 East			
NO3	35.86	0.14	0.40
PO4	2.50	0.01	0.59
SIL	148.1	0.51	0.34

Table 8: P02 RMNS batch BX

Parameter	Conc (umol/kg)	Stdev	%CV	Assigned value	Difference
P02 West					
NO3	43.08	0.16	0.37	43.00	-0.08
PO4	2.90	0.02	0.69	2.906	0.006
SIL	138.7	0.55	0.40	136.0	-2.7
P02 East					
NO3	43.13	0.12	0.27	43.00	-0.13
PO4	2.89	0.02	0.60	2.906	0.016
SIL	138.8	0.56	0.40	136.0	-2.8

As was noted for P06, S04P, A22, and A20 the intra cruise variability for nitrate, phosphate, and silicate were all close to or less than one percent. The differences in the observed and assigned RMNS values for nitrate and phosphate are again less than the intra cruise variability. The differences between the observed and assigned RMNS values for silicate were still greater than the intra cruise variability. An initial comparison to data from the P02 cruise done in 2004 does not indicate major differences in deep nutrient values between the two data sets.

Conclusions:

The need for RMNS to attain comparability of nutrient data sets collected on global repeat cruises has well been established (Bindoff et al., 2007, Jutterstrom et al 2008, Tanhua 2010). Participation in comparison studies and use of the RMNS on cruises has allowed the laboratory at SIO/STS to improve at-sea accuracy and precision as well as compare temporally separated data sets. The intra cruise variability for all the nutrient parameters is close to or within the stated goals of the Go-SHIP manual (1%). The inter cruise data set comparisons for nitrate and phosphate are also within this goal. This is backed up by the agreement between the observed and assigned RMNS values. Silicate data set comparisons are not as straight forward. There is good agreement between cruise data sets when the SIO/STS laboratory was responsible for the onboard analysis. For example, 2003 vs 2012 A22 and A20 data as well as the P02 2004 vs 2013 data sets. However, when comparing the P06 2003 (Japan) vs 2009/2010 (SIO/STS) data, the SIO/STS silicate data is not in close agreement with the data from the Japanese cruise. A similar difference is seen in the silicate RMNS analysis. The differences between labs and between the observed vs assigned values for RMNS are on the order of 2% and are consistent. These results suggest that the differences lie in standardization and lack of a primary silicate standard. Data sets, particularly nitrate and phosphate, that are referenced to analysis of RMNS can now be directly compared.

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