ANALYTICAL SCIENCES
SEPTEMBER 2007, VOL. 23

Recent Comparability of Oceanographic Nutrients Data: Results of a 2003 Intercomparison Exercise Using Reference Materials

Michio Aoyama,[*1†] Susan Becker,[*2] Minhan Dai,[*3] Hideshi Daimon,[*4] Louis I. Gordon,[*5] Hiromi Kasai,[*6] Roger Kerouel,[*7] Nurit Kress,[*8] Doug Masten,[*2] Akihiko Murata,[*9] Naoki Nagai,[*10] Hiroshi Ogawa,[*11] Hidekazu Ota,[*12] Hiroaki Saito,[*13] Kazuhiro Saito,[*14] Takao Shimizu,[*15] Hiroyuki Takano,[*16] Atsushi Tsuda,[*6] Katsumi Yokouchi,[*17] And Agnes Youenou[*18]

*1 Meteorological Research Institute, Japan Meteorological Agency, 1-1 Nagamine, Tsukuba 305–0052, Japan
*2 Scripps Institution of Oceanography, STS/ODF, 8855 Biological Grade 216 Isaacs Hall La Jolla, CA 92037, USA
*3 State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China
*4 Kobe Marine Observatory, 1-4-3 Wakihamakaigandori, Chuo, Kobe 651–0073, Japan
*5 College of Oceanic and Atmospheric Sciences, Oregon State University, 104 Ocean Admin Bldg., Corvallis, OR 97331-5503, USA
*6 Hokkaido National Fisheries Research Institute, 116 Katsurakoi, Kushiro, Hokkaido 085–0802, Japan
*7 French Research Institute for Exploitation of the Sea (IFREMER) (Centre de Brest), BP 70, 29280 Plouzane, France
*8 Israel Oceanographic & Limnological Res., P. O. Box 8030, Tel Shikmona, 31080, Haifa, Israel
*9 Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237–0061, Japan
*10 Marine Division, Climate and Marine Department, Japan Meteorological Agency, 1-3-4 Otemachi, Chiyoda, Tokyo 100–8122, Japan
*11 Ocean Research Institute, The University of Tokyo, 1-15-1 Minamidai, Nakano, Tokyo 164–8639, Japan
*12 The General Environmental Technos Co., Ltd. (KANSO), 3-1-1 Higashikurazi, Katano, Osaka 576–0061, Japan
*13 Tohoku National Fisheries Research Institute, 3-27-5 Shinhama-cho, Shiogama, Miyagi 985–0001, Japan
*14 Hakodate Marine Observatory, 3-4-4 Mihara, Hakodate, Hokkaido 041–0806, Japan
*15 Nagasaki Marine Observatory, 11-51 Minamiote-machi, Nagasaki 850–0931, Japan
*16 Maizuru Marine Observatory, 901 Shimofukui, Maizuru, Kyoto 624–0946, Japan
*17 Seikai National Fisheries Research Institute, 3-30 Kokubu-machi, Nagasaki 850–0951, Japan
*18 French Research Institute for Exploitation of the Sea, IFREMER DEL/EC/PP, BP 70, 29280 Plouzane, France

To whom correspondence should be addressed. E-mail: maoyama@mri-jma.go.jp

Abstract:

An intercomparison exercise was conducted using the recently developed Reference Material for Nutrients in Seawater (RMNS). Discrepancies of reported values among laboratories were greater than the homogeneity of RMNS samples and the reported analytical precision of nutrients. The variability of in-house standards of the participating laboratories might be the most likely source of interlaboratory discrepancies. Therefore, the use of common reference materials, *i.e.* certified RM, is essential to establish and improve the comparability of nutrient data of the world's oceans

Introduction

Measurements of nutrients in seawater have a long history, but neither widely used reference material for nutrients in seawater in our oceanographic community nor an internationally agreed scale of nutrients in seawater is available. Therefore, it is difficult to discern small changes in nutrient concentrations between laboratories, which might be important to clarify oceanic carbon and nutrient cycles. There is an urgent need to develop certified reference materials for nutrients in seawater[1-3] and to establish comparability of nutrient data of the world's oceans provided by different laboratories.

Efforts to establish comparability of nutrients in seawater have been carried out for over 30 years. The International Council for the Exploration of the Sea (ICES) conducted intercomparison exercises for nutrients in seawater five times from 1965 to 1993.[4-9] The exercises resulted in considerable improvements in techniques for both measuring nutrients in seawater and producing reference materials. The National Oceanic and Atmospheric Administration (NOAA) and the National Research Council (NRC) conducted intercomparison studies in 2000 and 2002 using reference material.[10-11] The reference material they used was certified based on consensus concentrations obtained by the intercomparison exercise and was provided as MOOS-1 from the National Research Council of Canada in 2003[12]; it became the first certified reference material for nutrients in seawater in a seawater matrix. A second set of certified reference materials for nutrients in seawater were provided as QC-SW3.1, 3.2, 4.1, and 4.2 by EUROFINS in 2006 (http://www.eurofins.dk/index_en.asp). However, the nutrient concentrations of MOOS-1 and QC-SW3.1, 3.2, 4.1, and 4.2 were too low to encompass the nutrient concentrations in the Pacific Ocean and some other oceans.

An intercomparison study using the newly produced Reference Material for Nutrients in

Seawater (RMNS) was conducted in 2003 to examine interlaboratory comparability for nutrients in seawater with one of the authors (M. Aoyama) as a coordinator. This study utilized two improvements in sample treatment compared with the previous studies.[4-11] One improvement was that the RMNS sample concentrations almost covered the ranges of concentrations in the Pacific Ocean, in which the peak concentrations are the highest of the world's oceans; the RMNS concentrations were 0–38μmol kg^{-1} for nitrate, 0.0 to 0.9μmol kg^{-1} for nitrite, 0.1 to 2.7μmol kg^{-1} for phosphate, and 2 to 136μmol kg^{-1} for silicic acid. The other improvement was that the four determinants—nitrate, nitrite, phosphate, and silicic acid—could be analyzed in a single bottle under the same conditions as those for natural seawater samples.

We first describe in this paper the experiment methods for the intercomparison, together with the process of RMNS production. We then examine whether consensus values, which are important for future certification of RMNS, can be obtained from the reported values of the intercomparison exercise. Finally, we discuss the effectiveness of the RMNS for establishing comparability of nutrient data in the world's oceans. A detailed report of the "2003 Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix" was published by the coordinator of this intercomparison.[13]

Experimental

Sample preparation

Seawater with various nutrient concentrations was collected from the surface to deep water in the western North Pacific Ocean. The RMNS of specific concentrations of nutrients (one batch) was prepared as follows.

The seawater was gravity-filtered with a membrane filter with a 0.45μm pore size. We used a stainless steel container with 40-l volume for five of the six batches used in this intercomparison exercise and a stainless steel container with 100-l volume for one of the six batches, sample #3. The seawater was sterilized by autoclaving at 120°C for 2h; the autoclaving was then repeated twice. The autoclaving was based on previous studies,[15,16] the details of which are described elsewhere.[14] The seawater was cooled for a few days to room temperature, after which an aliquot (90ml) of autoclaved seawater in the stainless steel container was filtered through a 0.22μm pore size membrane into polypropylene (PP) bottles with 100ml volume. These bottles had been rinsed with pure water and exposed to UV-light before they were used. Each PP bottle was vacuum-sealed in a vinyl bag to prevent subsequent contamination from air and evaporation or condensation of water. The bottling process was conducted in a clean room of class 1000.[14]

Six batches (RMNS #1 – #6) of various nutrient concentrations were prepared for the intercomparison exercise using the autoclaving method described above. The nitrate concentrations did not change during the autoclaving. However, the phosphate concentrations decreased 7% and the silicate concentrations decreased 5%. The reasons for these nutrients concentration decreases are not yet clear.

Homogeneity

The homogeneities of 30 bottles of RMNS #3 for nitrate+nitrite, phosphate, and silicic acid, expressed as the coefficient of variation (CV), are provided in Table 1, together with the analytical precisions (CV) that were estimated from 30 unprocessed seawater samples with nutrient concentrations similar to those of RMNS #3. The homogeneities for nitrate+nitrite and silicic acid of RMNS #3 were almost equivalent to the analytical precision for unprocessed seawater, implying good homogeneity. The homogeneity for phosphate was only double the analytical precision, and therefore the coordinator considered the homogeneity of phosphate to be sufficient for the intercomparison exercise.

No analyses were conducted for other batches of RMNS due to a limited number of RMNS bottles. Nevertheless, we consider that the others had the same level of homogeneity as RMNS #3 since they were prepared following an identical procedure.

A long-term storage experiment demonstrated that the homogeneity and concentrations of nutrients are maintained near room temperature for about four years; details of the long-term storage experiment are available elsewhere.[14]

Sample shipment and responses

The six batches of RMNS used for the intercomparison exercise were produced in 2001 and 2002 and were sent to participants (eighteen laboratories from five countries) in the year 2002. Individual laboratories were provided with one sample from each batch, i.e., six samples in total. The shipping method to each laboratory was normal transport on a commercial basis by air for foreign laboratories and by surface for Japanese laboratories. No serious damage to RMNS during transport was reported, but one laboratory reported a shortage of samples 1 and 4.

One laboratory cancelled participation in the intercomparison exercise, which left 17 laboratories. All results from the 17 laboratories were received by April 2003. One group did not report nitrite. Four laboratories did not report nitrate; instead, they reported nitrate+nitrite. The

nitrate concentrations for those laboratories were calculated by subtracting the concentrations of nitrite from those of nitrate+nitrite. Four laboratories did not report silicic acid.

Results and Discussion

Consensus values

We defined the consensus value (mean and median) of a nutrient species based on the successive application of the *t*-test. We calculated the modes as being statistically equal to the values reported from the majority of the laboratories.

A *t*-test at the 95% confidence level was applied to each species (nitrate, nitrite, nitrate+nitrite, phosphate, and silicic acid) of each batch (RMNS #1 through #6) before calculating the consensus means and medians. This selection procedure was repeated until a stable mean was reached. The stable means were obtained by a second iteration.

The means computed from the selected data are listed in Table 2, together with standard deviations, medians, and modes. The medians were calculated from the original reported values, while the modes were estimated from frequency distributions with 2*n* classes as follows:

$$(\bar{x} + i\sigma) - \frac{1}{2}\sigma \le i\text{th class} < (\bar{x} + i\sigma) + \frac{1}{2}\sigma,\ \ i = -n, \Lambda\ , -1, 0, 1, \Lambda\ , n \qquad (1),$$

where $\bar{x}$ and σ are the consensus mean and standard deviation. Values in parentheses represent the *i*th class.

The means, medians, and modes were in excellent overall agreement for all species and for all batches (Table 2), which implies that the means could be treated as consensus values (concentrations).

Discrepancies in reported values

The standard deviations (expressed as CV) of the reported value filtered by the successive application of a *t*-test, as described above for RMNS #3, were compared with the homogeneities of RMNS #3 (Table 1) to estimate the overall discrepancies between the reported and consensus values.

The standard deviation of the consensus values for nitrate+nitrite was only double the homogeneity, which suggests that the interlaboratory comparability is high. Therefore, our community now has an analytical technique suitable for producing nitrate+nitrite data of high reproducibility. In contrast, the consensus standard deviation for phosphate was 4.5 times greater than that of the homogeneity, and the consensus standard deviation for silicic acid was more than 10 times greater than that of the homogeneity.

Several participating laboratories also reported their analytical precision. This information is important for discussions regarding the cause of discrepancies of reported values. Table 3 presents the medians, the range of analytical precision at participating laboratories, and consensus standard deviations for sample #3. The analytical precision for nitrate+nitrite was 0.2% as the median among the laboratories and ranged from 0.1% to 0.6%, while the consensus standard deviation of nitrate+nitrite for sample #3 was 1.0%. The consensus standard deviation for nitrate+nitrite was five times greater than the analytical precision. The analytical precision for phosphate and silicic acid was 0.9% and 0.4% as the median among the laboratories, while the consensus standard deviations of phosphate and silicic acid were 3.5% and 1.7%. Therefore, the consensus standard deviations for phosphate and silicic acid were four times greater than the analytical precision. These results indicate that interlaboratory comparability for nitrate+nitrite, phosphate, and silicic acid is relatively low when we consider the homogeneity of the RMNS sample and the reported analytical precision of participating laboratories. These results also

indicate that variability in in-house standards of the participating laboratories may be the source of interlaboratory discrepancy.

A close inspection of Table 2 reveals discrepancies between means and modes, particularly for an RMNS of low concentration. The difficulty with blank determination is the most likely source of these discrepancies.

We calculated the *Z*-score (Z_{spc}) to evaluate discrepancies in the reported values among laboratories as follows:

$$Z_{sp\,c} = | (C_{spc} - C_{con}) / P_{sp} | \qquad (2)$$

where C_{spc} and C_{con} are the concentrations of RMNS measured by individual laboratories for each species and the consensus mean (Table 2). P_{spc} is the standard deviation of each species (Table 2).

Z-scores were calculated for each reported value (24 values; 6 RMNS × 4 species at most), but they were averaged for each species: Z_{NO_3}, Z_{NO_2}, Z_p, and Z_s (Table 4). Even if anomalous values such as $Z_{NO_3} = 22.97$ were excluded from consideration, laboratories reporting nutrient values with large discrepancies ($Z_{spc} > 1.00$) remained. However, the averaged *Z*-scores (($Z_{NO_3} + Z_p$)/2 or $Z_{NO_3} + Z_p + Z_s$)/3) indicate that some laboratories, for example laboratories 3, 4, 5, 6, 7, 8, 10, 13, 15, and 18, consistently reported values with small discrepancies ($Z_{spc} \leq 1.00$) throughout the range of nutrient concentrations.

Conclusions

The results of the intercomparison exercise revealed the existing interlaboratory comparability of nutrients data. The standard deviation for phosphate (silicic acid), which

represents the overall discrepancy of reported values, exceeded 4.5 times (10 times) the homogeneity of the RMNS prepared for the intercomparison exercise. The standard deviation for nitrate was only about double the homogeneity. These results demonstrate that our community has an analytical technique for nitrate that is sufficient to provide data of high comparability. The consensus standard deviations for nitrate+nitrite, phosphate, and silicic acid were four to five times greater than the analytical precision. These results also indicate that the variability in in-house standards of the participating laboratories might be the most likely source of interlaboratory discrepancies. Therefore, use of common reference materials, i.e. certified RM, for nutrients in seawater is essential to improve and establish comparability of nutrient data in the world's oceans.

Acknowledgements

The authors thank Wenjing Zhu and Mayako Shimizu for data management and the preparation of tables.

References

1. National Research Council, "*Chemical Reference Materials*", **2002**, National Academic Press, Washington, D.C.
2. UNESCO, "*IOC-IAEA-UNEP Group of experts on standards and reference material (GESREM), 2nd session*", **1991**, UNESCO.
3. UNESCO, "*IOC-IAEA Group of experts on standards and reference materials (GESREM),* 3rd session", **1992**, UNESCO.
4. UNESCO Technical Papers in Marine Science, No.3, "*Report on the intercalibration measurements in Copenhagen, 9-13 June 1965*", **1965**, UNESCO.
5. UNESCO Technical Papers in Marine Science, No.9, "*Report on intercalibration measurements Leningrad, 24-28 May 1966, Copenhagen, September 1966*", **1966**, UNESCO.
6. ICES, 1966. 1967. ICES CM 1967/C:20, "*Report on the analysis of phosphate at the ICES intercalibration trials of chemical methods held at Copenhagen*", **1967**, International Council for the Exploration of the Sea, Denmark.
7. ICES Cooperative Research Report, No.67, "*The International Intercalibration Exercise for Nutrient Methods*", **1977,** International Council for the Exploration of the Sea, Denmark.
8. D. S. Kirkwood, A. Aminot, and M. Perttila; ICES Cooperative Research Report, No. 174, "*Report on the results of the ICES fourth intercomparison exercise for nutrients in sea water*", **1991**, International Council for the Exploration of the Sea, Denmark.
9. A. Aminot and D.S. Kirkwood, ICES Cooperative Research Report, No.213, "*Report on the results of the fifth ICES intercomparison exercise for nutrients in sea water*", **1995**, International Council for the Exploration of the Sea, Denmark.

10. S. Willie and V. Clanay, NOAA Technical Memorandum NOS NCCOS CCMA 143, "*NOAA/NRC Intercomparison for Nutrients in Seawater*", **2000**, National Oceanic and Atmospheric Administration, USA.
11. V. Clanay and S. Willie, NOAA Technical Memorandum NOS NCCOS CCMA 158 "*NOAA/NRC Intercomparison for Nutrients in Seawater*", **2003**, National Oceanic and Atmospheric Administration, USA.
12. V. Clanay and S. Willie, *Anal. Bioanal. Chem.*, **2004**, *378 (5)*, 1239.
13. Technical Reports of the Meteorological Research Institute No. 50, "2003 Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix", **2006**, Meteorological Research Institute, Japan.
14. M. Aoyama, H. Ota, Y. Arii, S. Iwano, H. Kamiya, M. Kimura, T. Kitao, S. Masuda, N. Nagai, and K. Saito, *Papers in Meteorology and Geophysics*, **2006,** submitted.
15. A. Aminot and R. Kerouel, *Anal. Chim. Acta*, **1991**, *248*, 277.
16. A. Aminot and R. Kerouel, *Mar. Chem.*, **1995**, *49*, 221.

Table 1. Analytical precision estimated from unprocessed seawater and the homogeneity and consensus standard deviation (s.d) of RMNS #3, all expressed by CV.

Nutrients	Analytical precision		Homogeneity		Consensus s.d.	
	n	%	n	%	n	%
Nitrate+nitrite	30	0.34	30	0.44	15	1.0
Phosphate	30	0.32	30	0.80	17	3.5
Silicic acid	30	0.16	30	0.15	13	1.7

Note: Concentrations of nutrients in unprocessed seawater were 43μmol $kg^{-1}$for nitrate+nitrite, 3.1μmol kg^{-1} for phosphate, and 148μmol kg^{-1} for silicic acid.

Table 2. Means, standard deviations (s.d.), medians, and modes of nitrite, nitrate, nitrate+nitrite, phosphate, and silicic acid for six kinds of RMNS

Nutrient	RMNS	n	Mean μmol kg^{-1}	s.d. μmol kg^{-1}	Median μmol kg^{-1}	Mode μmol kg^{-1}
Nitrite	1	15(15)	0.02	0.01	0.02	0.02
	2	15(16)	0.13	0.06	0.15	0.13
	3	13(14)	0.01	0.01	0.01	0.01
	4	14(14)	0.02	0.02	0.02	0.00
	5	14(16)	0.91	0.02	0.90	0.89
	6	16(16)	0.23	0.09	0.24	0.23
Nitrate	1	13(15)	0.04	0.03	0.05	0.01
	2	16(16)	17.6	0.6	17.6	17.6
	3	13(15)	35.3	0.4	35.5	35.3
	4	13(15)	0.03	0.03	0.03	0.00
	5	13(16)	13.1	0.2	13.1	12.9
	6	15(16)	38.1	1.1	38.1	38.1
Nitrate+Nitrite	1	15(16)	0.06	0.04	0.07	0.10
	2	17(17)	17.6	0.6	17.7	17.6
	3	14(17)	35.3	0.3	35.4	35.3
	4	14(16)	0.03	0.03	0.04	0.03
	5	14(17)	14.0	0.2	13.9	13.7
	6	16(17)	38.6	1.0	38.5	38.6

Table 2 (Continued)

Nutrient	RMNS	n	Mean µmol kg^{-1}	s.d. µmol kg^{-1}	Median µmol kg^{-1}	Mode µmol kg^{-1}
Phosphate	1	14(17)	0.09	0.02	0.09	0.07
	2	15(17)	1.25	0.04	1.25	1.25
	3	16(17)	2.14	0.07	2.14	2.14
	4	16(17)	0.09	0.03	0.09	0.09
	5	17(17)	1.10	0.04	1.10	1.10
	6	16(17)	2.74	0.10	2.74	2.74
Silicic acid	1	11(13)	2.06	0.23	2.03	2.06
	2	12(13)	66.4	2.0	66.6	66.4
	3	11(13)	136	2	136	136
	4	12(13)	2.09	0.31	2.08	2.09
	5	13(13)	73.8	2.4	73.4	73.8
	6	11(13)	134	3	134	131

Note: n represents the number of data used to calculate the consensus mean and standard deviations after successive application of a *t*-test at the 95% confidence level. The number in parentheses represents the number of results reported by the participant.

Table 3. Median and range of analytical precision of participating laboratories and consensus standard deviation (s.d.) for sample #3

Nutrients	Analytical precision of participating laboratory		Consensus standard deviation	
	n	Median (range)	n	C.V.
Nitrate+nitrite	9	0.2% (0.1-0.6)	15	1.0%
Phosphate	8	0.9% (0.5-2.5)	17	3.5%
Silicic acid	6	0.4% (0.2-0.7)	13	1.7%

Table 4. *Z*-scores of individual laboratories for nitrate, nitrite, phosphate, and silicic acid

Labnum	Z_{NO_3}	Z_{NO_2}	Z_P	Z_S	$(Z_{NO_3} + Z_P)/2$	$(Z_{NO_3} + Z_P + Z_S)/3$
1	22.97	2.07	1.02	1.34	11.99	8.44
2	3.71	1.43	1.24	6.04	2.48	3.66
3	0.37	0.44	0.41	0.65	0.39	0.48
4	1.02	0.63	0.97	0.52	1.00	0.84
5	0.46	0.41	0.95	n.d.	0.71	n.d.
6	0.33	0.39	0.63	0.08	0.48	0.35
7	0.71	0.77	1.16	0.96	0.93	0.94
8	0.90	0.97	0.38	1.02	0.64	0.77
9	0.58	0.77	2.12	1.80	1.35	1.50
10	1.02	1.46	0.68	n.d.	0.85	n.d.
11	2.13	0.96	0.77	0.63	1.45	1.18
13	0.69	0.50	0.61	1.27	0.65	0.86
14	0.86	1.08	1.19	n.d.	1.02	n.d.
15	0.89	0.81	0.78	n.d.	0.83	n.d.
16	1.19	0.84	2.28	0.79	1.74	1.42
17	2.65	1.84	0.65	0.53	1.65	1.27
18	0.36*	n.d.	0.96	2.58	0.66	1.30

* Z_{NO_3} was not available for this lab and therefore the *Z*-score of nitrate+nitrite was used.

n.d.: No data available.