

3. 3. 4 Nutrients

(1) Personnel

Ryosuke MAKABE (NIPR): Principal investigator

Yasuhiro ARII (MWJ): Operation leader

(2) Objectives

To monitor the long-term variability of nutrient concentrations along 110° E transect.

(3) Parameters

The determinants are nitrate, nitrite, silicate and phosphate.

(4) Summary of nutrients analysis

We made 4 QuAAtro runs for the water columns sample at 6 stations during UM-15-08. The total amount of layers of the seawater sample reached up to 98. We made duplicate sampling at 6 layers and measurement.

The Reference Material for Nutrients in Seawater was used for calibration standard in UM-15-08. This calibration method was same as CLIVER cruise by JAMSTEC.

(5) Instrument and method

Analytical detail using QuAAtro 2-HR systems (BL-Tech).

Nitrate + nitrite and nitrite are analyzed according to the modification method of Grasshoff (1970). The sample nitrate is reduced to nitrite in a cadmium tube inside of which is coated with metallic copper. The sample stream with its equivalent nitrite is treated with an acidic, sulfanilamide reagent and the nitrite forms nitrous acid which reacts with the sulfanilamide to produce a diazonium ion. N-1-Naphthylethylene-diamine added to the sample stream then couples with the diazonium ion to produce a red, azo dye. With reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured; without reduction, only nitrite reacts. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by difference.

The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1983), wherein silicomolybdic acid is first formed from the silicate in the sample and added molybdic acid; then the silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue", using ascorbic acid as the reductant. The analytical methods of the nutrients, nitrate, nitrite, silicate and phosphate, during this cruise are same as the methods used in (Kawano et al. 2009).

The phosphate analysis is a modification of the procedure of Murphy and Riley (1962). Molybdic acid is added to the seawater sample to form phosphomolybdic acid which is in turn reduced to phosphomolybdous acid using L-ascorbic acid as the reductant.

The flow diagrams and reagents for each parameter are shown in appendix.

a. Nitrate + Nitrite reagents

Imidazole (buffer), 0.06 M (0.4 % w/v)

Dissolve 4 g imidazole, $C_3H_4N_2$, in ca. 1000 ml DIW; add 2 ml concentrated HCl. After mixing, 1 ml TritonTM X-100 (50 % solution in ethanol) is added.

Sulfanilamide, 0.06 M (1 % w/v) in 1.2M HCl

Dissolve 10 g sulfanilamide, $4-NH_2C_6H_4SO_3H$, in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml TritonTM X-100 (50 % solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v)

Dissolve 1 g NED, $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$, in 1000 ml of DIW and add 10 ml concentrated HCl. After mixing, 1 ml TritonTM X-100 (50 % solution in ethanol) is added.

b. Nitrite reagents

Sulfanilamide, 0.06 M (1 % w/v) in 1.2 M HCl

Dissolve 10 g sulfanilamide, $4-NH_2C_6H_4SO_3H$, in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml TritonTM X-100 (50 % solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v)

Dissolve 1 g NED, $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$, in 1000 ml of DIW and add 10 ml concentrated HCl. After mixing, 1 ml TritonTM X-100 (50 % solution in ethanol) is added.

c. Silicate reagents

Molybdic acid, 0.06 M (2 % w/v)

Dissolve 15 g Disodium Molybdate(VI) Dihydrate, $Na_2MoO_4 \cdot 2H_2O$, in 980 ml DIW, add 8 ml concentrated H_2SO_4 . After mixing, 20 ml sodium dodecyl sulphate (15 % solution in water) is added.

Oxalic acid, 0.6 M (5 % w/v)

Dissolve 50 g Oxalic Acid Anhydrous, $HOOC:COOH$, in 950 ml of DIW.

Ascorbic acid, 0.01 M (3 % w/v)

Dissolve 2.5 g L (+)-Ascorbic Acid, $C_6H_8O_6$, in 100 ml of DIW. Freshly repared before every measurement.

d. Phosphate reagents

Stock molybdate solution, 0.03 M (0.8 % w/v)

Dissolve 8 g Disodium Molybdate(VI) Dihydrate, $Na_2MoO_4 \cdot 2H_2O$, and 0.17 g Antimony Potassium Tartrate, $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, in 950 ml of DIW and add 50 ml concentrated H_2SO_4 .

Mixed Reagent

Dissolve 1.2 g L (+)-Ascorbic Acid, $C_6H_8O_6$, in 150 ml of stock molybdate solution. After mixing, 3 ml sodium dodecyl sulphate (15 % solution in water) is added. Freshly prepared before every measurement.

e. Sampling procedures

Sampling of nutrients followed that oxygen and salinity. Samples were drawn into a virgin 10 ml polyacrylates vials without sample drawing tubes. These were rinsed three times before filling and vials were capped immediately after the drawing. The vials are put on room temperature, ca. 23 ± 2 deg C, before use to stabilize the temperature of samples.

No transfer was made and the vials were set an auto sampler tray directly. Samples were analyzed after collection basically within 24 hours.

f. Data processing

Raw data from QuAAtro 2-HR was treated as follows:

- Checked baseline shift.
- Checked the shape of each peak and positions of peak values taken, and then changed the positions of peak values taken if necessary.
- Carry-over correction and baseline drift correction were applied to peak heights of each samples followed by sensitivity correction.
- Baseline correction and sensitivity correction were done basically using liner regression.
- Loaded pressure and salinity from CTD data to calculate density of seawater.
- Calibration curves to get nutrients concentration were assumed second order equations.

(6) Nutrients standards

a. Volumetric laboratory ware of in-house standards

All volumetric polypropylene (PP) ware used was gravimetrically calibrated. Plastic volumetric flasks were gravimetrically calibrated at the temperature of use within 0 to 4 K.

Volumetric flasks

Volumetric flasks of PP are used because their nominal tolerances are 0.05 % or less over the size ranges likely to be used in this work. Because of their larger temperature coefficients of cubical expansion and lack of tables constructed for these materials, the plastic volumetric flasks were gravimetrically calibrated over the temperature range of intended use and used at the temperature of calibration within 0 to 4 K. The weights obtained in the calibration weightings were corrected for the density of water and air buoyancy.

Pipettes and pipettors

All pipettes have nominal calibration tolerances of 0.1 % or better. These were gravimetrically calibrated in order to verify and improve upon this nominal tolerance.

b. Reagents, general considerations

Specifications

For nitrate standard, “potassium nitrate 99.995 suprapur®” provided by Merck, Lot. B0771365211, CAS No.: 7757-91-1, was used.

For nitrite standard, we used “nitrous acid iron standard solution (NO_2^- 1000) provided by Wako, Lot ECP4122, Code. No. 140-06451”. This standard solution was certified by Wako using Ion chromatograph method. Calibration result is 999 mg/L at 20 deg C. Expanded uncertainty of calibration ($k = 2$) is 0.7 % for the calibration result.

For the silicate standard, we use “Silicon standard solution SiO_2 in NaOH 0.5 mol/l CertiPUR®” provided by Merck, CAS No.: 1310-73-2, of which lot number is HC54715536 are used. The silicate concentration is certified by NIST-SRM3150 with the uncertainty of 0.5 %. HC54715536 is certified as 1005 mg L^{-1} .

For phosphate standard, “potassium dihydrogen phosphate anhydrous 99.995 suprapur®” provided by Merck, Lot B0691108204, CAS No.: 7778-77-0, was used.

Treatment of silicate standard due to high alkalinity

Since the silicon standard solution Merck CertiPUR® is in NaOH 0.5 mol/l, we need to dilute and neutralize to avoid make precipitation of MgOH_2 etc. When we make B standard, silicon standard solution is diluted by factor 12 with pure water and neutralized by HCl 1.0 mol L^{-1} to be about 7. After that B standard solution is used to prepare C standards.

Ultra pure water

Ultra pure water (Milli-Q) freshly drawn was used for preparation of reagent, standard solutions and for measurement of reagent and system blanks.

Low-nutrients seawater (LNSW)

Surface water having low nutrient concentration was taken and filtered using 0.20 μm pore size capsule cartridge filter. This water is stored in 20 liter cubitainer with paper box. The concentrations of nutrient of this water were measured carefully in October 2014.

c. Concentrations of nutrients for A, D, B and C standards

Concentrations of nutrients for A, D, B and C standards are set as shown in Table 3.3.4-1. The C standard is prepared according recipes as shown in Table 3.3.4-2. All volumetric laboratory tools were calibrated prior the cruise as stated in chapter (6) a. Then the actual concentration of nutrients in each fresh standard was calculated based on the ambient, solution temperature and determined factors of volumetric laboratory wares.

The calibration curves for each run were obtained using 6 levels, C-1, C-2, C-3, C-4, C-5 and C-6. C-1, C-2, C-3, C-4 and C-6 were the Reference Material for Nutrients in Seawater and C-5 was in-house standard.

Table 3.3.4-1 Nominal concentrations of nutrients for A, D, B and C standards.

	A	D	B	C-1	C-2	C-3	C-4	C-5	C-6
NO_3 (μM)	22500	900	900	BY	CD	CA	BW	36	BZ
NO_2 (μM)	21700	870	26	BY	CD	CA	BW	1.0	BZ
SiO_2 (μM)	35000		2800	BY	CD	CA	BW	112	BZ
PO_4 (μM)	3000		60	BY	CD	CA	BW	2.4	BZ

Table 3.3.4-2 Working calibration standard recipes.

C Std.	B-1 Std.	B-2 Std.
C-5	20 ml	20 ml

B-1 Std.: Mixture of nitrate, silicate and phosphate

B-2 Std.: Nitrite

d. Renewal of in-house standard solutions

In-house standard solutions as stated in paragraph c were renewed as shown in Table 3.3.4-3(a) to (c).

Table 3.3.4-3(a) Timing of renewal of in-house standards.

NO ₃ , NO ₂ , SiO ₂ , PO ₄	Renewal
A-1 Std. (NO ₃)	maximum a month
A-2 Std. (NO ₂)	commercial prepared solution
A-3 Std. (SiO ₂)	commercial prepared solution
A-4 Std. (PO ₄)	maximum a month
B-1 Std. (mixture of NO ₃ , SiO ₂ , PO ₄)	maximum a week
B-2 Std. (NO ₂)	maximum a week

Table 3.3.4-3(b) Timing of renewal of working calibration standards.

Working standards	Renewal
C-5 Std. (mixture of B-1 , B-2 Std.)	every 24 hours

Table 3.3.4-3(c) Timing of renewal of in-house standards for reduction estimation.

Reduction estimation	Renewal
D-1 Std. (NO ₃)	maximum a week
D-2 Std. (NO ₂)	maximum a week
36 µM NO ₃	when C Std. renewed
35 µM NO ₂	when C Std. renewed

(7) Reference Material for Nutrients in Seawater

To get the more accurate and high quality nutrients data to achieve the objectives stated above, huge numbers of the bottles of the Reference Material for Nutrients in Seawater (hereafter RMNS) are prepared (Aoyama et al., 2006, 2007, 2008, 2009). In the previous worldwide expeditions, such as WOCE cruises, the higher reproducibility and precision of nutrients measurements were required (Joyce and Corry, 1994). Since no standards were available for the measurement of nutrients in seawater at that time, the requirements were described in term of reproducibility. The required reproducibility was 1 %, 1 to 2 %, 1 to 3 % for nitrate, phosphate and silicate, respectively. Although nutrient data from the WOCE one-time survey was of unprecedented quality and coverage due to much care in sampling and measurements, the differences of nutrients concentration at crossover points are still found among the expeditions (Aoyama and Joyce, 1996, Mordy et al., 2000, Gouretski and Jancke, 2001). For instance, the mean offset of nitrate concentration at deep waters was 0.5 µmol kg⁻¹ for 345 crossovers at world oceans, though the maximum was 1.7 µmol kg⁻¹ (Gouretski and Jancke, 2001). At the 31 crossover points in the Pacific WHP one-time lines, the WOCE

standard of reproducibility for nitrate of 1 % was fulfilled at about half of the crossover points and the maximum difference was 7 % at deeper layers below 1.6 deg C in potential temperature (Aoyama and Joyce, 1996).

a. RMNS for this cruise

RMNS Lots BY, CD, CA, BW and BZ which cover full range of nutrients concentrations in the Antarctic Ocean were prepared for UM-15-08.

RMNS Lot CB are prepared for this cruise. Lot CB was analyzed at all station to keep the comparability.

These RMNS assignment were completely done based on random number. The RMNS bottles were stored at the laboratory (C), where the temperature was maintained around ca. 23 deg C.

b. Assigned concentration for RMNSs

We used nutrients concentrations for RMNS Lots BY, CD, CA, BW, CB and BZ as shown in appendix.

(8) Quality control

a. Precision of nutrients analyses during the cruise

Precision of nutrients analyses during the cruise was evaluated based on the 4 measurements, which are measured every 6 to 9 samples, during a run at the concentration of C-5 std. Summary of precisions are shown as shown in Table 3.3.4-4. During this cruise, analytical precisions were 0.09% for nitrate, 0.13% for nitrite, 0.07% for silicate and 0.12% for phosphate in terms of median of precision, respectively.

Table 3.3.4-4 Summary of precision based on the replicate analyses.

	Nitrate	Nitrite	Silicate	Phosphate
	CV%	CV%	CV%	CV%
Median	0.09	0.13	0.07	0.12
Mean	0.11	0.14	0.09	0.13
Maximum	0.19	0.22	0.18	0.20
Minimum	0.07	0.08	0.03	0.09
N	4	4	4	4

b. Carry-over

We can also summarize the magnitudes of carry-over throughout the cruise as shown in

Table 3.3.4-5.

Table 3.3.4-5 Summary of carry-over throughout UM-15-08.

	Nitrate	Nitrite	Silicate	Phosphate
	CV %	CV %	CV %	CV %
Median	0.17	0.11	0.16	0.09
Mean	0.17	0.10	0.13	0.09
Maximum	0.18	0.13	0.20	0.17
Minimum	0.15	0.06	0.00	0.02
N	4	4	4	4

(9) Data archive

All raw data were attached in appendix of this cruise report. Quality checked data are archived in National Institute of Polar Research (NIPR), and published as JARE data reports within one year.

(10) References

- Aminot, A. and Kerouel, R. 1991. Autoclaved seawater as a reference material for the determination of nitrate and phosphate in seawater. *Anal. Chim. Acta*, 248: 277-283.
- Aminot, A. and Kirkwood, D.S. 1995. Report on the results of the fifth ICES intercomparison exercise for nutrients in sea water, ICES coop. Res. Rep. Ser., 213.
- Aminot, A. and Kerouel, R. 1995. Reference material for nutrients in seawater: stability of nitrate, nitrite, ammonia and phosphate in autoclaved samples. *Mar. Chem.*, 49: 221-232.
- Aoyama M., and Joyce T.M. 1996, WHP property comparisons from crossing lines in North Pacific. In Abstracts, 1996 WOCE Pacific Workshop, Newport Beach, California.
- Aoyama, M., 2006: 2003 Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix, Technical Reports of the Meteorological Research Institute No.50, 91pp, Tsukuba, Japan.
- Aoyama, M., Susan B., Minhan, D., Hideshi, D., Louis, I. G., Kasai, H., Roger, K., Nurit, K., Doug, M., Murata, A., Nagai, N., Ogawa, H., Ota, H., Saito, H., Saito, K., Shimizu, T., Takano, H., Tsuda, A., Yokouchi, K., and Agnes, Y. 2007. Recent Comparability of Oceanographic Nutrients Data: Results of a 2003 Intercomparison Exercise Using Reference Materials. *Analytical Sciences*, 23: 1151-1154.
- Aoyama M., J. Barwell-Clarke, S. Becker, M. Blum, Braga E. S., S. C. Coverly, E. Czobik, I. Dahllöf, M. H. Dai, G. O. Donnell, C. Engelke, G. C. Gong, Gi-Hoon Hong, D. J. Hydes, M. M. Jin, H. Kasai, R. Kerouel, Y. Kiyomono, M. Knockaert, N. Kress, K. A. Kroglund, M.

- Kumagai, S. Leterme, Yarong Li, S. Masuda, T. Miyao, T. Moutin, A. Murata, N. Nagai, G.Nausch, M. K. Ngirchchol, A. Nybakk, H. Ogawa, J. van Ooijen, H. Ota, J. M. Pan, C. Payne, O. Pierre-Duplessix, M. Pujo-Pay, T. Raabe, K. Saito, K. Sato, C. Schmidt, M. Schuett, T. M. Shammon, J. Sun, T. Tanhua, L. White, E.M.S. Woodward, P. Worsfold, P. Yeats, T. Yoshimura, A.Youenou, J. Z. Zhang, 2008: 2006 Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix, Technical Reports of the Meteorological Research Institute No. 58, 104pp.
- Gouretski, V.V. and Jancke, K. 2001. Systematic errors as the cause for an apparent deep water property variability: global analysis of the WOCE and historical hydrographic data • REVIEW ARTICLE, *Progress In Oceanography*, 48: Issue 4, 337-402.
- Grasshoff, K., Ehrhardt, M., Kremling K. et al. 1983. *Methods of seawater anylysis*. 2nd rev. Weinheim: Verlag Chemie, Germany, West.
- Joyce, T. and Corry, C. 1994. Requirements for WOCE hydrographic programmed data reporting. WHPO Publication, 90-1, Revision 2, WOCE Report No. 67/91.
- Kawano, T., Uchida, H. and Doi, T. WHP P01, P14 REVISIT DATA BOOK, (Ryoin Co., Ltd., Yokohama, 2009).
- Kirkwood, D.S. 1992. Stability of solutions of nutrient salts during storage. *Mar. Chem.*, 38 : 151-164.
- Kirkwood, D.S. Aminot, A. and Perttila, M. 1991. Report on the results of the ICES fourth intercomparison exercise for nutrients in sea water. ICES coop. Res. Rep. Ser., 174.
- Mordy, C.W., Aoyama, M., Gordon, L.I., Johnson, G.C., Key, R.M., Ross, A.A., Jennings, J.C. and Wilson. J. 2000. Deep water comparison studies of the Pacific WOCE nutrient data set. *Eos Trans-American Geophysical Union*. 80 (supplement), OS43.
- Murphy, J., and Riley, J.P. 1962. *Analytica chim. Acta* 27, 31-36.
- Uchida, H. & Fukasawa, M. WHP P6, A10, I3/I4 REVISIT DATA BOOK Blue Earth Global Expedition 2003 1, 2, (Aiwa Printing Co., Ltd., Tokyo, 2005).