Nutrients

(1) Personnel

    Takahiro IIDA (NIPR): Principal investigator

    Kenichiro SATO (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, phosphate and silicate.

(4) Summary of nutrients analysis

    We made 6 AACS runs for the water columns sample at 6 stations during UM-12-08. The total amount of layers of the seawater sample reached up to 139. We made duplicate sampling and measurement. The station locations for nutrients measurement is shown in Figure 1.

    The reference material of nutrients in seawater was used for calibration standard in UM-12-08. This calibration method was same as CLIVER cruise by JAMSTEC. The reference material was supplied by Dr. Aoyama, Meteorological Research Institute / Japan Meteorological Agency.
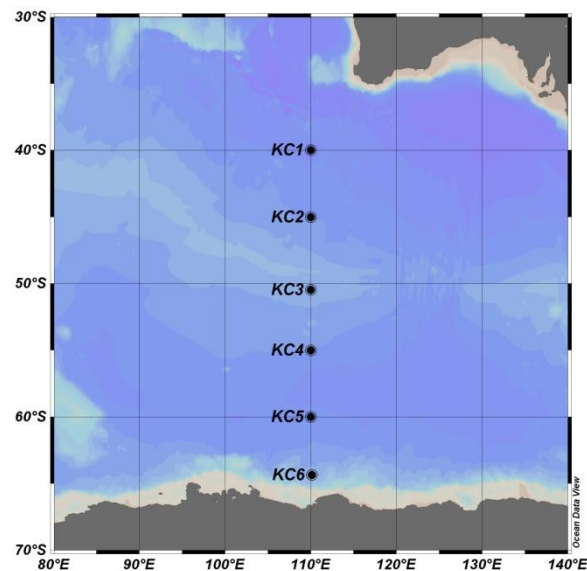

Figure 1 Sampling positions of nutrients sample.

(5) Instrument and Method

Analytical detail using AACS-III system for nutrients analysis.

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.

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 flow diagrams and reagents for each parameter are shown in Figures 2 to Figure 5.
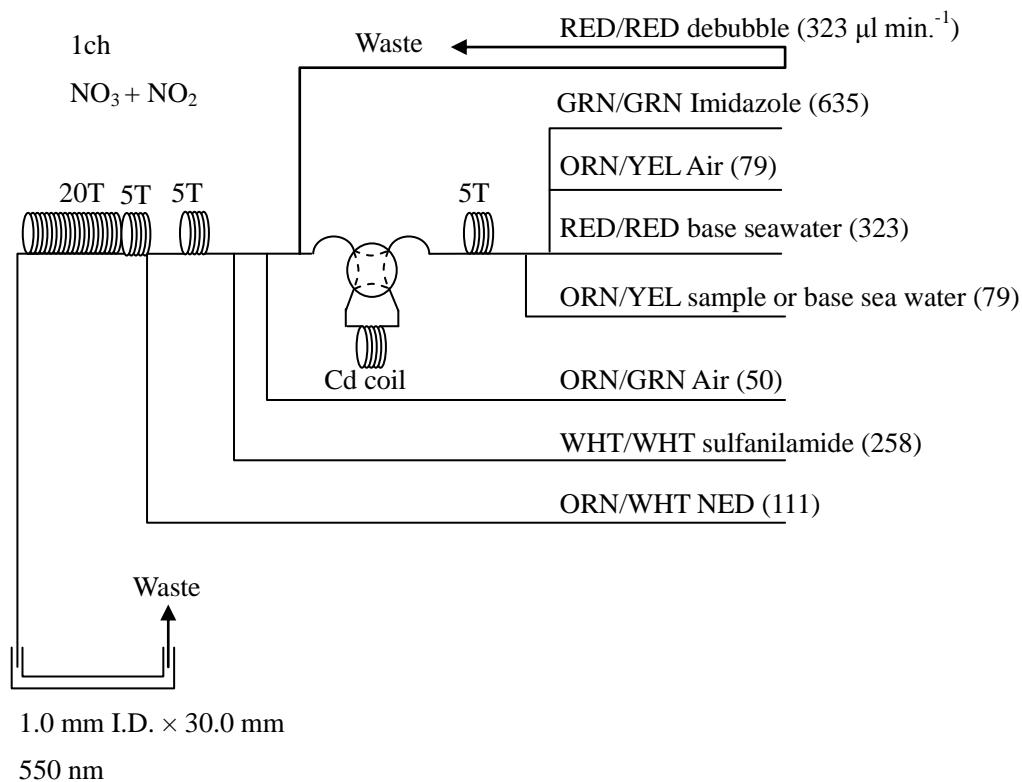

c. Nitrate + Nitrite Reagents

1ch

$NO_3 + NO_2$

RED/RED debubble (323 µl min.$^{-1}$)

Waste

GRN/GRN Imidazole (635)

ORN/YEL Air (79)

20T 5T 5T

5T

RED/RED base seawater (323)

ORN/YEL sample or base sea water (79)

Cd coil

ORN/GRN Air (50)

WHT/WHT sulfanilamide (258)

ORN/WHT NED (111)

Waste

1.0 mm I.D. $\times$ 30.0 mm

550 nm

Figure 2 NO_3+NO_2 (1ch.) Flow diagram.

Imidazole (buffer)

Dissolve 6 g imidazole, $C_3H_4N_2$, in ca. 1000 ml DIW; add 1 ml concentrated H_2SO_4. After mixing, 2 ml Triton®X-100 (50 % solution in ethanol) is added.

Sulfanilamide

Dissolve 10 g sulfanilamide, $4-NH_2C_6H_4SO_3H$, in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 1 ml Triton®X-100 (50 % solution in ethanol) is added.

N-1-Napthylethylene-diamine dihydrochloride

Dissolve 1 g NED, $C_{10}H_7NHCH_2CH_2NH_2•2HCl$, in 1000 ml of DIW and add 10 ml concentrated HCl.
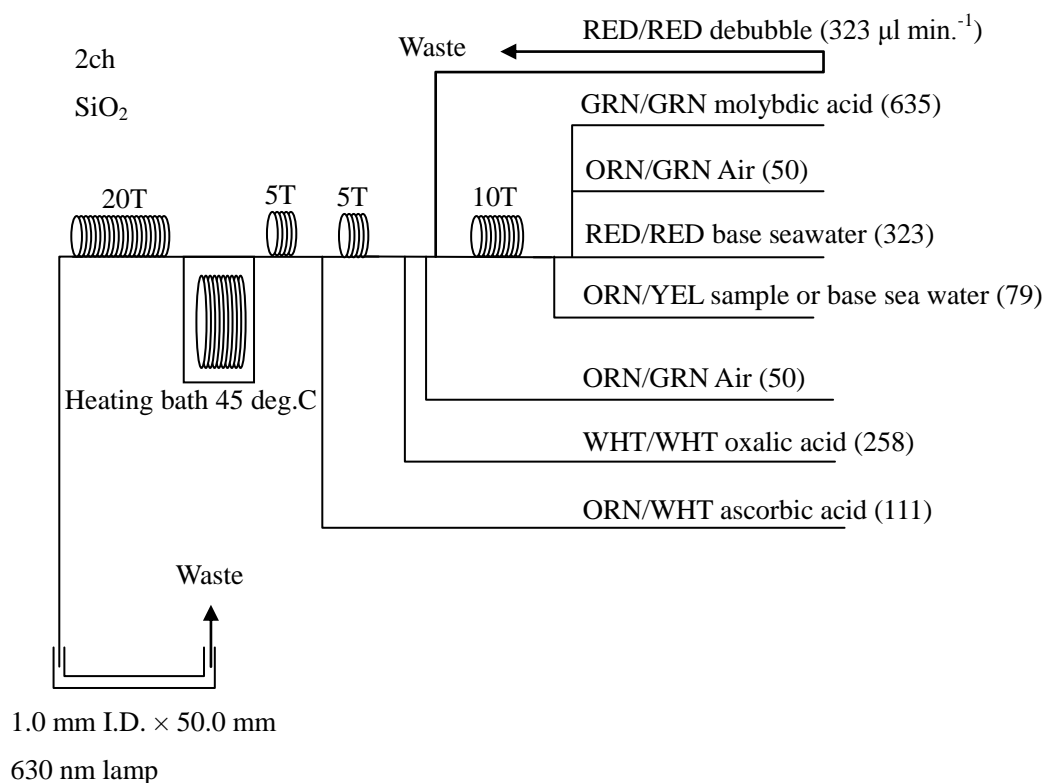
d. Silicate Reagents

2ch
SiO$_2$

20T   5T   5T   10T

RED/RED debubble (323 μl min.$^{-1}$)

Waste

GRN/GRN molybdic acid (635)

ORN/GRN Air (50)

RED/RED base seawater (323)

ORN/YEL sample or base sea water (79)

ORN/GRN Air (50)

WHT/WHT oxalic acid (258)

ORN/WHT ascorbic acid (111)

Heating bath 45 deg.C

Waste

1.0 mm I.D. × 50.0 mm

630 nm lamp

Figure 3 SiO$_2$ (2ch.) Flow diagram.

Molybdic acid

Dissolve 15 g disodium molybdate(VI) dihydrate, Na$_2$MoO$_4$•2H$_2$O, in ca. 1000 ml DIW, add 6 ml concentrated H$_2$SO$_4$. After mixing, 20 ml sodium dodecyl sulphate (15 % solution in water) is added.

Oxalic acid

Dissolve 20 g oxalic acid anhydrous, HOOC: COOH, in 400 ml of DIW.

Ascorbic acid

Dissolve 5 g L (+)-ascorbic acid, C$_6$H$_8$O$_6$, in 200 ml of DIW.

e. Phosphate Reagents

3ch.

PO$_4$

10T 20T 5T

Waste ← RED/RED debubble (323 μl min.$^{-1}$)

GRN/GRN base seawater (635)

ORN/GRN Air (50)

RED/RED sample or base seawater (323)

ORN/YEL base seawater (79)

ORN/GRN Air (50)

WHT/WHT ascorbic acid (258)

ORN/WHT mixed reagent (111)

Heating bath 45 deg.C

Waste
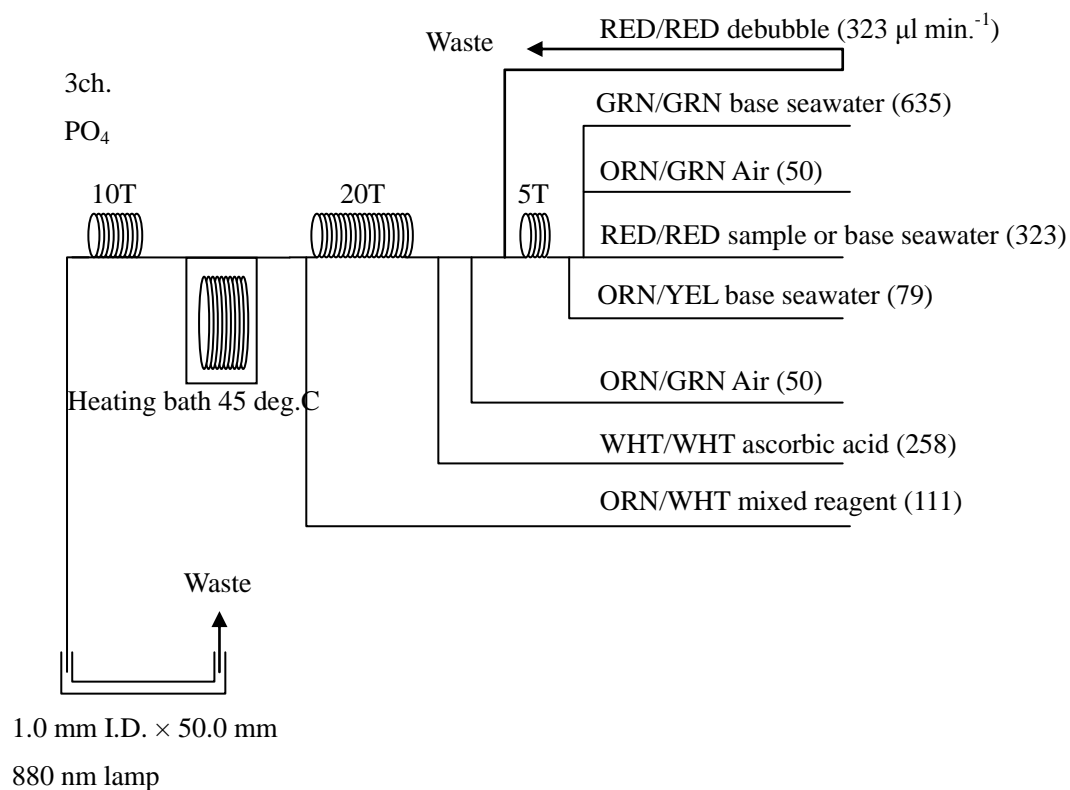
1.0 mm I.D. × 50.0 mm

880 nm lamp

Figure 4 PO$_4$ (3ch.) Flow diagram.

Stock antimony solution

Dissolve 2.3 g antimony potassium tartrate, $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, in 100 ml of DIW.

Mixed Reagent

Dissolve 1.23 g ammonium molybdate(VI) dihydrate, $(NH4)_2MoO_4 \cdot 2H_2O$, and 2 ml of stock antimony solution in 198 ml of DIW and add 14.8 ml concentrated H_2SO_4.

Ascorbic acid

Dissolve 0.94 g L (+)-ascorbic acid, $C_6H_8O_6$, in 500 ml of DIW. After mixing, 1 ml sodium dodecyl sulphate (15 % solution in water) is added.
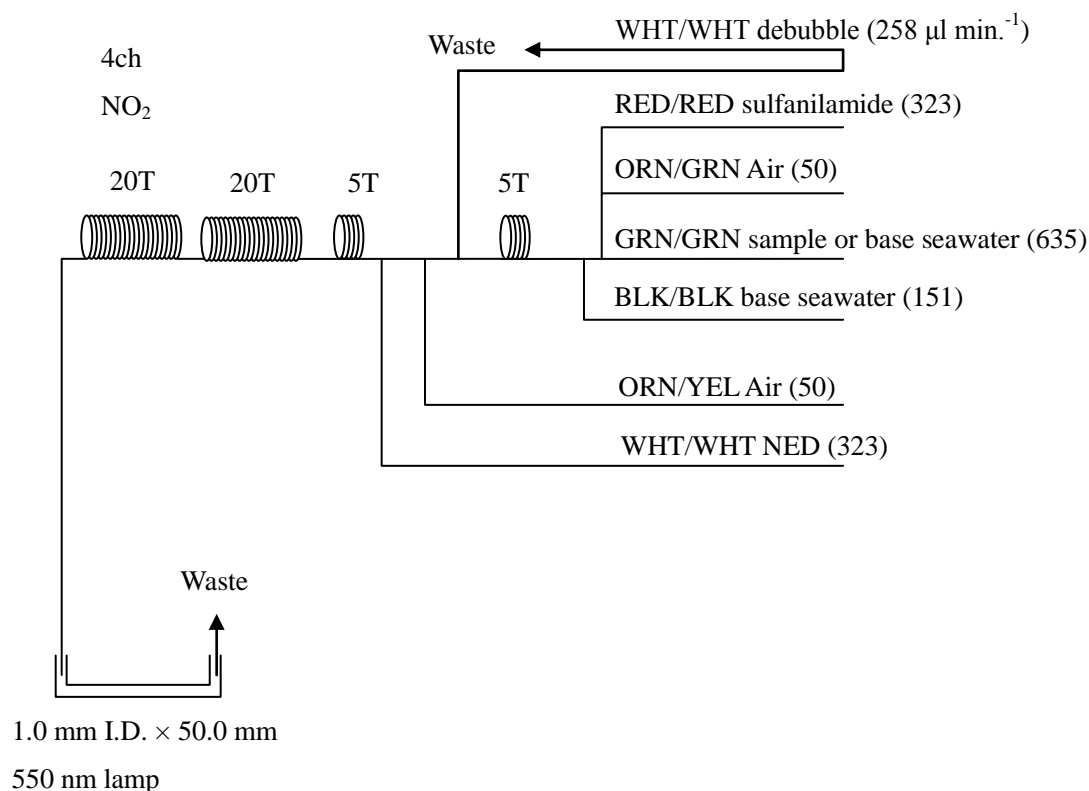
f. Nitrite Reagents

4ch

NO_2

Waste ← WHT/WHT debubble (258 μl min.$^{-1}$)

RED/RED sulfanilamide (323)

ORN/GRN Air (50)

20T   20T   5T   5T   GRN/GRN sample or base seawater (635)

BLK/BLK base seawater (151)

ORN/YEL Air (50)

WHT/WHT NED (323)

Waste

1.0 mm I.D. × 50.0 mm

550 nm lamp

Figure 5 NO_2 (4ch.) Flow diagram.

Sulfanilamide

Dissolve 10g sulfanilamide, 4-$NH_2C_6H_4SO_3H$, in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 1 ml Triton®X-100 (50 % solution in ethanol) is added.

N-1-Napthylethylene-diamine dihydrochloride

Dissolve 1 g NED, $C_{10}H_7NHCH_2CH_2NH_2$·2HCl, in ca. 1000 ml of DIW and add 10 ml concentrated HCl.

i. 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. 22 ± 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.

j. Data processing

Raw data from AACS were treated using BRAATEC as follows:

- Check baseline shift.

- Check the shape of each peak and positions of peak values taken, and then change 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.

- Calibration curves to get nutrients concentration were assumed second order equations.

(6) Nutrients standards

a. Volumetric laboratory ware of in-house standards

All volumetric polymethylpentene (PMP) 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 PMP 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 phosphate standard, "potassium dihydrogen phosphate anhydrous 99.995 suprapur®" provided by Merck, Lot. B0691108204, CAS No.: 7778-77-0, was used.

For nitrite standard, "sodium nitrate" provided by Wako, CAS No.: 7632-00-0, was used. And assay of nitrite was determined according JIS K8019 and assays of nitrite salts were 98.73 %. We use that value to adjust the weights taken.

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 HC122701 are used. The silicate concentration is certified by NIST-SRM3150 with the uncertainty of 0.5 %. HC122701 is certified as 1000+-5 mg L-1, however, our direct comparison between two Merck standards and estimation based on 5 lots of RMNS gave us a factor of 975 mg L-1 for HC247279 which was exceed 5 mg of certification by Merck. We use this factor throughout UM-12-08 to keep comparability for silicate concentration.

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 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 0.45 µm pore size membrane filter. This water is stored in 20 liter cubitainer with paper box. The concentrations of nutrient of this water were measured carefully in August 2012.

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

Concentrations of nutrients for A, B and C standards are set as shown in Table 1. The C standard is prepared according recipes as shown in Table 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-5 were the reference material of nutrients in seawater and C-6 was in-house standard.

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

	A	B	C-1	C-2	C-3	C-4	C-5	C-6
NO$_3$ (µM)	22000	680	BS	BU	BT	BD	BF	45

NO_2 (μM)	4000	20	BS	BU	BT	BD	BF	1.0
SiO_2 (μM)	36000	1400	BS	BU	BT	BD	BF	106
PO_4 (μM)	3000	60	BS	BU	BT	BD	BF	3.0

Table 2 Working calibration standard recipes.

C Std.	B-1 Std.	B-2 Std.
C-6	25 ml	25 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(a) to (c).

Table 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)	maximum a month
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(b) Timing of renewal of working calibration standards.

Working standards	Renewal
C-6 Std. (mixture of B-1 , B-2 and B-3 Std.)	every 24 hours

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

Reduction estimation	Renewal
D-1 Std. (3600 μM NO_3)	maximum a week
14 μM NO_3	when C Std. renewed
16 μM NO_2	when C Std. renewed

(7) Reference material of 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 of 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 BS, BU, BT, BD and BF which cover full range of nutrients concentrations in the Antarctic Ocean were prepared for UM-12-08. 10 sets of BS, BU, BT, BD and BF were supplied by Dr. Aoyama, Meteorological Research Institute / Japan Meteorological Agency. .

Twenty bottles of RMNS lot BV are prepared for this cruise. Lot BE 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 22 deg. C.

b. Assigned concentration for RMNSs

We assigned nutrients concentrations for RMNS lots BS, BU, BT, BD and BF as shown in Table 4.

Table 4 Assigned concentration of RMNSs.

unit: μmol kg^{-1}

	Nitrate	Nitrite	Phosphate	Silicate	Assigned year
BS	0.07	0.02	0.064	1.61	2011
BU	3.97	0.07	0.379	20.30	2011
BT	18.21	0.47	1.320	41.00	2011
BD	29.86	0.05	2.194	64.39	2011
BF	41.39	0.02	149.71	2.809	2011*

* The value of silicate was re-assigned in 2012

(8) Quality control

a. Precision of nutrients analyses during the cruise

Precision of nutrients analyses during UM-12-08 was evaluated by the standard deviation from the difference of set of duplicate sample. The duplicate sample was collected all layer at all station.

Summary of precisions are shown as shown in Table 5. During in this cruise, analytical precisions were 0.10 μmol/L for nitrate, 0.01 μmol/L for nitrite, 0.03 μmol/L for phosphate and 0.43 μmol/L for silicate, respectively.

Table 5 Summary of precision based on the standard deviation from difference of set of duplicate sample.

	Nitrate μmol/L	Nitrite μmol/L	Phosphate μmol/L	Silicate μmol/L
Standard deviation	0.10	0.01	0.03	0.43
Maximum difference	0.43	0.05	0.17	2.02
Minimum difference	0.00	0.00	0.00	0.00
N	139	139	139	139

c. Carry-over

We can also summarize the magnitudes of carry-over throughout the cruise as shown in Table 6.

Table 6 Summary of carry-over throughout UM-12-08.

	Nitrate %	Nitrite %	Phosphate %	Silicate %
Median	0.52	0.50	2.22	0.16
Mean	0.56	0.50	2.23	0.22
Maximum	0.78	0.92	2.91	0.46
Minimum	0.45	0.05	1.26	0.05
N	6	5	6	3

(10) References

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3.3.5 表層モニタリングシステムによる水温塩分蛍光値の連続測定

航路に沿った表面海水の水温、塩分、クロロフィル *a* 濃度（in-vivo 蛍光）をモニターするため、2011 年 12 月 31 日から 2013 年 1 月 23 日にかけて表層環境モニタリングシステム（CT&C 社製）の運用を行った。海水を専用ポンプにより船底海水採取口よりくみ上げ、その水温・塩分を FSI 社製 CT センサー、in-vivo 蛍光を WETLabs 社製 蛍光光度計 WETStar 及び Turnar 社 AU-10 を使用して測定した。また、同時に SATLANTIC 社製 光量子センサー（PAR LIN）を使用して、光合成有効放射を測定した。

また、海鷹丸航走中に船底からポンプで連続的にくみ上げた表面海水（研究用海水）を採取した。海水採取時には、表層環境モニタリング装置に供給される海水の流速が 0.9~1.2 L min^{-1} で安定していることを確認した。また、船位、表層水温・塩分 (C-T) およびクロロフィル蛍光 (Wet Star) を記録した。