



R/V Kaimei Cruise Report
KM22-02

Understanding the actual condition of marine pollutants
and their impact on marine ecosystems

Off Nansei Islands

Jan. 31 – Feb. 11, 2022

Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)

Acknowledgements

We are grateful to captain Naoto Kimura, and the crew of *R/V Kaimei* for their outstanding efforts to make this scientific program successful. We also thank Morifumi Takaesu, Misaki Horiuchi Nippon Marine Enterprises, Ltd., Rei Ito, Tun Htet Aung, Yuki Miyajima, Shiori Ariga, Tohru Koizumi, Tomomi Sone, Marine Works Japan Ltd. for their supports. We also thank all the JAMSTEC people who have supported us.

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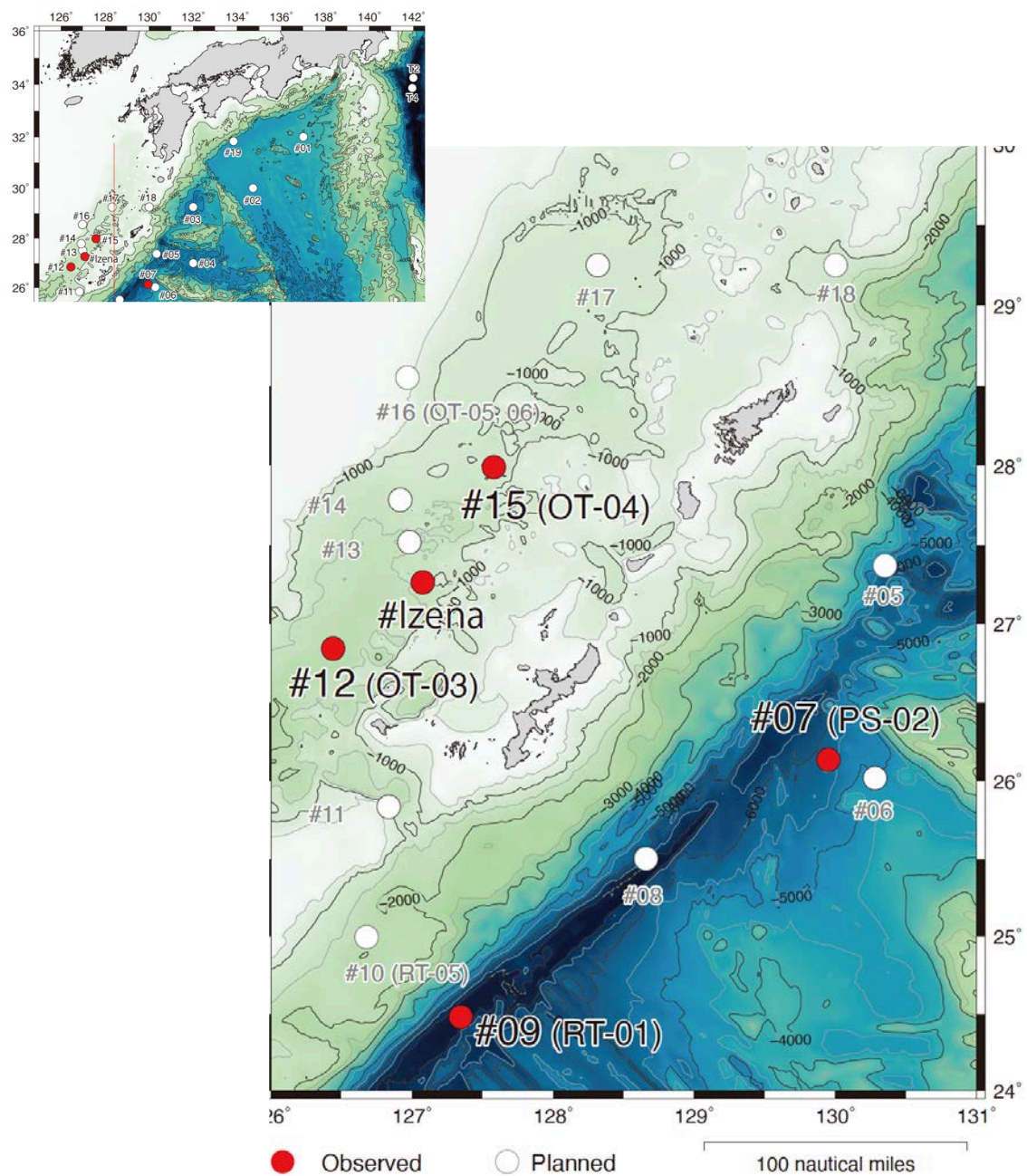
Bathymetry at each sampling site

Beam trawl: system configuration

Multiple core: system configuration

Cruise information

Cruise ID: KM22-02
Vessel: KAIMEI
Title of the cruise: Understanding the actual condition of marine pollutants and their impact on marine ecosystems
Chief Scientist: Masashi Tsuchiya, JAMSTEC
Cruise period: Jan. 31–Feb. 11, 2022
Port of call: Yokosuka–Yokosuka (Yokosuka, Kanagawa, Japan)
Research Area: off Nansei Islands
Research map:



List of participants

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[Marine Works Japan Ltd.]

Tomomi SONE

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Wataru KUROSE

[1st Engineer]

Shohei MIYAZAKI

[2nd Engineer]

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[Jr. 2nd Engineer]

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[2nd Electronic Op.]

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[Boat Swain]

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Takuya MIYASHITA	[Able Seaman]
Toru NAKANISHI	[Able Seaman]
Kenta NASU	[Able Seaman]
Kazuya SUMOMOZAWA	[Sailor]
Keito SEGUCHI	[Sailor]
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Ryota SUZUKI	[Oiler]
Kazuya ANDO	[Oiler]
Marina SHIMIZU	[Assistant Oiler]
Tatsunari ONOUE	[Chief Steward]
Yoshio OKADA	[Steward]
Yoshie HIDAKA	[Steward]
Yuma FUJIMOTO	[Steward]

1. Introduction and objectives of the cruise

The purpose of this cruise is to clarify the distribution and behavior of plastic debris, including microplastics (MPs), in the deep sea, and to understand their impact on deep-sea organisms and ecosystems. We will clarify the actual situation of plastic pollution in the open ocean and optimize the environmental impact assessment method and develop tools for it.

It is an urgent global issue to understand the changes in marine biodiversity, which is one of the important indicators of global environmental change, and to obtain knowledge that will contribute to the assessment of the impact of human activities on ecosystems. It is an urgent issue to understand the actual status of MPs pollution in the ocean, and it is essential to identify the sources and routes of plastic waste discharge to appropriately regulate plastics and take measures against ocean discharge. For deep-sea ecosystems, where there is little information on the effects of changes in the marine environment, it is necessary to conduct comprehensive biological surveys using multiple methods and to promote integrated analysis with environmental data. In addition, to obtain knowledge that contributes to the understanding and evaluation of the impact of human activities on deep-sea ecosystems and their diversity, as well as knowledge for conservation, it is necessary to develop new measurement technologies, conduct surveys of marine plastic litter and deep-sea biodiversity, and improve and optimize methods for assessing impacts on the marine environment. To achieve these goals, a research cruise was conducted in the waters surrounding Japan to investigate the actual distribution of marine plastic litter and the distribution and habitat of deep-sea biological communities incorporating the developed methods. In this research voyage, we will focus on the sources of litter to the Western Pacific litter patch area, which was conducted in YK19-11 and KM20-08 and elucidate the extent of litter accumulation in the waters around the Nansei Islands near the sources of litter in Southeast Asia and East Asia. Some of the observation points set for this voyage were reported by Shimanga & Yanagi (2016) ("Hakuho Maru" KH-05-1), and samples were collected using a beam trawl. The results of this voyage can be compared with the situation 16 years after the observation of Shimanga & Yanagi (2016) by using the same method of collection used then.

To improve and optimize the impact assessment methods in the marine environment, the baseline survey using microbial ecosystems as indicators were expanded to include microbial communities that are unique to each water mass. The baseline data on microbial ecosystems were also expanded to use changes in metabolic activity and community composition that respond quickly to environmental changes. To demonstrate the usefulness of indicators for environmental impact assessment.

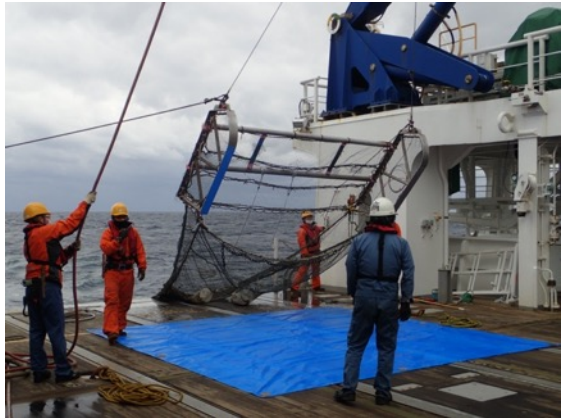
We carried out various observations including collection of microplastics from the ocean surface using a Neuston net, survey of the vertical structure of the ocean using a CTD multiple corer and water sampling for microbial community survey, and continuous observation of various chemical parameters governing the distribution of microbial communities and dissolved gas concentrations such as mercury, methane, and carbon dioxide in the ocean surface layer. Multiple corer observations were used to collect samples of seafloor sediments, and beam trawls were used to collect plastic debris and attached organisms. In addition, visual observation, and video recording of meso to macro plastic debris floating on the surface layer was carried out from the ship.

2. Survey items

Multiple corer



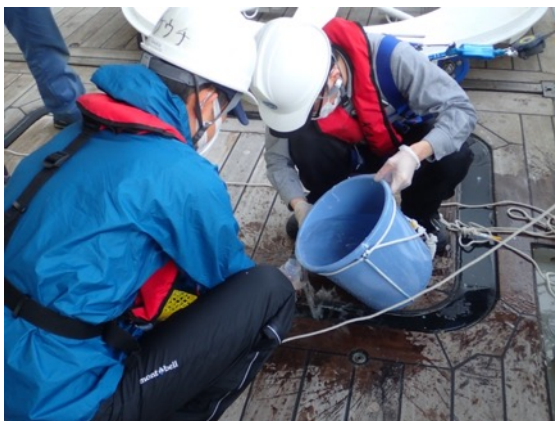
Beam trawl



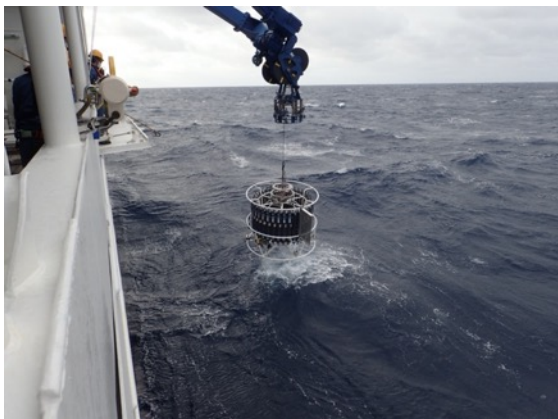
Neuston net



Water sampling with a bucket & sampler



CTD/Niskin



3. Summary and the cruise results

3.1. Actual condition of microplastics and its impact on marine ecosystems

The distribution of microplastic in the open ocean of the western Pacific is largely undocumented. Substantial numbers of studies on floating microplastics have been reported in the Eastern Pacific and Atlantic Ocean, yet fewer data are available in the Western Pacific. In the present study, we conducted microplastic surveys off Nansei Islands to fill gaps in the Western Pacific.

3.1.1. Marine plastic observations off Nansei Islands

Ryota Nakajima, Tomo Kitahashi, Masashi Tsuchiya (JAMSTEC)

Neuston net sampling for surface microplastic analysis

From February 3rd to February 7th 2022, a total of 11 surface net tows were carried out at Sts. 7, 9, 12 and 15 (Table 3.1.1). Floating microplastic samples were collected using a neuston net with a 333 µm opening mesh and a collecting net (100 µm) at the cod-end. The net without cod-end was rinsed from the outside with seawater prior to use. At each station, the net was towed two to three times at 1-2 knot for 15-20 min from the starboard side. A flow meter (RIGO) was installed at the net mouth to estimate the volume of water filtered during each tow. The collected samples were fixed with 3-5% formalin and stored at room temperature until analysis.

Table 3.1.1. Sampling locations of the neuston net towing.

Station	Year	Month	Date	Latitude			Longitude		
7	2022	02	03	26	07.8521	N	129	56.9905	E
15	2022	02	04	27	59.5592	N	127	35.4343	E
12	2022	02	05	26	53.3776	N	126	25.8051	E
9	2022	02	07	24	28.4756	N	127	20.4067	E

Floating macro-debris observation

Macro-debris floating on the surface water were recorded using a digital camera (GoPro) from the starboard side of the vessel throughout the cruise. The GoPro was installed to a handling bar of the ship at 7.7 m height from the sea surface. The GoPro was connected to an external mobile battery allowing a continuous recording. The video was taken during daytime for 7-9 hours at each site.

3.1.2. Additional theme 1: Plastics pollution changes over the years around the Nansei-shoto area

Haruhiko Nakata, Nurlatifah, Junpei Okita (Kumamoto University)

Ryota Nakajima, Tomo Kitahashi, Masashi Tsuchiya (JAMSTEC)

Macro-debris samplings lying on the sea-bottom were made at Sts. 12 and 15 using a beam trawling net. No debris was collected at St. 15 but one debris (empty can) at St. 12. Plastic additives will be analyzed in the inner coated materials of the can to understand their temporal pollution off Nansei Islands.

3.1.3. Microplastics in the sediment off Nansei Islands (Kitahashi, Tsuchiya)

Sediment samples were collected, by the multiple corer (covering 43.0 cm² seafloor surface area) at Stations #12 (Kerama Strait, 1882 m water depth) and #9 (Ryukyu Trench, 7347 m water depth). We used two types of the core, acrylic and aluminum-made core tubes. Aluminum-made tubes were used in this study for microplastic analysis to prevent plastic contamination. Three sediment samples collected with aluminum-made core tube were used for microplastic analysis, and one or two sediment samples collected with acryl made core tubes were used for meiofaunal analysis at each

station. On board, sediment samples were sliced every 1 cm down to about 30 cm below surface for microplastic analysis. In addition, for one sediment sample collected with aluminum-made core tube will be analyzed pollutants including plastic additives. For meiofauna analysis, on board, sediment samples were sliced into 6 layers (0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5 cm) and fixed with formalin.

Sediment samples will be treated according to the procedure described by Nakajima et al. (2019) and extracted microplastics will be counted under a fluorescence microscope of FTIR microscopy. Sediment samples will be treated according to the procedure described by Danovaro (2010) and meiofauna specimens will be counted and identified under a binocular stereoscopic microscope. Also, we will conduct smear slide descriptions, X-CT analysis, grain size measurements to decipher the sediment source and transport processes using these data.

3.1.4. Additional theme 4: Understanding biofouling assemblages of marine plastics

Naoto Jimi (Nagoya University)

Kensuke Yanagi (CMNH)

Akito Ogawa (JAMSTEC)

Hiroki Kise (AIST)

Gregorius Altius Pratama, Natsumi Hookabe (The University of Tokyo)

Marine organisms are often attached to marine debris. It is also known that floating debris on the sea surface were transferred to the bottom by attached organisms. Therefore, grasping the biofouling fauna is important for understanding the dynamics of marine debris. We investigated the biota that is attached to benthic debris by sampling with an Oregon type beam trawl of 3 m span in the waters off the Nansei Islands.

Samples

Beam trawls were carried out at three sites, #15 (1350 m), #12 (1861 m) and #9 (7339 m). As a result, the sediments were successfully collected at #15 and #12. At #9 we could not collect any sediments. This will be discussed later. The sediments were collected with an Oregon type beam trawl of 3 m span (see appendix figure 1) and the supernatant was filtered through a 350 µm net. The remaining sediments were then separated using a 1 mm or 2 mm mesh sieves. The organisms were then sorted under microscopes or naked eye on board. Some marine debris was recovered, but the only organisms attached were white calcareous egg-like ones on a cola can collected at #12. We also collected biological specimens from sediments: #15, Annelida, Mollusca, Nematoda, Crustacea, Nemertea, Echinodermata, Cnidaria, Vertebrata; #12, Annelida, Nematoda, Mollusca, Echinodermata, Cnidaria, Vertebrata. #9 also contained some shrimps, but these specimens were probably derived from pelagic zone.

3.1.5. Beam trawl operation

This was the first attempt on the research vessel *Kaimei* to operate a beam trawl survey. As the operation was carried out using PC ropes (the cable for Piston Core sampler), the situation was different from a normal protocol. The detailed configuration of the beam trawl was shown in Appendix figure 1. In this configuration, nets, weight and transponder were lowered in this order, and the rope was unrolled at a rate of 1 m/s to the same depth as the seabed. The vessel moved forward at 0.5 knots to the water during the unrolling and at 0.5 knots to the ground after the rope had been unrolled to the same depth. The beam trawl was judged to have landed on bottom when the transponder was at 200 m above the water depth. After landing, the beam trawl was towed for 30 minutes (#15 and #12) or one hour (#9). After the tow, the vessel was hoisted in slow speed (0.2–0.3 m/s) until the beam trawl left off bottom and then reeled in at a speed of 1 m/s. The trawl was considered to have left off bottom when the transponder was at 400 m above the water depth. In #15 and #12, we were able to collect sediments using the above procedure, with a lot of deeper layer sediments collected in #12, as indicated by the number of the biota collected. We could not collect any sediments in #9, as the lead-in wire came up with a complex tangle, entangled in the weight. This was probably due to low towing speed that the vessel used during the PC rope winch unrolling

to the same depth, fearing that the beam trawl would float due to the resistance of the PC rope, which caused it to deflect. For resolving these problems, we suggest bellows: [for #12] keep towing speed faster than 0.5 knots to the ground; [for #9] keep the vessel speed faster than 0.5 knots to the water during unrolling. Adding weight for the beam trawl is needed to solve this problem, however, the present dredge winch's rolling power is not enough for hoisting heavy weight gears.

3.2. CTD

3.2.1 CTDO₂

27 February 2022

Hiroshi Uchida (JAMSTEC) (Principal Investigator, not on board)

Taichi Yokokawa (JAMSTEC)

Tun Htet Aung (MWJ) (Operation Leader)

Rei Ito (MWJ)

Instruments

Instruments used in this cruise are as follows:

Winch and cable system

14.7 kN trawler with AHC (Active Heave Compensation) winch system (MacArtney A/S, Esbjerg, Denmark) for 11,000 m synthetic fiber cable (OCC Co., Yokohama, Japan)

The CTD package was deployed from starboard by using a folding a-frame

Frame and water sampler

Aluminum frame for 36-position 12-L water sample bottles with an aluminum rectangular fin (54×90 cm)

Compact underwater slip ring swivel (Hanayuu Co., Ltd., Shizuoka, Japan) (Uchida et al., 2018)

36-position carousel water sampler, SBE 32 (Sea-Bird Scientific, Washington, USA)

12-L Niskin-X water sample bottle, model 1010X (General Oceanic, Inc., Florida, USA) (TEFLON coating) [for #16-20 and #33-36]

12-L sample bottle, model OTE 110 (OceanTest Equipment, Inc., Florida, USA) (TEFLON coating) [for #1-15]

12-L sample bottle, model OTE 110 (OceanTest Equipment, Inc., Florida, USA) (No TEFLON coating) [for #21-32]

Deck unit

SBE 11plus (Sea-Bird Scientific)

Serial no. 90876-1033

Underwater unit

Pressure sensor, SBE 9plus (Sea-Bird Scientific)

Serial no. 09P84583-1235 (134402) (calibration date: March 4, 2020)

Deep standard reference thermometer, SBE 35 (Sea-Bird Scientific)

Serial no. 0045 (calibration date: July 21, 2020)

Temperature sensor, SBE 3 (Sea-Bird Scientific)

Primary serial no. 034818 (calibration date: Jun 5, 2021)

Secondary serial no. 031464 (calibration date: Jun 5, 2021)

Conductivity sensor, SBE 4C (Sea-Bird Scientific)

Primary serial no. 044450 (calibration date: Jun 8, 2021)

Secondary serial no. 041172 (calibration date: September 17, 2021)

Dissolved oxygen sensor

Primary, RINKO III (JFE Advantech Co., Ltd., Hyogo, Japan)

Serial no. 0252, Sensing foil no. 201238

Secondary, SBE 43 (Sea-Bird Scientific)

Serial no. 433161 (calibration date: May 25, 2021)

Transmissometer, C-Star, WET Labs, Inc., Philomath, Oregon, USA

Serial no. 1726DR (calibration date: December 23, 2021)

[at CTD station 001]

Serial no. 1727DR (calibration date: May 15, 2021)

[at CTD stations 002, 003, 004 and 005]

Chlorophyll fluorometer (Seapoint Sensors Inc., New Hampshire, USA)
Serial no. 3701, Gain: 30X (0-5 ug/L)
Ultraviolet fluorometer (Seapoint Sensors Inc., New Hampshire, USA)
Serial no. 6246, Gain 30X (0-50 QSU)
Turbidity meter (Seapoint Sensors Inc., New Hampshire, USA)
Serial no. 14954, Gain: 100X (0-25 FTU)
PAR sensor, PAR-Log ICSW (Satlantic, LP, Nova Scotia, Canada)
Serial no. 1026 (calibration date: July 6, 2015)
Altimeter, PSA-916T (Teledyne Benthos, Inc.)
Serial no. 66557
Pump, SBE 5T (Sea-Bird Scientific)
Primary serial no. 058088
Secondary serial no. 058145
Bottom contact switch (Sea-Bird Scientific)
[at all CTD stations]

Additional stand-alone underwater unit

Pressure/temperature/Conductivity recorder, A7CT-USB (JFE Advantech, Co.)
Serial no. 0743, 0745 and 0746 [at CTD stations 001 and 004]
Turbidity recorder, ATU6-CMP (JFE Advantech, Co.)
Serial no. 03 [at CTD stations 001, 002, 003, and 005]
Absolute Salinity sensor (NMIJ, AIST)
Prototype 1 [at all CTD stations]

Software

Data acquisition software, SEASAVE-Win32, version 7.26.7.121
Data processing software, SBEDataProcessing-Win32, version 7.26.7.129

Data Collection

The CTD system was powered on at least 20 minutes in advance of the data acquisition to stabilize the pressure sensor. The data was acquired at least two minutes before and after the CTD cast to collect atmospheric pressure data on the ship's deck.

The CTD package was lowered into the water from the starboard side and held 10 m beneath the surface to activate the pump. After the pump was activated, the package was lifted to the surface and lowered to 35 m then the package was stopped to operate the active heave compensator of the winch. The package was lowered again at a rate of 1.2 m/s (maximum) to the bottom. For the up cast, the package was lifted at a rate of 1.2 m/s (maximum) except for bottle firing stops. As a rule, the bottle was fired after waiting from the stop for more than 30 seconds and the package was stayed at least 5 seconds for measurement of the SBE 35 at each bottle firing stops. For depths where vertical gradient of water properties was expected to be large (from surface to thermocline), the bottle was fired after waiting from the stop for 60 seconds to enhance exchanging the water between inside and outside of the bottle. At 35 m from the surface, the package was stopped to stop the active heave compensator of the winch.

Data Collection Problems

There were several Modulo Error Count when using the synthetic fiber cable at depths between 500 and 600 m).

After all CTD casts were done and during CTD system disassembly and post cruise sensor condition checking, it was found that there was a crack on the conductivity cell of primary conductivity sensor (serial no. 044450).

Data Processing

The following are the data processing software (SBEDataProcessing-Win32) and original software data processing module sequence and specifications used in the reduction of CTD data in this cruise.

DATCNV converted the raw data to engineering unit data. DATCNV also extracted bottle information where scans were marked with the bottle confirm bit during acquisition. The duration was set to 4.4 seconds, and the offset was set to 0.0 second. The hysteresis correction for the SBE 43 data (voltage) was applied for both profile and bottle information data.

RINKOCOR (original module, version 1.0) corrected the time-dependent, pressure-induced effect (hysteresis) of the RINKO for both profile data.

RINKOCORROS (original module, version 1.0) corrected the time-dependent, pressure-induced effect (hysteresis) of the RINKO for bottle information data by using the hysteresis-corrected profile data.

BOTTLESUM created a summary of the bottle data. The data were averaged over 4.4 seconds.

ALIGNCTD compensated the delay of the RINKO data by 1 second and SBE43 data by 5 seconds advancing sensor output (voltage) relative to the temperature data.

WILDEDIT marked extreme outliers in the data files. The first pass of WILDEDIT obtained an accurate estimate of the true standard deviation of the data. The data were read in blocks of 1000 scans. Data greater than 10 standard deviations were flagged. The second pass computed a standard deviation over the same 1000 scans excluding the flagged values. Values greater than 20 standard deviations were marked bad. This process was applied to pressure, temperature and conductivity data.

CELLTM used a recursive filter to remove conductivity cell thermal mass effects from the measured conductivity. Typical values used were thermal anomaly amplitude $\alpha = 0.03$ and the time constant $1/\beta = 7.0$.

FILTER performed a low pass filter on pressure and depth with time constant of 0.15 seconds. In order to produce zero phase lag (no time shift) the filter runs forward first then backwards.

WFILTER performed as a median filter to remove spikes in transmissometer, chlorophyll fluorometer and ultraviolet fluorometer data. A median value was determined by 49 scans of the window. For the ultraviolet fluorometer data, an additional box-car filter with a window of 361 scans was applied to remove noise.

SECTIONU (original module, version 1.1) selected a time span of data based on scan number in order to reduce a file size. The minimum number was set to be the start time when the CTD package was beneath the sea-surface after activation of the pump. The maximum number was set to be the end time when the depth of the package was 1 dbar below the surface. The minimum and maximum numbers were automatically calculated in the module.

LOOPEDIT marked scans where the CTD was moving less than the minimum velocity of 0.0 m/s (traveling backwards due to ship roll).

DESPIKE (original module, version 1.0) removed spikes of the data. A median and mean absolute deviation was calculated in 1-dbar pressure bins for both down- and up-cast, excluding the flagged values. Values greater than 4 mean absolute deviations from the median were marked bad for each bin. This process was performed 2 times for temperature, conductivity and RINKO output.

DERIVE was used to compute oxygen (SBE 43).

BINAVG averaged the data into 1-dbar pressure bins. The center value of the first bin was set equal to the bin size. The bin minimum and maximum values are the center value plus and minus half the bin size. Scans with pressures greater than the minimum and less than or equal to the maximum were averaged. Scans were interpolated so that a data record exist every dbar.

BOTTOMCUT (original module, version 0.1) deleted the deepest pressure bin when the averaged scan number of the deepest bin was smaller than the average scan number of the bin just above.

DERIVE was re-used to compute salinity, potential temperature, and density

SPLIT was used to split data into the down cast and the up cast.

Station List

The CTD station information for this cruise is summarized in Table 3.2.1.1.

Table 3.2.1.1. Station summary of the CTD/water sampling measurement.

KM22-02 CTD cast table												
Stnno	Castno	Date(UTC)	Time(UTC)		BottomPosition		Depth	Wire	HT Above	Max	Max	CTD
		(mmddyy)	Start	End	Latitude	Longitude	(m)	Out (m)	Bottom (m)	Depth	Pressure	Filename
001	1	020222	19:17	23:23	26-08.07N	129-56.96E	5403.9	5485.1	10.9	5383.4	5488.0	001M001
002	1	020322	23:15	01:05	27-59.31N	127-34.74E	1337.5	1344.0	11.0	1316.9	1330.0	002M001
003	1	020422	19:26	22:15	26-50.87N	126-26.39E	1856.5	1913.8	10.1	1852.4	1873.0	003M001
004	1	020522	21:13	02:03	24-29.12N	127-21.04E	7342.8	7462.0	9.1	7315.2	7489.0	004M001
005	1	020722	22:16	00:00	27-16.01N	127-04.18E	1634.4	1659.1	10.6	1620.8	1638.0	005M001

Pre- and post-cruise calibrations

Pressure sensor

Pre-cruise sensor calibration for linearization was performed at Sea-Bird Scientific. The time drift of the pressure sensor was adjusted by periodic recertification corrections by using electric dead-weight testers (model E-DWT-H A70M and A200M, Fluke Co., Phoenix, Arizona, USA) and a barometer (model RPM4 BA100Ks, Fluke Co.):

Serial no. 181 (A70M) (for 10-70 MPa) (calibration date: January 19, 2019)

Serial no. 1305 (A200M) (for 90 to 100 MPa) (calibration date: January 19, 2019)

Serial no. 1453 (BA100Ks) (for 0 MPa) (calibration date: January 15, 2019)

These reference pressure sensors were calibrated by Ohte Giken, Inc. (Ibaraki, Japan) traceable to National Institute of Standards and Technology (NIST) pressure standards. The pre-cruise correction was performed at JAMSTEC (Kanagawa, Japan) by Marine Works Japan Ltd. (MWJ) (Kanagawa, Japan).

The CTD pressure sensor offset in the period of the cruise was estimated from the pressure readings on the ship's deck. For best results the Paroscientific sensor was powered on for at least 20 minutes before the operation. To obtain the calibration data for the pre- and post-cast pressure sensor drift, the CTD deck pressure was averaged over first and last one minute, respectively. Then the atmospheric pressure deviation from a standard atmospheric pressure (1013.25 hPa) was subtracted from the CTD deck pressure to check the pressure sensor time drift.

The CTD pressure sensor offset was estimated from the deck pressure. Mean of the pre- and the post-casts data over the whole period gave an estimation of the pressure sensor offset (-0.04 ± 0.05 dbar) from the pre-cruise calibration. The post-cruise correction of the pressure data is not deemed necessary for the pressure sensor.

Temperature sensors

Pre-cruise sensor calibrations of the SBE 3s were performed at Sea-Bird Scientific. Pre-cruise sensor calibration of the SBE 35 for linearization was also performed at Sea-Bird Scientific. The slow time drift of the SBE 35 was adjusted by periodic recertification corrections by measurements in thermodynamic fixed-point cells (water triple point [0.01 °C] and gallium melt point [29.7646 °C]) (Uchida et al., 2015). Since 2016, pre-cruise calibration was performed at JAMSTEC by using fixed-point cells traceable to National Metrology Institute of Japan (NMIJ) temperature standards.

The CTD temperature sensors (SBE 3) were calibrated with the SBE 35 (Uchida et al., 2007; Uchida et al., 2015) as

$$\text{Calibrated temperature} = T - (c_0 \times P + c_1 \times t + c_2)$$

where T is CTD temperature in °C, P is pressure in dbar, t is time in days from pre-cruise calibration date of the CTD temperature and c₀, c₁, and c₂ are calibration coefficients. The coefficients were determined using the data for the depths deeper than 1950 dbar. Since the SBE 35 data obtained at only one station (001) were available for the calibration, the calibration coefficient c₁ was set to zero.

The results of the post-cruise calibration for the CTD temperature are shown in Table 3.2.1.2, and the calibration coefficients are as follows:

$$c_0 = 1.32483370 \times 10^{-7}, c_1 = 0.0, c_2 = 5.9214 \times 10^{-4} \quad [\text{for primary}]$$

$$c_0 = 2.36598822 \times 10^{-7}, c_1 = 0.0, c_2 = -9.0835 \times 10^{-4} \quad [\text{for secondary}]$$

Table 3.2.1.2. Difference between the CTD temperature and the SBE 35 after the post-cruise calibration. Mean and standard deviation (Sdev) are calculated for the data below and above 1950 dbar. Number of data used is also shown.

Sensor	Pressure $\geq$ 1950 dbar			Pressure < 1950 dbar		
	Number	Mean (mK)	Sdev (mK)	Number	Mean (mK)	Sdev (mK)
Primary	12	-0.0	0.1	117	0.9	3.0
Secondary	12	-0.0	0.1	117	0.3	2.2

Conductivity sensors

Pre-cruise sensor calibrations were performed at Sea-Bird Scientific.

The discrepancy between the CTD conductivity and the conductivity calculated from the bottle salinity data with the CTD temperature and pressure data is considered to be a function of conductivity and pressure. The CTD conductivity was calibrated as

Calibrated conductivity =

$$C - (c_0 \times C + c_1 \times P + c_2 \times C \times P + c_3 \times P^2 + c_4 \times P^2 \times C + c_5)$$

where C is CTD conductivity in S/m, P is pressure in dbar, t is time in days and c_0 , c_1 , c_2 , c_3 , c_4 and c_5 are calibration coefficients. The best fit sets of coefficients were determined by a least square technique to minimize the deviation from the conductivity calculated from the bottle salinity data.

The primary conductivity data created by the software module ROSSUM were used after the post-cruise calibration for the temperature data. The results of the post-cruise calibration for the CTD salinity are summarized in Table 3.2.1.3. The calibration coefficients are as follows:

$$c_0 = 0.9991512824087, c_1 = 4.034789901316e-08, c_2 = -8.010335584951e-08, \\ c_3 = 1.328678113416e-10, c_4 = -3.392138030216e-11, c_5 = 2.460417309123e-03$$

Table 3.2.1.3. Difference between the CTD salinity and the bottle sampled salinity after the post-cruise calibration. Mean and standard deviation (Sdev) are calculated for the data below and above 1950 dbar. Number of data used is also shown.

Sensor	Pressure $\geq$ 1950 dbar			Pressure < 1950 dbar		
	Number	Mean (10^{-3})	Sdev (10^{-3})	Number	Mean (10^{-3})	Sdev (10^{-3})
Primary	75	0.1	0.5	75	-0.1	4.3

Dissolved oxygen sensors

Pre-cruise sensor calibration of RINKO was not performed. Pre-cruise sensor calibration of SBE 43 was performed at Sea-Bird Scientific.

Data from the RINKO can be corrected for the time-dependent, pressure-induced effect by means of the same method as that developed for the SBE 43 (Edwards et al., 2010). The calibration coefficients, H1 (amplitude of hysteresis correction), H2 (curvature function for hysteresis), and H3 (time constant for hysteresis) were determined to minimize the down-cast and up-cast data.

$$H1 = 0.0062, H2 = 4000.0, H3 = 2000.0$$

Outputs from RINKO are the raw phase shift data. The RINKO can be calibrated by the modified Stern-Volmer equation slightly modified from a method by Uchida et al. (2010):

$$O_2 (\mu\text{mol/l}) = [(V_0 / V)^E - 1] / K_{sv}$$

where V is voltage, V_0 is voltage in the absence of oxygen, K_{sv} is Stern-Volmer constant. The coefficient E corrects nonlinearity of the Stern-Volmer equation. The V_0 and the K_{sv} are assumed to be functions of temperature as follows.

$$K_{sv} = c_0 + c_1 \times T + c_2 \times T^2$$

$$V_0 = 1 + d_0 \times T$$

$$V = d1 + d2 \times V_b$$

where T is CTD temperature (°C) and V_b is raw output (volts). V_0 and V are normalized by the output in the absence of oxygen at 0°C. The oxygen concentration is calculated using accurate temperature data from the CTD temperature sensor instead of temperature data from the RINKO. The pressure-compensated oxygen concentration O_{2c} can be calculated as follows.

$$O_{2c} = O_2 (1 + cp \times P / 1000)$$

where P is CTD pressure (dbar) and cp is the compensation coefficient. Since the sensing foil of the optode is permeable only to gas and not to water, the optode oxygen must be corrected for salinity. The salinity-compensated oxygen can be calculated by multiplying the factor of the effect of salt on the oxygen solubility (Garcia and Gordon, 1992).

The post-cruise calibrated temperature and salinity data were used for the calibration. The results of the post-cruise calibration for the RINKO oxygen are summarized in Table 3.2.1.4. The calibration coefficients are as follows:

$$c0 = 4.560130921839303e-03, c1 = 1.559434729622180e-04$$

$$c2 = 2.920327262869676e-06, c3 = -4.110299732888129e-03$$

$$c4 = -0.1262419088547563, c5 = 0.2986279801172353$$

$$E = 1.1, cp = 0.026$$

Table 3.2.1.4. Difference between the CTD oxygen and the bottle sampled oxygen after the post-cruise calibration. Mean and standard deviation (Sdev) are calculated for the data below and above 1950 dbar. Number of data used is also shown.

Sensor	Pressure $\geq$ 1950 dbar			Pressure < 1950 dbar		
	Number	Mean ($\mu\text{mol/kg}$)	Sdev ($\mu\text{mol/kg}$)	Number	Mean ($\mu\text{mol/kg}$)	Sdev ($\mu\text{mol/kg}$)
Primary	73	-0.04	0.26	73	-0.39	0.86

Transmissometer

Light transmission (T_r in %) and beam attenuation coefficient c_p are calibrated as

$$T_r = (V - V_d) / (V_r - V_d) \times 100$$

$$c_p = -(1 / 0.25) \ln(T_r / 100)$$

where V is the measured signal (voltage), V_d is the dark offset for the instrument, and V_r is the signal for clear water. V_d can be obtained by blocking the light path. The calibration coefficients (V_d and V_r) estimated from the previous cruise were used, because periodic recertification was not performed by the manufacturer for the transmissometer.

For the post-cruise calibration, V_d was measured on deck before each cast. V_r is estimated from the measured maximum signal in the deep ocean at each cast.

$$V_d = 0.0036, V_r = 4.7021 \text{ (for station 001 (SN 1726DR))}$$

$$V_d = 0.0012, V_r = 4.7607 \text{ (for stations 002-005 (SN 1727DR))}$$

PAR

Periodic recertification was not performed by the manufacturer for PAR sensor.

PAR is expected to be zero in the deep ocean. Therefore, the offset measured in the deep ocean was corrected. The corrected data (PARc) is calculated from the raw data (PAR) as follows:

$$\text{PARc} [\mu\text{E m}^{-2} \text{ s}^{-1}] = \text{PAR} - 0.098.$$

Turbidity meter, chlorophyll fluorometer, ultraviolet fluorometer

Periodic recertification was not performed by the manufacturer for these sensors.

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3.2.2. Water sampling and analysis Chemical parameter

3.2.2.1 Salinity

19 February 2022

Hiroshi Uchida (JAMSTEC) (Principal Investigator, not on board)

Kei Kondo (JAMSTEC)

Water samples for salinity measurements were collected in 250 mL brown borosilicate glass bottles with screw caps (PTFE packing) at all CTD/water sampling stations, from surface water monitoring system once in a day, and from Niskin bottles of the multiple corers sampling system. Each bottle was rinsed three times with sample water and was filled to the shoulder of the bottle. The samples were stored in the refrigerated container on the ship and in the air-conditioned (~24 °C) laboratory for a few days after the cruise. Salinity of water samples were measured with a salinometer (Autosal model 8400B, Guildline Instruments Ltd., Ontario, Canada, serial no. 60132). The salinometer was operated in the laboratory at a bath temperature of 24 °C, based on a method by Kawano (2010).

The IAPSO Standard Seawater (batch P164, $S_p = 34.9941$) (Ocean Scientific International Ltd., Havant, UK) was measured to correct the salinity measurements at the beginning, the middle, and the end of each day of measurements. Ultra-pure water (Milli-Q water, Millipore, Billerica, Massachusetts, USA) was also measured with the IAPSO Standard Seawater to monitor time drift of the offset of the salinometer at the beginning and the end of each day of measurements. Measurements were taken on February 13 and 14, 2022.

Time drift of the slope of the salinometer was estimated from the IAPSO Standard Seawater (SSW) measurement and corrected for each day of measurements based on a method by Uchida et al. (2020). Average (standard deviation) of the standards after time drift correction was 34.9941 (0.0002) and 0.0002 (0.0002) for IAPSO SSW and ultra-pure water, respectively.

A total of 10 pairs of replicate samples was collected from the same Niskin bottle, and the root-mean-square for the replicate samples was 0.0003. Results for samples obtained at 1 m above the bottom from Niskin bottles of the multiple corers sampling system are shown in Table 3.2.2.1.1. Measured salinity data are consistent with the salinity data obtained at 10 m above the bottom from the CTD measurement except for station 003 probably due to miss trip of the Niskin bottle.

Table 3.2.2.1.1. Results for samples obtained from Niskin bottles of the multiple corers sampling system. Density salinity and refractive index anomaly data (see Section 3.2.2.2) are also shown.

Station_#	Practical Salinity	Density Salinity	dS _A	Refractive index anomaly	note
	(PSS-78)	(g/kg)	(g/kg)	(nD)	
001_#1	34.6823	34.8633	0.0175	640.59	

001_#2	34.6809	34.8610	0.0166	640.59	
003_#1	34.5480	34.7109	-0.0000	637.99	Miss trip?
004_#1	34.6819	34.8621	0.0167	640.59	

References

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3.2.2.2 Density

18 February 2022

Hiroshi Uchida (JAMSTEC) (Principal Investigator, not on board)
Yohei Kayukawa (NMIJ, AIST)

Density of seawater samples was measured after the cruise with an oscillation-type density meter (DMA 5000M, serial no. 80570578, Anton-Paar GmbH, Graz, Austria) with a sample changer (Xsample 122, serial no. 80548492, Anton-Paar GmbH), based on a method by Uchida et al. (2011). The sample changer was used to load samples automatically from up to ninety-six 12-mL glass vials. Two seawater samples of the 12-mL glass vials were taken from the 250-mL glass bottle collected for the salinity measurement immediately before the salinity measurement. Refractive index was also simultaneously measured for the same seawater samples for the density measurements with a laboratory refractometer (Abbemat 650, serial no. 99058548, Anton-Paar GmbH) by connected to the density meter.

Density and refractive index of the samples were measured at 20 °C. Time drift of the density meter was monitored by periodically measuring the density of ultra-pure water (Milli-Q water, Millipore, Billerica, Massachusetts, USA) prepared from Yokosuka (Japan) tap water in October 2014. The true density at 20 °C of the Milli-Q water was estimated to be 998.2041 kg m⁻³ from the isotopic composition ($\delta D = -8.86$ ‰, $\delta^{18}O = -59.9$ ‰) and International Association for the Properties of Water and Steam (IAPWS)-95 standard. An offset correction was applied to the measured density by using the Milli-Q water measurements ($\rho_{\text{Milli-Q}}$) with a slight modification of the density dependency (Uchida et al., 2011). The offset (ρ_{offset}) of the measured density (ρ) was evaluated for the serial no. 80570578 as follows:

$$\rho_{\text{offset}} = (\rho_{\text{Milli-Q}} - 998.2041) - (\rho - 998.2041) \times 0.000411 \text{ [kg m}^{-3}\text{]}.$$

The offset correction was verified by measuring the IAPSO Standard Seawater (batch P164). The IAPSO Standard Seawater was measured at the beginning, the middle, and the end of each day of measurement. The measured densities are listed in Table 3.2.2.2.1.

For the refractometer, refractive index anomalies from the ultra-pure water measurement were calculated for each day of measurement. Refractive index for pure water is about 1.332987 nD.

Table 3.2.2.2.1. Measured density at 20 °C of the IAPSO Standard Seawater. True density estimated from practical salinity with the batch offset correction (Uchida et al., 2020) and composition of seawater using TEOS-10 is also shown.

Standard Seawater	True density (kg/m ³)	Measured density (kg/m ³)	Number of measurements
P164	1024.7626	1024.7624±0.0011	19

Density salinity was back calculated from measured density and temperature (20 °C) with TEOS-10. The measured refractive index data will be used to estimate density salinity of the sample.

A total of 10 pairs of replicate samples was measured. The root-mean square of the absolute difference of replicate samples was 0.0018 g/kg and 0.04 nD for density and refractive index, respectively.

The density meter during the measurement for station 001 was unstable probably due to small bubble. Therefore, the data quality flag for the density data of bottle no. #2, 4, 5, 6, 12, 13, 14, 15, 16_2, 18 at station 001 was set to 4 (bad measurement).

References

- Uchida, H., T. Kawano, M. Aoyama, and A. Murata (2011): Absolute salinity measurements of standard seawaters for conductivity and nutrients. *La mer*, 49, 119-126.
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3.2.2.3 Dissolved Oxygen

16 February 2022

Hiroshi Uchida (JAMSTEC) (Principal Investigator) (non-boarding)
Shiori Ariga (MWJ) (Operation Leader)

Dissolved oxygen in seawater samples was determined by an iodometric titration following a procedure based on Winkler's method (Dickson 1996; Culbertson 1991; Kumamoto et al. 2020).

Instruments

Burette for sodium thiosulfate and potassium iodate:

Automatic piston burette (APB-620) (Kyoto Electronics Manufacturing Co., Ltd.)
10 cm³ of titration vessel

Detector:

Automatic photometric titrator (DOT-15X) manufactured by Kimoto Electric Co., Ltd.

Software:

DOT_Terminal Ver. 1.3.0

Reagents

Pickling Reagent I:

Manganese(II) chloride solution (3 mol dm⁻³)

Pickling Reagent II:

Sodium hydroxide (8 mol dm⁻³) / Sodium iodide solution (4 mol dm⁻³)

Sulfuric acid solution (5 mol dm⁻³)

Sodium thiosulfate (0.025 mol dm⁻³)

Potassium iodate (0.001667 mol dm⁻³)

Sampling

Seawater samples were collected with sampling bottle attached to the CTD/Carousel Water Sampling System (CTD system). Seawater for oxygen measurement was transferred from the bottle to a volume calibrated flask (ca. 100 cm³), and three times volume of the flask was overflowed. Temperature was simultaneously measured by digital thermometer during the overflowing. After transferring the sample, two reagent solutions (Reagent I and II) of 1 cm³ each were added immediately and the stopper was inserted carefully into the flask. The sample flask was then shaken vigorously to mix the contents and to disperse the precipitate finely throughout. After the precipitate has settled at least halfway down the flask, the flask was shaken again vigorously to disperse the precipitate. The sample flasks containing pickled samples were stored in a laboratory until they were titrated.

Sample measurement

For over two hours after the re-shaking, the pickled samples were measured on board. Sulfuric acid solution with its volume of 1 cm³ and a magnetic stirrer bar were put into the sample flask and the sample was stirred. The samples were titrated by sodium thiosulfate solution whose morality was determined by potassium iodate solution. Temperature of sodium thiosulfate during titration was recorded by a digital thermometer. Dissolved oxygen concentration (μmol kg⁻¹) was calculated by sample temperature during seawater sampling, salinity of the Bottle Salinity, flask volume, and titrated volume of sodium thiosulfate solution without the blank.

Standardization and determination of the blank

Concentration of sodium thiosulfate titrant was determined by potassium iodate solution. Pure potassium iodate was dried in an oven at 130 °C, and 1.7835 g of it was dissolved in deionized water and diluted to final weight of 5 kg in a flask. After 10 cm³ of the standard potassium iodate solution was added to an another flask using a volume-calibrated dispenser, 90 cm³ of deionized water, 1 cm³ of sulfuric acid solution, and 1 cm³ of pickling reagent solution II and I were added in order. Amount of titrated volume of sodium thiosulfate for this diluted standard potassium iodate solution (usually 5 times measurements average) gave the morality of sodium thiosulfate titrant.

The oxygen in the pickling reagents I (1 cm³) and II (1 cm³) was assumed to be 7.6×10^{-8} mol (Murray et al. 1968). The blank due to other than oxygen was determined as follows. First, 1 and 2 cm³ of the standard potassium iodate solution were added to each flask using a calibrated dispenser. Then 100 cm³ of deionized water, 1 cm³ of sulfuric acid solution, 1 cm³ of pickling II reagent solution, and same volume of pickling I reagent solution were added into the flask in order. The blank was determined by difference between the first (1 cm³ of potassium iodate) titrated volume of the sodium thiosulfate and the second (2 cm³ of potassium iodate) one. The titrations were conducted for 3 times and their average was used as the blank value (Table 3.2.2.3-1).

Table 3.2.2.1-1 Results of the standardization and the blank determinations during cruise

Date (yyyy/mm/dd)	Potassium iodate ID	Sodium thiosulfate ID	DOT-15X (Kaimei)		Station number
			E.P. (cm ³)	Blank (cm ³)	
2022/2/1	K21F01	T-21Y	3.929	-0.002	001,002
2022/2/4	K21F02	T-21Y	3.930	0.001	003,004,005
2022/2/9	K21F03	T-21Y	3.928	0.001	-

Repeatability of sample measurement

Replicate samples were taken at every CTD casts. The standard deviation of the replicate measurement (Dickson et al. 2007) was 0.17 μmol kg⁻¹ (n=9).

Data archives

These data obtained in this cruise will be submitted to the Data Management Group (DMG) of JAMSTEC, and will be opened to the public via “Data Research System for Whole Cruise Information in JAMSTEC (DARWIN)” in JAMSTEC web site (<http://www.godac.jamstec.go.jp/darwin/e>).

References

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3.2.2.4. Nutrients

Hiroshi UCHIDA (JAMSTEC): Principal Investigator, non-boarding
Kei Kondo (JAMSTEC)
Tomomi SONE (MWJ): Operation Leader

(2) Objectives

The objective of nutrients analyses during the R/V Kaimei KM22-02 cruise, of which EXPOCODE are 490T20220131, is as follows:

- Describe the present status of nutrients concentration with excellent comparability using certified reference material of nutrient in seawater.

(3) Parameters

The determinants are nitrate, nitrite, silicate, phosphate and ammonia off Nansei Islands.

(4) Instruments and methods

(4.1) Analytical detail using QuAatro39 systems (BL TEC K.K.)

Nitrate + nitrite and nitrite are analyzed following a modification of the method of Grasshoff (1976). The sample nitrate is reduced to nitrite in a cadmium tube the inside of which is coated with metallic copper. The sample stream after reduction is treated with an acidic, sulfanilamide reagent to produce a diazonium ion. N-1-naphthylethylenediamine dihydrochloride is added to the sample stream to produce a red azo dye. With the reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by the difference between nitrate+nitrite concentration and nitrite concentration,

The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1999). Silicomolybdic acid is first formed from the silicate in the sample and molybdic acid. The silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using ascorbic acid.

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 ammonia in seawater is mixed with an alkaline containing EDTA, ammonia as gas state is formed from seawater. The ammonia (gas) is absorbed in sulfuric acid by way of 0.5 µm pore size membrane filter (ADVANTEC PTFE) at the dialyzer attached to the analytical system. The ammonia absorbed in sulfuric acid is determined by coupling with phenol and hypochlorite to form indophenols blue.

The details of a modification of analytical methods for four parameters, nitrate, nitrite, silicate and phosphate, used in this cruise are also compatible with the methods described in nutrients section in the new GO-SHIP repeat hydrography nutrients manual (Becker et al., 2019) which is a revised version of the GO-SHIP repeat hydrography nutrients manual (Hydes et al., 2019), while an analytical method of ammonium is compatible with the determination of ammonia in seawater using a vaporization membrane permeability method (Kimura, 2000). The flow diagrams and reagents for each parameter are shown in Figures 3.2.2.4-1 to 3.2.2.4-5.

(4.2) Nitrate + Nitrite Reagents

50 % Triton solution

50 mL Triton™ X-100 provided by Sigma-Aldrich Japan G. K. (CAS No. 9002-93-1) were mixed with 50 mL ethanol (99.5 %).

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

Dissolve 4 g imidazole (CAS No. 288-32-4), in 1000 mL ultra-pure water, add 2 mL hydrogen chloride (CAS No. 7647-01-0). After mixing, 1 mL 50 % triton solution is added.

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

Dissolve 10 g 4-aminobenzenesulfonamide (CAS No. 63-74-1), in 900 mL of ultra-pure water, add 100 mL hydrogen chloride (CAS No. 7647-01-0). After mixing, 2 mL 50 % triton solution is added.

NED, 0.004 M (0.1 % w/v)

Dissolve 1 g N-(1-naphthalenyl)-1,2-ethanediamine, dihydrochloride (CAS No. 1465-25-4), in 1000 mL of ultra-pure water and add 10 mL hydrogen chloride (CAS No. 7647-01-0). After mixing, 1 mL 50 % Triton solution is added. This reagent was stored in a dark bottle.

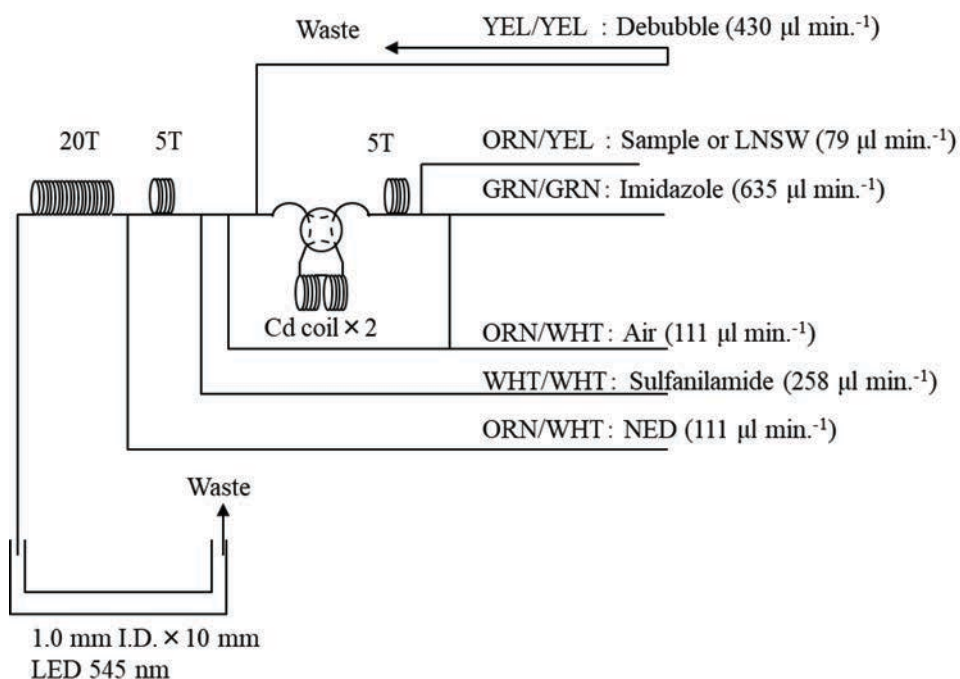


Figure 3.2.2.4-1 NO_3+NO_2 (1ch.) flow diagram.

(4.3) Nitrite Reagents

50 % Triton solution

50 mL Triton™ X-100 provided by Sigma-Ardrich Japan G. K. (CAS No. 9002-93-1) were mixed with 50 mL ethanol (99.5 %).

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

Dissolve 10 g 4-aminobenzenesulfonamide (CAS No. 63-74-1), in 900 mL of ultra-pure water, add 100 mL hydrogen chloride (CAS No. 7647-01-0). After mixing, 2 mL 50 % triton solution is added.

NED, 0.004 M (0.1 % w/v)

Dissolve 1 g N-(1-naphthalenyl)-1,2-ethanediamine, dihydrochloride (CAS No. 1465-25-4), in 1000 mL of ultra-pure water and add 10 mL hydrogen chloride (CAS No. 7647-01-0). After mixing, 1 mL 50 % triton solution is added. This reagent was stored in a dark bottle.

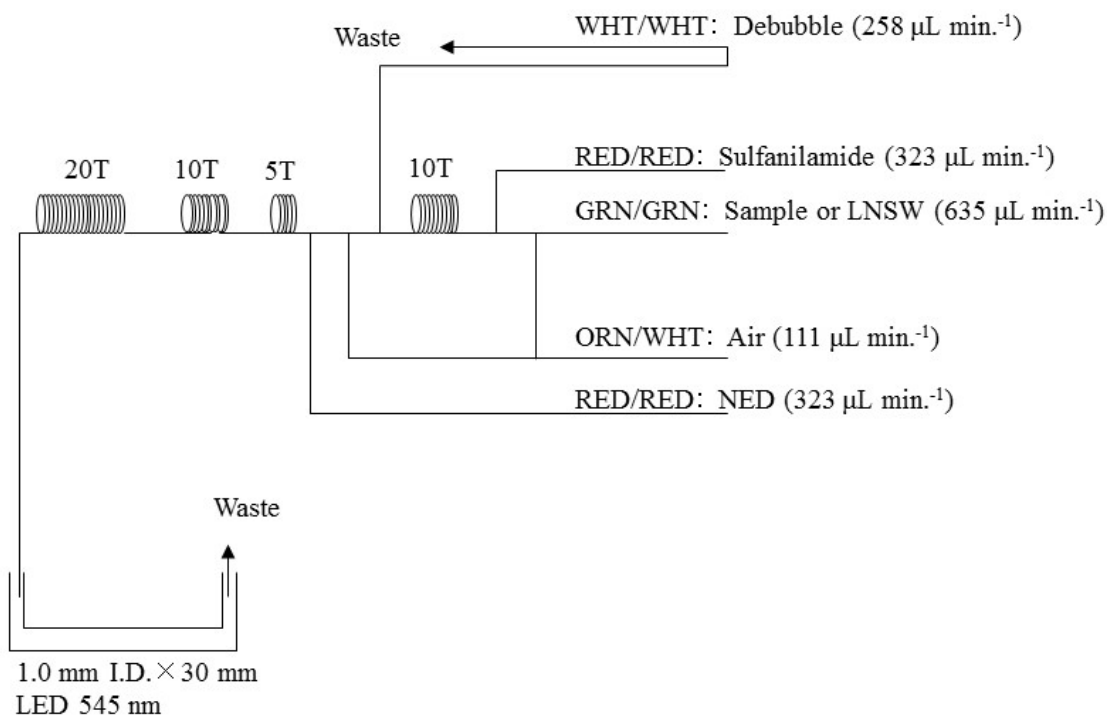


Figure 3.2.2.4-2 NO_2 (2ch.) flow diagram.

(4.4) Silicate Reagents

15 % Sodium dodecyl sulfate solution

75 g sodium dodecyl sulfate (CAS No. 151-21-3) was mixed with 425 mL ultra-pure water.

Molybdic acid, 0.03 M (1 % w/v)

Dissolve 7.5 g sodium molybdate dihydrate (CAS No. 10102-40-6), in 980 mL ultra-pure water, add 12 mL 4.5M sulfuric acid. After mixing, 20 mL 15 % sodium dodecyl sulfate solution is added. Note that the amount of sulfuric acid is reduced from previous reports because we readjusted to Grasshoff et al. (1999).

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

Dissolve 50 g oxalic acid (CAS No. 144-62-7), in 950 mL of ultra-pure water.

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

Dissolve 2.5 g L-ascorbic acid (CAS No. 50-81-7), in 100 mL of ultra-pure water. This reagent was freshly prepared every day.

Alkaline phenol

Dissolve 10 g phenol (CAS No. 108-95-2), 5 g sodium hydroxide (CAS No. 1310-73-2) and 2 g sodium citrate dihydrate (CAS No. 6132-04-3), in 200 mL ultra-pure water. Stored in a dark bottle and prepared a week approximately.

NaClO solution

Mix 3 mL sodium hypochlorite (CAS No. 7681-52-9) in 47 mL ultra-pure water. Stored in a dark bottle and freshly prepared before every measurement. This reagent is prepared 0.3 % available chlorine.

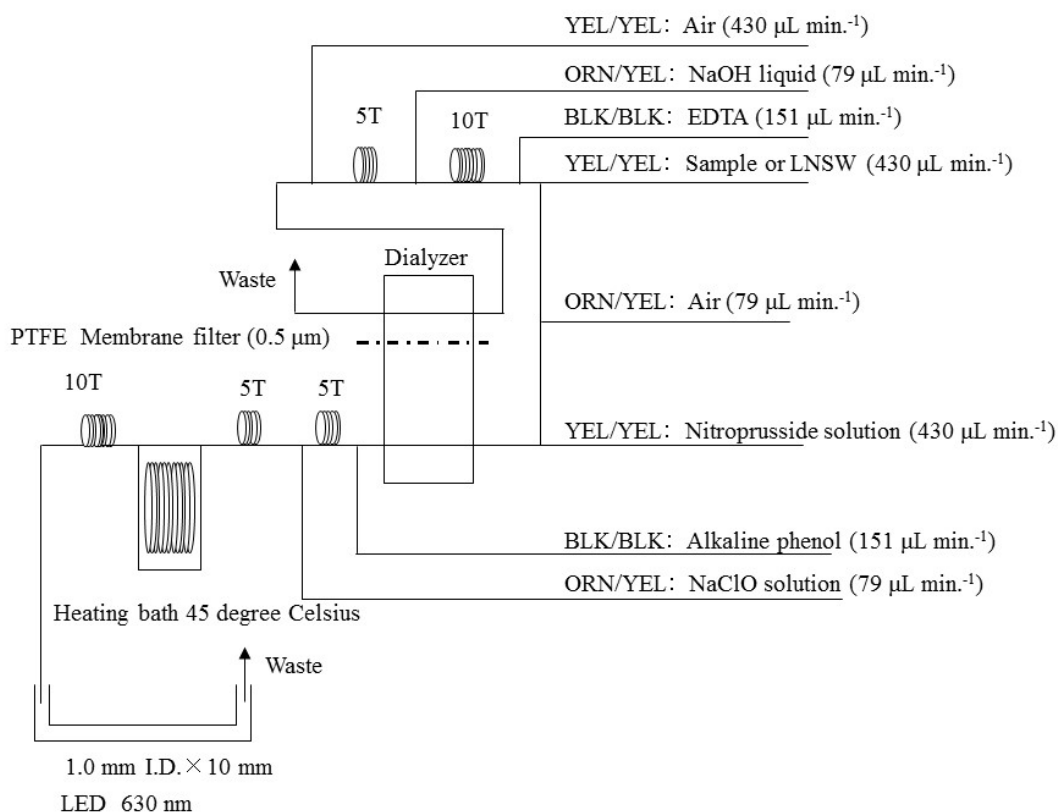


Figure 3.2.2.4-5 NH_4 (5ch.) flow diagram.

(4.7) Sampling procedures

Sampling of nutrients followed that oxygen, salinity and trace gases. Samples were drawn into a new 10 mL polyacrylates vials without sample drawing tubes. These were rinsed three times before filling and the vials were capped immediately after the drawing without headspace.

No transfer from the vial to another container was made and the vials were set an autosampler tray directly. Samples were analyzed after collection basically within 24 hours.

(4.8) Data processing

Raw data from QuAAtro 39 were treated as follows:

- Checked if there were any baseline shift.
- Checked the shape of each peak and positions of peak values. If necessary, a change was made for the positions of peak values.
- Conducted carry-over correction and baseline drift correction followed by sensitivity correction to apply to the peak height of each sample.
- Conducted baseline correction and sensitivity correction using the linear regression.

- Using the pressure and the salinity from uncalibrated CTD data and the laboratory room temperature (20 degree Celsius), the density of each sample had been calculated tentatively. The obtained density was used to calculate the final nutrient concentration with the unit of $\mu\text{mol kg}^{-1}$.
- Calibration curves to obtain the nutrients concentrations were assumed second order equations.

(4.9) Summary of nutrients analysis

We made 6 QuAatro runs for the samples collected by 5 stations as shown in Table 3.2.2.4-1 during KM22-02. The total amount of layers of the seawater sample reached to 302. We made duplicate measurements at all the sampling layers. The station locations for nutrients measurement is shown in Figure 3.2.2.4-6.

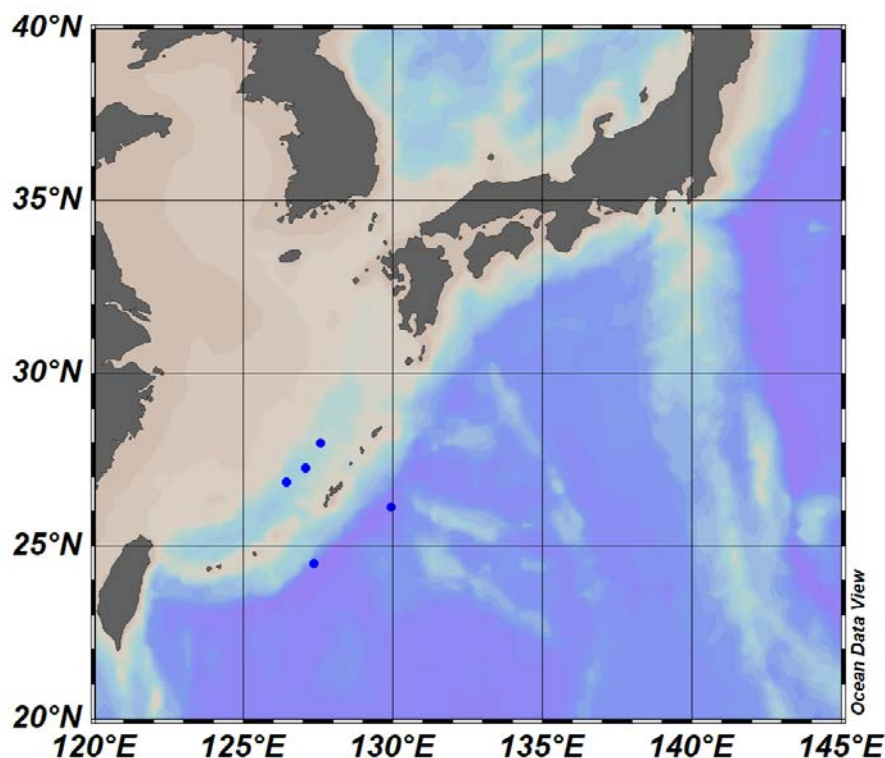


Figure 3.2.2.4-6 Sampling positions of nutrients sample.

(5) Station list

The sampling station list for nutrients is shown in Table 3.2.2.4-1.

Table 3.2.2.4-1 List of stations

Run serial	Station	Cast	Date (UTC)	Position*		Depth (m)
			(mm/dd/yyyy)	Latitude	Longitude	
1	001	1	02/02/2022	26-08.08N	129-56.96E	5404
2, 4	002	1	02/03/2022	27-59.32N	127-00.00E	1338
3	003	1	02/04/2022	26-50.87N	126-00.04E	1857
5	004	1	02/05/2022	24-29.12N	127-21.05E	7343
6	005	1	02/07/2022	27-16.01N	127-04.19E	1634

* Position indicates latitude and longitude where CTD reached maximum depth at the cast.

(6) Certified Reference Material of nutrients in seawater

KANSO certified reference materials (CRMs, Lot: CE, CG, CF) were used to ensure the comparability and traceability of nutrient measurements during this cruise. The details of CRMs are shown below.

Production

KANSO CRMs for inorganic nutrients in seawater were produced by KANSO Co.,Ltd. This CRM has been produced using autoclaved natural seawater based on the quality control system under ISO Guide 34 (JIS Q 0034).

KANSO Co.,Ltd. has been accredited under the Accreditation System of National Institute of Technology and Evaluation (ASNITE) as a CRM producer since 2011. (Accreditation No.: ASNITE 0052 R)

(6.1) CRM concentration

Nutrients concentrations for the CRM lots CE, CG and CF are shown in Table 3.2.2.4-2.

Table 3.2.2.4-2 Certified concentration and uncertainty (k=2) of CRMs.

unit: $\mu\text{mol kg}^{-1}$

Lot	Nitrate**	Nitrite	Silicate	Phosphate	Ammonia***
CE*	0.01 ± 0.03	0.031 ± 0.03	0.06 ± 0.09	0.012 ± 0.006	0.69
CG	23.70 ± 0.20	0.073 ± 0.03	56.40 ± 0.50	1.700 ± 0.020	0.61
CF	43.40 ± 0.40	0.093 ± 0.02	159.7 ± 1.00	3.060 ± 0.030	0.46

*Nitrate, silicate and phosphate values of CRM lot CE are below quantifiable detection limit and shown as only reference values.

**Nitrite concentration values are measured on the ship before MR21-04 cruise in July 2021.

***Ammonia values are not certified and shown as only reference values.

(7) Nutrients standards

(7.1) Volumetric laboratory-ware of in-house standards

All volumetric glassware and polymethylpentene (PMP)-ware used were gravimetrically calibrated. Plastic volumetric flasks were gravimetrically calibrated at the temperature of use within 3 K.

(7.1.1) Volumetric flasks

Volumetric flasks of Class quality (Class A) are used because their nominal tolerances are 0.05 % or less over the size ranges likely to be used in this work. Class A flasks are made of borosilicate glass, and the standard solutions were transferred to plastic bottles as quickly as possible after they are made up to volume and well mixed in order to prevent the excessive dissolution of silicate from the glass. PMP volumetric flasks was gravimetrically calibrated and used only within 5 K of the calibration temperature.

The computation of volume contained by glass flasks at various temperatures other than the calibration temperatures were done by using the coefficient of linear expansion of borosilicate crown glass.

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 5 K. The weights obtained in the calibration weightings were corrected for the density of water and air buoyancy.

(7.1.2) Pipettes

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

(7.2) Reagents, general considerations

(7.2.1) Specifications

For nitrate standard, “potassium nitrate 99.995 suprapur®” provided by Merck, Batch B1706365, CAS No. 7757-79-1, was used.

For nitrite standard solution, we used a nitrite ion standard solution (NO_2^- 1000) provided by Wako, Lot ESG1055, Code. No. 146-06453. This standard solution was certified by Wako using the ion chromatography method. Calibration result is 1004 mg L^{-1} at 20 degree Celsius. Expanded uncertainty of calibration ($k=2$) is 0.8 % for the calibration result.

For the silicate standard solution, we used our in-house Si standard solution “exp96” which was produced by alkali fusion technique from 5N SiO_2 powder produced jointly by JAMSTEC and KANSO. The mass fraction of Si in the “exp96” solution was calibrated based on NMIJ CRM 3645-a02 Si standard solution.

For phosphate standard, we used a potassium dihydrogen phosphate anhydrous 99.995 suprapur®” provided by Merck, Batch B1781408, CAS No.: 7778-77-0, was used.

For ammonia standard, ammonium chloride (CRM 3011-a) provided by NMIJ, CAS No. 12125-02-9 was used. The purity of this standard was reported as >99.9 % by the manufacture. Expanded uncertainty of calibration ($k=2$) was 0.022 %.

(7.2.2) Ultra-pure water

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

(7.2.3) Low nutrients seawater (LNSW)

Surface water having low nutrient concentration was taken and filtered using $0.20 \mu\text{m}$ pore capsule cartridge filter at MR19-04 cruise in February 2020. This water is stored in 20 L cubitainer with cardboard box.

Nutrients concentrations in LNSW were measured on August 2020.

(7.2.4) 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 3.2.2.4-3. All volumetric laboratory tools were calibrated prior the cruise as stated in chapter (7.1) 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 for nitrate, nitrite, silicate and phosphate were obtained using 5 levels, C-2, C-3, C-4, C-5 and C-6. For ammonia, that was obtained using 3 levels, C-1, C-4, C-6. C-2, C-3 and C-5 were the CRM of nutrients in seawater, C-4 and C-6 were diluting using the B standard solution, and C-1 was LNSW.

The D standard solutions were made to calculate the reduction rate of Cd coil. The D standard was diluted from the A standard solution into the pure water, final concentrations were shown in Table 3.2.2.4-3.

Table 3.2.2.4-3 Nominal concentrations of nutrients for A, D, B and C standards.

	A	B	D	C-1	C-2	C-3	C-4	C-5	C-6
	Unit: $\mu\text{mol kg}^{-1}$								
NO_3	45000	900	900	-	CE	CG	27	CF	54
NO_2	21800	26	870	-	CE	CG	0.8	CF	1.5
SiO_2	35600	2850	-	-	CE	CG	87	CF	172
PO_4	6000	60	-	-	CE	CG	1.9	CF	3.7
NH_4	4000	40	-	LNSW	-	-	1.2	-	2.4

(7.2.5) Renewal of in-house standard solutions

In-house standard solutions as stated in paragraph (7.2.4) were renewed as shown in Table 3.2.2.4-4(a) to (c).

Table 3.2.2.4-4(a) Timing of renewal of in-house standards.	
NO ₃ , NO ₂ , SiO ₂ , PO ₄ , NH ₄	Renewal
A-1 Std. (NO ₃)	maximum a month
A-2 Std. (NO ₂)	commercial prepared solution
A-3 Std. (SiO ₂)	JAMSTEC-KANSO Si standard solution
A-4 Std. (PO ₄)	maximum a month
A-5 Std. (NH ₄)	maximum a month
D-1 Std.	maximum 8 days
D-2 Std.	maximum 8 days
B Std. (mixture of A-1, D-2, A-3, A-4 and A-5 std.)	maximum 8 days

Table 3.2.2.4-4 (b) Timing of renewal of working calibration standards.	
Working standards	Renewal
C Std. (dilute B Std.)	every 24 hours

Table 3.2.2.4-4 (c) Timing of renewal of in-house standards for reduction estimation.	
Reduction estimation	Renewal
36 µM NO ₃ (dilute D-1 Std.)	when C Std. renewed
35 µM NO ₂ (dilute D-2 Std.)	when C Std. renewed

(8) Quality control

(8.1) The precision of nutrients analyses during the cruise

The precision of nutrients analyses during this cruise was evaluated based on the 11 to 12 measurements, which are measured every 7 to 12 samples, during a run at the concentration of C-5 std. Summary of precisions is shown in Table 3.2.2.4-5. During this cruise, analytical precisions were 0.13 % for nitrate, 0.17 % for nitrite, 0.08 % for silicate, 0.16 % for phosphate and 0.66 % for ammonia in terms of a median of precision, respectively.

Table 3.2.2.4-5 Summary of precision based on the replicate analyses. (<i>k</i> =1)					
	Nitrate CV %	Nitrite CV %	Silicate CV %	Phosphate CV %	Ammonia CV %
Median	0.13	0.17	0.08	0.16	0.66
Mean	0.14	0.18	0.10	0.18	0.64
Maximum	0.21	0.30	0.15	0.27	0.85
Minimum	0.06	0.07	0.06	0.15	0.41
N	5	5	5	5	5

(8.2) CRM lot. CF measurement during this cruise

CRM lot. CF was measured every run to evaluate the comparability throughout the cruise. The

results of lot. CF during this cruise are shown as Figures 3.2.2.4-7 to 3.2.2.4-11. All of the measured concentrations of CRM lot. CF was within the uncertainty of certified values for nitrate, nitrite, silicate and phosphate.

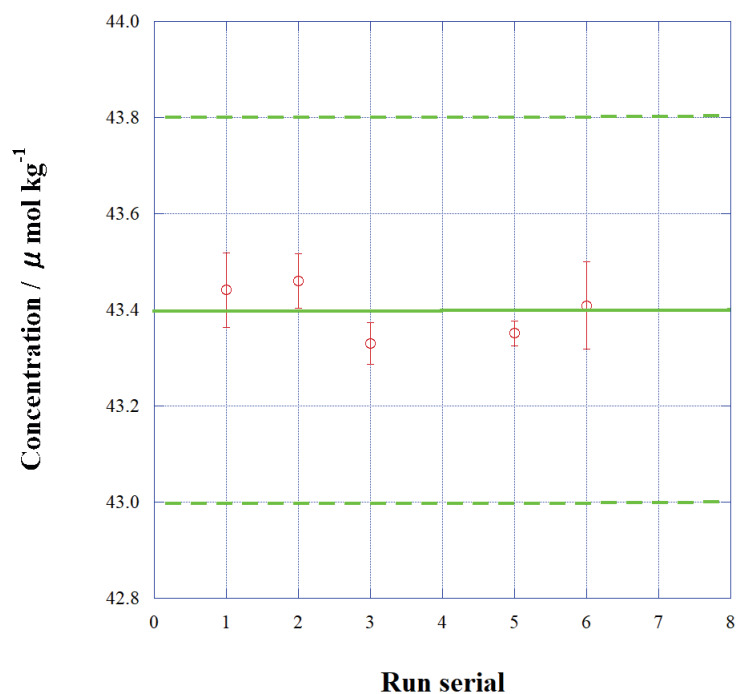


Figure 3.2.2.4-7 Time series of CRM-CF of nitrate in KM22-02. Solid green line is certified nitrate concentration of CRM and dotted green line show uncertainty of certified value at $k=2$.

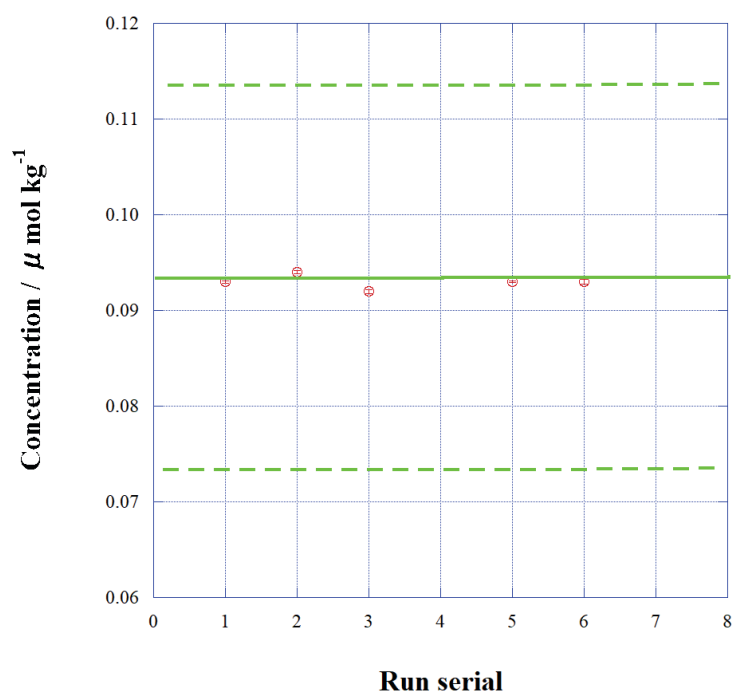


Figure 3.2.2.4-8 Same as Figure 3.2.2.4-7, but for nitrite.

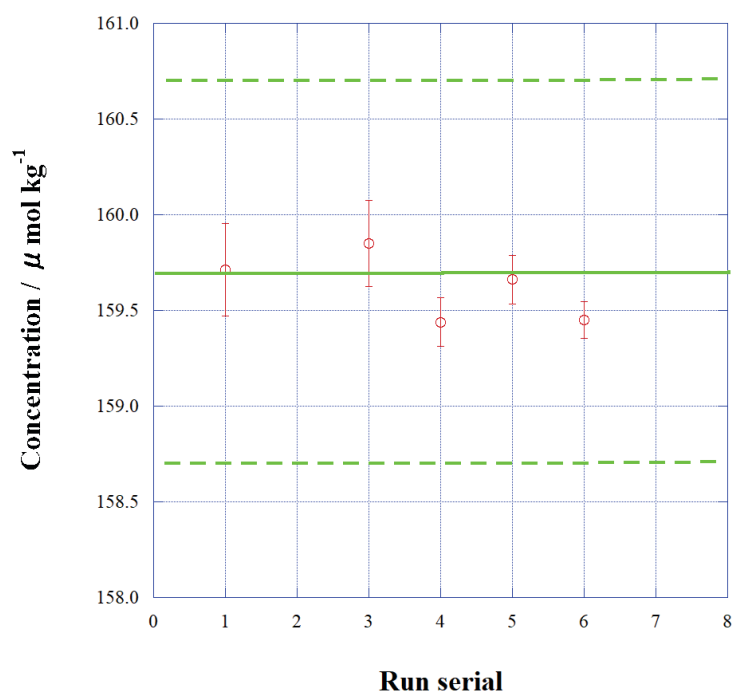


Figure 3.2.2.4-9 Same as Figure 3.2.2.4-7, but for silicate.

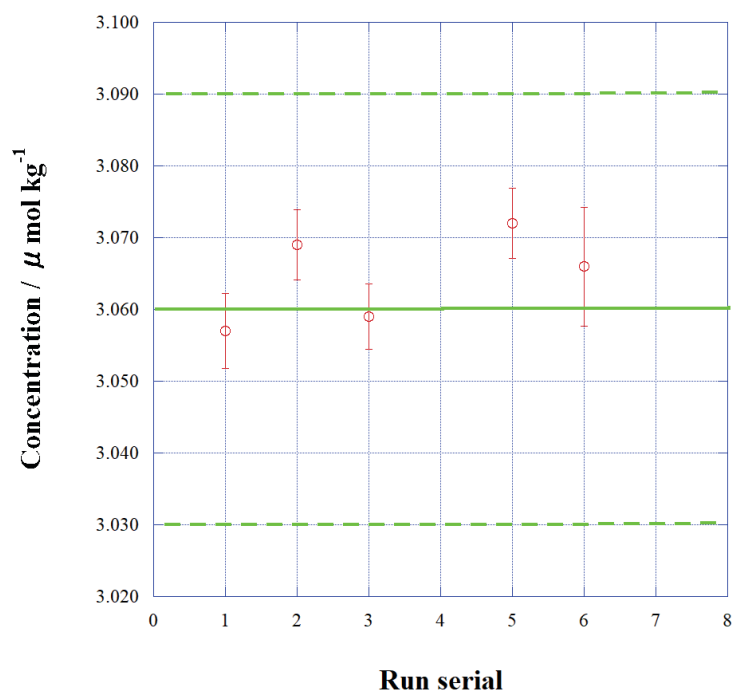


Figure 3.2.2.4-10 Same as Figure 3.2.2.4-7, but for phosphate.

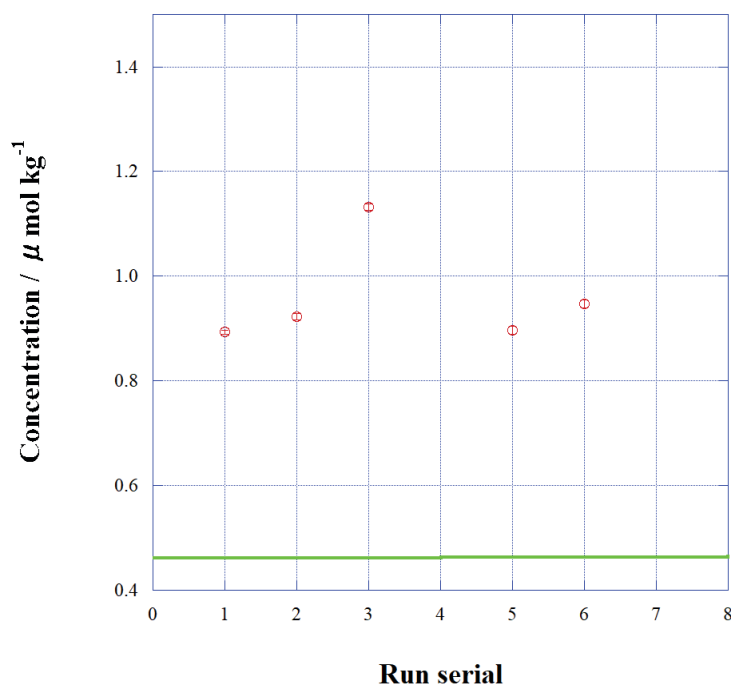


Figure 3.2.2.4-11 Time series of CRM-CF of ammonia in KM22-02. Green line is reference value for ammonia concentration of CRM-CF.

(8.3) The standard deviation of absolute difference of replicate samples

The standard deviation of absolute difference of replicate samples was obtained based on 151 pairs of replicate samples taken from the same water sampling bottle. The calculation method is described in SOP23, Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water. Summary of standard deviation is shown in Table 3.2.2.4-6.

Table 3.2.2.4-6 Summary of standard deviation of absolute difference among replicate samples.

	Nitrate $\mu\text{mol kg}^{-1}$	Nitrite $\mu\text{mol kg}^{-1}$	Silicate $\mu\text{mol kg}^{-1}$	Phosphate $\mu\text{mol kg}^{-1}$	Ammonia $\mu\text{mol kg}^{-1}$
standard deviation of absolute difference	0.05	0.002	0.14	0.005	0.02
N	151	151	151	151	151

(9) Data archives

These data obtained in this cruise will be submitted to the Data Management Group of JAMSTEC, and will be opened to the public via “Data Research System for Whole Cruise Information in JAMSTEC (DARWIN)” in JAMSTEC web site.

<<http://www.godac.jamstec.go.jp/darwin/e>>

(10) Problems and our actions/solutions

During the run serial #2 as stated in chapter (5), we had faced significant baseline and sensitivity drift issues for silicate analysis. The normal baseline correction procedure could not apply to this data set since the obtained baseline and sensitivity drift was too large, so we measured these samples again on the next day (run serial #4) and adopted the values obtained from the re-measurement for silicate.

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3.2.2.5 Carbon Items

3 June 2022

Kei Kondo (JAMSTEC) (Principal Investigator)
Hiroshi Uchida (JAMSTEC)

The carbon parameter was sampled from all layers at 5 stations (07, 12, 15, 09 and Izena). Samples for carbon parameters (dissolved inorganic carbons [hereafter DIC] and total alkalinity [hereafter TA]) were drawn with a plastic drawing tube (PFA tubing connected to silicone rubber tubing) into 250 ml DURAN® glass bottle. The glass bottle was filled with seawater smoothly from the bottom following a rinse with seawater of a full bottle volume. At the container on ship, a volume of about 3 ml seawater was removed with a plastic pipette from sampling bottles to have a headspace. After adding 100 µl of saturated mercuric chloride to poison seawater samples, the bottle closed by an inner cap fitted tightly to the bottle mouth and an outer cap. The seawater samples for carbon parameters were kept at 5 °C in a refrigerator on the board, and the analysis was carried out at our laboratory after the cruise in February-March 2022.

Analytical method and Reference materials

The measurements of DIC and TA were carried out from the same bottle. The analytical methods of the carbon parameters is same as the methods used in Uchida et al. (2018). Certified reference materials (CRM, batch 195) provided by Prof. A. G. Dickson of Scripps Institution of Oceanography and two kinds of reference materials (RM) made from seawater were used for the standardization. One of the RM, in-house RM (batch Q41), was produced by JAMSTEC. Another RM, KRM (batch AV), was provided by KANSO Co., LTD.

The KRM was measured to check consistency between the CRM and KRM. DIC values of the KRM measured with reference to the CRM agreed well with the label value of the KRM. However, TA values of the KRM measured with reference to the CRM significantly disagreed with the label value of the KRM (about 6 µmol/kg). TA values for the deep water at stations 001 and 004 were compared with TA values obtained from the other cruises near these stations (Table 3.5.2.2.5-1).

Result of the comparison suggests that the TA value of the CRM might be larger than true value. Therefore, the CRM and KRM were used to standardize measurements of DIC and TA, respectively.

Table 3.5.2.2.5-1 Comparison of mean TA for the deep water (2500-5000 dbar) in the Philippine Sea.

Cruise no.	Station no.	Mean±SD for TA [μmol/kg]	Data source
MR05-05	353, 357	2412.8±1.5	https://cchdo.ucsd.edu/cruise/49NZ20051127
RF13-06	RF4789	2413.2±4.2	https://www.data.jma.go.jp/gmd/kaiyou/db/vessel_obs/data-report/html/ship/cruisedata.php?id=RF1306
RF21-06	RF6865	2410.8±0.9	https://www.data.jma.go.jp/gmd/kaiyou/db/vessel_obs/data-report/html/ship/cruisedata.php?id=RF2106
KM22-02	001, 004	2417.7±1.8	This cruise, Calibrated with CRM
KM22-02	001, 004	2411.1±1.8	This cruise, Calibrated with KRM

Quality control

The in-house RM were measured to correct the DIC and TA measurements at the beginning and the end of each day of measurements. During time of measurement, analytical precisions were estimated from the in-house RM as 0.21 % for DIC (n=40) and 0.08% for TA (n=36).

Repeatability of sample measurements

A total of 10 pairs of replicate samples was taken from all CTD casts. For DIC and TA, the standard deviations of the replicate measurements (Dickson et al. 2007) were 1.4 μmol kg⁻¹ and 1.3 μmol kg⁻¹, respectively.

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3.2.2.6 DOC/CDOM/FDOM

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Masahito Shigemitsu (JAMSTEC) (Principal Investigator for DOC/FDOM)
Akiko Makabe, Taichi Yokokawa (JAMSTEC)

Hadal trench waters harbor characteristic microbiological assemblages. To date, resuspension of trench sediments followed by the decomposition of organic matter in the resuspension is considered to be the main mechanism to explain the microbiological characteristics (e.g., Nunoura et al., 2015). Shigemitsu et al. (2021) demonstrated the sedimentary supply of dissolved organic matter (DOM) in the Izu-Ogasawara trench and suggested that the supply might contribute to the unique microbiological community structure in the hadal waters. However, whether the same mechanism applies to other trenches is unknown.

Hydrothermal systems are known to serve as an important source or sink for various elements in the ocean (e.g., Elderfield and Schultz, 1996). However, the information about DOM is extremely

scarce. A few studies indicate that hydrothermal systems act as a sink of DOM (Hawkes et al., 2016; Yang et al., 2017), while some studies indicate that the systems behave as a source of DOM (Yang et al., 2012; Sarma et al., 2018).

In this cruise, we try to gain insights into the dynamics of dissolved organic matter (DOM) in the extremely special environments: Ryukyu trench and waters affected by hydrothermal fluids. To this end, we measure the vertical distributions of dissolved organic carbon (DOC), chromophoric dissolved organic matter (CDOM) and fluorescent fractions of CDOM (FDOM) at the Nansei islands and the surrounding stations (#12, #9, and Izena). CDOM and FDOM can be useful tracers for evaluating DOM quantity and quality (e.g., Siegel et al., 2002; Nelson et al., 2010; Catala et al., 2015a; Catala et al., 2015b).

Methods

DOC

Seawater samples for DOC were obtained from Niskin bottles. Each sample taken was filtered using a pre-combusted (450°C for 4 hours) 47-mm Whatman GF/F filter. The filtration was carried out by connecting a spigot of the Niskin bottle through silicone tube to an inline plastic filter holder. Filtrates were collected in acid-washed 60 mL High Density Polyethylene (HDPE) bottles, and were immediately stored frozen until analysis. Non-filtered samples were also collected to evaluate the effect of particulate organic carbon on DOC quantification.

The frozen samples will be thawed at room temperature, and acidified to pH < 2 with hydrochloric acid. Then, the concentrations of DOC will be measured by using a Shimadzu TOC-L system coupled with a Shimadzu Total N analyzer at JAMSTEC.

CDOM

Seawater samples (filtered and non-filtered) for absorption coefficient of CDOM ($a_{\text{cdom}}(\lambda)$) were collected in 60 ml pre-combusted glass vials with acid-washed Teflon-lined caps using the same way as DOC. Optical densities of CDOM ($\text{OD}_{\text{cdom}}(\lambda)$) in seawaters between 190 and 750 nm were measured at 0.5 nm intervals by an UV-VIS recording spectrophotometer (MPS-2400, Shimadzu Co.), using 10-cm pathlength quartz cells. Milli-Q water was used as a base line. The absorption coefficient of CDOM ($a_{\text{cdom}}(\lambda)$ (m^{-1})) was calculated from measured optical densities ($\text{OD}_{\text{cdom}}(\lambda)$) as follows:

$$a_{\text{cdom}}(\lambda) = 2.303 \times \text{OD}_{\text{cdom}}(\lambda) / 0.1 \text{ (0.1 is the cuvette path-length (m))}.$$

FDOM

Seawater samples (filtered and non-filtered) for FDOM were collected in 40 ml pre-combusted glass vials with acid-washed Teflon-lined caps using the same way as DOC. EEM fluorescence spectra were measured using a benchtop fluorometer (Aqualog, Horiba Scientific, Japan) after the samples were acclimated to laboratory temperature in the dark. The measurements were conducted within 2 days of sampling. Emission scans from 248–829 nm were obtained at 2.33 nm intervals for sequential excitations from 240–560 nm at 5 nm intervals, with an integration time of 12 s and using the high Charge-Coupled Device (CCD) gain mode.

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3.2.3. Additional theme 2: Seawater environment monitoring by an optical absolute salinity sensor

Yohei Kayukawa (NMIJ, AIST)

Hiroshi Uchida (JAMSTEC) (not on board)

Absolute salinity and/or density of seawater is one of the most important property to investigate the global-scale sea-water dynamics. The conventional technique to obtain salinity is to measure electrical conductivity of the seawater, however, there are some drawbacks such as an anomaly in density and the limited measurement performance. Refractive index is one of the most accurate property to obtain the absolute salinity due to its linear relation with the absolute salinity or density.

A refractive index sensor was developed in joint research by AIST and JAMSTEC (Uchida, 2019). The AIST version of the sensor consists of a commercial laser interferometer (KEYENCE SI-F80U, S/N: 9D710089) and a Michelson interferometer cell. The whole sensor system including a micro-computer (Raspberry Pi 3B+) and Li ion batteries are enclosed in a pressure tight housing ($\phi 210\text{ mm} \times 500\text{ mm}$, up to 10 500 m depth). Fig. 3.2.3.1 shows a photo of the sensor attached to the CTD frame.



Fig. 3.2.3.1 Absolute salinity sensor attached to the CTD frame.

As shown in Fig. 3.2.3.1, the absolute salinity sensor was attached to the CTD frame with a grating board and stainless steel bands then immersed into the water. The sensor was charged until a few hours before the CTD cast. Consequently, the sensor module was powered on. During the sensor are on, it automatically measures the optical path length and the module temperatures. The data are logged in every 1 second and saved as a text file in the micro-computer. After the CTD cast, it takes about 2 or 3 hours for water samplings, rinsing, etc., then the sensor was recharged by connecting a charging cable assembly. The micro-computer can be remotely accessed via wifi connection. The saved data file was transmitted to a PC after the CTD cast.

The objectives of this study are, a) to measure refractive index of sea-water in the full depth and compare the results with density calculated by the pressure, temperature and practical salinity based on the thermodynamic equation of state for sea-water (TEOS-10); b) to investigate an effectiveness of the Si crystal cells with the reference optical path of pure-water; c) to accumulate experiences of the sensor operation including the data transmission, recharging of the batteries, exchanging the interferometer cells, etc. In addition to those for the absolute salinity sensor, we aimed d) to test a new type of temperature-conductivity sensors to compare the results with the absolute salinity sensor.

a) Refractive index measurements in the full depth

Unfortunately, measurement data for refractive index was not obtained in this cruise due to an unknown trouble of the sensor module. It is planned to investigate the cause and the solution of the trouble at AIST lab within a few weeks.

b) Silicon single crystal cell with the reference optical path of pure water

Table 3.2.3.1 summarize the specification of the cells prepared for this cruise. Concerning the silicon crystal cells, the laser beam passes through the hole inside the silicon crystal spacer. Pure water (Milli-Q water) was filled in the hole and sealed with a fluorocarbon-rubber sheet packing.

Table 3.2.3.1 Specification of the Michelson interferometer cells

	Optical path length for sea-water (mm)	Spacer material	Reference optical path	
			Optical path length (mm)	Material
Glass cell	34.1	Quartz glass	23.0	Quartz glass
Si crystal cell (v1)	60.0	Si single crystal	60.0	Milli-Q water
Si crystal cell (v2)	60.0	Si single crystal	60.0	Milli-Q water

The optical interferometer cell (ver. 1) was used at the station number 001 (station #7, 5 403 m), 002 (station #15, 1 344 m) and 003 (station #12, 1 852 m). After the CTD cast at the station number 001 and 003, the pure water filled in the reference path was extracted by a plastic syringe and its refractive index was measured by a compact refractometer (Atago, XXXX). From the measurement results, it is found that there were no refractive-index change in pure water. As a result, it is confirmed that the pure water inside the reference path are well sealed.

c) Operation of the sensor

Through the whole measurements, useful knowledge about the operation of the sensor including the data acquisition and the battery recharging. After the casts at the station number 001, 003 and 004, the sensor was uninstalled from the CTD frame and the housing of the sensor was opened to check the water leak. There found a small amount of water leak, however, it was absorbed by a polymer sheet. Those used sheets were packed in a plastic bag then the leak amount of water will be measured after the cruise.

d) Temperature-conductivity sensor

A new type of temperature-conductivity sensor (JFE Advantec, A7CT-USB, S/N: 0743, 0745 and 0746) was tested. They were attached to the CTD frame then scheduled to turned on 1 hour before the cast. They measure and record the temperature and the conductivity at every 1 second. After each cast the lid of the sensors was opened to collect the measured data and program a new schedule. The measurements were successfully conducted at the CTD stations 001 and 004. The detailed results will be obtained by comparing it with other under-water sensor results.

Uchida, H., Y. Kayukawa, and Y. Maeda (2019): Ultra high-resolution seawater density sensor based on a refractive index measurement using the spectroscopic interference method. *Sci. Rep.*, 9:15482, doi:10.1038/s41598-019-52020-z.

3.2.4. Additional theme 3: Distribution of mercury species in marine environments

Koji Marumoto, Yuya Tada (National Institute for Minamata Disease)
Akinori Takeuchi, Nobuaki Okabe (National Institute for Environmental Studies)
Chenchen Tang (Meiji University)
Fumiyoshi Kondo (Japan Coast Guard Academy)

Information about distribution of total mercury (THg), monomethylmercury (MMHg), and dimethylmercury (DMHg) concentrations in marine environments is critical for understanding the Hg biogeochemical cycle and bioaccumulation in marine food webs. In this cruise, we investigated the full-depth distribution of these mercury species including dissolved gaseous mercury (DGM) in the pelagic water column and continuously measured air-sea exchange of gaseous Hg, CH₄ and CO₂ using a gas-liquid equilibrium instrument. In addition, we evaluated the potential of MMHg and DMHg production and degradation in the oceanic seawater using isotope dilution technique.

The seawater samples for the analyses of dissolved THg and MMHg were filtered directly from the Niskin bottles with 0.2 µm-pore size cartridge filter. In addition, the samples were also filtered with 0.2 µm-pore size polycarbonate filters using a suction pump to analyze the dissolved THg and MMHg, and particulate Hg. Then filtrates were treated with HCl and H₂SO₄ addition, respectively, and stored at 4°C until further analyses. The filters were stored at -80°C until further analysis. Moreover, the filtered seawater was bubbled with Hg-free N₂ gas to trap the dissolved Hg⁰ and DMHg using gold amalgam and carbon resin traps. These traps were stored at room temperature and -20°C, respectively. On the other hand, gaseous Hg, CH₄ and CO₂ concentrations in surface seawaters and the atmosphere were continuously measured to obtain their fluxes in air-sea interfaces.

To assess the potential of the MMHg and DMHg production and degradation (Hg methylation and demethylation rate), we spiked enriched two Hg isotopes to the unfiltered seawater and incubated them more than 24-hr. After incubation, the DMHg and Hg⁰ were collected by purging with gold amalgam and carbon resin traps, and stored at room temperature and -25°C, respectively. Dissolved MeHg and Hg²⁺ samples were treated with H₂SO₄ addition and stored at the 4°C until further analysis.

3.3. Microbial parameters

3.3.1. Spatial patterns of prokaryotic abundance, community composition and prokaryotic processes related nitrogen compounds in relation to the water masses off Nansei Islands

Taichi Yokokawa, Akiko Makabe, Eiji Tasumi (JAMSTEC)

Introduction

Prokaryotes (Bacteria and Archaea) play a major role in marine biogeochemical fluxes. Biogeochemical transformation rates and functional diversity of microbes are representative major topics in marine microbial ecology. However, the link between prokaryotes properties and biogeochemistry in the meso- and bathypelagic layers has not been explained systematically despite of the recent studies that highlight the role of microbes in the cycling of organic and inorganic

matter. Moreover, microbial community composition and biogeography in meso- and bathypelagic ocean and its relationship with upper layers and deep-water circulation have also not been well studied.

The objectives of this study, which analyze the water columns from the sea surface to 10m above the bottom of the research area, were 1) to determine the abundance of microbes; 2) to assess the community composition of prokaryotes, and 3) to determine the nitrogen compounds related with the prokaryotic activities.

Methods

Microbial abundance

Samples for microbial abundances (prokaryotes, eukaryotes and viruses) were collected in Stations: #07, #15, #12, #9, and Izena. Samples were fixed with glutaraldehyde (final concentration 0.5%) and frozen at -80°C. The abundance and relative size of microbes and viruses will be measured by a flow cytometry in JAMSTEC after nucleic acid staining with SYBR-Green I.

Microbial diversity

Microbial cells in water samples were filtrated on cellulose acetate filter (0.2µm) and stored at -80°C. Environmental DNA or RNA will be extracted from the filtrated cells and used for 16S/18S rRNA gene tag sequencing using MiSeq, quantitative PCR for genes for 16S rRNA, and/or metatranscriptomics. Samples for microbial diversity were taken at stations, where #07, #15, #12, #9, and Izena, in the CTD casts.

Nitrate isotope ratio

Samples for nitrate stable isotope analysis were collected into a 50 mL plastic syringe with a filter (pore size: 0.45µm) and filtered immediately after sampling on board the ship. We will measure both nitrogen and oxygen stable isotope ratio of nitrate using the bacterial method at JAMSTEC. Samples for nitrate stable isotope ratio were collected in the stations: #07, #15, #12, #9, and Izena.

N₂O and CH₄ concentration and isotope ratio

Samples for N₂O and CH₄ stable isotope analysis were collected into 100 mL glass vials, respectively. To inhibit microbial activities, HgCl₂ solution was added to seawater samples immediately after sampling. Nitrogen and oxygen stable isotope ratio of N₂O and carbon stable isotope ratio of CH₄ will be measured by GC-IRMS at JAMSTEC. Samples for N₂O and CH₄ stable isotope ratio were collected in the stations: #07, #15, #12, #9, and Izena

Ammonium concentration

Samples for ammonium concentration analysis were collected into 25 mL PP bottles. Concentration of ammonium was measured by the fluorometric method on board. Samples for ammonium concentration were collected in the stations: #07, #15, #12, #9, and Izena

4. Notice on Using

This cruise report is a preliminary documentation as of the end of cruise.

This report is not necessarily corrected even if there is any inaccurate description (i.e. taxonomic classifications). This report is subject to be revised without notice. Some data on this report may be raw or unprocessed. If you are going to use or refer the data on this report, it is recommended to ask the Chief Scientist for latest status.

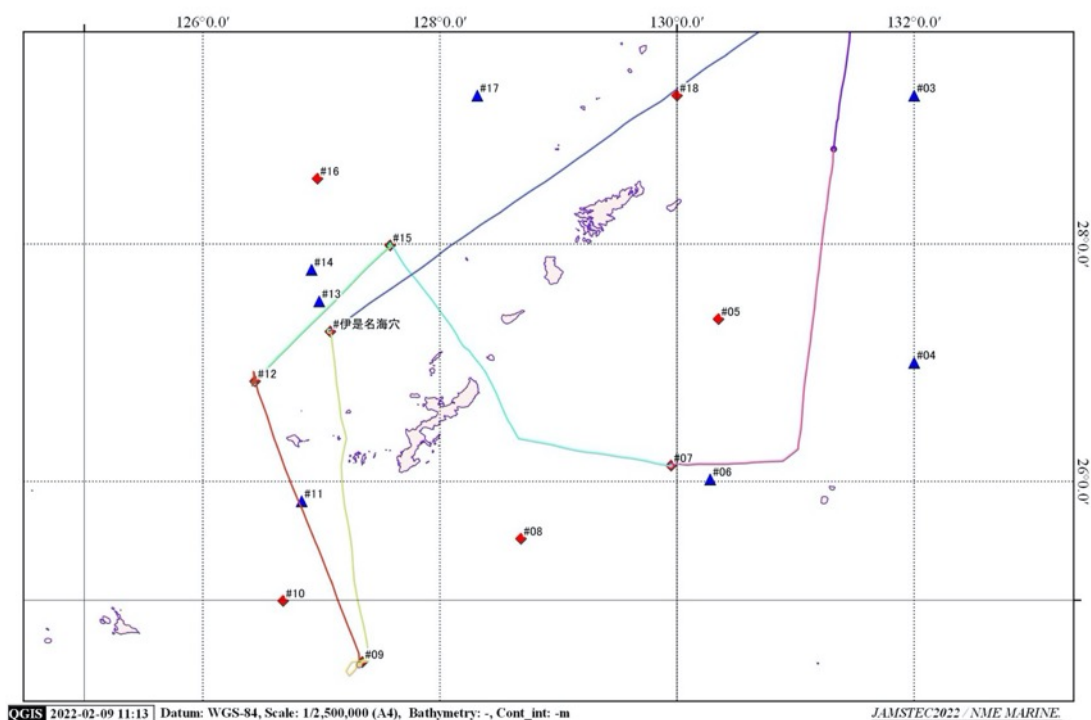
Users of information on this report are requested to submit Publication Report to JAMSTEC.

<http://www.godac.jamstec.go.jp/darwin/explain/1/e#report>

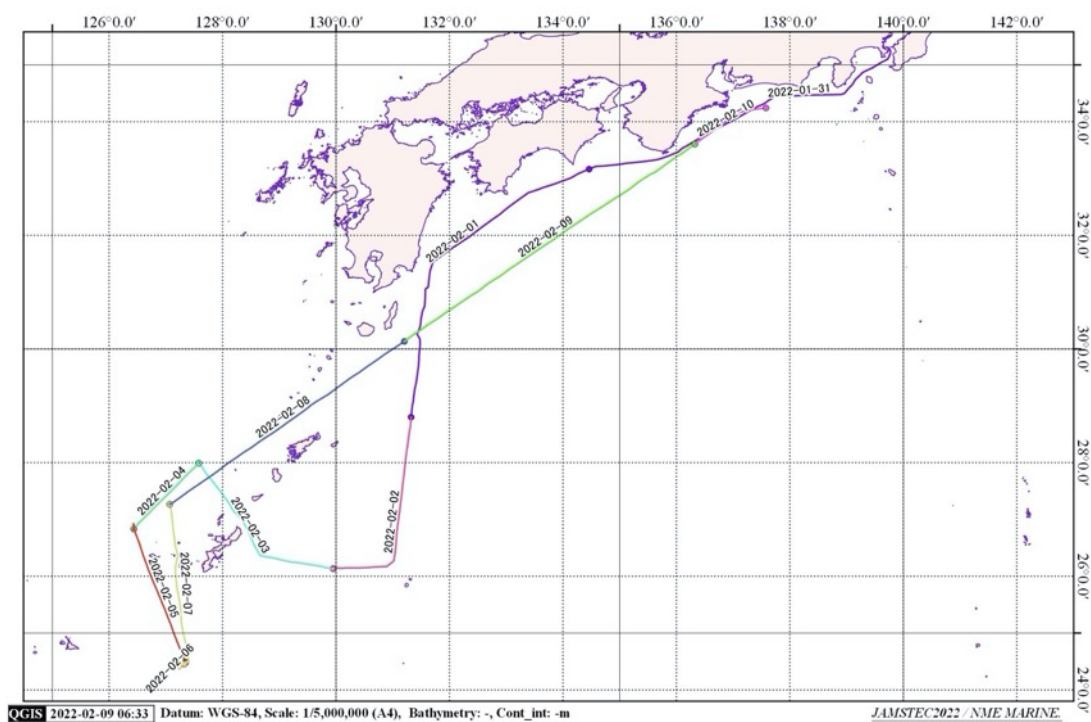
E-mail: submit-rv-cruise@jamstec.go.jp

Appendix: Navigation

KM22-02, R/V KAIMEI, Cruise Track, NANSEI Shoto



KM22-02, R/V KAIMEI, Cruise Track, NANSEI Shoto



Appendix: Cruise log

Date & Time	Description	Weather / Wind / Sea Condition
2022/01/31 Mon.	Noon Position: 34-53.0N, 139-19.3E (South ward Off OOSHIMA)	bc / NNE / 4
07:30	Onboarded	
08:30	Let go all shore lines and left YOKOSUKA KO for research area	
09:00	Scientists Meeting	
10:00-11:00	Carried out on-board education for scientists	
11:30	Briefing with crews	
2022/02/01 Tue.	Noon Position: 32-52.6N, 133-45.4E (Southwest Of MUROTO Misaki)	bc / West / 5
	Bound for research area	
2022/02/02 Wed.	Noon Position: 28-07.3N, 131-14.4E (East ward off AMAMIOSHIMA)	o / NE / 3
	Bound for research area	
2022/02/03 Thu.	Noon Position: 26-08.0N, 129-57.0E (East ward Of OKINAWA)	o / South / 7
00:45	Arrived at research area, Ryukyu Trench, site #07	
03:00	Released XBT at <26-08.4N, 129-58.0E>	
03:23-03:31	Carried out MBES mapping survey	
04:13	Carried CTD sampling operation(site #07)	
08:24	Finished CTD sampling operation	
09:23	Launched multiple corer	
09:30	Multiple corer landed on the sea bottom (w.o.=5632m)	
11:35-11:55	Carried out towing NEUSTON NET sampling #1	
12:02-12:22	Carried out towing NEUSTON NET sampling #2	
12:26-12:46	Carried out towing NEUSTON NET sampling #3	
13:35	Recovered multiple corer	
14:30	Com'ced proceeding to site #15	
2022/02/04 Fri.	Noon Position: 35-00.4N, 139-22.7E (Northwest Of OKINAWA)	r / North / 6
06:25	Arrived at research area, Okinawa Trough, site #15	
06:27	Released XBT	
06:53-07:00	Carried out MBES mapping survey	
08:11-10:08	Carried out CTD sampling operation (site #15)	
10:53	Launched Beam Trawl and com'ced towing it	
12:16	Beam Trawl landed on the sea bottom	
12:43	Finished towing Beam Trawl (w.o.=1340m)	
14:38	Recovered Beam Trawl	
15:00-15:20	Carried out towing NEUSTON NET sampling #1	
15:24-15:44	Carried out towing NEUSTON NET sampling #2	
16:35	Com'ced proceeding to site #12	

Date & Time	Description	Weather / Wind / Sea Condition
2022/02/05 Sat.	Noon Position: 26-28.4N, 126-25.2E (Northwest Of OKINAWA island)	c / North / 4
00:15	Arrived at research area, Kerama Strait, site #12	
03:00	Released XBT at <26-52.3N, 126-26.4E>	
03:20-03:29	Carried out MBES mapping survey	
04:22-07:18	Carried out CTD sampling operation (site #12)	
08:35	Launched multiple corer	
09:48	Multiple corer landed on the sea bottom (w.o.=1960m)	
10:49	Recovered multiple corer	
11:34	Launched Beam Trawl and com'ced towing it	
13:00	Beam Trawl landed on the sea bottom (w.o.=1938m)	
13:31	Finished towing Beam Trawl	
14:55	Recovered Beam Trawl	
15:14-15:34	Carried out towing NEUSTON NET sampling #1	
15:39-15:59	Carried out towing NEUSTON NET sampling #2	
16:03-16:23	Carried out towing NEUSTON NET sampling #3	
16:30	Com'ced proceeding to site #9	
2022/02/06 Sun.	Noon Position: 24-28.1N, 127-20.0E (South ward Of OKINAWA island)	o / NNE / 5
04:50	Arrived at research area, south ward of Okinawa island, site #9	
05:00	Released XBT	
05:25-05:37	Carried out MBES mapping survey	
06:07-11:06	Carried out CTD sampling operation (site #9)	
08:09-08:22	Carried out fluoro measurement	
11:48	Launched multiple corer	
14:25	Multiple corer landed on the sea bottom (w.o.=7630m)	
16:52	Recovered multiple corer	
2022/02/07 Mon.	Noon Position: 24-28.5N, 127-20.4E (South ward Of OKINAWA island)	c / East / 4
08:09	Launched Beam Trawl and com'ced towing it	
11:19	Beam Trawl landed on the sea bottom (w.o.=7527m)	
12:19	Finished towing Beam Trawl	
12:59-13:19	Carried out towing NEUSTON NET sampling #1	
13:24-13:39	Carried out towing NEUSTON NET sampling #2	
13:41-13:56	Carried out towing NEUSTON NET sampling #3	
16:30	Recovered Beam Trawl	
16:50	Com'ced proceeding to site #Izena	
2022/02/08 Tue.	Noon Position: 27-33.9N, 127-30.1E (East ward Of OKINOERABU Island)	o / North / 7
05:00	Arrived at research area, Izena hole, site #Izena	
06:00	Released XBT	
06:30-06:39	Carried out MBES mapping survey	
7:14-09:03	Carried out CTD sampling operation (site #Izena)	
09:30	Com'ced proceeding to YOKOSUKA KO	
2022/02/09 Wed.	Noon Position: 30-35.4N, 131-52.0E (East ward Of TANEGASHIMA)	c / NNE / 5
	Bound for YOKOSUKA KO	
2022/02/10 Thu.	Noon Position: 34-00.4N, 136-53.0E (Southwest Of DAIOSAKI)	r / North / 7
	Bound for YOKOSUKA KO	
2022/02/11 Fri.		
09:00	Sent out 1st shore line, arrived at JAMSTEC at YOKOSUKA, then completed voy. No.KM22-02	

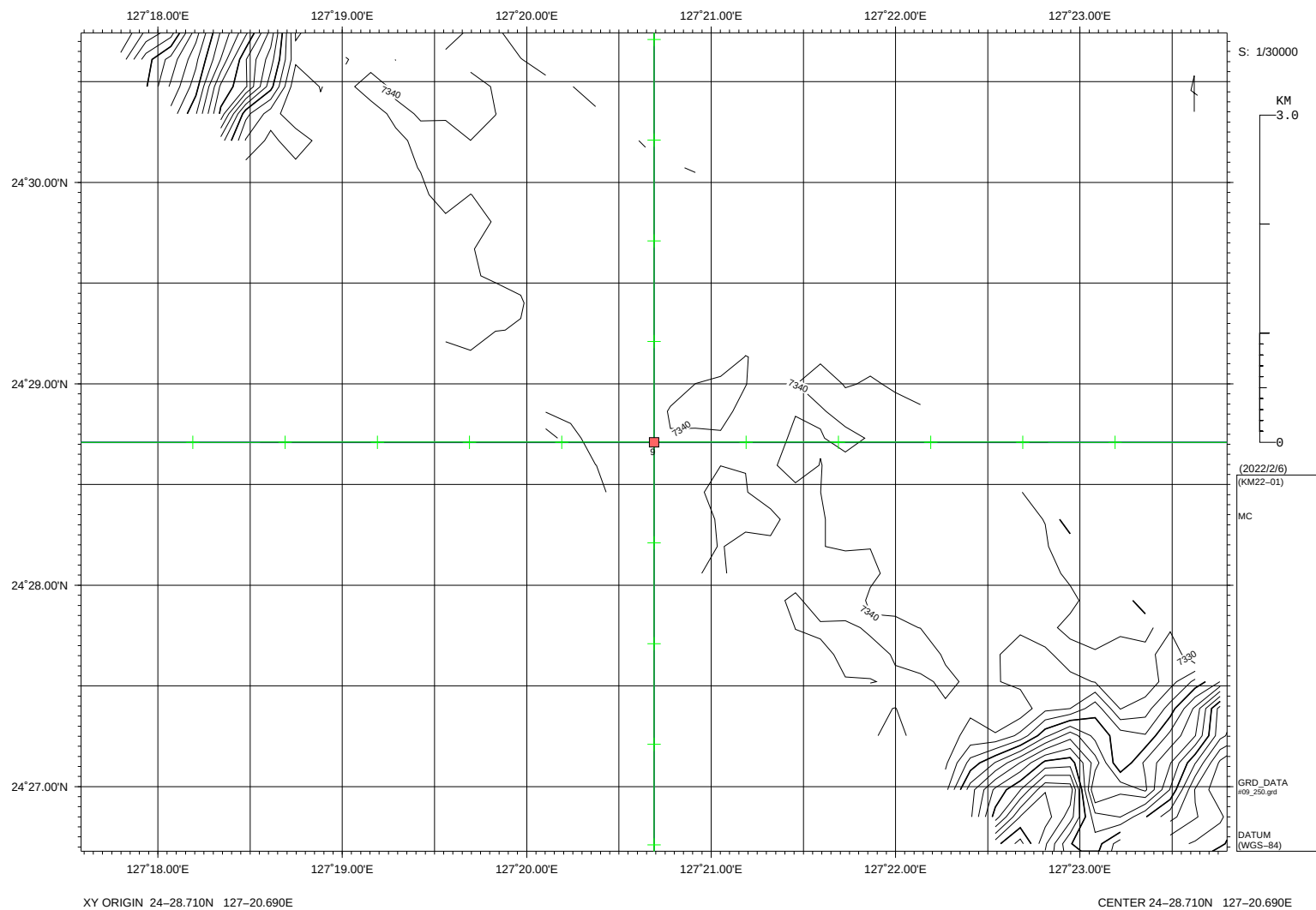
Appendix: Operation log

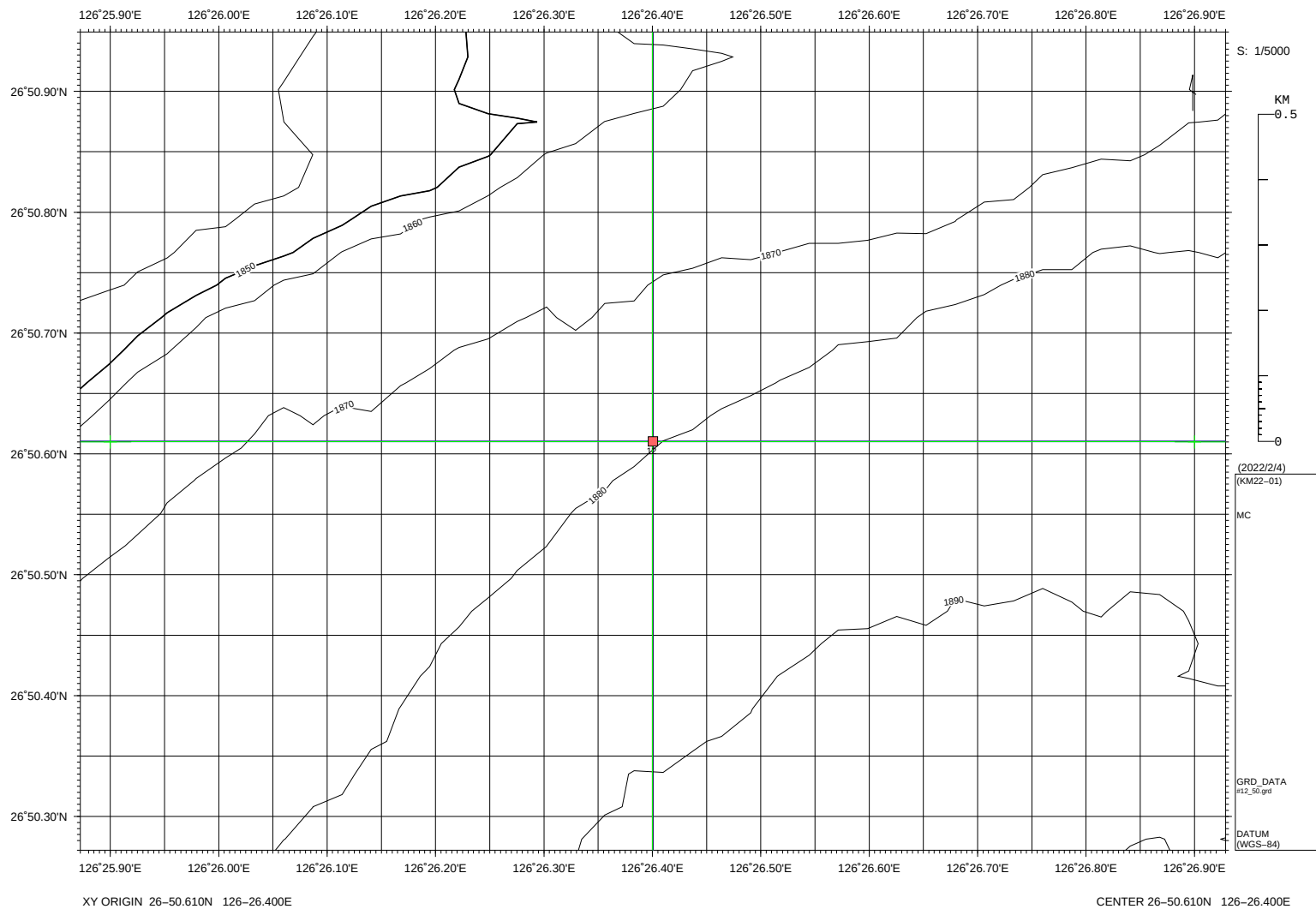
Site,日付	時刻 (JST)	母船緯度	母船経度	GYRO (°)	風向 (°)	風速 (m/s)	流向 (°)	流速 (kt)	水深 (m)	音響緯度	音響経度	深度 (m)	comment
2月3日													
#7_CTD													
吊上げ	4:09:39	26-08.0113N	129-56.9878E	165	172	10.6	320	0.5	5390	29.18.2115N	138.40.2790E	0	
着水	4:13:03	26-08.0116N	129-56.9873E	166	164	10.5	325	0.5	5386	29.18.2115N	138.40.2790E	0	
水切り	8:21:16	26-08.2316N	129-56.7990E	175	186	12.0	321	0.6	5376	30.33.3735N	130.43.2891E	54	
揚収	8:23:59	26-08.2447N	129-56.7841E	180	186	13.2	317	0.6	5376	30.33.3735N	130.43.2891E	54	
#7_MC													
吊上げ	9:21:23	26-07.9790N	129-57.0021E	179	185	11.3	336	0.7	5395	30.33.3735N	130.43.2891E	54	
本体着水	9:23:12	26-07.9789N	129-57.0017E	180	193	12.1	337	0.7	5391	30.33.3735N	130.43.2891E	54	
ウェイト着水	9:35:10	26-07.9795N	129-57.0016E	179	192	12.4	330	0.7	5265	30.33.3735N	130.43.2891E	54	
トラッキング開始(8秒)	9:36:42	26-07.9790N	129-57.0017E	180	189	13.9	331	0.7	5393	30.33.3735N	130.43.2891E	54	
トラボﾝ取付	9:46:02	26-07.9791N	129-57.0016E	186	194	13.0	332	0.7	5389	26.05.9687N	129.58.7591E	2118	
トラボﾝ着水	9:46:07	26-07.9790N	129-57.0016E	186	195	12.8	332	0.7	5389	26.05.9687N	129.58.7591E	2118	トラボﾝ応答回数
T-8S→10S	9:58:40	26-07.9797N	129-57.0022E	184	186	12.6	329	0.7	5394	26.08.0086N	129.57.0058E	613	95
ウィンチ停止, AHC停止	11:15:55	26-07.9816N	129-56.9909E	191	186	10.6	3	0.7	5388	26.07.9964N	129.56.9803E	5025	
着底	11:30:24	26-07.9817N	129-56.9901E	192	190	11.6	6	0.6	7193	26.08.0086N	129.56.9654E	5333	
離底	11:32:48	26-07.9815N	129-56.9905E	189	195	13.0	6	0.6	7291	26.07.9935N	129.56.9700E	5330	
ニューストネット1回目着水	11:35:28	26-07.9789N	129-56.9900E	191	182	12.1	5	0.6	5390	26.08.0109N	129.56.9725E	5199	
ニューストネット1回目揚収	11:57:52	26-07.8521N	129-56.9650E	191	191	11.6	20	0.6	5392	26.07.9769N	129.56.9725E	0	
ニューストネット2回目着水	12:02:30	26-07.8287N	129-56.9613E	189	192	12.9	21	0.6	5382	26.07.9605N	129.56.9708E	3710	
ニューストネット2回目揚収	12:24:19	26-07.7217N	129-56.9386E	191	193	12.9	10	0.5	5250	26.07.8592N	129.56.9566E	2520	
ニューストネット3回目着水	12:26:00												
ニューストネット3回目揚収	12:47:42	26-07.6034N	129-56.9159E	191	184	11.7	41	0.5	5153	26.07.6736N	129.56.9428E	0	
トラボﾝ水切り,トラッキング停止	13:11:13	26-07.6044N	129-56.9174E	194	196	12.3	0	0.0	5317	26.07.0483N	129.57.2348E	514	1155
ウェイト水切り	13:17:17	26-07.6030N	129-56.9154E	197	192	11.9	54	0.4	5312	26.07.0483N	129.57.2348E	514	
MC揚収完了	13:35:38	26-07.6029N	129-56.9170E	206	193	12.1	52	0.5	5296	26.07.0483N	129.57.2348E	514	
2月4日													
#15_CTD													
吊上げ	8:09:01	27-59.4173N	127-34.8201E	011	009	8.6	177	1.1	1337	26.07.0483N	129.57.2348E	514	
着水	8:10:58	27-59.3903N	127-34.8137E	009	010	11.5	176	1.1	1336	26.07.0483N	129.57.2348E	514	
水切り	10:05:38	27-59.3106N	127-34.6215E	005	358	9.5	181	0.8	1347	26.07.0483N	129.57.2348E	514	
揚収	10:08:14	27-59.2736N	127-34.6161E	003	006	11.0	176	0.8	1342	26.07.0483N	129.57.2348E	514	
#15_BT													
コッドエンド着水	10:47:11	27-57.7746N	127-34.5035E	010	015	12.3	161	1.2	1330	26.07.0483N	129.57.2348E	514	
ウェイト着水	11:26:02	27-58.1355N	127-34.6490E	360	358	8.4	154	1.3	1333	26.07.0483N	129.57.2348E	514	
トラッキング開始(8秒)	11:26:38	27-58.1419N	127-34.6516E	357	359	11.2	154	1.3	1335	26.07.0483N	129.57.2348E	514	
トラボﾝ取付	11:35:57	27-58.2405N	127-34.6951E	355	351	10.3	151	1.3	1339	27.58.9342N	127.33.8614E	0	
トラボﾝ着水	11:37:10	27-58.2529N	127-34.7043E	354	334	7.3	150	1.3	1336	27.58.6524N	127.34.1013E	0	866
着底,曳航開始	12:16:36	27-58.7499N	127-34.7285E	351	003	9.8	148	1.4	1347	27.59.0957N	127.34.3934E	324	
曳航終了,巻き上げ開始	12:43:50	27-59.0051N	127-34.7563E	350	009	9.8	147	1.5	1348	27.58.7871N	127.34.7480E	1082	
トラボﾝ水切り	13:32:39	27-59.3985N	127-34.7921E	354	002	12.1	146	1.4	1333	27.59.3623N	127.34.7977E	0	
トラボﾝ揚収	13:34:50	27-59.4013N	127-34.8073E	000	007	10.3	147	1.4	1333	27.59.3623N	127.34.7977E	0	
ウェイト揚収	13:47:41	27-59.4707N	127-34.9065E	001	006	9.5	144	1.3	1334	27.59.3623N	127.34.7977E	0	
コッドエンド揚収	14:38:12	27-59.6882N	127-35.2505E	000	356	7.2	148	1.3	1339	27.59.3623N	127.34.7977E	0	
2月5日													
#12_MC													
トラッキング開始(8秒)	8:22:44	26-49.1675N	126-25.8945E	002	015	10.9	23	1.1	1902	27.59.3623N	127.34.7977E	0	
吊上げ	8:33:30	26-49.1973N	126-25.8540E	009	019	10.0	18	1.0	1900	26.49.2129N	126.25.8587E	0	
ネット着水	8:35:11	26-49.2333N	126-25.8672E	011	019	9.9	20	1.0	1901	26.49.2469N	126.25.8716E	0	
ウェイト着水	8:47:09	26-49.5189N	126-25.9992E	010	023	11.5	30	1.1	1892	26.49.6785N	126.25.5319E	0	
トラボﾝ取付	8:56:55	26-49.7656N	126-26.0452E	011	359	9.9	29	1.0	1890	26.49.6757N	126.26.3763E	0	
トラボﾝ着水	8:56:55	26-49.7656N	126-26.0452E	011	359	9.9	29	1.0	1890	26.49.6757N	126.26.3763E	0	
ウィンチ停止	9:42:51	26-50.4144N	126-26.3038E	009	008	9.3	38	1.1	1880	26.50.3856N	126.26.2817E	0	
ウィンチ繰出し	9:45:00	26-50.4278N	126-26.3067E	012	005	6.4	35	1.1	1882	26.50.3907N	126.26.2841E	0	
着底	9:48:35	26-50.4438N	126-26.3223E	006	345	8.3	33	1.1	1880	26.50.4065N	126.26.2861E	0	
ウィンチ巻き取り	9:50:52	26-50.4438N	126-26.3134E	005	001	7.8	34	1.1	1880	26.50.4091N	126.26.2866E	0	
離底	9:53:32	26-50.4870N	126-26.3183E	006	009	7.5	34	1.0	1883	26.50.4056N	126.26.2884E	1801	
トラボﾝ水面,測位停止	10:29:28	26-50.9593N	126-26.4513E	009	021	10.5	28	0.8	1864	26.50.9383N	126.26.4478E	0	
ウェイト揚収	10:42:21	26-51.2377N	126-26.5602E	012	017	8.9	33	0.9	1862	26.50.9383N	126.26.4478E	0	694
MC揚収完了	10:49:22	26-51.3934N	126-26.6475E	010	346	8.5	36	1.0	1846	26.50.9383N	126.26.4478E	0	
#12_BT													
トラッキング開始(8秒)	11:32:04	26-48.4149N	126-25.0157E	002	003	6.6	24	1.0	1897	26.50.9383N	126.26.4478E	0	
ネット着水	11:39:34	26-48.6340N	126-25.0589E	006	348	8.3	28	0.9	1900	26.48.6262N	126.25.0266E	0	
ウェイト着水	12:08:27	26-49.4778N	126-25.2511E	005	010	8.7	29	1.0	1887	26.49.5379N	126.25.2793E	0	
トラボﾝ取付	12:16:49	26-49.6975N	126-25.2863E	006	006	8.1	30	0.8	1877	26.49.7001N	126.25.2863E	0	
トラボﾝ着水	12:16:57	26-49.7012N	126-25.2867E	006	350	9.1	31	0.9	1878	26.49.7001N	126.25.2863E	0	
ウィンチ巻き出し停止	12:48:51	26-50.3802N	126-25.6997E	006	020	10.0	18	0.7	1860	26.50.1228N	126.25.5064E	1285	
巻き出し再開	12:51:47	26-50.4159N	126-25.6960E	015	353	8.3	27	0.7	1861	26.50.1428N	126.25.5206E	0	
着底	13:00:52	26-50.4820N	126-25.7481E	011	353	7.3	17	0.8	1863	26.50.2026N	126.25.5506E	1711	
巻き上げ開始	13:31:16	26-50.7054N	126-25.8719E	005	358	7.2	10	0.8	1839	26.50.4215N	126.25.6878E	1722	
離底	13:45:33	26-50.8087N	126-25.8744E	001	349	7.6	7	0.7	1830	26.50.5624N	126.25.7872E	1396	
トラボﾝ水面,測位停止	14:10:00												
ウェイト揚収	14:22:13	26-51.3100N	126-25.8754E	009	350	8.9	349	0.6	1842	26.51.1289N	126.25.6789E	5366	847
コッドエンド揚収	14:55:33	26-52.1575N	126-25.9391E	004	009	7.2	345	0.8	1848	26.51.1289N	126.25.6789E	5366	
NN1回目着水	15:14:54	26-52.5145N	126-25.8916E	000	340	7.4	343	0.8	1841	26.51.1289N	126.25.6789E	5366	
NN1回目揚収	15:36:30	26-53.3776N	126-25.8051E	000	023	7.5	348	1.0	1829	26.51.1289N	126.25.6789E	5366	
NN2回目着水	15:39:16	26-53.4986N	126-25.7880E	000	358	8.7	344	1.0	1827	26.51.1289N	126.25.6789E	5366	
NN2回目揚収	16:01:32	26-54.5019N	126-25.7157E	000	355	7.8	351	1.1	1828	26.51.1289N	126.25.6789E	5366	
NN3回目着水	16:03:11	26-54.5295N	126-25.7149E	000	346	6.6	351	1.1	1824	26.51.1289N	126.25.6789E	5366	
NN3回目揚収	16:24:32	26-55.4548N	126-25.7027E	000	341	8.5	355	1.2	1800	26.51.1289N	126.25.6789E	5366	

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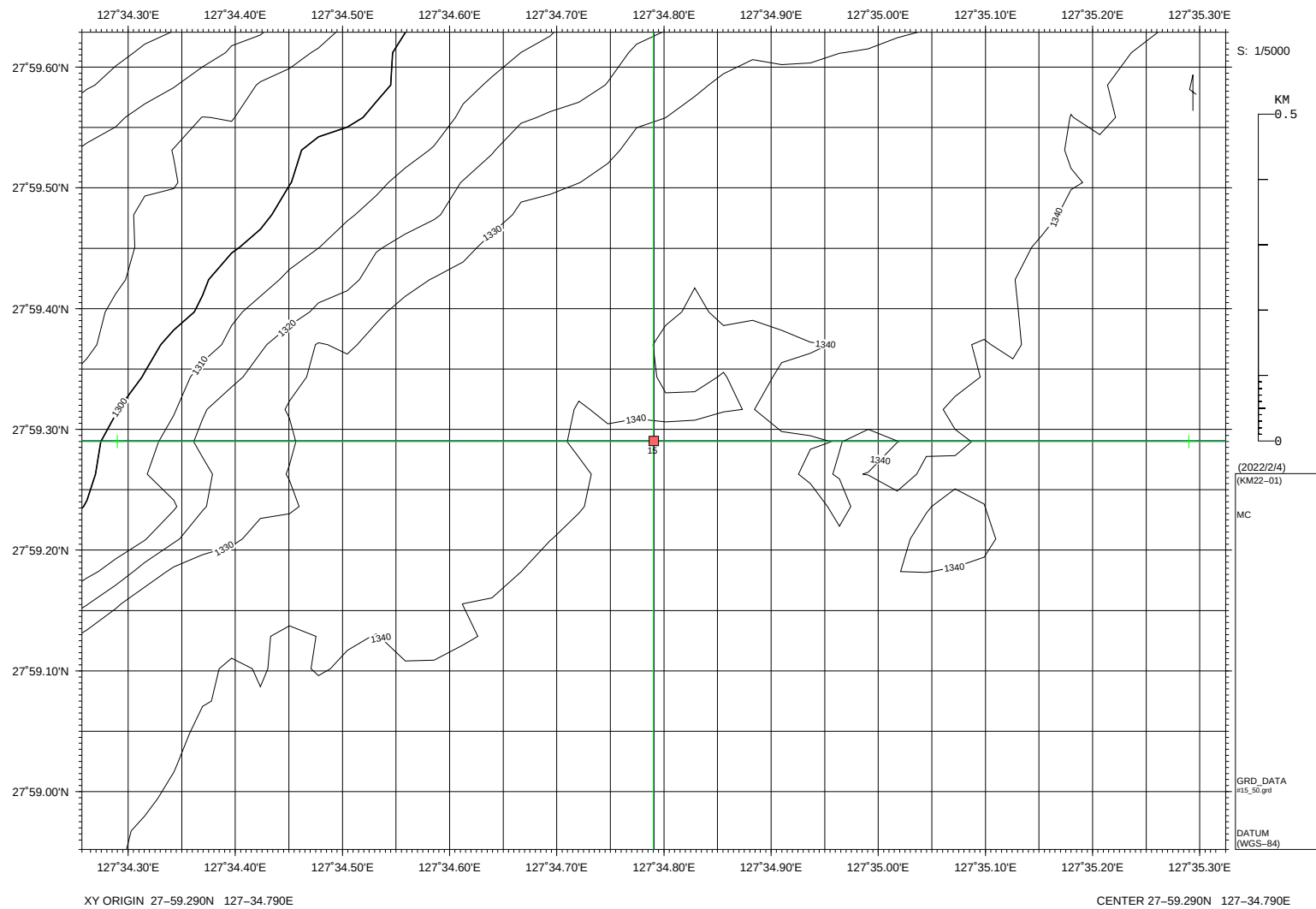
Appendix: Bathymetry at each sampling site

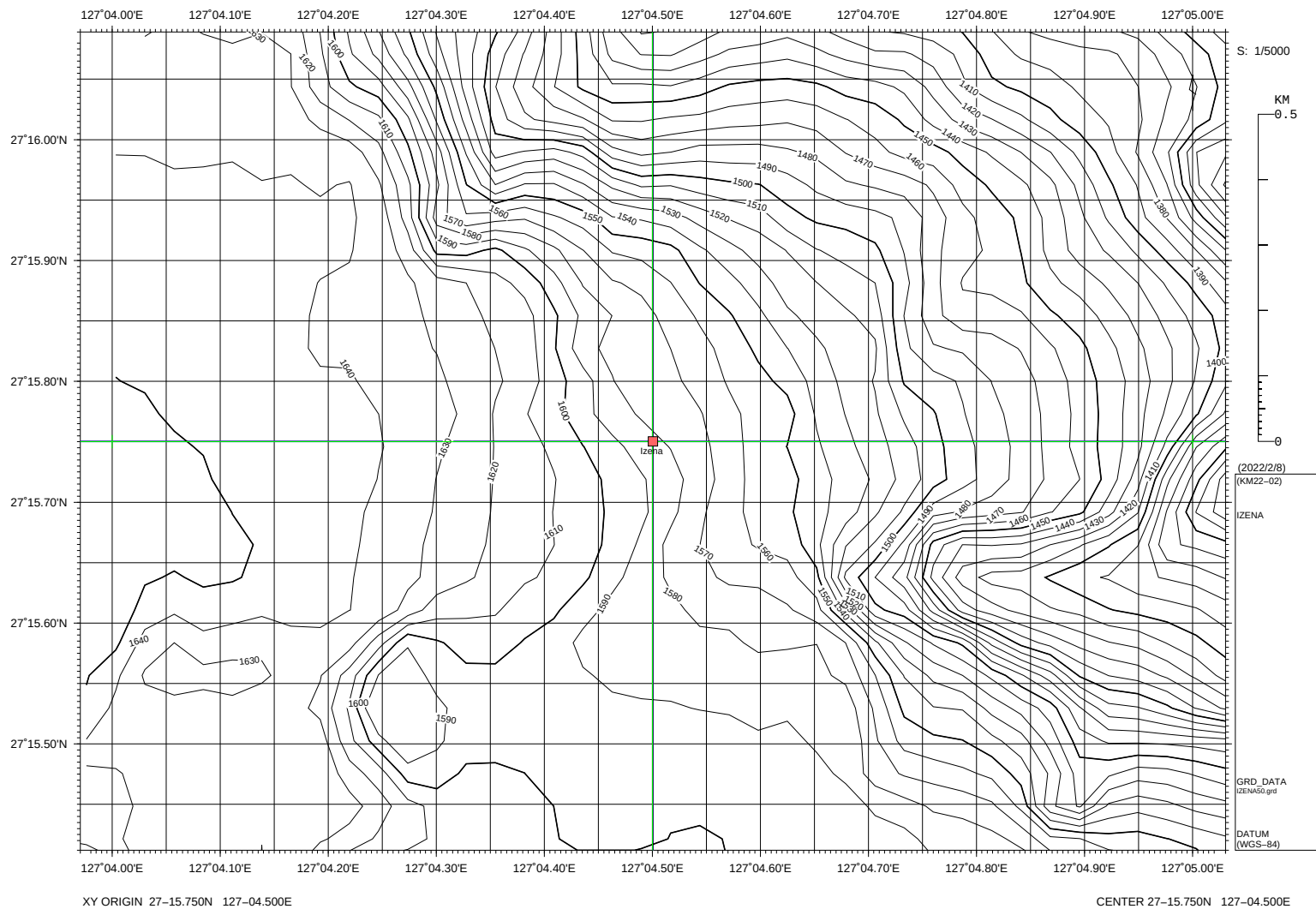
Station #09



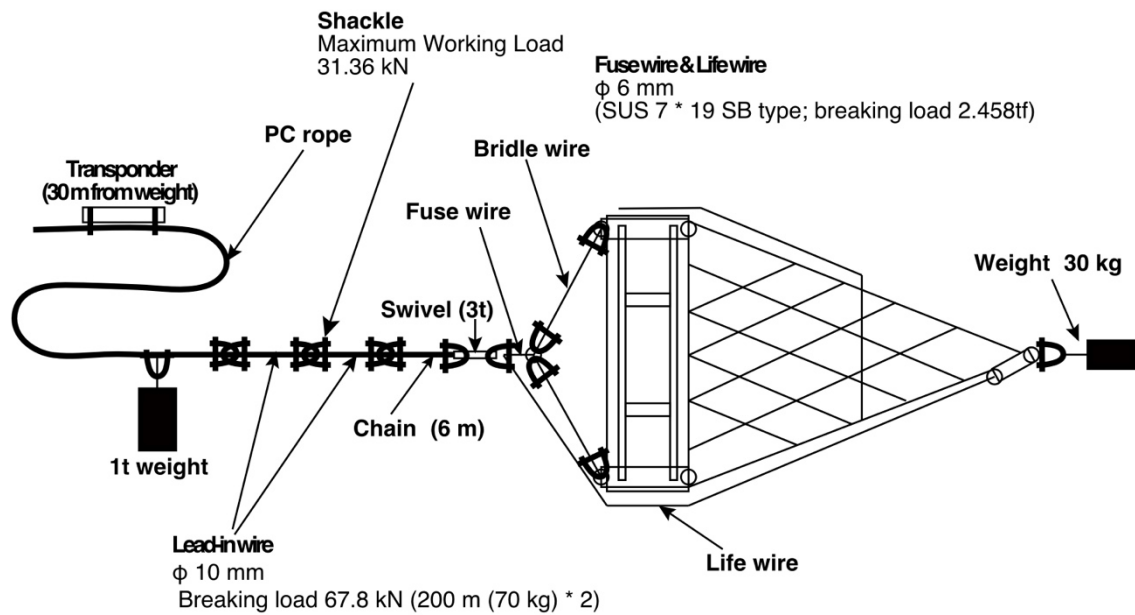


Station #12

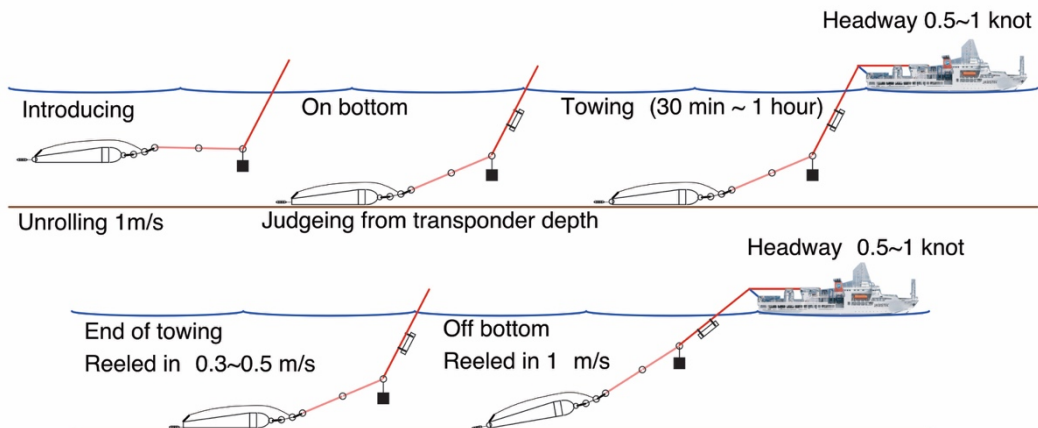




Appendix: Beam trawl_system configuration



General procedure of beam trawl operation



Appendix: Multiple core_system configuration

