



R/V Kaimei Cruise Report
KM20-08

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

Sagami Bay, off Boso Peninsula, east off Izu-Ogasawara
Islands

Oct. 25 – Nov. 11, 2020

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 Mitsuyasu Katagiri Nippon Marine Enterprises, Ltd., Rei Ito, Yuta Shinomiya, Yasuhiro Arie, Katsunori Sagishima, Tun Htet Aung, Taiki Kawada, Marine Works Japan Ltd. for their supports. We also thank all the JAMSTEC people who have supported us.

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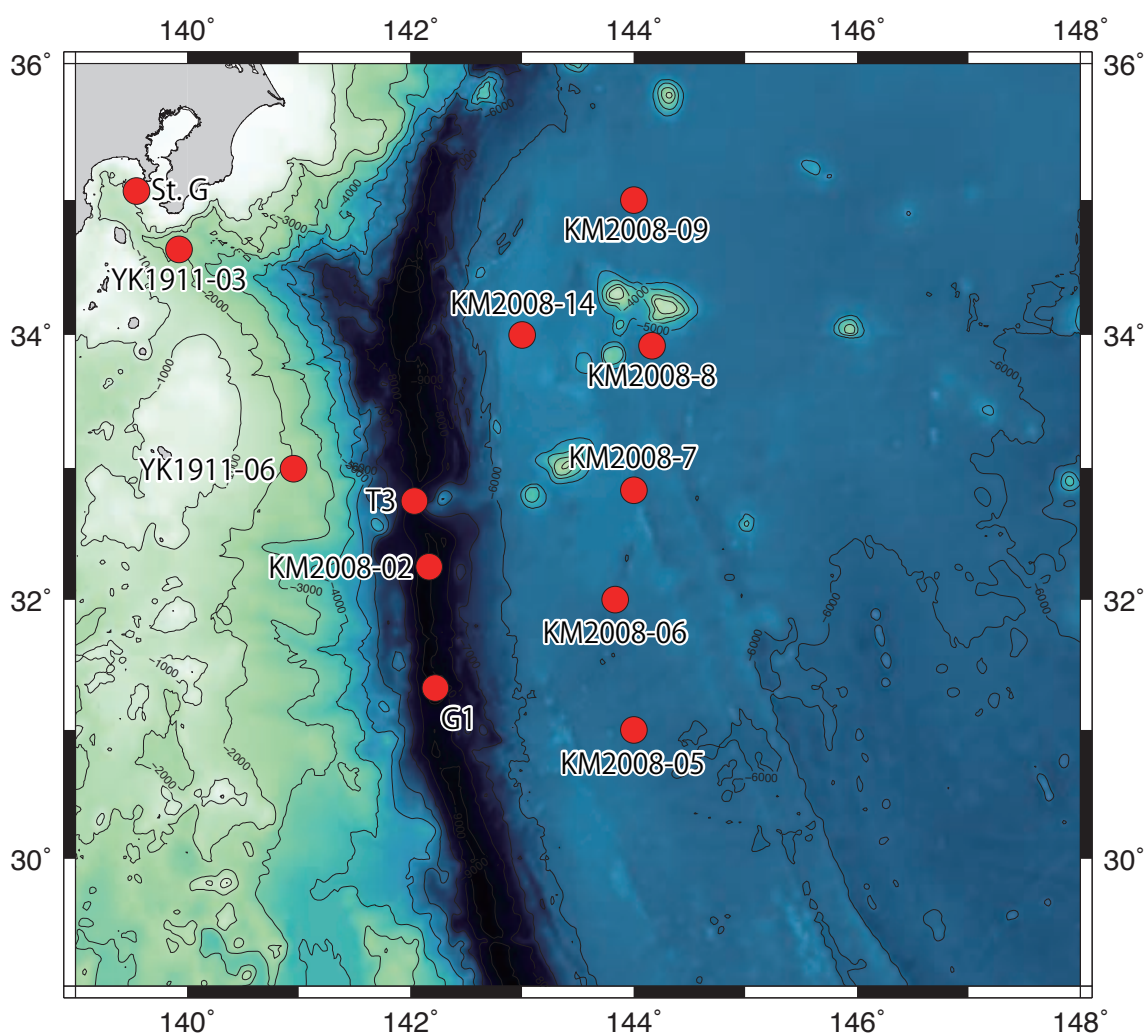
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Multiple core: system configuration

Cruise information

Cruise ID: KM20-08
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: Oct. 25–Nov. 11, 2020
Port of call: Yokosuka–Yokosuka (Yokosuka, Kanagawa, Japan)
Research Area: Sagami Bay, off Boso Peninsula, east off Izu-Ogasawara Islands
Research map:



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2nd Officer	Ryo Yamaguchi
3rd Officer	Asami Kikuchi
Chief Engineer	Kazuhiko Kaneda
1st Engineer	Wataru Kurose
Jr. 1st Engineer	Shuichi Hashide
2nd Engineer	Yoichi Yasue
3rd Engineer	Naoyuki Hamakawa
Chief Electronic Operator	Tokinori Nasu
2nd Electronic Operator	Misato Taki
3rd Electronic Operator	Kohei Maeda
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Able Seaman	Masashige Okada
Able Seaman	Yukito Ishii
Able Seaman	Yuta Ohjiri
Able Seaman	Sho Suzuki
Able Seaman	Hideaki Tamotsu
Sailor	Yuki Sato
No.1 Oiler	Masanori Ueda
Oiler	Ryota Suzuki
Oiler	Makoto Kozaki
Assistant Oiler	Masakazu Ishida
Assistant Oiler	Ruki Saito
Chief Steward	Yukihide Chikuba
Steward	Yoshitaka Yamamoto
Steward	Shinobu Ohyu
Steward	Koichiro Kashiwagi
Steward	Koki Yamamoto

1. Introduction and objectives of the cruise

It is an urgent global issue to understand the changes in marine biodiversity, which is an important indicator of global environmental change, and to gain knowledge that can contribute to the assessment of the impact of human activities on ecosystems. In particular, deep-sea ecosystems, for which little information is available on the effects of changes in the marine environment, must be comprehensively surveyed using a multidisciplinary approach and analyzed in conjunction with environmental data. For this reason, we have to obtain knowledge that will contribute to the understanding and assessment of the impact of human activities on deep-sea ecosystems and diversity, as well as knowledge that will contribute to their conservation. This requires the development of new measurement techniques including marine plastic debris and environmental assessment. There is also a need to upgrade and optimize impact assessment methods in the marine environment.

In order to achieve these goals, we will conduct a research cruise in the waters around Japan, especially in the Kuroshio Extension Recirculation zone (KER), to investigate the actual condition of marine plastic debris (both micro and macroplastics) on the sea surface and on the seafloor. This cruise was interpolating the assumed area of the western Pacific Garbage Patch (WPGP), which was conducted in the YK19-11 cruise, in order to clarify the extent of debris accumulation in the WPGP in the KER. We also conducted to improve and optimize impact assessment methods in the marine environment. The baseline survey, which uses microbial ecosystems as an indicator, expands the baseline data on microbial ecosystems by targeting microbial communities that are characteristic of each water mass. We presented the usefulness of environmental impact assessment indicators is demonstrated by using metabolic activity and changes in community composition that respond rapidly to environmental changes.

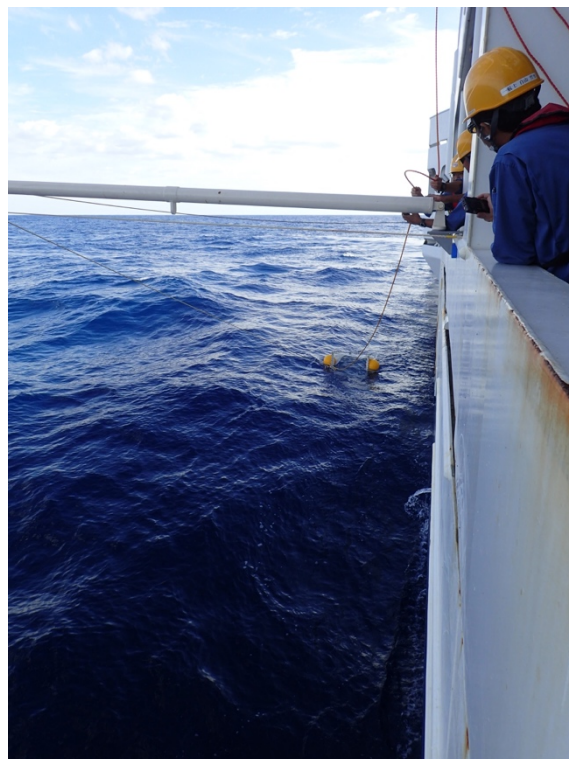
In this study, we conducted to clarify the distribution and behavior of plastic debris including microplastics (MP) in deep-seas, the impact of MPs on deep-sea organisms and ecosystems, the actual condition of plastic pollution in the open ocean, and the optimization of the environmental impact assessment method and development of tools. We conducted the Kaimei cruise to collect microplastics from the ocean surface using Neuston net, the use of multiple corers to collect samples of seafloor sediments, and visual observation and imaging of meso to macro plastic debris floating on the surface from the ship. We also conducted CTD/Niskin sampling to investigate the vertical structure of the ocean and microbial communities, and chemical properties. To carry out above purpose, we conducted following subjects: 1) identification of WPGP and understanding of its actual condition of marine pollutants (e.g., mass and variety of plastic debris there) at surface, 2) understanding of the distribution of sunken microplastics at the abyssal to bathyal zone, 3) acquiring basic biological (e.g., protist and meiobenthos), physical and chemical data development of environmental assessment methods. The cruise started from 25th October at JAMSTEC, Yokosuka and ended on 11th November 2020 at JAMSTEC, Yokosuka (18 days in total) and the scientific party was composed of 29 persons.

2. Survey items

Multiple corer



Neuston net



Floating macro-debris observation



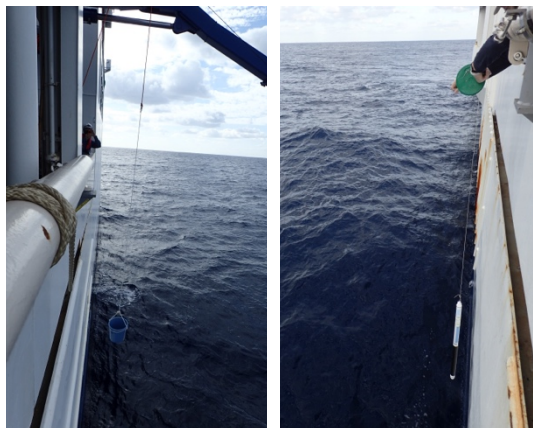
Subsurface microplastic sampling



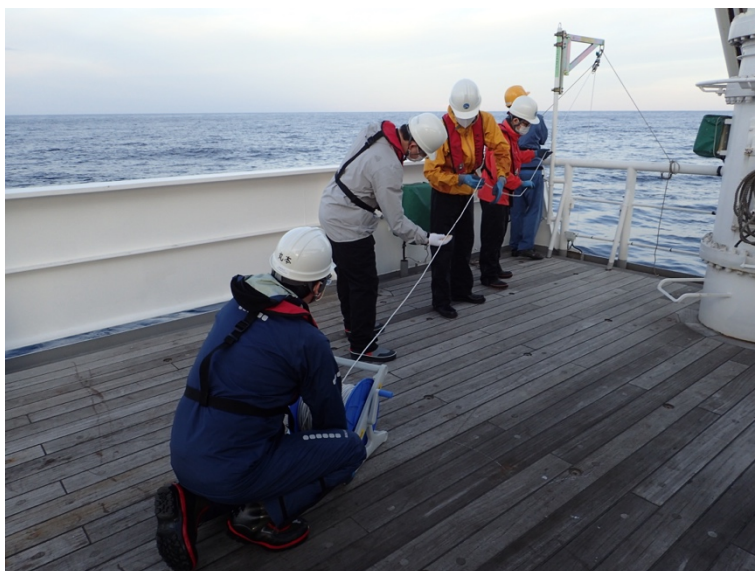
CTD/Niskin



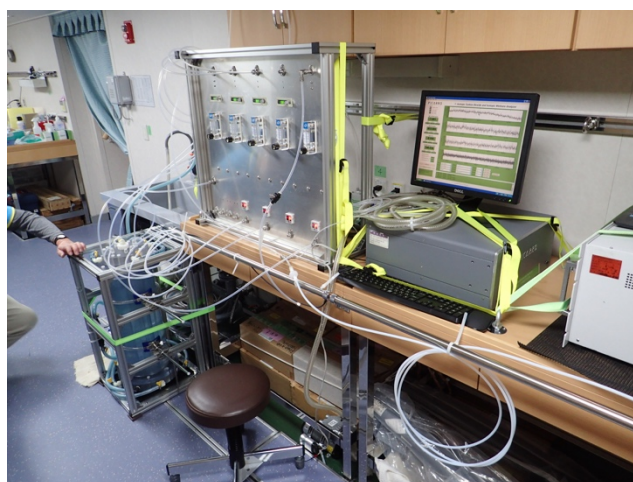
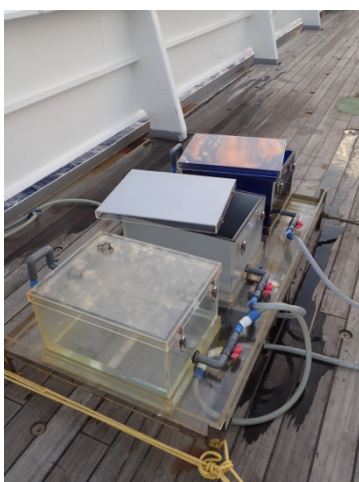
Water sampling with a bucket & sampler



RINKO profiler deployment



RINKO profiler



3. Summary and the cruise results

3.1. Understanding of the actual condition of microplastics and its impact on marine ecosystems

3.1.1. *Microplastics in the Kuroshio Extension and its recirculation gyre*

Ryota Nakajima, Tomo Kitahashi, Akinori Yabuki, Masashi Tsuchiya (JAMSTEC), Kengo Nakano, Kiichiro Kawamura (Yamaguchi University)

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 very few data are available in the Western Pacific. In the present study, we conducted microplastic surveys on the Kuroshio Extension and its recirculation gyre off the coast of Boso Peninsula to fill gaps in the Western Pacific.

Neuston net sampling for surface microplastic analysis

From October 25th to November 11th 2020, a total of 36 surface net tows were carried out between 30°N-35°N and 139°W- 146°W by R/V Kaimei. Floating microplastic samples were collected using a neuston net with a rectangular mouth opening of 75 cm height and 100 cm width, equipped a 333 µm mesh opening net with a collecting bottle 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 three times at ca. 1 knot for 20 min from the starboard side. A flow meter 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.

Seawater sampling for subsurface microplastic analysis

Subsurface microplastic samples were collected at ca. 5 m depth below the sea surface using a pumped seawater system of the vessel throughout the cruise. The pumped unfiltered seawater was continuously filtered through a 333 µm mesh screen and then 100 µm mesh screen at flow rates of 7 -12 L/min. These meshes were placed in the system upon arrival to a sampling site and then collected when the vessel leaves the site. The collected meshes were wrapped with an aluminum foil and stored at 4°C until analysis.

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 handrail of the ship at 7.7 m height from the sea surface with a camera angle of ca. 60 degree. The GoPro was connected to the electricity of the ship allowing a continuous recording. The video was taken during daytime for 7-9 hours at each site.

Multiple core sampling

Sedimentology

We collected multiple core samples for sedimentological study at Stations G, T3, KM2008-02, G1, KM2008-07, KM2008-08, KM2008-06, YK1911-03, and YK1911-14.

We collected core samples in surface sediment layers from the north-west Pacific regions related to microplastic pollution. We collected totally 10 multiple core samples for sedimentological study. We observed all the core samples briefly by naked eyes from the outside and described those sediment core facies of the core barrel and smear slides by a polarized microscope as below.

We will conduct smear slide descriptions, X-CT analysis, grain size measurements to decipher the sediment source and transport processes using these data.

Microplastic and meiofaunal analysis (Kitahashi, T., Tsuchiya, M.)

Sediment samples were collected, by the multiple corer (covering 43.0 cm² seafloor surface area) at Stations G, T3, KM2008-02, G1, KM2008-07, KM2008-08, KM2008-06, YK1911-03, and

YK1911-14. 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 acrylic made core tubes were used for meiofaunal analysis at each station. On board, sediment samples were sliced every 1 cm down to 10 cm or 20 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.

3.1.2. Acquiring reference data for eDNA analysis targeting marine protists

Akinori Yabuki (JAMSTEC)

Surface sea water that was regularly pumped up in the TSG room was filtered through a filter of 75 µm mesh and then the dense plankton samples were subsequently substituted in a RNA-later solution. The filtration was basically conducted at the following stations: KM2008-02, 06, 07, 09, YK1911-03 and G1, and then stored in the cold room (ca. 4 to 5°C)

The collected samples are subjected to the following analyses: the members Diplonemea and Alveolate that are focused as the possible biological indicator on the environmental changes are searched for in the samples and the detected ones are isolated for more careful observation and sequencing analysis. The acquired data will be utilized as the reference data for the eDNA analysis that has been conducted in monitoring the marine ecosystems.

3.1.3. Pore-water chemistry

Hidetaka Nomaki, Masahito Shigemitsu, Tomo Kitahashi, Masashi Tsuchiya (JAMSTEC)

Sediment sample was collected at St. KM2008-06 using Multiple corer. Each sediment layers were sliced into 1 cm ~ 5 cm and were transferred to each 50 ml falcon tube. Approximately 50 ml per 1cm depth of sediment samples were put into 50 ml falcon tube and were centrifuged at 2600 × g for 5 min. Supernatant were gently decanted with a 20 ml plastic syringe (pre-washed with Milli Q water) and filtered through a 0.45-µm membrane filter. The porewater nutrient samples were kept frozen prior to nutrient analyses on land, DOC samples were immediately analyzed onboard.

Nutrients: same methods as water chemistry described in section 3.2.2.4.

DOC/CDOM/FDOM: Samples with high levels of CDOM/FDOM will be individually diluted using ultrapure water and measured with the same methods as described in section 3.2.2.6. The levels measured will finally be corrected with each dilution factor. DOC and acetate concentrations will also be measured at Kochi Institute for Core Sample Research of JAMSTEC.

Dissolved oxygen

Dissolved oxygen (DO) concentrations at the sediment surface and within the sediment profile were measured using a microelectrode (Unisense A/S, Aarhus, Denmark). Measurement of DO began immediately after the recovery of sediment cores on deck. The first measurements were from 5 mm above the sediment surface and were collected at 500-mm intervals over 4 cm (Fig. 3.1.3).

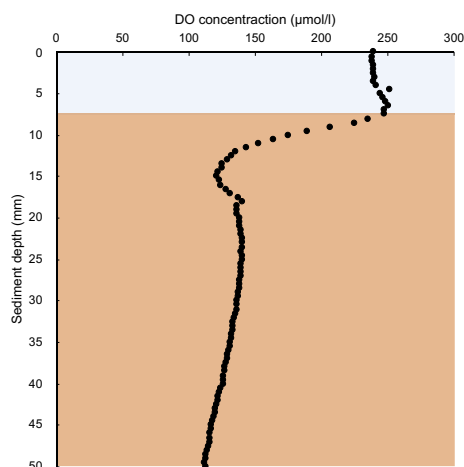


Fig. 3.1.3. Vertical profile of dissolved oxygen (DO) concentration at KM2008-06 site.

3.2. CTD

3.2.1 CTDO₂

3 December 2020

Hiroshi Uchida (JAMSTEC) (Principal Investigator), Taichi Yokokawa (JAMSTEC), Rei Ito (MWJ) (Operation Leader), Tun Htet Aung (MWJ)

Instruments

Instruments used in this cruise are as follows:

Winch and cable system

14.7 kN traction with AHC (Active Heave Compensation) winch system (MacArtney A/S, Esbjerg, Denmark) for 11,000 m synthetic fiber cable (OCC Co., Yokohama, Japan) [at CTD stations 001, 002, 003, 004, 007, 011, 012]

39.2 kN traction with AHC winch system (MacArtney A/S) for 8,000 m steel armored cable (Rochester Wire & Cable, LLC, Virginia, USA) [at CTD stations 006, 005, 016, 013]

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) [the fin was used at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]

Compact underwater slip ring swivel (Hanayuu Co., Ltd., Shizuoka, Japan) (Uchida et al., 2018) [at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]

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

Serial no. 324510-1085 [at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]

Serial no. 324510-1086 [at CTD stations 016, 013]

12-L Niskin-X water sample bottle, model 1010X (General Oceanic, Inc., Florida, USA)

(TEFLON coating) [for #27-36 at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]

12-L sample bottle, model OTE 110 (OceanTest Equipment, Inc., Florida, USA)
(No TEFLON coating)

Deck unit

SBE 11plus (Sea-Bird Scientific)
Serial no. 90876-1033

Underwater unit

Pressure sensor, SBE 9plus (Sea-Bird Scientific)
Serial no. 09P84583-1236 (134404) (calibration date: March 5, 2020)
[at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Serial no. 09P84583-1235 (134402) (calibration date: March 4, 2020)
[at CTD stations 016, 013]

Deep standard reference thermometer, SBE 35 (Sea-Bird Scientific)
Serial no. 0022 (calibration date: July 21, 2020)

Temperature sensor, SBE 3 (Sea-Bird Scientific)
Primary serial no. 03P5982 (calibration date: January 22, 2020)
Secondary serial no. 03P4815 (calibration date: April 25, 2019)
[at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Primary serial no. 03P2730 (calibration date: December 28, 2019)
Secondary serial no. 03P4811 (calibration date: April 25, 2019)
[at CTD stations 016, 013]

Conductivity sensor, SBE 4C (Sea-Bird Scientific)
Primary serial no. 044254 (calibration date: January 22, 2020)
Secondary serial no. 043264 (calibration date: March 1, 2012)
[at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Primary serial no. 044450 (calibration date: January 10, 2020)
Secondary serial no. 043261 (calibration date: March 1, 2012)
[at CTD stations 016, 013]

Dissolved oxygen sensor
Primary, RINKO III (JFE Advantech Co., Ltd., Osaka, Japan)
Serial no. 037, Sensing foil no. 182822
Secondary, RINKO III (JFE Advantech Co., Ltd.)
Serial no. 024, Sensing foil no. 182822
Serial no. 079, Sensing foil no. 182822
[at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Primary, RINKO III (JFE Advantech Co., Ltd.)
Serial no. 221

Secondary, SBE 43 (Sea-Bird Scientific)
Serial no. 430205 (calibration date: December 13, 2019)
[at CTD stations 016, 013]

Chlorophyll fluorometer (Seapoint Sensors Inc., New Hampshire, USA)
Serial no. 3701, Gain: 30X (0-5 ug/L)
[at CTD stations 007, 011, 012, 006, 005, 016, 013]
Ultraviolet fluorometer (Seapoint Sensors Inc.)
Serial no. 6223, Gain 30X (0-50 QSU)
[at CTD stations 001, 007, 011, 012, 006, 005, 016, 013]
Serial no. 6238, Gain 30X (0-50 QSU)
[at CTD stations 007, 011, 012, 006, 005, 016, 013]

Ultraviolet fluorometer, ECO Fluorometer (WET Labs, Inc., Oregon, USA)
Serial no. 2014 (calibration date: January 9, 2015)
[at CTD stations 007, 011, 012, 006, 005, 016, 013]

PAR sensor, PAR-Log ICSW (Satlantic, LP, Nova Scotia, Canada)
Serial no. 1025 (calibration date: July 6, 2015)
[at CTD stations 001, 007, 011, 012, 006, 005, 016, 013]
Serial no. 1026 (calibration date: July 6, 2015)
[at CTD stations 016, 013]

Altimeter, PSA-916T (Teledyne Benthos, Inc.)

Serial no. 66557 [at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Serial no. 66558 [at CTD stations 016, 013]
Pump, SBE 5T (Sea-Bird Scientific)
Primary serial no. 054032
Secondary serial no. 052627
[at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]
Primary serial no. 058145
Secondary serial no. 058088
[at CTD stations 016, 013]
Bottom contact switch (Sea-Bird Scientific)
[at all CTD stations]

Additional stand-alone underwater unit

Turbidity recorder, ATU6-CMP (JFE Advantech, Co.)
Serial no. 9 [at CTD stations 002, 003, 004, 012, 011, 006]
Refractive index density sensor (Uchida et al., 2019)
Prototype 1 [at CTD stations 001, 002, 003, 007, 012, 011, 006, 005]
Pressure/temperature recorder, RBRduet3 T.D.deep (RBR Ltd., Ottawa, Canada)
Serial no. 095974 [at CTD stations 001, 002, 003, 004, 007, 011, 012, 006, 005]

Software

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

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 many Modulo Error Count when using the synthetic fiber cable (at depths between 400 and 900 m) and the steel armored cable (during the up-cast). Data missed at occurrence of the errors were estimated from the pressure change and linearly interpolated for the raw (24 Hz) data. At the CTD stations 016 and 013, there was no Modulo Error Count by replacing the slip ring of the winch.

The synthetic fiber cable was broken due to dropping out of the cable from the sheave of the traction winch and the CTD package was lost at the beginning of the CTD cast 014 (between the CTD station 005 and 016). The synthetic fiber cable was cut about 1,000 m from the tip after the cruise as measures for the error.

At the CTD station 002, the bottle #3 did not close correctly, and therefore, the latch assembly of #3 was replaced after the cast.

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 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 with a 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 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, SBE 43, 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.

Post-cruise Calibration

The CTD data will be calibrated after the cruise using in situ reference data (atmospheric pressure for pressure, the SBE 35 data for temperature, bottle sampled salinity, dissolved oxygen and FDOM data for conductivity sensors, dissolved oxygen sensors and ultraviolet fluorometer, respectively) (e.g. Uchida et al. 2010; Uchida et al. 2015).

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.

Stnno	Castno	Date(UTC)	Time(UTC)		BottomPosition		Depth (m)	Wire Out (m)	HT Above Bottom (m)	Max Depth	Max Pressure	CTD Filename	Remark
		(mmddyy)	Start	End	Latitude	Longitude							
001	2	102620	04:58	07:37	32-59.19N	140-57.10E	2266.3	2308.1	12.4	2255.6	2284.0	001M002	YK1911-06_2nd
002	1	102620	19:10	01:44	32-53.16N	141-59.48E	8374.7	8571.0	9.7	8423.0	8650.0	002M001	T3
003	1	102720	19:09	01:45	32-14.65N	142-09.95E	9421.9	9528.0	10.4	9388.2	9661.0	003M001	KM2008-02
004	1	102820	19:11	01:46	31-19.34N	142-13.30E	9630.5	9761.4	11.6	9592.7	9875.0	004M001	G1
007	1	102920	22:03	02:45	32-49.61N	144-00.06E	5886.6	5973.2	11.7	5853.7	5977.0	007M001	KM2008-07
012	1	103120	03:00	07:35	34-51.60N	144-03.74E	5831.3	5961.5	11.7	5812.1	5935.0	012M001	KM2008-09
011	1	103120	22:04	02:32	33-54.77N	144-09.81E	5488.7	5576.0	12.2	5455.6	5566.0	011M001	KM2008-08
006	1	110120	22:04	03:08	32-00.01N	143-49.99E	5825.7	5813.5	13.3	5801.9	5923.0	006M001	KM2008-06
005	1	110220	19:06	23:42	31-00.01N	144-00.00E	5836.0	5825.4	13.3	5813.0	5934.0	005M001	KM2008-05
016	1	110620	22:09	00:43	34-37.99N	139-55.62E	2215.0	2203.0	11.3	2183.6	2211.0	016M001	YK1911-03
013	1	110720	21:11	01:10	34-00.49N	142-59.98E	5174.6	5173.6	11.7	5148.4	5249.0	013M001	KM2008-14

References

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- 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.
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3.2.2. Water sampling and analysis Chemical parameter

3.2.2.1. Salinity

10 November 2020

Hiroshi Uchida (JAMSTEC) (Principal Investigator)

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. Each bottle was rinsed three times with sample water and was filled to the shoulder of the bottle. The samples were stored more than 24 hours in the air-conditioned (~23 °C) salinity-measurement room on the ship. 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 P162, $S_p = 34.9933$) (Ocean Scientific International Ltd., Havant, UK) was measured to correct the salinometer measurements at the beginning 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. The Multiparametric Standard Seawater (MSSW) (batch Pre19) (Kanso Technos Co., Ltd., Osaka, Japan), currently under development, was also measured to monitor and correct the batch-to-batch offset of the IAPSO Standard Seawater (Uchida et al. 2020).

Time drift of the slope of the salinometer was estimated and corrected from the IAPSO Standard Seawater (SSW) measurement (Fig. 3.2.2.1). Average (standard deviation) of the standards

after time drift correction was 34.9933 (0.0003), 34.2746 (0.0002), and 0.0006 (0.0002) for IAPSO SSW, Pre19, and ultra-pure water, respectively.

A total of 22 pairs of replicate samples was collected from the same Niskin bottle, and the root-mean-square for the replicate samples was 0.0002.

References

- Kawano, T. (2010): Salinity. The GO-SHIP Repeat Hydrography Manual: A collection of Expert Reports and Guidelines, IOCCP Report No. 14, ICPO Publication Series No. 134, Version 1.
- Uchida, H., T. Kawano, T. Nakano, M. Wakita, T. Tanaka, and S. Tanihara (2020): An expanded batch-to-batch correction for IAPSO standard seawater. *J. Atmos. Oceanic Technol.*, 37, 1507-1520, doi:10.1175/JTECH-D-19-0184.1.

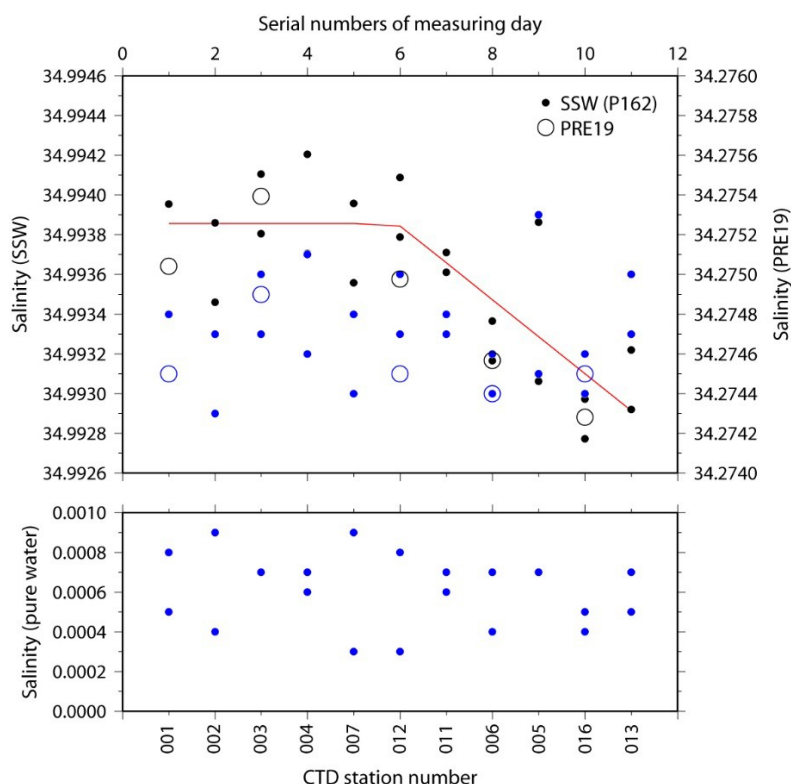


Fig. 3.2.2.1. Result of standard seawaters (IAPSO Standard Seawater [SSW] and Pre19) measurements before (black) and after (blue) time drift correction (red lines).

3.2.2.2 Density

29 October 2021

Hiroshi Uchida (JAMSTEC) (Principal Investigator), Yohei Kayukawa (NMIJ, AIST) (not onboard), Tomoya Muramoto (NMIJ, AIST) (not onboard)

Density of seawater samples was measured in the cruise with an oscillation-type density meter (DMA 5000M, serial no. 80570578, Anton-Paar GmbH, Graz, Austria) with a sample change (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 reevaluated for the serial no. 80570578 in August 2021 as follows:

$$\rho_{\text{offset}} = (\rho_{\text{Milli-Q}} - 998.2041) - (\rho - 998.2041) \times 0.000341 \text{ [kg m}^{-3}\text{]}.$$

The offset correction was verified by measuring Multiparametric Standard Seawater (MSSW) (batch Pre19) (Uchida et al. 2020) along with the IAPSO Standard Seawater (batch P162). The IAPSO Standard Seawater was measured at the beginning and the end of each day of measurement. The MSSW was also measured at each day of measurement. The measured densities are listed in Table 3.2.2.1.

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 3.2.2.1. Measured density at 20 °C of the IAPSO Standard Seawater and the Multi-parametric Standard Seawater (MSSW). True densities estimated from practical salinity and composition of seawater using TEOS-10 are also shown.

Standard Seawater	True density (kg/m ³)	Measured density (kg/m ³)	Number of measurements
P162	1024.7622	1024.7618±0.0016	22
PRE19	1024.2171	1024.2183±0.0011	61

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 22 pairs of replicate samples were measured. The root-mean square of the absolute difference of replicate samples was 0.0011 g/kg and 0.04 nD for density and refractive index, respectively.

Density of seawater was also measured in situ by a seawater density sensor (Uchida et al. 2019) attached to the CTD/water sampling frame, except for the CTD stations 004, 006 and 005. Density data measured for the seawater samples will be used for an in situ calibration of the density sensor.

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.
- 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.
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3.2.2.3 Dissolved Oxygen

1 December 2020

Hiroshi Uchida (JAMSTEC) (Principal Investigator), Katsunori Sagishima (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).

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. 1.2.0

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)	
2020/10/26	K20I01	T19L	3.854	0.001	001, 002, 003, 004, 007
2020/10/31	K20I02	T19L	3.871	0.001	012, 011, 006, 005
2020/11/04	K20I03	T19L	3.869	0.001	016, 013
2020/11/09	K20I04	T19L	3.868	0.000	

Repeatability of sample measurement

Replicate samples were taken at every CTD casts. The standard deviation of the replicate measurement (Dickson et al. 2007) was $0.17 \mu\text{mol kg}^{-1}$ ($n=22$).

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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- Dickson, A. G., Sabine, C. L., & Christian, J. R. (Eds.) (2007): *Guide to best practices for ocean CO₂ measurements*, *PICES Special Publication 3*: North Pacific Marine Science Organization.
- Murray, C. N., Riley, J. P., & Wilson, T. R. S. (1968): The solubility of oxygen in Winkler reagents used for the determination of dissolved oxygen. *Deep Sea Res.*, 15, 237-238.

3.2.2.4. Nutrients

2 December 2020

Hiroshi UCHIDA (JAMSTEC): Principal Investigator, Yasuhiro ARII (MWJ): Operation Leader

Parameters

The determinants are nitrate, nitrite, silicate, phosphate, and ammonia in the Pacific Ocean.

Instruments and methods

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 $0.5 \mu\text{m}$ 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.

Nitrate + 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 %).

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.

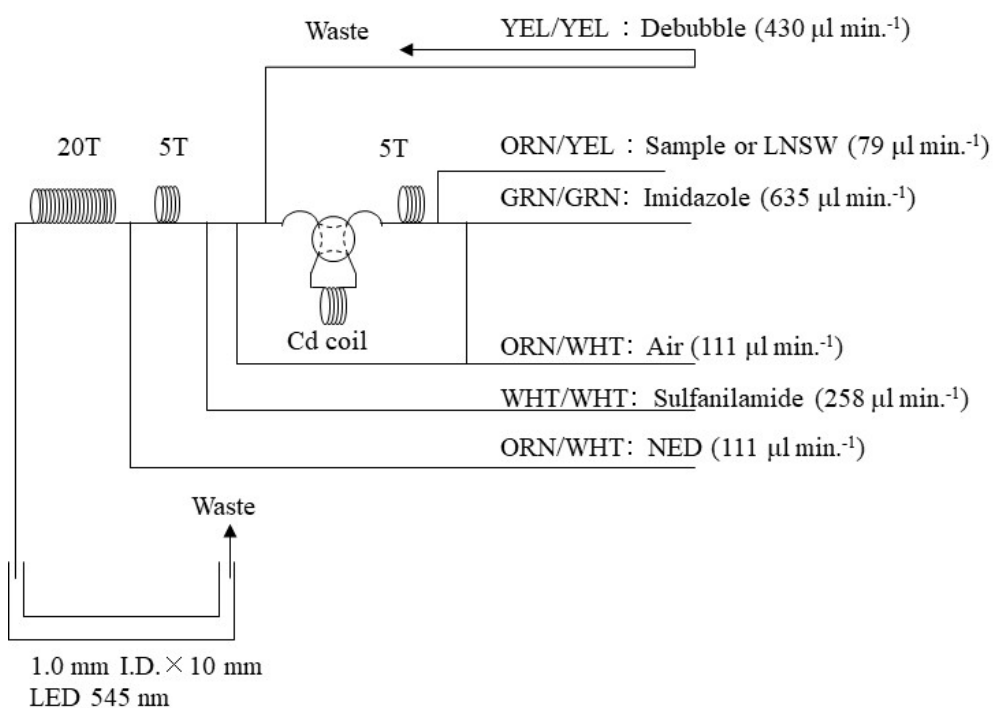


Figure 3.2.2.4-1 NO₃+NO₂ (1ch.) flow diagram.

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.

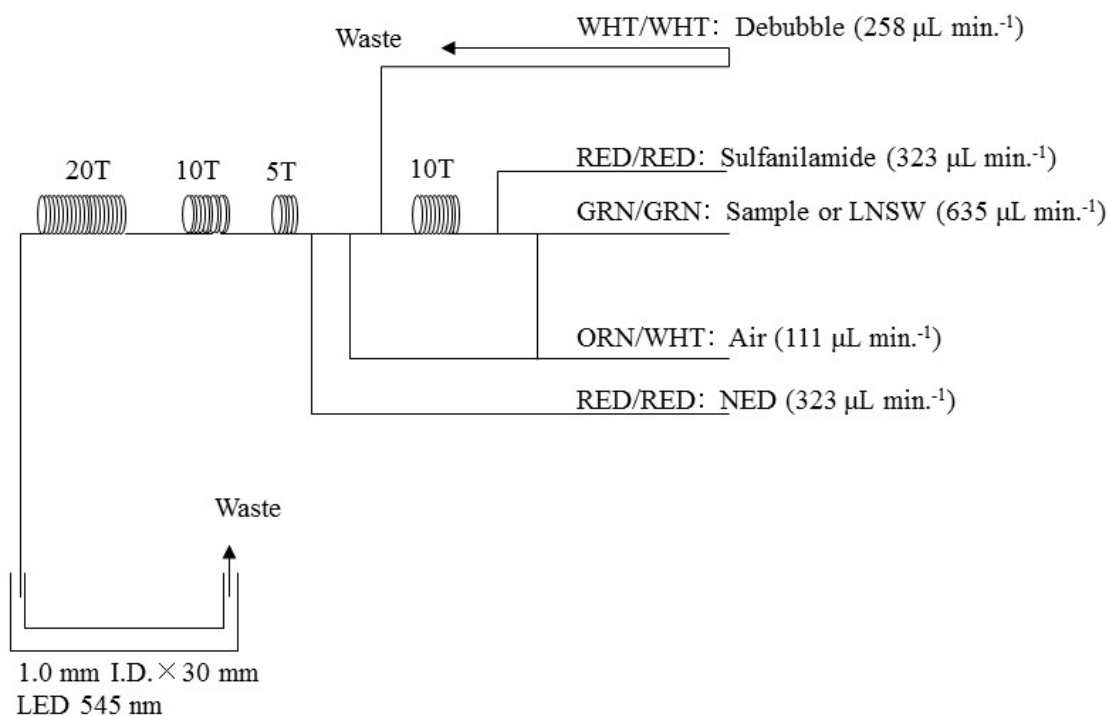


Figure 3.2.2.4-2 NO₂ (2ch.) flow diagram.

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.

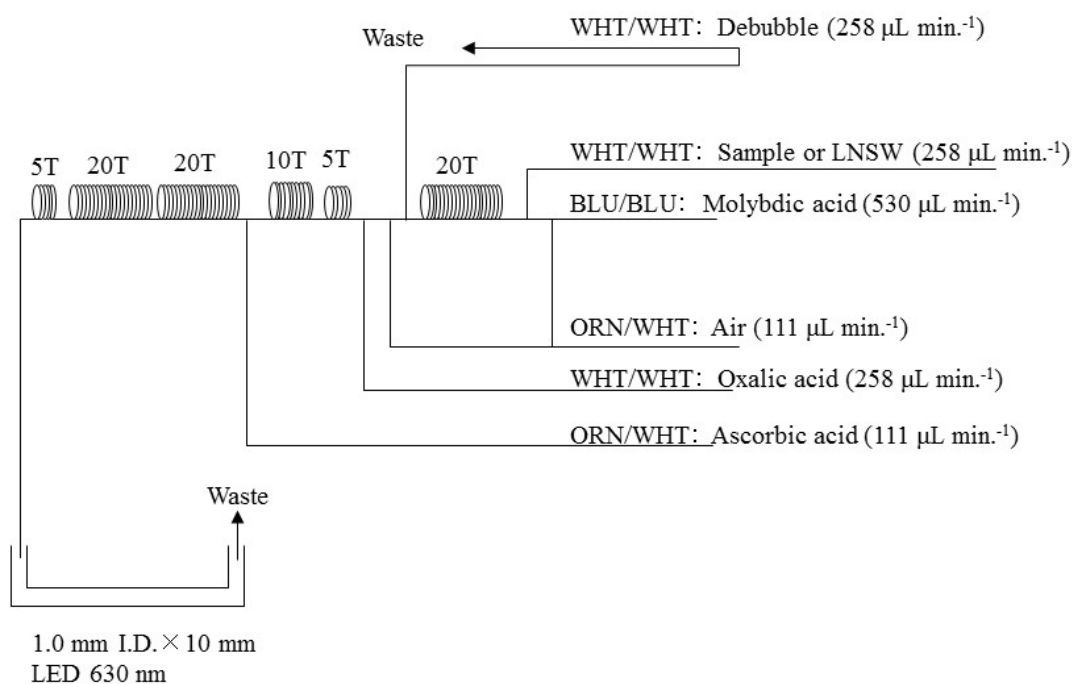


Figure 3.2.2.4-3 SiO_2 (3ch.) flow diagram.

Phosphate Reagents

15 % Sodium dodecyl sulfate solution

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

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

Dissolve 8 g sodium molybdate dihydrate (CAS No. 10102-40-6), and 0.17 g antimony potassium tartrate trihydrate (CAS No. 28300-74-5), in 950 mL of ultra-pure water and added 50 mL sulfuric acid (CAS No. 7664-93-9).

PO_4 color reagent

Dissolve 1.2 g L-ascorbic acid (CAS No. 50-81-7), in 150 mL of stock molybdate solution. After mixing, 3 mL 15 % sodium dodecyl sulfate solution is added. This reagent was freshly prepared before every measurement.

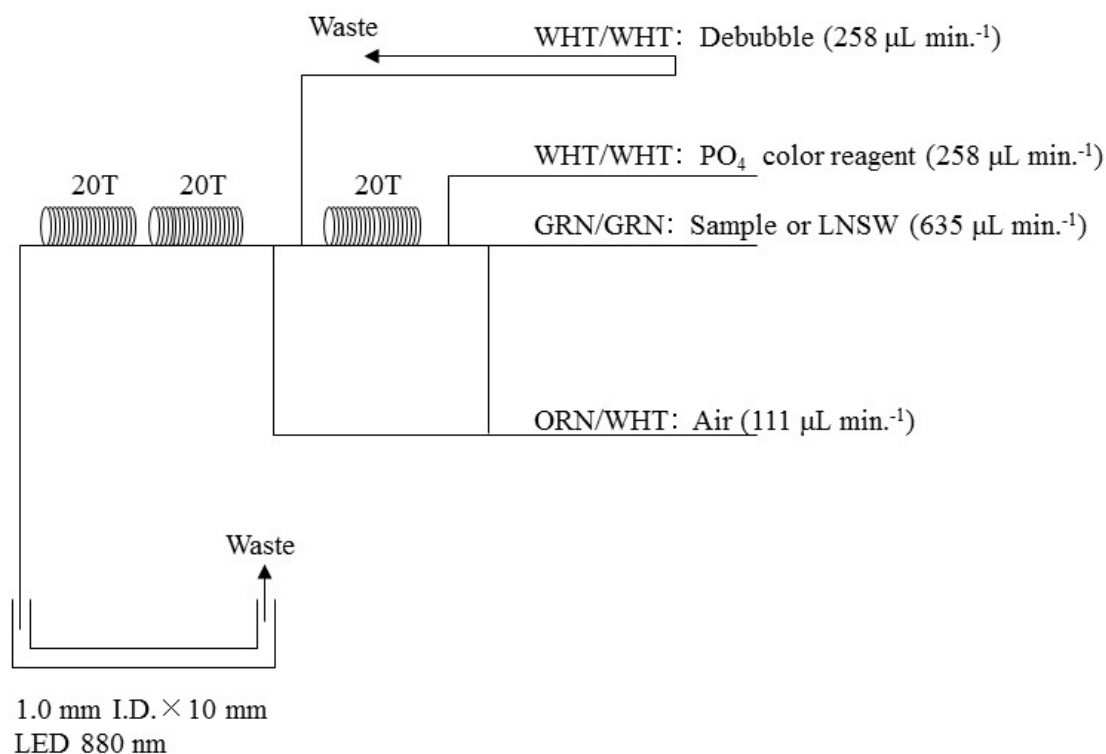


Figure 3.2.2.4-4 PO_4 (4ch.) flow diagram.

Ammonia Reagents

30 % Triton solution

30 mL TritonTM X-100 provided by Sigma-Ardrich Japan G. K. (CAS No. 9002-93-1) .were mixed with 70 mL ultra-pure water.

EDTA

Dissolve 41 g tetrasodium;2-[2-[bis(carboxylatomethyl)amino]ethyl-(carboxylatomethyl)amino]acetate;tetrahydrate (CAS No. 13235-36-4), and 2 g boric acid (CAS No. 10043-35-3), in 200 mL of ultra-pure water. After mixing, a 1 mL 30 % triton solution is added. This reagent is prepared a week approximately.

NaOH liquid

Dissolve 1.5 g sodium hydroxide (CAS No. 1310-73-2), and 16 g tetrasodium;2-[2-[bis(carboxylatomethyl)amino]ethyl-(carboxylatomethyl)amino]acetate;tetrahydrate (CAS No. 13235-36-4) in 100 mL of ultra-pure water. This reagent is prepared a week about. Note that we reduced the amount of sodium hydroxide from 5 g to 1.5 g because pH of C standard solutions lowered 1 due to the change of recipe of B standards solution.

Stock nitroprusside

Dissolve 0.25 g sodium nitroferrocyanide dihydrate (CAS No. 13755-38-9) in 100 mL of ultra-pure water and add 0.2 mL 1M sulfuric acid. Stored in a dark bottle and prepared a month approximately.

Nitroprusside solution

Mix 4 mL stock nitroprusside and 4 mL 1M sulfuric acid in 500 mL of ultra-pure water. After mixing, 2 mL 30 % triton solution is added. This reagent is stored in a dark bottle and prepared every 2 or 3 days.

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.

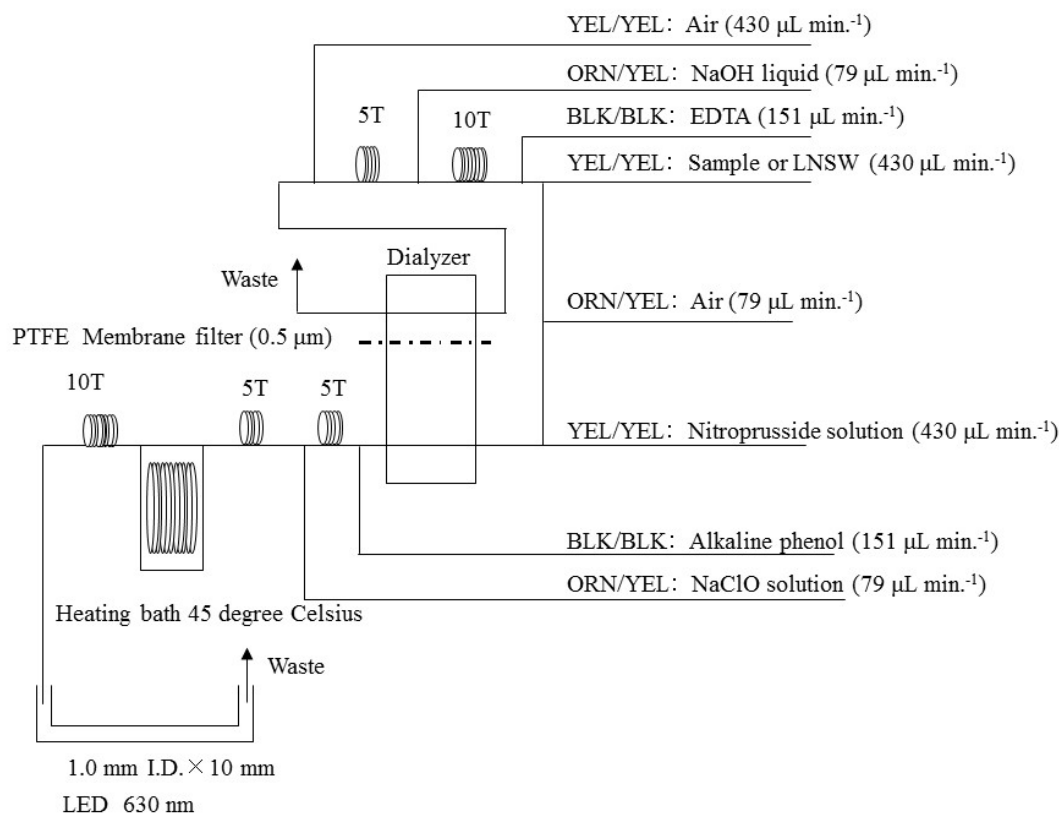


Figure 3.2.2.4-5 NH₄ (5ch.) flow diagram.

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 within 7 hours.

Data processing

Raw data from QuAAtro39 were treated as follows:

- Check the baseline shift.
- Check the shape of each peak and positions of peak values taken, and then change the positions of peak values taken if necessary.
- Carry-over correction and baseline drift correction were applied to peak heights of each sample followed by sensitivity correction.
- Baseline correction and sensitivity correction were done basically using linear regression.

- Load pressure and salinity from uncalibrated CTD data to calculate density of seawater tentatively. To calculate the final nutrient concentration, we used salinity data from calibrated CTD conductivity sensor data.
- Calibration curves to get nutrients concentration were assumed second order equations.

Summary of nutrients analysis

We made 11 QuAAtro runs for the samples collected by 11 stations as shown in Table 3.2.2.4-1 during KM20-08. The total amount of layers of the seawater sample reached to 381. We made basically duplicate measurements at all the sampling layers. The station locations for nutrients measurement is shown in Figure 3.2.2.4-6.

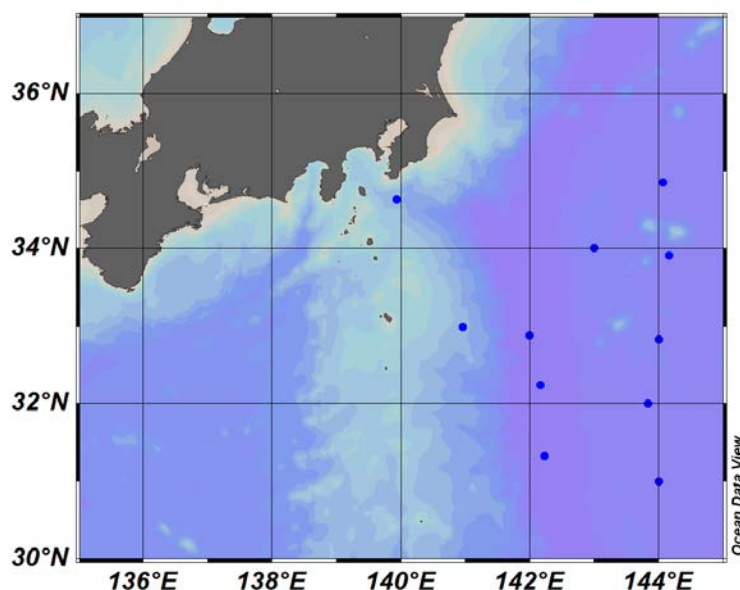


Figure 3.2.2.4-6 Sampling positions of nutrients sample.

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

Station	Cast	Date (UTC)	Position*		Depth (m)
		(mm/dd/yyyy)	Latitude	Longitude	
001	002	10/26/2020	32.9865	140.9517	2266
002	001	10/26/2020	32.8861	141.9913	8375
003	001	10/27/2020	32.2442	142.1658	9422
004	001	10/28/2020	31.3224	142.2216	9631
007	001	10/29/2020	32.8268	144.0011	5887
012	001	10/31/2020	34.8600	144.0624	5831
011	001	10/31/2020	33.9128	144.1636	5489
006	001	11/01/2020	32.0001	143.8332	5826
005	001	11/02/2020	31.0002	144.0001	5836
016	001	11/06/2020	34.6331	139.9270	2215
013	001	11/07/2020	34.0081	142.9997	5175
MC09**	-	11/02/2020	31.9998	143.8339	5826

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

** Position and Depth indicates where MC hit the bottom.

Certified Reference Material of nutrients in seawater

KANSO certified reference materials (CRMs, Lot: CL, 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)

CRM concentration

Nutrients concentrations for the CRM lots CL, 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: mmol kg ⁻¹					
Lot	Nitrate	Nitrite*	Silicate	Phosphate	Ammonia**
CL	5.47 ± 0.15	0.02 ± 0.01	13.8 ± 0.3	0.425 ± 0.019	1.68
CG	23.7 ± 0.2	0.06 ± 0.03	56.4 ± 0.5	1.70 ± 0.02	0.61
CF	43.4 ± 0.4	0.07 ± 0.02	159.7 ± 1.0	3.06 ± 0.03	0.46

* Nitrite value increasing trend (0.004 ± 0.002 µmol/kg per year) has been confirmed.

**For ammonia values are not certified and shown as only reference values.

Nutrients standards

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 4 K.

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 4 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 4 K. The weights obtained in the calibration weightings were corrected for the density of water and air buoyancy.

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.

Reagents, general considerations

Specifications

For nitrate standard, “potassium nitrate 99.995 suprapur®” provided by Merck, Batch B1452165, CAS No. 7757-79-1, was used.

For nitrite standard solution, we used “nitrite ion standard solution (NO₂⁻ 1000) provided by Wako, Lot ESQ6029, Code. No. 140-06451.”. This standard solution was certified by Wako using ion chromatography method. Calibration result is 1001 mg L⁻¹ at 20 degree Celsius. Expanded uncertainty of calibration (k=2) is 0.8 % for the calibration result.

For the silicate standard, we used in-house Si standard solution exp64 which was produced by alkali fusion technique from 5N SiO₂ powder produced jointly by JAMSTEC and KANSO. The mass fraction of Si in the exp64 solution was calibrated based on NMIJ CRM 3645-a02 Si standard solution.

For phosphate standard, “potassium dihydrogen phosphate anhydrous 99.995 suprapur®” provided by Merck, Batch B1642908, CAS No.: 7778-77-0, was used.

For ammonia standard, “Ammonium Chloride (CRM 3011-a)” provided by NMIJ, CAS No. 12125-02-9. The purity of this standard was greater than 99.9 %. Expanded uncertainty of calibration (k=2) is 0.022 %.

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.

Low nutrients seawater (LNSW)

Surface water having low nutrient concentration was taken and filtered using 0.20 µm 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.

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 (5.1) 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 were obtained using 5 levels, C-1, C-2, C-3, C-4, and C-5.

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

	Unit: µmol L ⁻¹							
	A	B	D	C-1	C-2	C-3	C-4	C-5
NO ₃	45000	900	1800	LNSW	9.0	18	36	54
NO ₂	21800	35	1740	LNSW	0.35	0.70	1.40	2.10
SiO ₂	35600	2850		LNSW	28.5	57.0	114	171
PO ₄	6000	60		LNSW	0.6	1.2	2.4	3.6
NH ₄	4000	80		LNSW	0.8	1.6	3.2	4.8

Renewal of in-house standard solutions

In-house standard solutions as stated in paragraph (5.2.4) 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

Quality control

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 11 to 13 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 0.11 % for nitrate, 0.14 % for nitrite, 0.09 % for silicate, 0.13 % for phosphate and 0.30 % for ammonia in terms of a median of precision, respectively.

Table 3.2.2.4-5 Summary of precision based on the replicate analyses.(*k*=1)

	Nitrate CV %	Nitrite CV %	Silicate CV %	Phosphate CV %	Ammonia CV %
Median	0.11	0.14	0.09	0.13	0.30
Mean	0.12	0.15	0.09	0.13	0.33
Maximum	0.16	0.20	0.15	0.22	0.57
Minimum	0.06	0.10	0.04	0.06	0.18
N	11	11	11	11	11

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, silicate and phosphate.

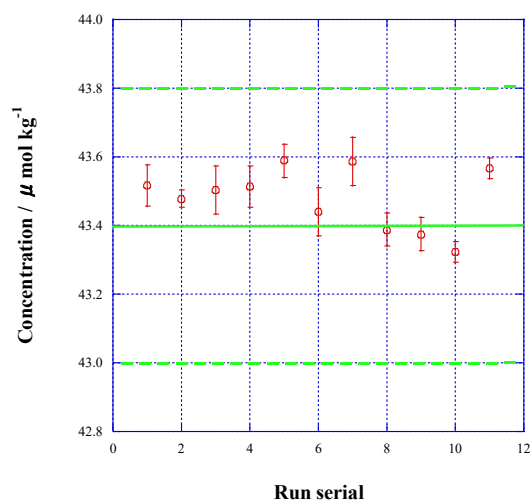


Figure 3.2.2.4-7 Time series of CRM-CF of nitrate in KM20-08. Solid green line is certified nitrate concentration of CRM and dotted green line show uncertainty of certified value at $k=2$.

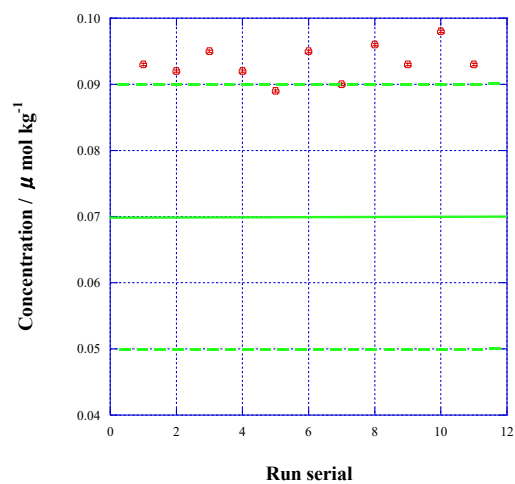


Figure 3.2.2.4-8 Same as Figure 3.2.2.4-7, but for nitrite.

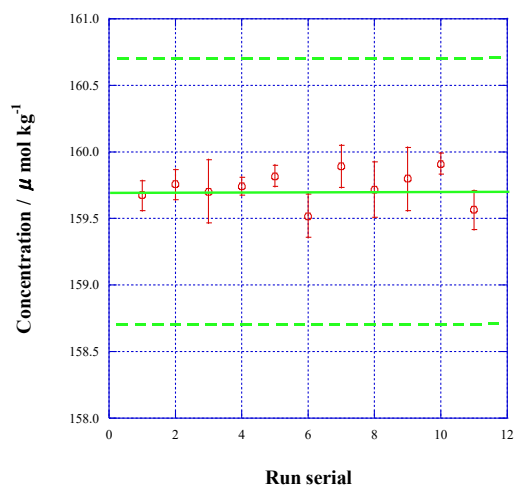


Figure 3.2.2.4-9 Same as Figure 3.2.2.4-7, but for silicate.

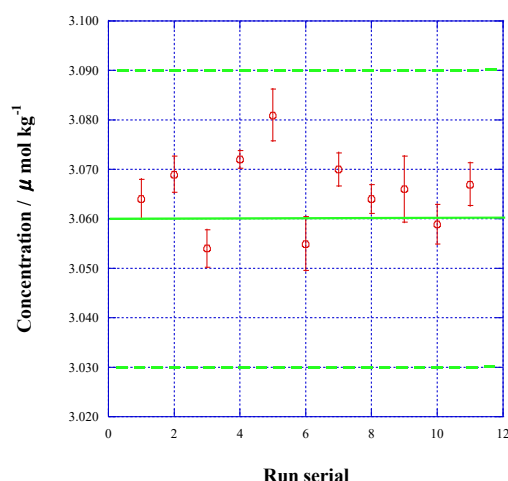


Figure 3.2.2.4-10 Same as Figure 3.2.2.4-7, but for phosphate.

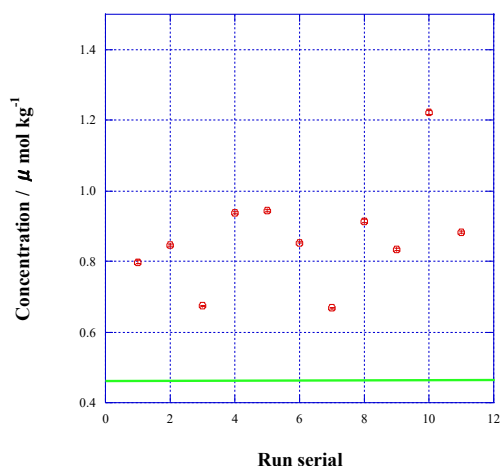


Figure 3.2.2.4-11 Time series of CRM-CF of ammonia in KM20-08. Green line is reference value for ammonia concentration of CRM-CF.

Estimation of uncertainty of nitrate, silicate, phosphate, nitrite and ammonia concentrations

Empirical equations, eq. (1), (2), (3), (4) and (5) to estimate the uncertainty of measurement of nitrate, nitrite, silicate, phosphate and ammonia are used based on duplicate measurements of the samples.

Nitrate Concentration C_{NO_3} in $\mu\text{mol kg}^{-1}$:

Uncertainty of measurement of nitrate (%) =

$$0.1024 + 1.0529 * (1 / C_{NO_3}) - 0.0085049 * (1 / C_{NO_3}) * (1 / C_{NO_3}) \quad \text{--- (1)}$$

where C_{NO_3} is nitrate concentration of sample.

Nitrite Concentration C_{NO_2} in $\mu\text{mol kg}^{-1}$:

Uncertainty of measurement of nitrite (%) =

$$1.1263 + 0.08922 * (1 / C_{NO_2}) \quad \text{--- (2)}$$

where C_{NO_2} is nitrite concentration of sample.

Silicate Concentration C_{SiO_2} in $\mu\text{mol kg}^{-1}$:

Uncertainty of measurement of silicate (%) =

$$0.08404 + 1.7185 * (1 / C_{SiO_2}) \quad \text{--- (3)}$$

where C_{SiO_2} is silicate concentration of sample.

Phosphate Concentration C_{PO4} in $\mu\text{mol kg}^{-1}$:
 Uncertainty of measurement of phosphate (%) =
 $0.075829 + 0.18348 * (1 / C_{PO4}) - 0.000071752 * (1 / C_{PO4}) * (1 / C_{PO4})$ --- (4)
 where C_{PO4} is phosphate concentration of sample.

Ammonia Concentration C_{NH4} in $\mu\text{mol kg}^{-1}$:
 Uncertainty of measurement of ammonia (%) =
 $22.792 + 0.63181 * (1 / C_{NH4})$ --- (5)
 where C_{NH4} is ammonia concentration of sample.

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>

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3.2.2.5 Carbon Items

29 October 2021

Minoru Hamana (JAMSTEC) (Principal Investigator), Hiroshi Uchida (JAMSTEC)

The carbon parameter was sampled from all layers at 8 stations (YK1911-06, T3, KM2008-02, G1, KM2008-09, KM2008-06, YK1911-03 and KM2008-14). 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 2 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 (from 26 November to 17 December, 2020).

The measurement of DIC and TA was carried out from the same bottle. The analytical methods of the carbon parameters were same as the methods used in Uchida et al. (2018). Certified reference materials (5

bottles of Batch 136) provided by Prof. A. G. Dickson of Scripps Institution of Oceanography and in-house reference materials (5 bottles of QRM, Marine Works Japan, Ltd.) made from seawater were used for the standardization.

The standard deviation of CRM measurements was 2.05 $\mu\text{mol/kg}$ and 0.68 $\mu\text{mol/kg}$ for DIC and TA, respectively. The average $\pm$ standard deviation of QRM measurements was 1967.19 $\pm$ 1.20 $\mu\text{mol/kg}$ and 2231.59 $\pm$ 2.71 $\mu\text{mol/kg}$ for DIC and TA, respectively.

A total of 6 pairs of replicate samples were measured. The standard deviation of replicate samples was 1.10 $\mu\text{mol/kg}$ and 0.76 $\mu\text{mol/kg}$ for DIC and TA, respectively.

Uchida, H., Katsumata, K. and Doi, T. WHP I10 REVISIT IN 2015 DATA BOOK, (Aiwa Enterprise, Ltd., Tokyo, 2018. <http://dx.doi.org/10.17596/00000002>)

3.2.2.6 DOC/CDOM/FDOM

10 November 2020

Kosei Sasaoka (JAMSTEC) (Principal Investigator for CDOM), Masaharu Shigemitsu (JAMSTEC) (Principal Investigator for DOC/FDOM), 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). However, there is no direct evidence for that.

In this cruise, we try to gain insights into spatial and vertical distributions of quality and quantity of organic matter in hadal trench waters which might be key factors to determine the microbial compositions there. To this end, we measure the distributions of dissolved organic carbon (DOC), chromophoric dissolved organic matter (CDOM) and fluorescent fractions of CDOM (FDOM) at the Izu-Ogasawara trench and the surrounding stations (St. T3, KM2008-02, G1, KM2008-06, and KM2008-14). CDOM and FDOM can be useful tracers for evaluating DOC 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.) onboard, 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:
$$a_{\text{cdom}}(\lambda) = 2.303 \times \text{OD}_{\text{cdom}}(\lambda) / 0.1$$
 (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 onboard 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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3.2.2.7 Inorganic Iodine

7 November 2020

Kazuhiko Takeda (Hiroshima University) (Principal Investigator [not onboard]), Hiroshi Uchida (JAMSTEC)

We collected water samples for analysis of inorganic iodine (iodide and iodate) in the Izu-Ogasawara trench and the Northwest Pacific Basin at the CTD/water sampling stations 002 (T3), 004 (G1), 006 (KM2008-06), and 012 (KM2008-09). The water samples were collected in 100-mL aluminum bottles (Mini Bottle Can, Daiwa Can Company, Japan). Each bottle was rinsed three times with sample water and was filled to the neck of the bottle. The samples were stored in a refrigerator. The iodide and iodate for the water samples will be determined by ion chromatography with ultraviolet detection (Takeda et al. 2017) in the laboratory at Hiroshima University after the cruise. The distribution and transformation of iodine in marine environments will be examined.

References

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3.3. Microbial parameters

3.3.1. *Spatial patterns of prokaryotic abundance, activity and community composition in relation to the water masses in the western north Pacific*

Taichi Yokokawa, Miho Hirai, Eiji Tasumi (JAMSTEC)

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 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.

Microbial abundance

Samples for microbial abundances (prokaryotes, eukaryotes and viruses) were collected in Station name: YK1911-06, T3, KM2008-02, G1, KM2008-07, KM2008-09, KM2008-08, KM2008-06, KM2008-05, YK1911-03 and KM2008-14. 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 YK1911-06, T3, KM2008-02, G1, KM2008-07, KM2008-09, KM2008-08, KM2008-06, KM2008-05, YK1911-03 and KM2008-14, in the routine casts.

3.3.2. Nitrogen cycle and biogeochemistry in the North Pacific Ocean

Akiko Makabe, Eiji Tasumi, Koji Miyakawa (JAMSTEC)

The nitrogen cycle in marine surface water is known to control biological activity in the ocean, because inorganic forms of nitrogen such as nitrate are indispensable nutrients for phytoplankton. Following the primary production, organic nitrogen compounds are metabolized into ammonium and low molecular organic nitrogen compounds that are energy source of nitrification and/or nitrogen source of microbes. Nitrous oxide (N₂O), known to be produced by microbial activity such as nitrification, is recognized as significant anthropogenic greenhouse gas and a stratospheric ozone destroyer. In low-nutrient region, nitrogen fixation is an important source of nitrogen for the ecosystem. On the other hand, biogeochemistry in hadal seawater is still not well known while we have conducted several scientific cruises with a full-depth CTD.

To understand transformation of nitrogen compound by (microbial) organisms and production processes of greenhouse gasses (N₂O), both natural abundance and tracer stable isotope technique are useful. We collected water samples to analyze natural abundance stable isotope ratio of dominant nitrogen species such as nitrate, nitrite, and nitrous oxide, which will have records of biochemical processes. On the other hand, tracer technique is relatively tough work but strongly useful tool to detect or determine the rates of each biochemical process. We conducted on-board incubation experiments to analyze nitrification and nitrogen fixation activities. To understand the biogeochemistry in both surface and hadal seawater, we collected the samples for analysis of CH₄, H₂, CO, and ammonium because those compounds are able to be produced and/or diffused by both biochemical and geochemical processes.

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 Station#: YK1911-06, T3, KM2008-02, G1, KM2008-08, KM2008-05, YK1911-03.

N₂O and CH₄ concentration and isotope ratio

Samples for N₂O and CH₄ concentration and stable isotope analysis were collected into 30 mL and 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 concentration were collected in Station#: YK1911-06, T3, KM2008-02, G1, KM2008-07, KM2008-09, KM2008-08, KM2008-06, KM2008-05, YK1911-03.

Samples for N₂O and CH₄ stable isotope ratio were collected in Station#: YK1911-06, T3, G1, YK1911-03.

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 Station#: YK1911-06, KM2008-02, G1, KM2008-08, KM2008-05, YK1911-03.

Nitrification activity

Samples for nitrification activity measurement were collected into 100 mL amber glass vials and 100 mL PE bottles without head space. Ammonium or urea (^{15}N 99 atom %) which are substrates of nitrification were added to the bottles. The seawater samples were incubated in dark at near in situ temperature on board. At the end of incubation period, the seawater samples were filtrated by a syringe filter (pore size: $0.45\mu\text{m}$) and frozen until analysis. The enrichment of ^{15}N in nitrite and nitrate will be determined by GC-IRMS after transformed to N_2O by denitrifier method at JAMSTEC. The transfer rate from substrates to nitrite and nitrate will be calculated by the enrichment of ^{15}N in nitrite and nitrate.

Samples for nitrification activity measurement were collected in Station#: YK1911-06, KM2008-02, KM2008-08, YK1911-03.

Nitrogen fixation activity

Samples for N_2 fixation activity measurement were collected into 250 mL PC bottles without head space. N_2 gas (^{15}N 99 atom %) was directly added to bottles. The PC bottles with/without ^{15}N enriched N_2 gas were incubated in seawater baths for 24 hours under appropriate screen to simulate the in situ temperature and light intensity. Each incubated sample was filtered with $2\mu\text{m}$ polycarbonate filters and $0.3\mu\text{m}$ glass fiber filters in sequence after the incubation. The filters and final filtrate were frozen until analysis. The enrichment of ^{15}N will be measured by GC-IRMS at JAMTEC.

Samples for N_2 fixation activity measurement were collected in Station#: G1, KM2008-05.

H_2 and CO concentration

Samples for H_2 and CO analysis were collected into 100 mL glass vials and added with HgCl_2 solution immediately after sampling. Each 20 mL of seawater was replaced to ultrapure N_2 gas to make head space in each vial. The vials were shaken and left with its head lowered in a refrigerator until achievement of equilibrium between the dissolved and head-space gases. Concentration of dissolved H_2 and CO were determined by measurement of the head-space gas using GC on board.

Samples for H_2 and CO concentration were collected in Station#: T3, G1, KM2008-05.

3.3.3. Full depth distribution of mercury species in the western North Pacific

Koji Marumoto (NIMD), Akinori Takeuchi (NIES), Nobuaki Okabe (NIES), Shogo Kawashima (The University of Tokyo), Yuya Tada (NIMD)

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, CH_4 and CO_2 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.

To obtain the dissolved THg and MMHg samples, seawater was filtered with $0.2\mu\text{m}$ -pore size cartridge filter. Then filtrates were treated with HCl and H_2SO_4 addition, respectively, and stored at 4°C until further analyses. In addition, the filtered seawater was bubbled with Hg-free N_2 gas to trap the dissolved Hg^0 and DMHg using gold amalgam and carbon resin traps. These traps were stored at room temperature and -20°C , respectively. As for particulate Hg samples, seawater was filtered with $0.2\mu\text{m}$ -pore size polycarbonate filters, and these filters were stored at -20°C until further analysis. Moreover, gaseous Hg, CH_4 and CO_2 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 Hg^0 were collected by purging with gold amalgam and carbon resin traps, and stored at room temperature and -25°C , respectively. Dissolved MeHg and Hg^{2+} samples were treated with H_2SO_4 addition and stored at the 4°C until further analysis.

4. AORI Cooperative Research Program

4.1. *Measurement of methylation potential of mercury in Kuroshio-area for improving a 3-D global mercury model*

Nobuaki Okabe (NIES)

In order to measure a mercury methylation potential in the Kuroshio-area, seawater samples at four different depth were collected at two locations. A certain amount of $\text{CH}_3^{199}\text{HgCl}$ and $^{201}\text{HgCl}_2$ solutions were spiked into the collected unfiltered seawater samples, and they were incubated up to approximately 36-hr. The same experiments were also performed on the filtered sample. Each sample was sparged by Hg-free N_2 gas immediately after the spikes and a certain period of time. The volatile components of mercury were collected on gold and carbon resin traps. The sparged seawater samples were acidified by addition of H_2SO_4 to preserve the dissolved components of mercury. All of the collected samples have been kept refrigerated and will be transferred to NIES to measure each component mercury.

4.2. *Geochemical evaluation of Methylmercury accumulation to marine seston using advanced X-ray spectroscopy*

Shogo Kawashima (The University of Tokyo)

In this cruise, we took samples to investigate the host phase of monomethylmercury (MMHg) in seawater. To obtain the seston samples, seawater was filtered with $0.4\mu\text{m}$ -pore size polycarbonate filters, then these filters were stored at -20°C . After taking the collected samples back to the laboratory, MMHg is added to the sample. After observing the sample with low vacuum SEM, MeHg is mapped by an X-ray microbeam having a resolution of about $1\mu\text{m}$ using the same sample holder. The host phase of MeHg in seawater is inferred by comparing with the distribution of plankton and POM.

4.3. *Estimation of the Effect of Ocean Acidification on N_2O Production by Simulated Experiments Using Seawater Samples*

Daiki Kawaguchi, Sakae Toyoda (Tokyo Institute of Technology), Akiko Makabe (JAMSTEC)

Nitrogen monoxide (N_2O) is a greenhouse gas and a contributor to global warming. It is also known to be optically active in the stratosphere and decomposes ozone. Therefore, its impact on the global environment is considered to be significant. N_2O is mainly produced by nitrification and denitrification reactions by microorganisms in soil and water. Generation from the oceans accounts for about 30% of the total. However, the mechanism and its dependence on environmental factors are not yet fully understood. In this experiment, seawater culture experiments were conducted to investigate how N_2O production from the ocean is changed by acidification.

Water sampling was performed with YK1911-03, YK1911-06, and KM2008-08. First, 1M hydrochloric acid was added to the samples, which were drawn and sealed in 200 ml vials, for acidification (Three conditions). Next, ^{15}N concentrated ammonium chloride and sodium nitrite ($^{15}\text{NH}_4\text{Cl} + \text{NaNO}_2$ or $\text{NH}_4\text{Cl} + \text{Na}^{15}\text{NO}_2$) were added as substrates and incubated in an incubator at a constant temperature for up to 36 hours. After measuring the pH of the finished samples, mercury was added to stop the bacterial activity.

As the culture experiment was completed without any problems at each site, we will measure isotope ratios using GC-IRMS in the future. We will determine the production rate and pathway of N₂O from the obtained isotope ratios and compare them with the results of other sea areas and conditions.

5. 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.

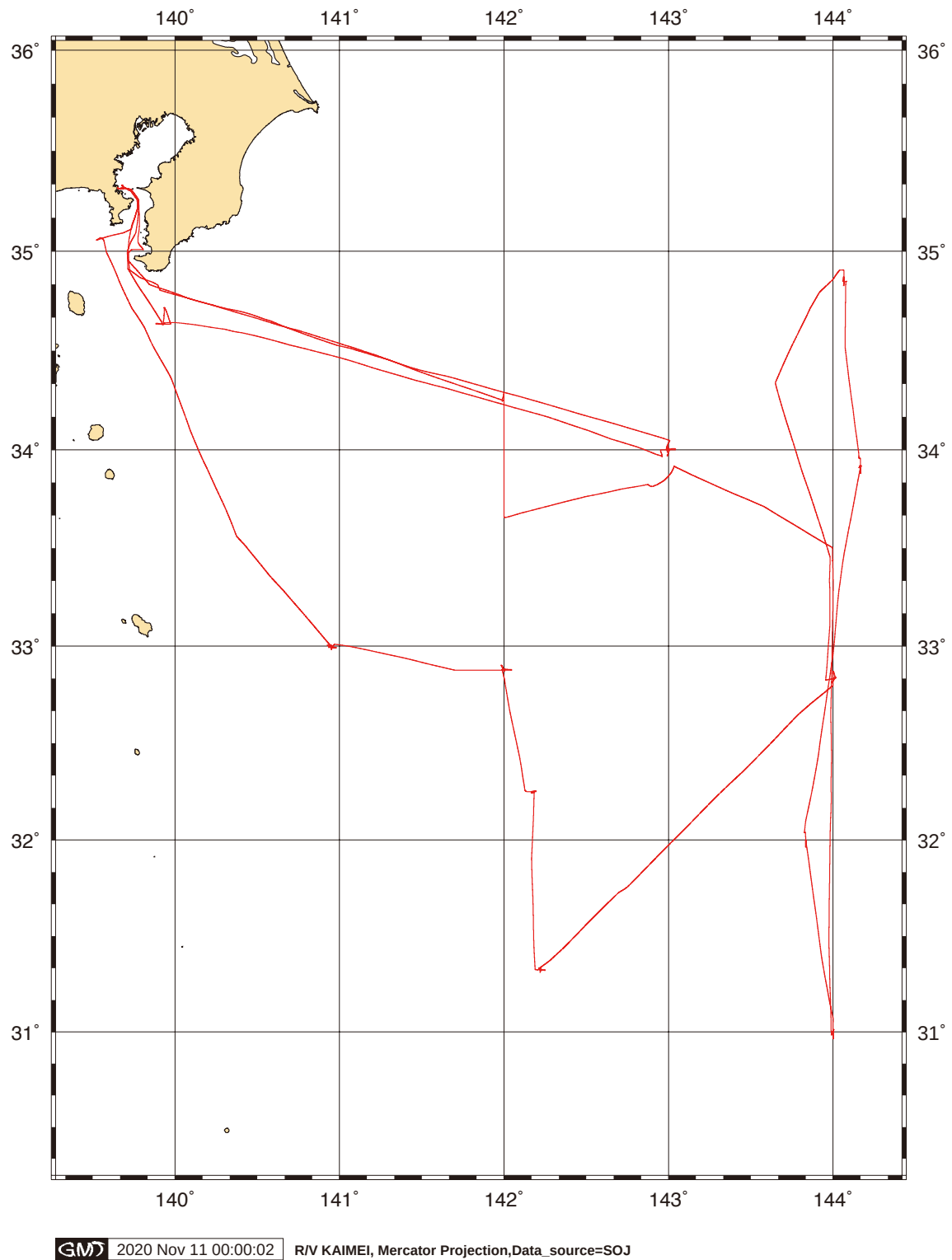
Users of information on this report are requested to submit Publication Report to JAMSTEC.

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

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

Appendix: Cruise log

KM20-08_NavTrack



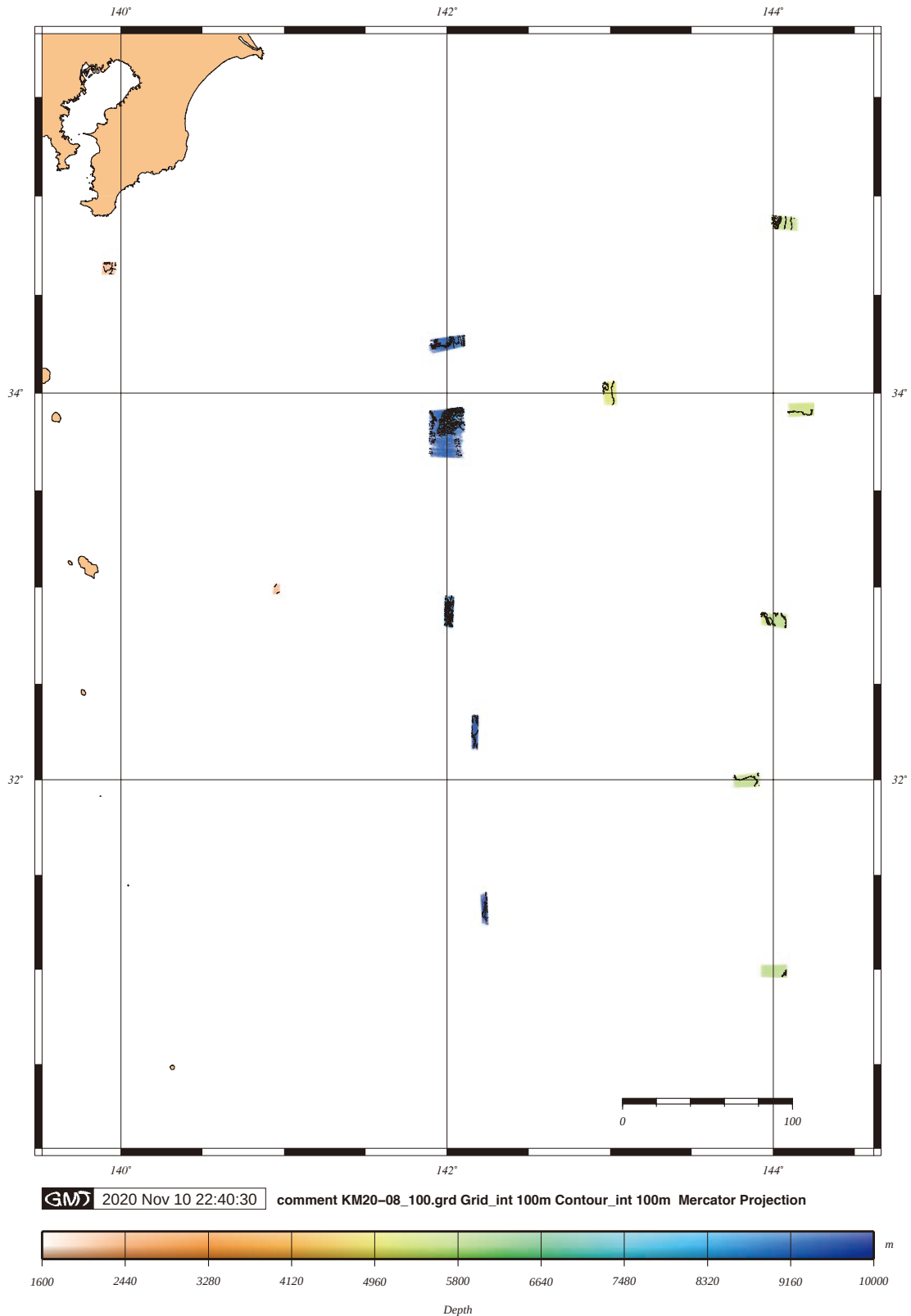
Date	Local Time (JST:UTC+9h)	Note	Noon Position/Weather/Wind/Sea condition
25-Oct-20		Left JAMSTEC YOKOSUKA & Arrived at Sagami-Wan	Sagami-Wan
	8:00	Scientists embarked	35-03.3N, 139-31.3E
	9:00	Left JAMSTEC YOKOSUKA for Research area	Fine but Cloudy
	9:10	Started Scientist Meeting	ENE-3 (Gentle breeze)
	9:30	Finished Scientist Meeting	2 (Sea Smooth)
	10:00	Started Safety Education & guidance for scientists	1 (Low Swell Short)
	11:00	Finished Safety Education & guidance for scientists	Visibly: 8'
	10:00	Arrived at Research area(Sagami-Wan)	
	10:30	Lunched XBT	
	11:55	Started Continuous Observation with Thermo Salino Graph	
	12:08	Started Sampling with Neuston Net	
	13:20	Finished Sampling with Neuston Net	
	13:40	Started Sampling with Multiple-Corer	
	16:53	Finished Sampling with Multiple-Corer	
26-Oct-20		Observation & Sampling with CTD,Neuston Net,M-Corerer & Bucket	East of Izu-Ogasawara-islands
	8:13	Started observation & sampling with CTD	32-59.6N, 140-57.3E
	8:57	Finished observation & sampling with CTD	Fine but Cloudy
	9:18	Started Sampling with Neuston Net	West-3 (Gentle breeze)
	10:33	Finished Sampling with Neuston Net	2 (Sea Smooth)
	11:08	Started Sampling with Multiple-Corer	1 (Low Swell Short)
	13:25	Finished Sampling with Multiple-Corer	Visibly: 8'
	13:54	Started observation & sampling with CTD	
	14:39	Finished observation & sampling with CTD	
	15:00	Started sampling with a Bucket	
	16:38	Finished sampling with a Bucket	
	17:41	Started observation & sampling with CTD	
	19:02	Finished observation & sampling with CTD	
27-Oct-20		Observation & Sampling with CTD,Neuston Net,M-Corerer & Bucket,R-Profil	East of Izu-Ogasawara-islands
	0:26	Started 8figure-running for STCM correction	32-52.3N, 142-00.0E
	0:50	Finished 8figure-running for STCM correction	Fine but Cloudy
	4:06	Started observation & sampling with CTD	WNW-2 (Light breeze)
	6:06	Started observation with RINKO-Profil	1 (Sea Calm Rippled)
	6:16	Finished observation with RINKO-Profil	1 (Low Swell Short)
	9:01	Finished sampling with bucket	Visibly: 7'
	9:11	Finished sampling with bucket	
	10:45	Finished observation & sampling with CTD	
	11:23	Started Sampling with Multiple-Corer	
	15:47	Started Sampling with Neuston Net	
	16:31	Finished Sampling with Neuston Net	
	17:09	Finished Sampling with Multiple-Corer	
28-Oct-20		Observation & Sampling with CTD,Neuston Net,M-Corerer & Bucket,R-Profil	East of Izu-Ogasawara-islands
	4:05	Started observation & sampling with CTD	32-15.0N, 142-10.0E
	6:03	Started observation with RINKO-Profil	Fine but Cloudy
	6:10	Finished observation with RINKO-Profil	ENE-5 (Fresh breeze)
	9:02	Finished sampling with bucket	4 (Sea Moderate)
	9:11	Finished sampling with bucket	2 (Low Swell Slong)
	10:45	Finished observation & sampling with CTD	Visibly: 8'
	11:12	Started Sampling with Multiple-Corer	
	15:12	Started Sampling with Neuston Net	
	16:21	Finished Sampling with Neuston Net	
	17:42	Finished Sampling with Multiple-Corer	
29-Oct-20		Observation & Sampling with CTD,Neuston Net,M-Corerer & Bucket,R-Profil	East of Izu-Ogasawara-islands
	0:17	Started 8figure-running for STCM correction	31-19.5N, 142-12.8E
	0:39	Finished 8figure-running for STCM correction	Fine but Cloudy
	4:07	Started observation & sampling with CTD	WNW-3 (Gentle breeze)
	6:04	Started observation with RINKO-Profil	3 (Sea Slight)
	6:12	Finished observation with RINKO-Profil	2 (Low Swell Slong)
	9:03	Finished sampling with bucket	Visibly: 8'
	9:15	Finished sampling with bucket	
	10:45	Finished observation & sampling with CTD	
	11:13	Started Sampling with Multiple-Corer	
	14:52	Started Sampling with Neuston Net	
	15:59	Finished Sampling with Neuston Net	
	17:14	Finished Sampling with Multiple-Corer	
30-Oct-20		Observation & Sampling with CTD,Neuston Net,M-Corerer & Bucket	East of Izu-Ogasawara-islands
	7:00	Started observation & sampling with CTD	32-50.3N, 144-00.7E
	8:59	Started sampling with bucket	Overcast
	9:08	Finished sampling with bucket	WSW-3 (Gentle breeze)
	11:45	Finished observation & sampling with CTD	2 (Sea Smooth)
	12:28	Started Sampling with Multiple-Corer	2 (Low Swell Slong)
	16:51	Finished Sampling with Multiple-Corer	Visibly: 7'
	16:58	Started Sampling with Neuston Net	
	17:18	Finished Sampling with Neuston Net	

Date	Local Time (JST-UTC+9h)	Note	Noon Position/Weather/Wind/Sea condition
31-Oct-20		Observation & Sampling with CTD & Bucket	East of Izu-Ogasawara-islands
	11:57	Started observation & sampling with CTD	34-52.3N, 144-03.8E
	14:00	Started sampling with bucket	Fine but Cloudy
	14:15	Finished sampling with bucket	North-6 (Strong breeze)
	16:37	Finished observation & sampling with CTD	5 (Sea Rough)
	16:48	Started Sampling with Neuston Net	3 (Moderate Short)
	17:57	Finished Sampling with Neuston Net	Visibly: 8'
01-Nov-20		Observation & Sampling with CTD, Neuston Net, M-Corer & Bucket	East of Izu-Ogasawara-islands
	7:01	Started observation & sampling with CTD	33-55.1N, 144-09.7E
	8:58	Started sampling with bucket	Fine but Cloudy
	9:08	Finished sampling with bucket	ENE-5 (Fresh breeze)
	11:32	Finished observation & sampling with CTD	4 (Sea Moderate)
	12:23	Started Sampling with Multiple-Corer	3 (Moderate Short)
	14:40	Started Sampling with Neuston Net	Visibly: 8'
02-Nov-20		Observation & Sampling with CTD, Neuston Net, M-Corer & Bucket	East of Izu-Ogasawara-islands
	7:00	Started observation & sampling with CTD	32-00.0N, 143-50.0E
	9:05	Started sampling with bucket	Fine but Cloudy
	9:13	Finished sampling with bucket	ESE-4 (Moderate breeze)
	12:09	Finished observation & sampling with CTD	3 (Sea Slight)
	12:26	Started Sampling with Multiple-Corer	3 (Moderate Short)
	14:49	Started Sampling with Neuston Net	Visibly: 8'
03-Nov-20		Observation & Sampling with CTD, Neuston Net, M-Corer & Bucket	East of Izu-Ogasawara-islands
	0:10	Started 8figure-running for STCM correction	31-21.0N, 143-58.7E
	0:32	Finished 8figure-running for STCM correction	Rain
	4:02	Started observation & sampling with CTD	ESE-7 (Near gale)
	7:04	Started sampling with bucket	4 (Sea Moderate)
	7:14	Finished sampling with bucket	3 (Moderate Short)
	8:42	Finished observation & sampling with CTD	Visibly: 1'
04-Nov-20		Restart observation with TSG & test cast with spare CTD	East of Izu-Ogasawara-islands
	11:55	Restart Continuous Observation with Thermo Salino Graph	33-49.4N, 142-56.3E
	13:25	Started observation & test cast with spare CTD	Cloudy
	16:57	Finished observation & test cast with spare CTD	North-6 (Strong breeze)
	11:56	Restarted Continuous Observation with Thermo Salino Graph	5 (Sea Rough)
			4 (Moderate Average)
			Visibly: 7'
05-Nov-20		Bound for JAMSTEC to Pick Up Spare CTD	Nojima-Saki-Oki
	4:06	Lost the CTD & Bound for JAMSTEC	34-44.0N, 140-11.6E
	14:50	Arrived at Tateyama-Wan	Fine but Cloudy
	14:58	Aborted Continuous Observation with Thermo Salino Graph for maintenance	North-4 (Moderate breeze)
			3 (Sea Slight)
06-Nov-20		Borded Spare CTD at JAMSTEC & CTD Test Cast	Tsurugi-Saki-Oki
	9:05	Arrived at JAMSTEC YOKOSUKA	35-07.5N, 139-44.5E
	10:20	Left JAMSTEC YOKOSUKA for Research Area	Fine but Cloudy
	14:32	Restarted Continuous Observation with Thermo Salino Graph	North-3 (Gentle breeze)
	15:12	Commenced CTD test Cast & Observation	2 (Sea Smooth)
	16:20	Finished CTD test Cast & Observation	1 (Low Swell Short)
	17:36	Finished MBES & SBP Line Survey	Visibly: 8'
07-Nov-20		Observation & Sampling with CTD, Neuston Net, M-Corer & Bucket	Nojima-Saki-Oki
	7:06	Started observation & sampling with CTD	34-37.9N, 139-55.8E
	8:10	Started sampling with bucket	Overcast
	8:23	Finished sampling with bucket	SW-6 (Strong breeze)
	9:43	Finished observation & sampling with CTD	4 (Sea Moderate)
	10:10	Started Sampling with Multiple-Corer	2 (Low Swell Slong)
	12:05	Finished Sampling with Multiple-Corer	Visibly: 7'
	13:18	Started Test for Multi-Pull-Corer on the deck	
	13:22	Finished Test for Multi-Pull-Corer on the deck	
	13:30	Started Sampling with Multiple-Corer	
	13:57	Started Sampling with Neuston Net	
	15:04	Finished Sampling with Neuston Net	
08-Nov-20		Observation & Sampling with CTD & Bucket	East of Izu-islands
	6:08	Started observation & sampling with CTD	34-01.2N, 142-59.7E
	8:03	Started sampling with bucket	Cloudy
	8:27	Finished sampling with bucket	WSW-5 (Fresh breeze)
	10:10	Finished observation & sampling with CTD	4 (Sea Moderate)
			3 (Moderate Short)
			Visibly: 7'

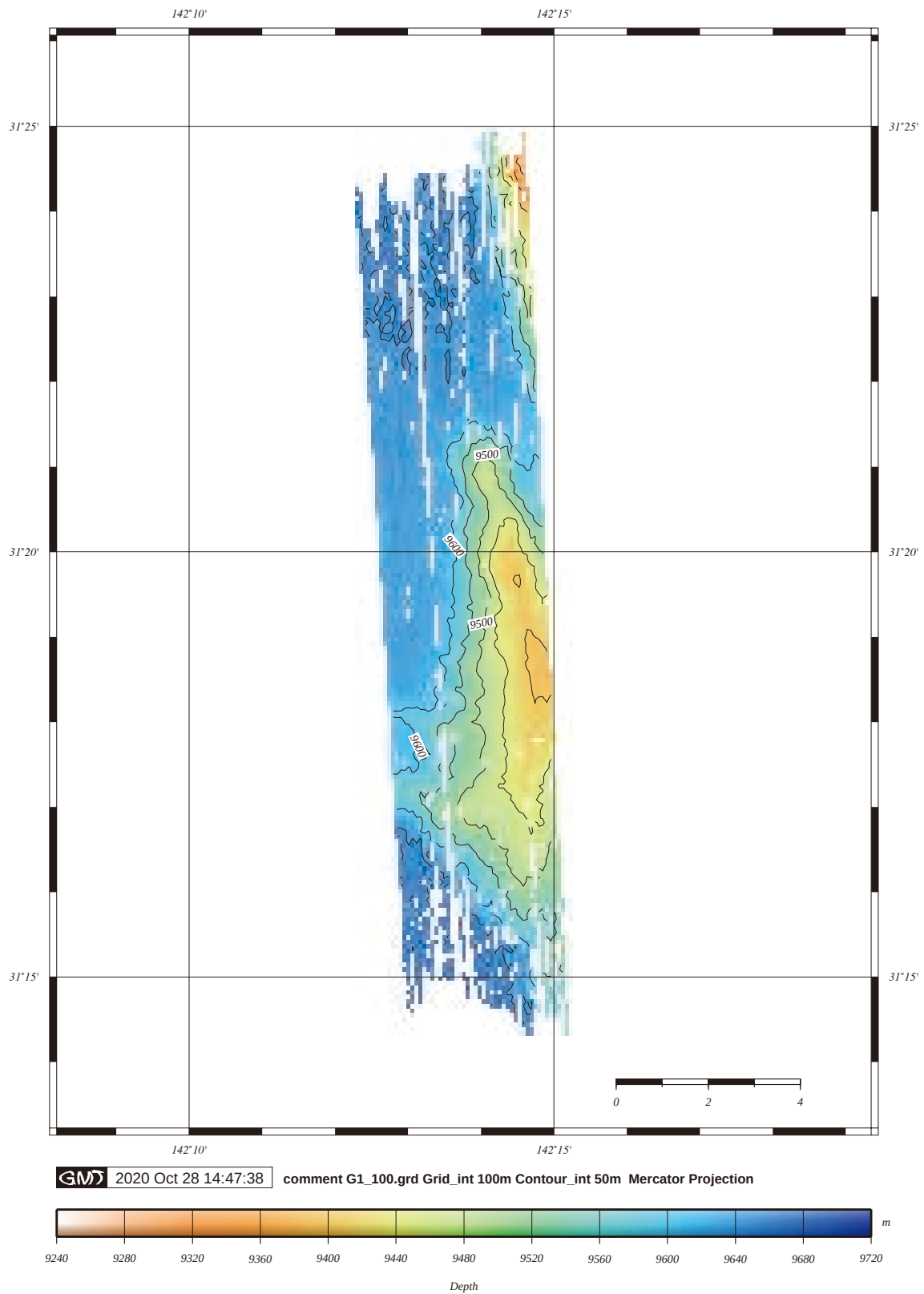
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09-Nov-20		Observation & Sampling with CTD, Neuston Net, M-Corer & Bucket	East of Izu-islands
	13:17	Started Sampling with Multiple-Corer	33-59.8N, 142-58.1E
	17:09	Started Sampling with Multiple-Corer	Fine but Cloudy
	17:18	Started Sampling with Neuston Net	NNE-6 (Strong breeze)
	18:25	Finished Sampling with Neuston Net	5 (Sea Rough)
	18:45	Left Research Area for JAMSTEC YOKOSUKA	4 (Moderate Average)
			Visibly: 8'
10-Nov-20		Bound for JAMSTEC YOKOSUKA	Yokosuka-Port No.4 ward
	8:30	Started Scientist Meeting	35-19.8N, 139-40.9E
	9:00	Finished Scientist Meeting	Fine but Cloudy
			NE-4 (Moderate breeze)
			3 (Sea Slight)
			1 (Low Swell Short)
			Visibly: 8'
11-Nov-20		Arrived at JAMSTEC	
	9:00	Arrived at JAMSTEC YOKOSUKA	
	12:00	Scientists disembarked	

Bathymetry at each sampling site

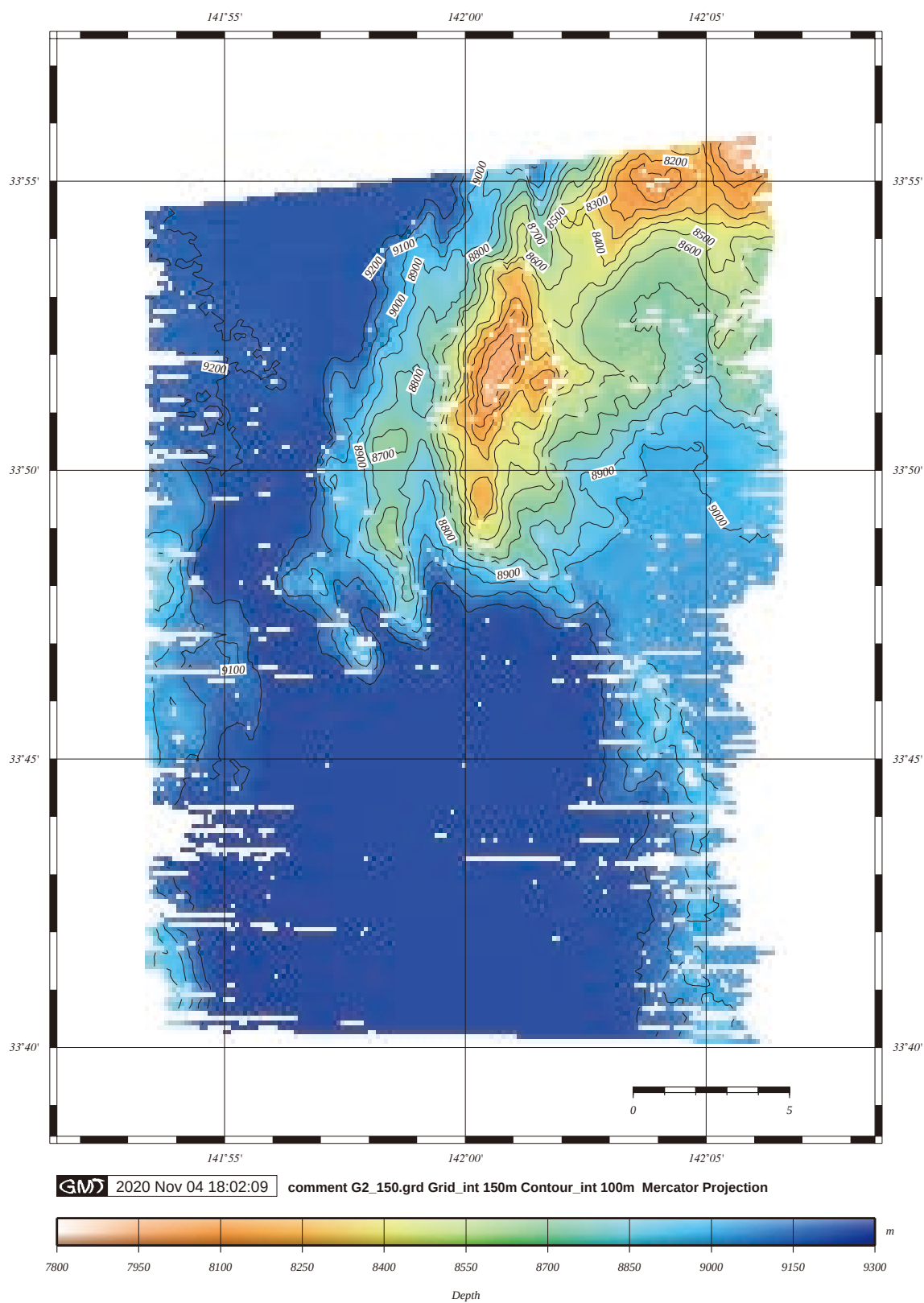
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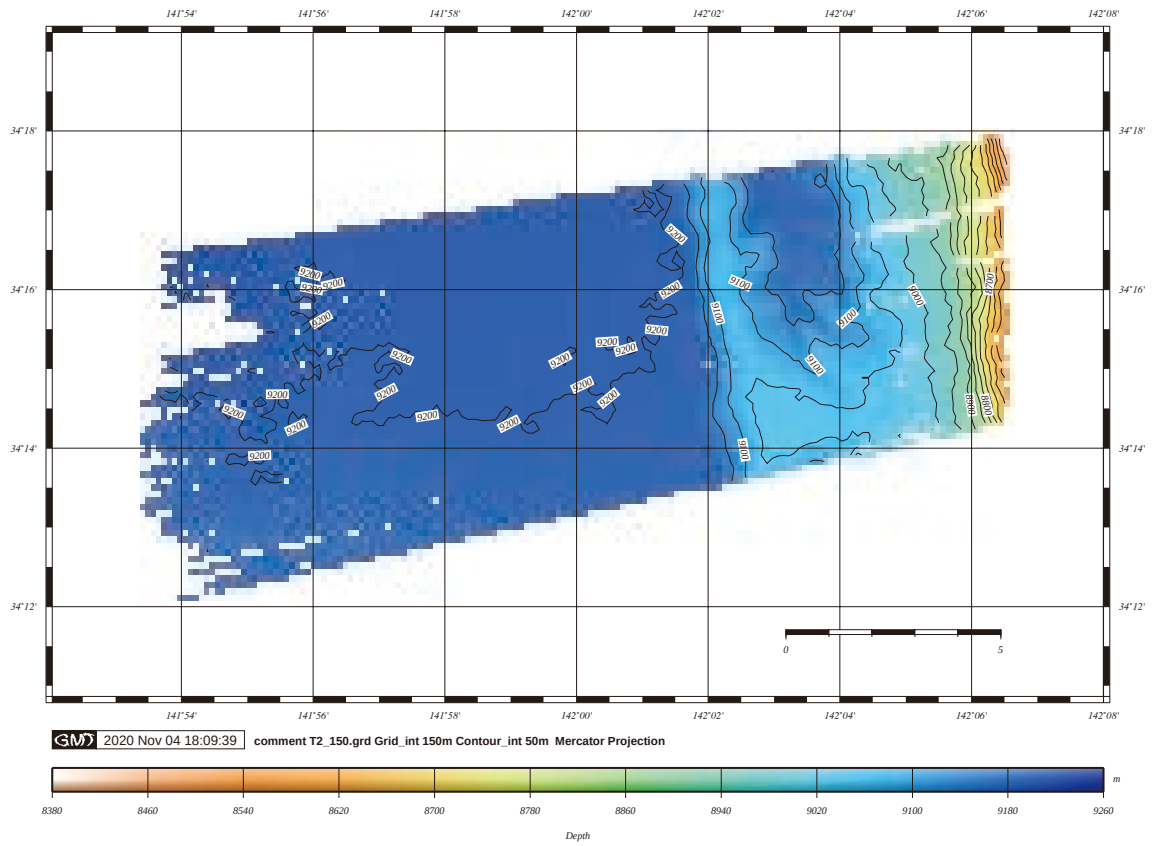
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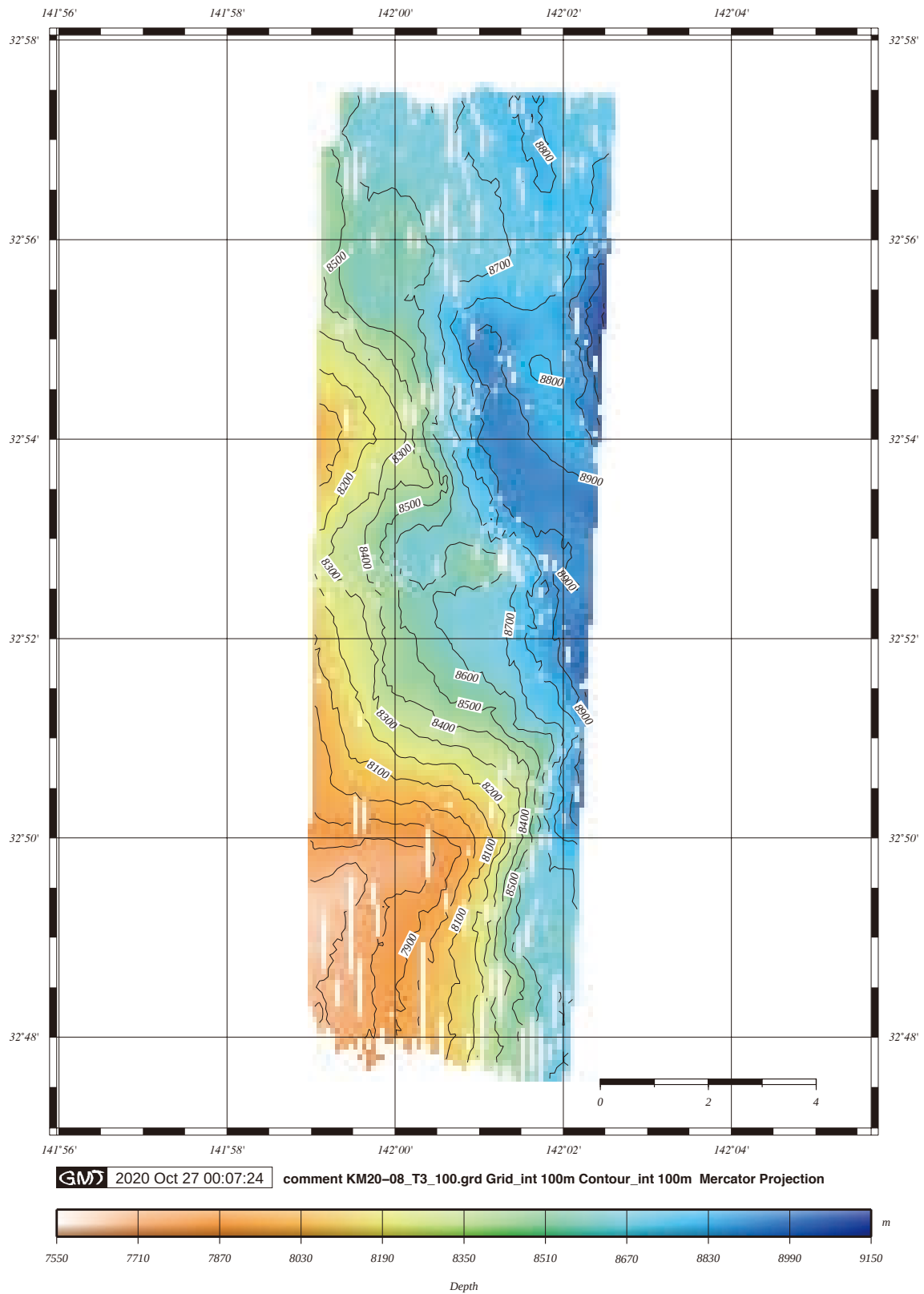
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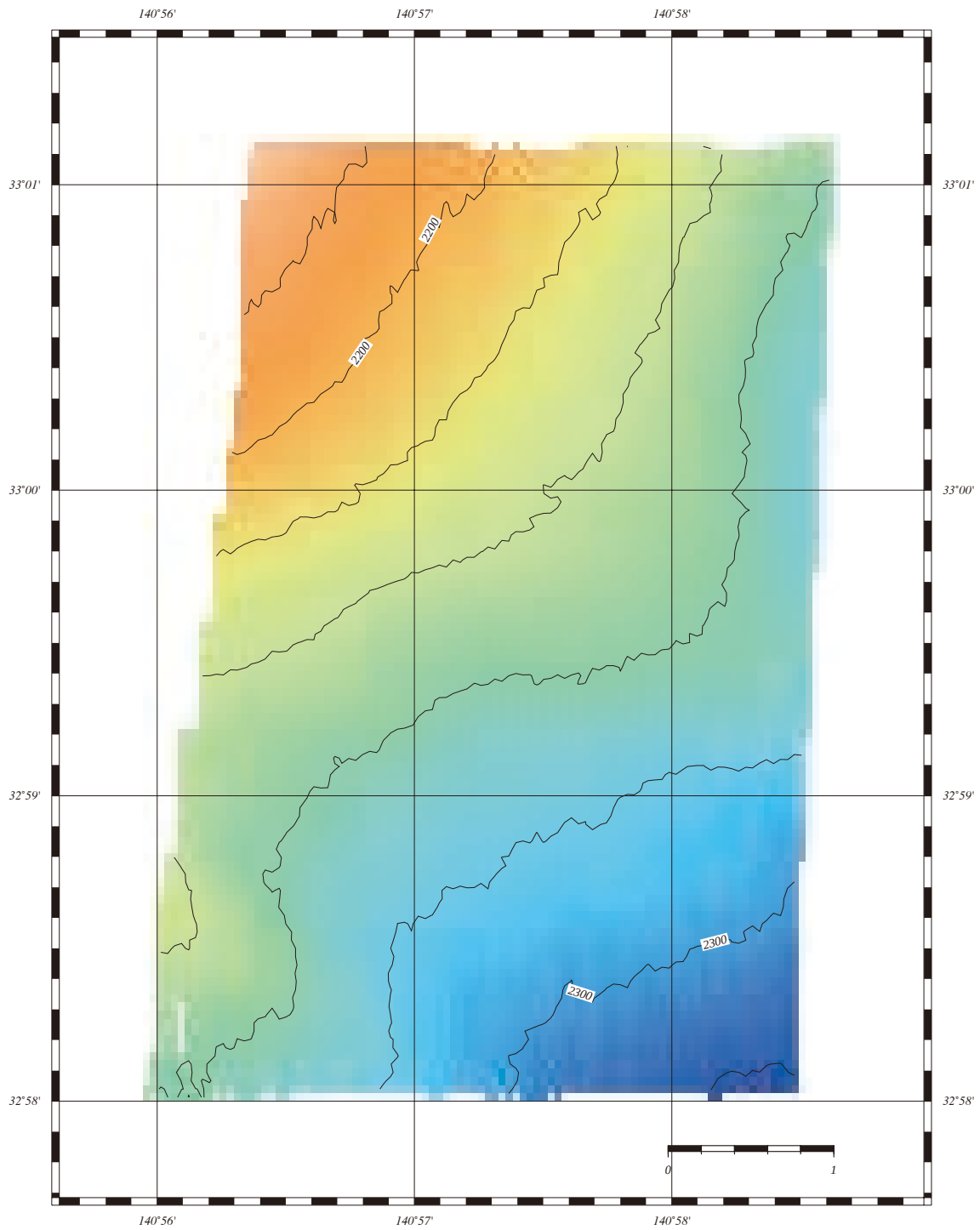
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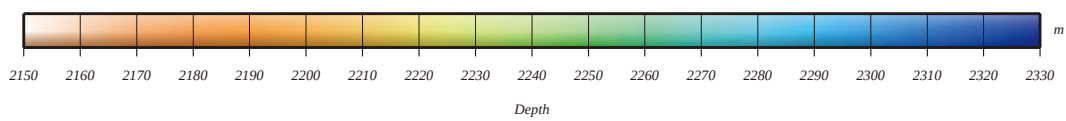
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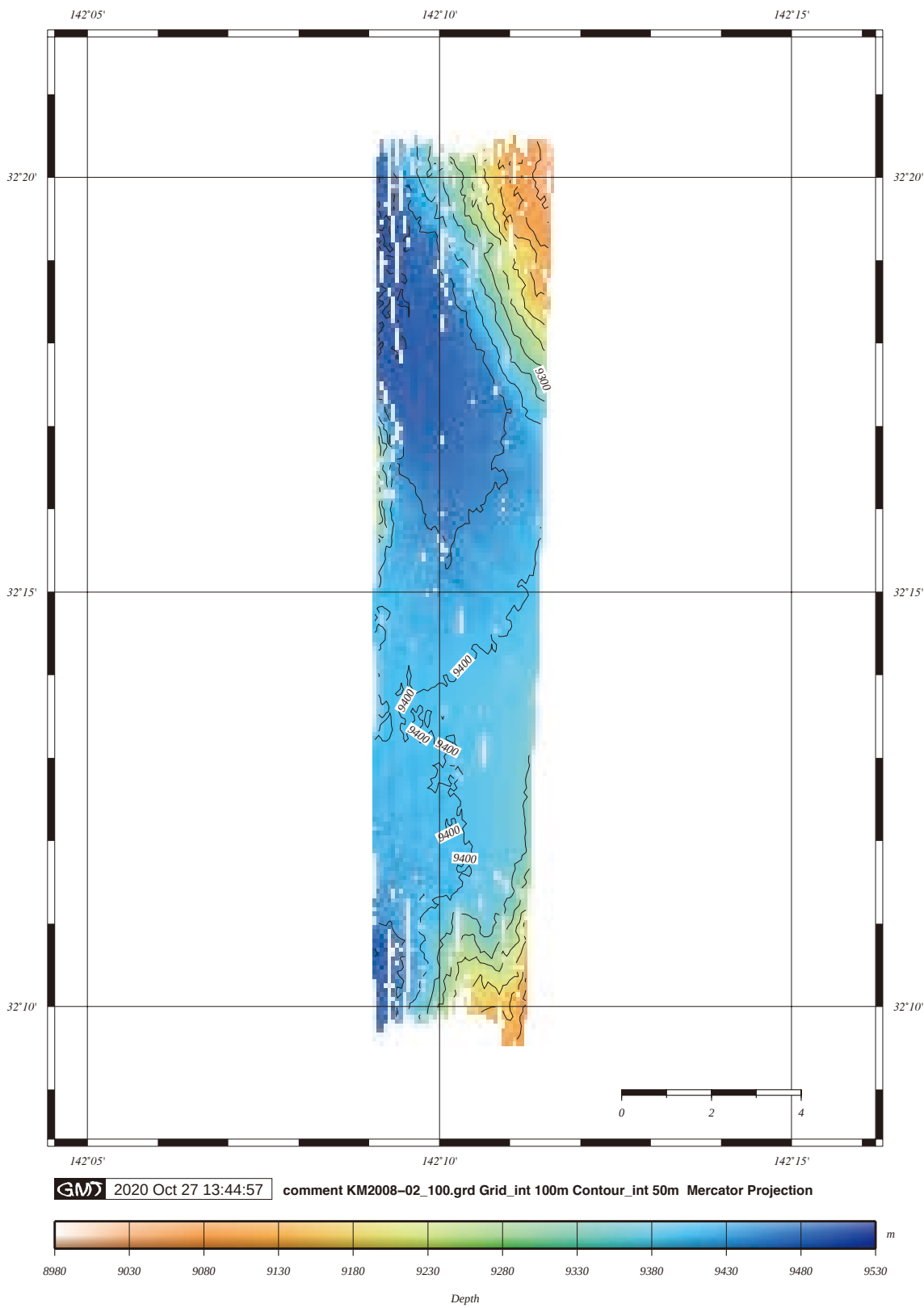
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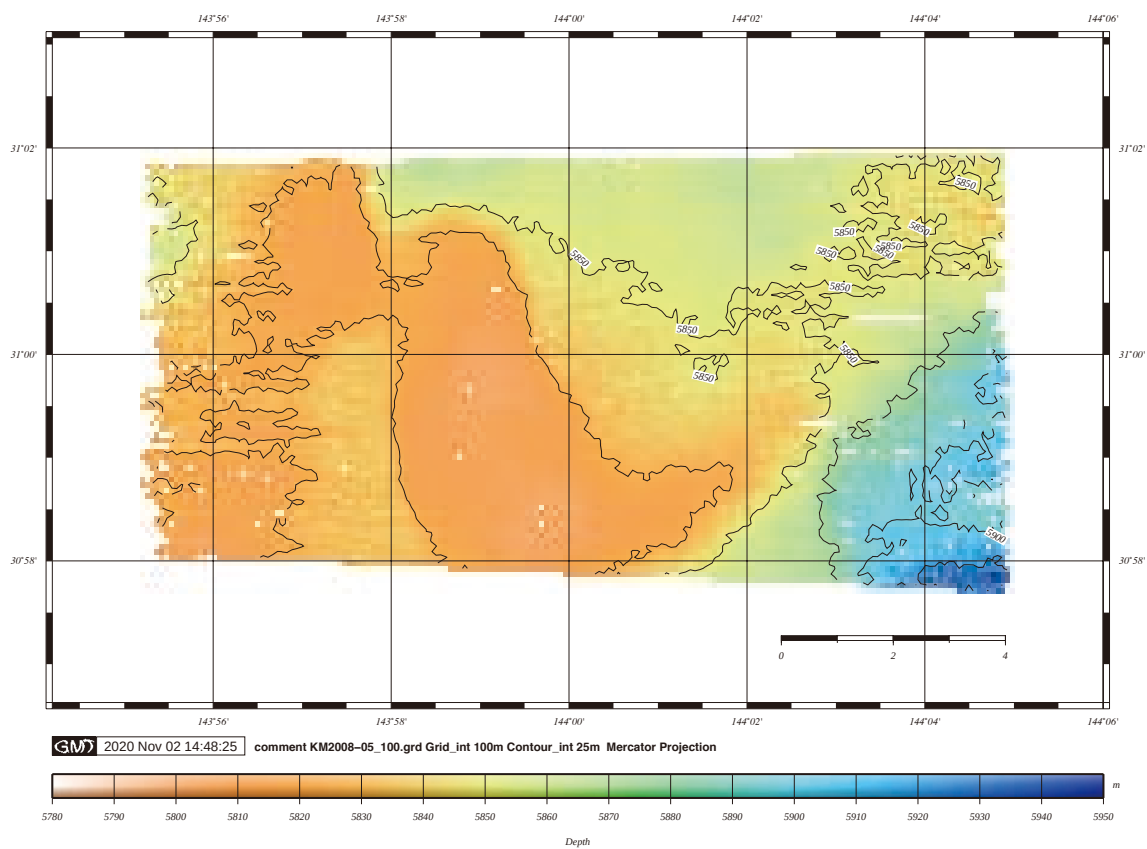
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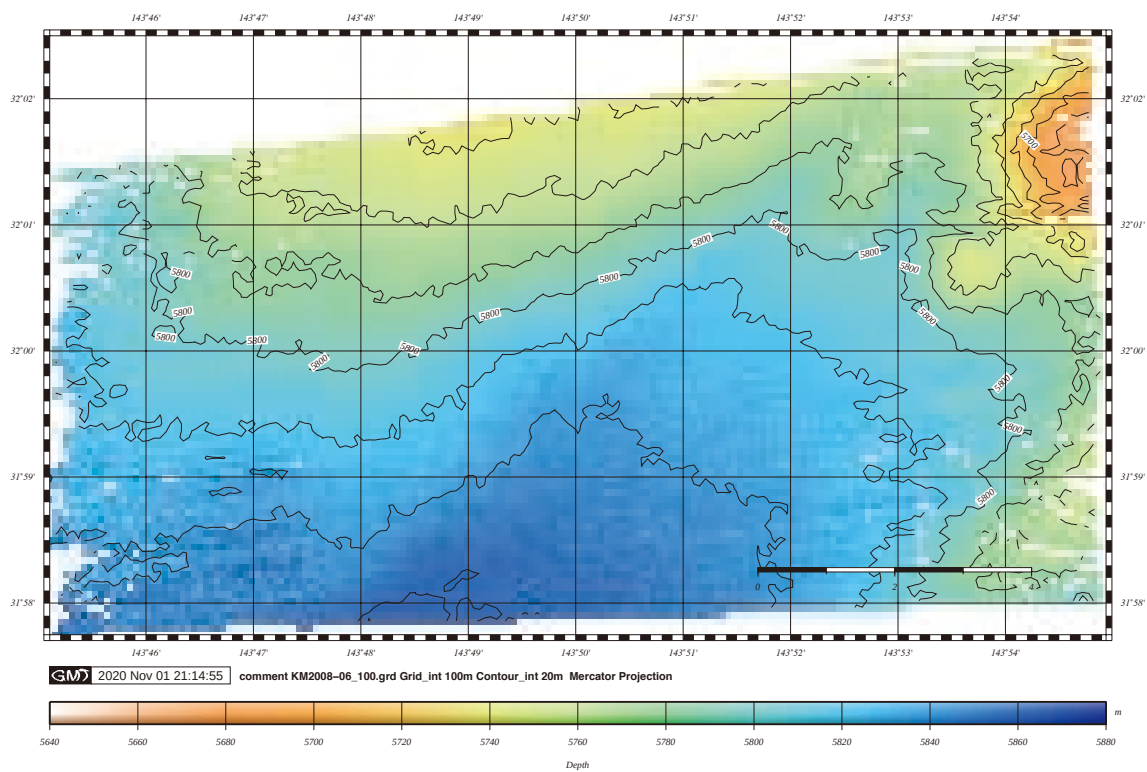
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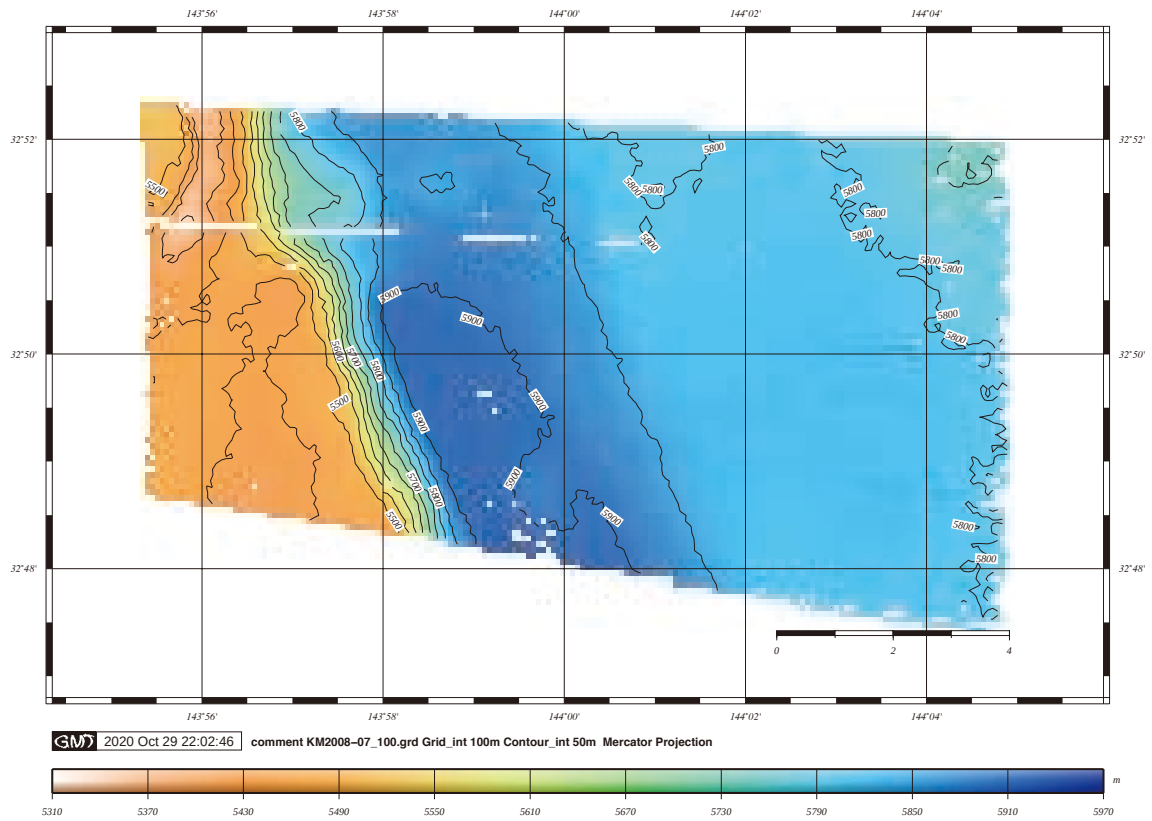
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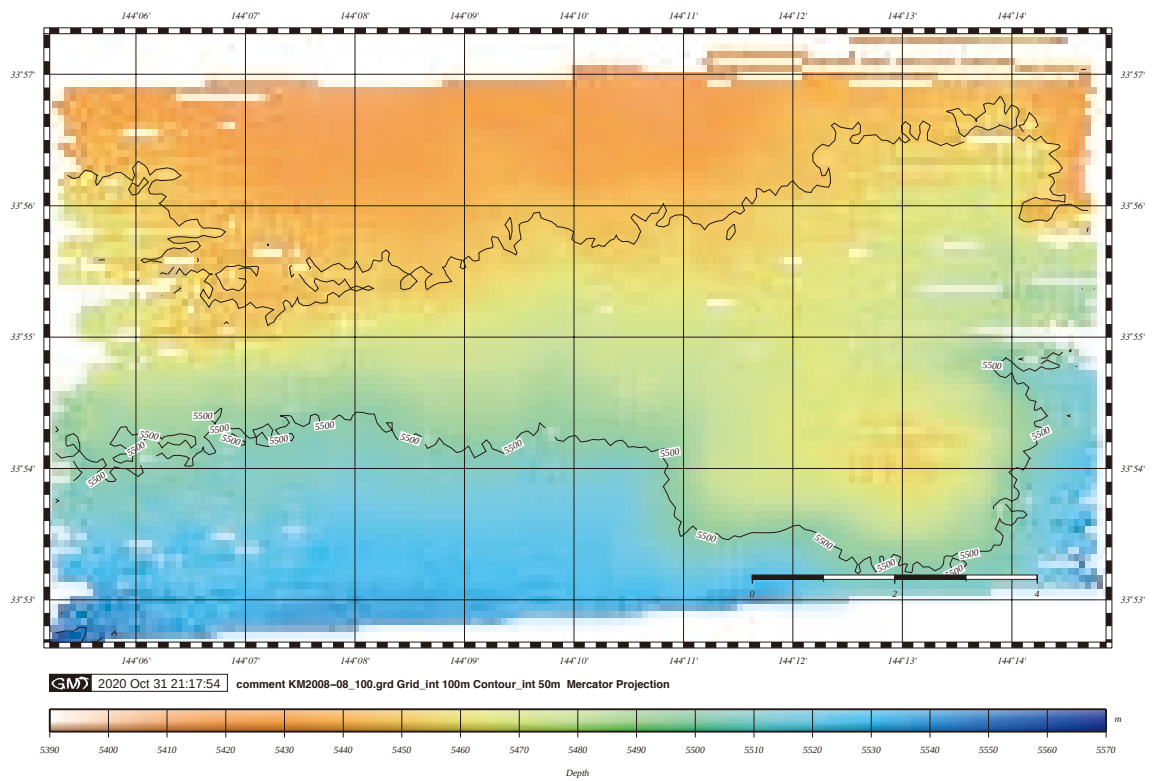
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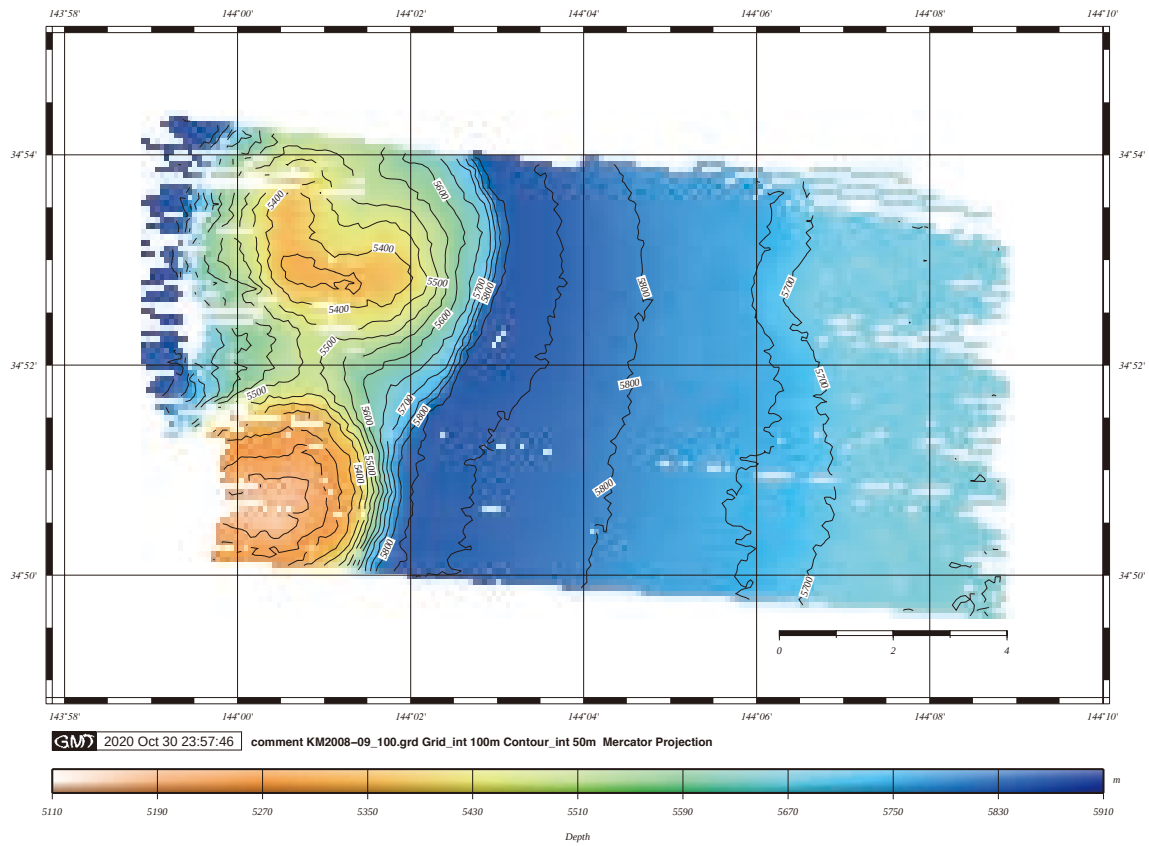
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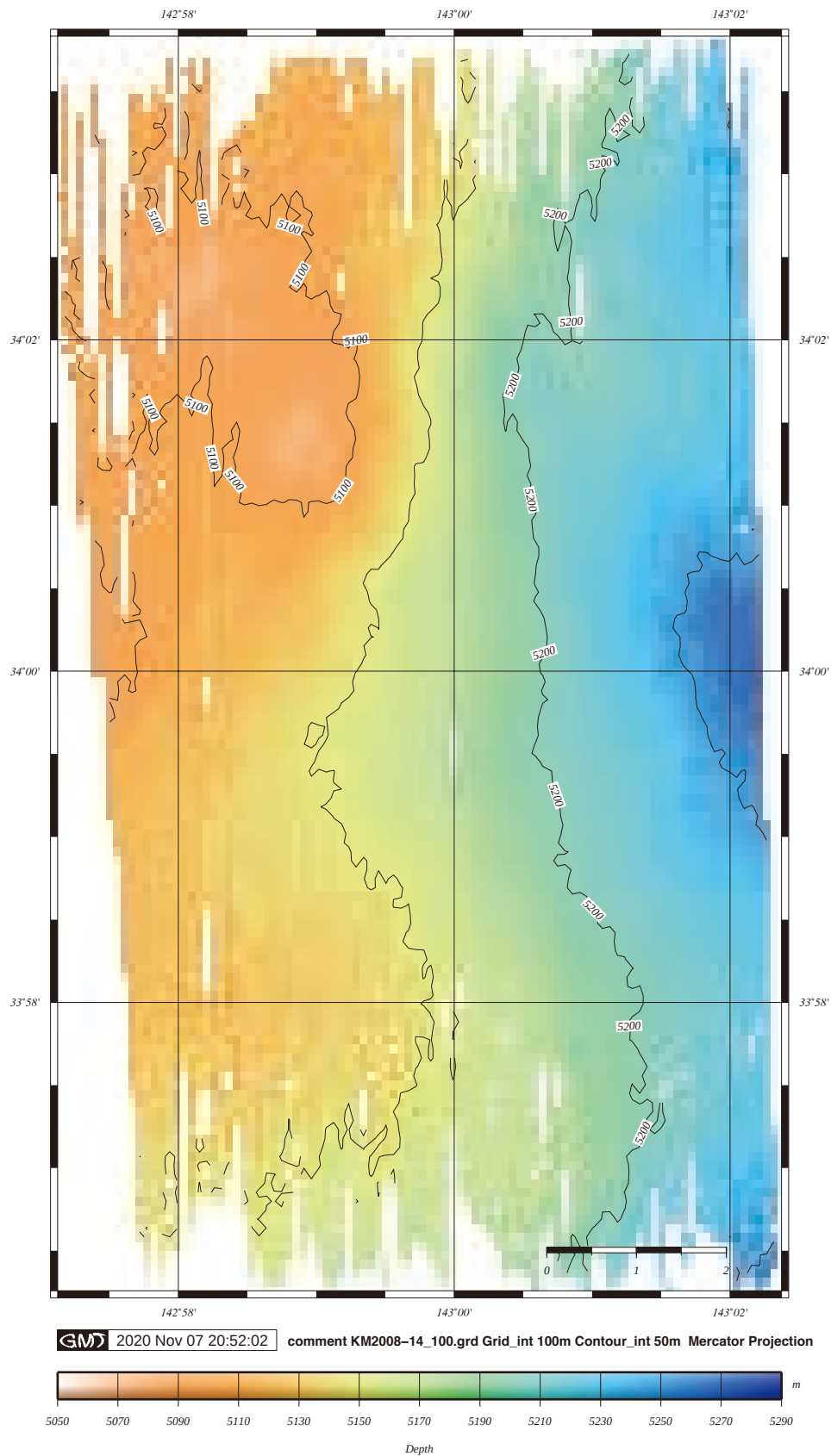
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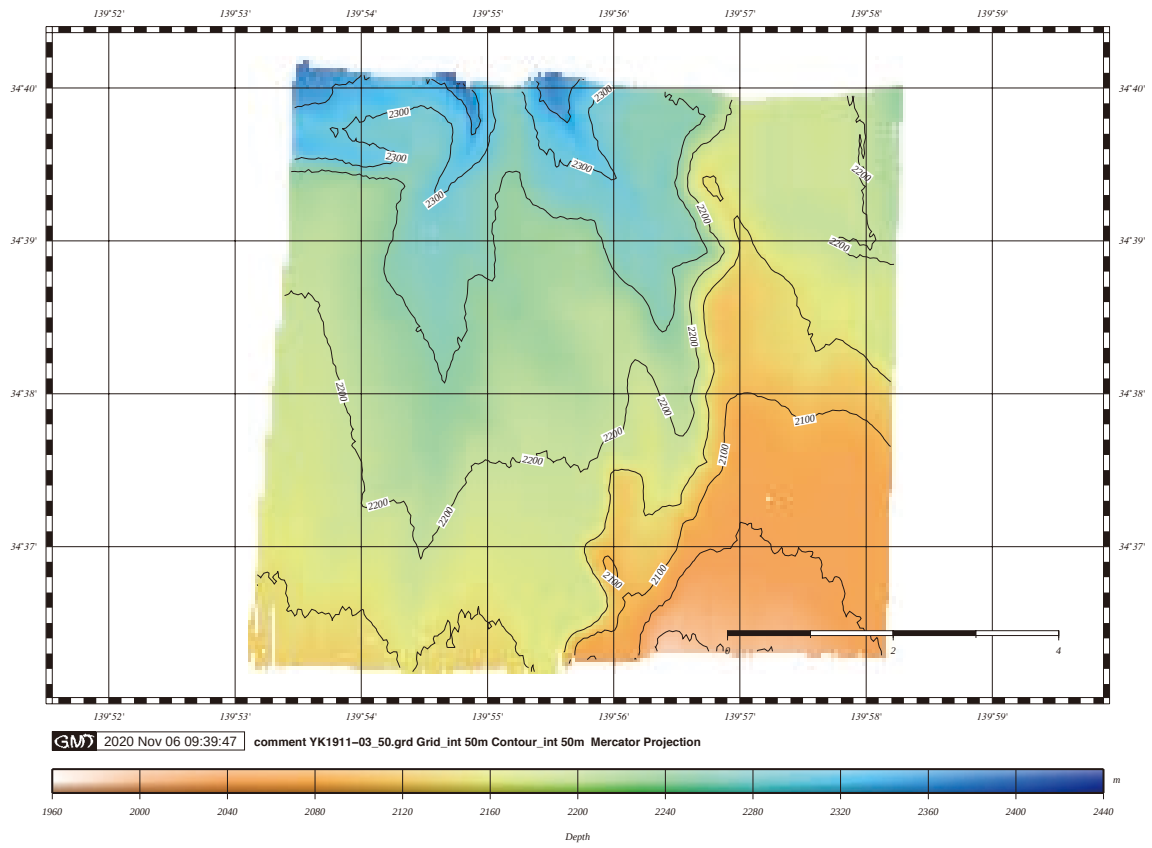
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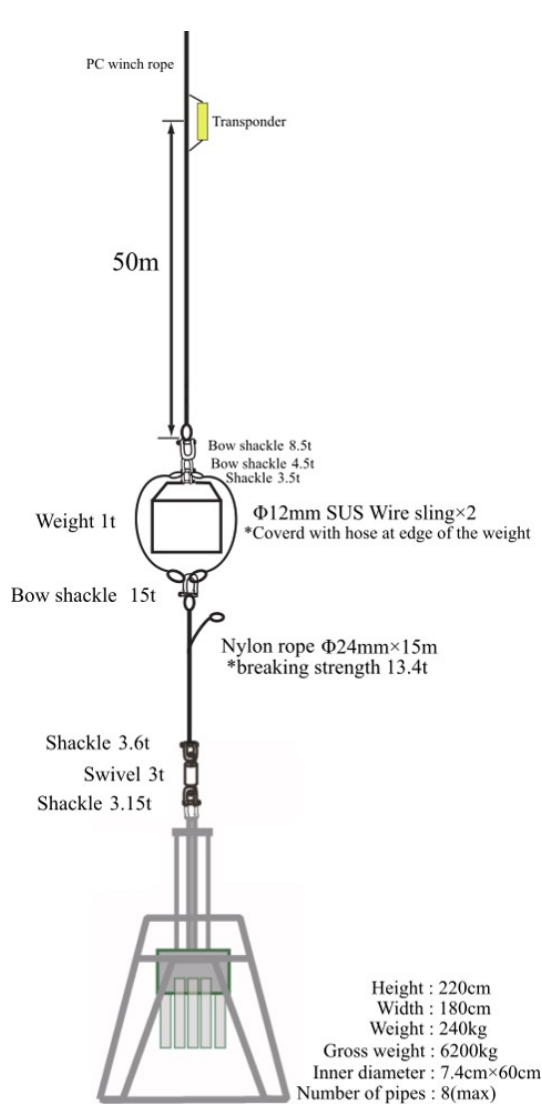
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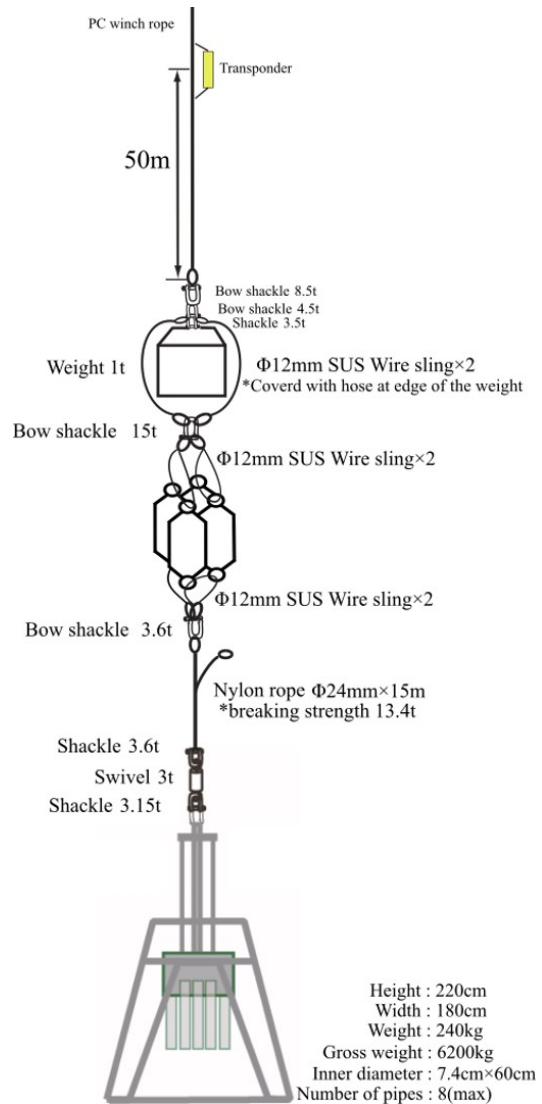
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Multiple core: system configuration



MULTIPLE CORER System



MULTIPLE CORER System