



Yokosuka Cruise Report YK24-11

Understanding the status of marine plastic pollution in the
deep-sea around Japan

Kuril-Japan Trench, Off Cape Erimo

June 30th- July 13th, 2024

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

Acknowledgements

We thank Captain Y. Nakamura and the crew of the R/V YOKOSUKA and the Shinkai 6500 team for organizing the cruise and sampling. We also thank marine technicians from Nippon Marine Enterprises (NME) for research support and ground staff from Japan Agency for Marine-Earth Science and Technology (JAMSTEC) for all the procedures to make this cruise possible.



A group photo of the science party YK24-11

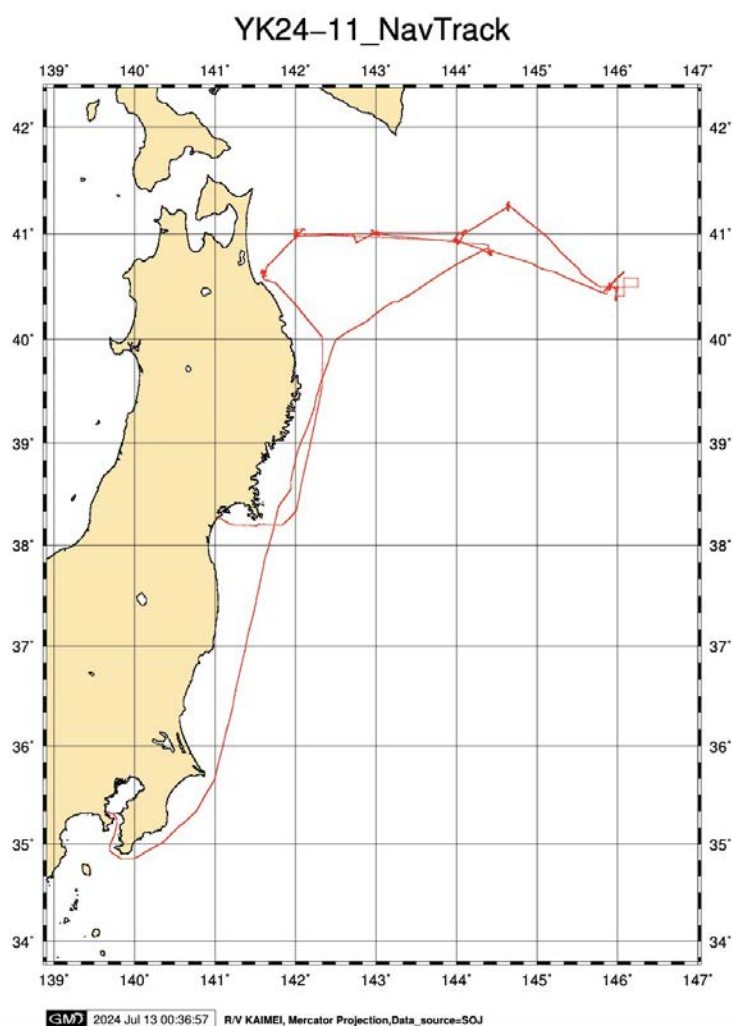
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1. Cruise Information

- Cruise ID: YK24-11
- Name of vessel: R/V Yokosuka
- Title of project/cruise: Understanding the status of marine plastic pollution in the deep-sea around Japan
- Chief Scientist: Ryota Nakajima [JAMSTEC]
- Cruise period: 30th June to 13th July, 2024
- Ports of departure/arrival: Sendai Raijin No. 1/JAMSTEC
- Research area: Kuril-Japan Trench and off Cape Erimo
- Research map
 - Research map (YK24-11 Navigation Track)



2. Science Party

- Title of proposal

Understanding the status of marine plastic pollution in the deep-sea around Japan

- Representative of Science Party [Affiliation]

Ryota Nakajima [Japan Agency for Marine-Earth Science and Technology]

- Science Party [Affiliation]

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Takahito Ikenoue	[Japan Agency for Marine-Earth Science and Technology]
Masashi Tsuchiya	[Japan Agency for Marine-Earth Science and Technology]
Tetsuro Ikuta	[Japan Agency for Marine-Earth Science and Technology]
Shiye Zhao	[Japan Agency for Marine-Earth Science and Technology]
Lixin Zhu	[East China Normal University]
Makoto Ito	[Nippon Marine Enterprise]
Natsune Iho	[Nippon Marine Enterprise]

- Crew

Captain	Y. Nakamura
Chief Officer	Y. Furukawa
2nd Officer	T. Kajisa
3rd Officer	R. Sakamoto
Jr. 3rd Officer	Y. Koyaishi
Chief Engineer	T. Ota
1st Engineer	K. Fujii
2nd Engineer	T. Inomoto
3rd Engineer	Y. Furuyama
Jr. 3rd Engineer	K. Shimizu
Chief Radio Op.	H. Ishiwata
2nd Radio Op.	T. Mabara
Boatswain	K. Hirosaki
Seaman	S. Inoue
Seaman	T. Matuda
Seaman	T. Chimoto
Seaman	N. Ishizuka
Seaman	T. Saga
Seaman	S. Kojima
No.1 Oiler	Y. Higashigawa
Oiler	K. Matsumura
Oiler	A. Dantsuka
Oiler	S. Manna
Oiler	D. Sato
Oiler	K. Ochiai
Chief Steward	K. Kosuji
Steward	T. Asano
Steward	Y. Fujimoto
Steward	T. Tanakaa
Steward	T. Shiraishi

- Shinkai 6500 operating team

Submersible Operation Manager	K. Matsumoto
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Deputy Submersible Operation Manager	T. Kondo
1st Submersible Tec. Officer	A. Ishikawa
1st Submersible Tec. Officer	T. Ohnishi
2st Submersible Tec. Officer	R. Saigo
2nd Submersible Tec. Officer	H. Ueki
2nd Submersible Tec. Officer	K. Suzuki
2nd Submersible Tec. Officer	S. Iijima
2nd Submersible Tec. Officer	G. Matsuzaka
2nd Submersible Tec. Officer	N. Minamino
3rd Submersible Tec. Officer	D. Ichinose
3rd Submersible Tec. Officer	K. Sato

3. Research Activities

3.1. Overall objectives and sampling sites

3.1.1. Objectives

The concentration of marine (floating) plastics in the waters surrounding Japan is known higher than the global average, making the assessment of their distribution, the estimation of their pathways, and the analysis of their behavior a pressing issue. Approximately half of the marine plastic debris that leaks into the ocean is believed to originate from East and Southeast Asian countries. A portion of this debris is carried northward by the Kuroshio Current and transported to the waters surrounding Japan, including the Sea of Japan and the Pacific side. Consequently, the density of microplastics (MPs) floating on the sea surface in these areas is reported to be about 30 times the global average, creating a hotspot for marine debris. A similar situation is anticipated on the seabed around Japan, as even lightweight plastics such as polyethylene and polypropylene, which have a lower specific gravity than seawater, are predicted to sink from the surface water within a short timescale of several months to several years due to biofouling, as evidenced by experiments and simulations.

Recent studies have revealed that the seabed in the recirculation area of the Kuroshio Extension, located about 500 km off the Boso Peninsula, Japan, at depths of approximately 6000m, exhibited higher densities of MPs and macroplastics than the global average due to the sinking of plastics carried by the Kuroshio Current (Nakajima et al. 2021, Tsuchiya et al. 2023). This suggests that the deep-sea floor around Japan forms a significant hotspot for marine debris. Such plastic debris hotspots are not limited to the debris off the Boso Peninsula but are expected to be found in seabeds that are prone to transport by ocean currents. One of the areas with a high likelihood of seeing such accumulations is the "Kuril-Japan Trench and off Cape Erimo." This cruise project for seabed debris survey is focused on this complex region where water masses from the Tsugaru Strait, originating from the Sea of Japan (Tsushima Warm Current), and those detached from the Kuroshio, as well as the Oyashio Current, converge. This convergence likely results in a high accumulation of marine debris originating from the Tsushima Warm Current and the Kuroshio.

The aims of this cruise were to clarify the distribution of MPs and macroplastics on the deep seabed (maximum depth 6500m) in this region, as well as the extent of contamination by hazardous chemicals derived from plastics in benthic organisms. The seabed of this region, where the Tsushima Warm Current and the Kuroshio Current merge, may contain unprecedented amounts of plastic debris, highlighting the need to elucidate the transport processes of debris and propose key monitoring sites.

3.1.2. Sampling sites

The observation of benthic plastic debris on the deep seafloor, as well as sea surface microplastics, was conducted at the abyssal plain west of the Kuril-Japan Trench (St. 1), the Kuril-Japan Trench area (Sts. 2, 3, 5), off Cape Erimo (St. 6) and off Shimokita Peninsula (St. 8) (Table 3.1.).

Table 3.1. Survey sites in the cruise YK24-11. Site numbers in parentheses indicate where no sampling was conducted.

Site	Site name	Depth (m)	Latitude	Longitude
St. 1 (Dive #1792)	West of the Kuril-Japan Trench	5185	40° 30.00'N	146° 00.00'E

St. 2 (Dive#1791)	Kuril-Japan Trench (Mussel site1)	4868	41° 15.2880'N	144° 38.5680'E
St. 3 (Dive#1793)	Kuril-Japan Trench	6454	40° 50.00'N	144° 25.00'E
(St. 4)	Kuril-Japan Trench	5411	41° 00.7308'N	144° 04.906'E
St. 5 (Dive#1790)	Kuril-Japan Trench	4320	40° 00'N	144° 00.00'E
St. 6 (Dive#1794)	Off Cape Erimo	1885	41° 00.00'N	143° 00.00'E
(St. 7)	Off Cape Erimo	961	42° 00.00'N	142° 00.00'E
St. 8 (Dive#1789)	Off Shimokita Peninsula	855	41° 00.00'N	142° 00.00'E
(St. 15)	Kuril-Japan Trench (Mussel site2)	6272	40° 06.50'N	144° 11.00'E

3.2. Observation of plastic debris on the seafloor and microplastic collection

3.2.1. Objectives

Debris is considered to gather at the survey area, the Kuril-Japan Trench area and off Cape Erimo, based on the simulation of the ocean currents, although it has not been well surveyed before. During this cruise, the plastic pollution in this area was thoroughly investigated. This section particularly focuses on macroplastic debris on the seafloor to understand the distribution, numerical density, and volume of the plastic debris. In addition, microplastics were collected during the dives to investigate the relationship between the amounts of macroplastic debris and microplastics.

3.2.2. Methods

Seafloor observations were carried out using JAMSTEC's human-occupied vehicle (HOV) *Shinkai 6500*. During HOV operations, video data were recorded with a forward-facing full HD fixed video camera (Fig. 3.2-1). The 3D mapping video camera was deployed during dives #1789 and #1794. The 3D mapping video camera has synchronized two cameras and can reconstruct 3D images through post-processing. A MASS Pump, a pump with 100 and 20 μ m mesh filters, was mounted on the Shinkai to collect microplastic particles during the dives. During the observations, debris of both natural and man-made origin were collected whenever possible using either manipulators or a suction sampler. Retrieved plastic debris were photographed on board. A portion of the debris was sub-sampled for further analysis of polymer type and associated organisms.



Fig. 3.2-1 Cameras and pump on the Shinkai.

3.2.3. Onboard results

Cans, PET bottles, and film-like fragments were common plastic debris observed during the dives. The production time could be estimated for some of the collected plastic debris, and it varied from recent to over 20 years old. Although the 3D mapping video camera worked fine during both dives, the water was unclear at St. 6 (#1794) due to a significant amount of particulate matter, which affected the image quality of the 3D mapping camera. Examples of the

image taken using the 3D mapping camera during #1789 are shown in Figure 3.2-2. Microplastics were successfully collected using the MASS Pump.



Fig. 3.2-2. Example of the mapping image. The object seen at the left bottom is a can.

3.2.4. Future plan

The videos from the full HD camera will be played onshore and all items of debris, both natural and man-made objects, will be identified through video analysis for the entirety of each dive. The obtained data will be submitted to JAMSTEC's Data Management Group (DMG). The 3D mapping camera images will be used for number density ($\#/m^2$) and volume analysis of the debris. Microplastics collected on the filters using the MASS Pump will be measured using Raman spectroscopy-based methods.

3.3. Distribution of microplastics in the deep-sea sediment

3.3.1. Objectives

The area off the coast of Shimokita Peninsula, the targeted area of this cruise, is located downstream of the Tsushima Current and is an important area for clarifying the extent and path of garbage deposition through the Japan Sea. Although garbage from Japan, Korea, China, and other countries washes ashore along the Japan Sea coast, the amount and source are different from those on the Pacific side. Therefore, the microplastics (MPs) in the sediments may also have different origins than those on the Pacific side, and may differ in quantity shape, and material type. In order to estimate the actual distribution and pathways of MPs compared to those under the influence of the Kuroshio Current, sediment core samples were collected using the H-type push corer and then analyzed for MPs.

3.3.2. Material and Methods

Sediment samples were collected by the H-type push corer (covering 52.8 cm² seafloor surface area) at Stations #1, 2, 3, 5, 6 and 8 (Table 3.3-1). We used two types of the core, polycarbonate and aluminum-made tubes. Aluminum-made tubes were used in this study for microplastic analysis to prevent plastic contamination.

3.3.3. Onboard results

Three sediment cores were collected with aluminum-made core tube for MP analysis, and two sediment cores collected with polycarbonate tube for sediment structure analysis with X-CT analysis and meiobenthos analysis, respectively. A sediment core sample was sliced every 1 cm down to the bottom of the core on board.

3.3.4. Future plan

Sediment samples will be treated according to the procedure described by Tsuchiya et al. (2024) and extracted MPs will be analyzed under a μ FTIR. For sediment structure analysis, we will

conduct X-CT analysis and grain size measurements to clarify the MPs source and transport processes using these data.

Table 3.3-1. List of sediment samples for MPs analysis.

St	Dive	Core		Date & time	Lat. (N)	Long. (E)	Depth (m)	Site	Length (cm) *
8	1789	Blue	1	Jul. 3, 2024 10:58 JST	40-59.927 0	142-0.7 188	860	Off Shimokit a Peninsula	19 (3)
		Blue	2						20 (20)
		Yellow w	1	Jul. 3, 2024 11:08 JST	40-59.927 0	142-0.7 153			20 (19)
		Yellow w	2						24 (23)
		Yellow w	3						21 (20)
5	1790	Blue	3	Jul. 4, 2024 12:10 JST	40-56.042 1	144-0.7 140	4323	Japan Trench, land side slope	23 (3)
		Blue	4						21 (21)
		Yellow w	1						22 (21)
		Yellow w	2						23 (22)
		Yellow w	3						21 (20)
2	1791	Blue	1	Jul. 5, 2024 11:56 JST	41-15.280 1	144-38. 5595	4821	Chishima Trench, land side slope	23 (3)
		Blue	2						21 (21)
		Yellow w	1						22 (21)
		Yellow w	2						23 (22)
		Yellow w	3						21 (20)
1	1792	Blue	1	Jul. 7, 2024 11:33 JST	40-29.927 3	145-54. 0184	5181	Off Shimokit a Peninsula , abyssal plain	19 (3)
		Blue	2						18 (18)
		Yellow w	1						17 (16)
		Yellow w	2						17 (16)
		Yellow w	3						16 (15)
3	1793	Blue	1	Jul. 8, 2024 12:05 JST	40-49.653 0	144-25. 6609	6413	North end of the Japan Trench, land side slope	20 (3)
		Blue	2						18 (18)
		Yellow w	1						21 (20)
		Yellow w	2						26 (25)
		Yellow w	3						24 (23)
6	1794	Blue	1	Jul. 9, 2024 11:12 JST	41-0.0637	143-1.7 637	1877	Off Erimomi	20 (3)
		Blue	2						18 (18)

		Yello w	1					saki	15 (14)
		Yello w	2						18 (17)
		Yello w	3						15 (14)

* Sampled length (Retrieved length)

3.4. Sediment, water and plastic sampling for eDNA extraction

3.4.1. Objectives

The sinking of plastic debris increasingly appears to be an important process in the global fate of plastic in the ocean. However, there is insufficient knowledge about the processes changing plastic debris and how these inputs of plastic substrate select pelagic microorganisms with specific features to colonize this new habitat, especially in deep-sea environments. Over the past decade, the number of studies on the plastisphere has increased. However, efforts have been concentrated mainly on pelagic systems or shallow benthic systems. Only a few studies, however, have analyzed the non-incubated plastisphere in deep-sea habitats. Furthermore, the differences in microbial communities on plastics versus other common deep-sea litter [e.g., metal and glass] are still unknown.

In this project, we collected deep-sea plastic debris and perform incubation experiments based on the Shinkai 6500 platform to test hypothesizes that plastic substrates could select unique microbes from surrounding environments compared to other compartments.

3.4.2. Methods

The search of plastic debris was conducted by Shinkai 6500. Once a piece of plastic was picked up by the manipulator of Shinkai 6500, the bottom seawater samples and sediment samples were collected with 4 Niskin bottles (two 12-L and two 5-L bottles) and 2 H-type push polycarbonate corer (covering 52.8 cm² seafloor surface area) at Stations #1, 2, 3, 5, 6 and 8. The plastic debris closely relate to the seawater and sediment samples were collected at Sts. 5, 1 and 6.

3.4.3. Onboard results

The plastic debris found was cut with the sterile scissors. One pieces of plastic was saved with RNAlater and another pieces of plastic was fixed with 4% Paraformaldehyde for 1-2 hours and then stored in a solution of PBS:Ethanol (1:1) at 4 °C. At each dive station, all the seawater collected in the 4 Niskin bottles was filtered through a 0.22-µm Sterivex filter and stored frozen until further analysis. The top 5 cm of sediments from two polycarbonate cores were collected at each station. The two sediment samples were placed into two sterile zip bags and frozen until DNA extraction.

3.4.4. Future plan

eDNA of the samples including plastic, seawater and sediment will be extracted in the laboratory and analyzed with the shotgun sequencing. Microbiomes in terms of community composition and function potential among three environmental compartments will be compared.

3.5. Distribution of microplastics in the surface water

3.5.1. Objectives

Monitoring the diffusion of microplastics (MPs) from the Sea of Japan through the Tsugaru Straits into the Pacific Ocean.

3.5.2. Materials and Methods

Floating MPs surveys were conducted from the surface water in the Kuril-Japan Trench (St. 1, 3, and 5), off Shimokita Peninsula (St. 8), and off Cape Erimo (St. 6). Floating MPs samples were collected using a neuston net with a perpendicular mouth opening of 75 cm height and 100 cm width, equipped with a 333 μ m mesh opening net. The net, without cod-end, was rinsed from the outside with running seawater prior to use. At each station, the net was towed three times at ca. 1-2 knots 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. Selected floating MPs samples were picked up and provided for environmental DNA analysis in the section 3.4. Surface seawater samples were collected by a bucket at the same stations as the neuston net tows to obtain background samples for DNA analysis of microbial community composition on MPs.

3.5.3. Onboard results

Locations and summaries of stations for this study are shown in Tables 3.5-1 and 3.5-2.

3.5.4. Future plan

Microplastic samples will be subjected to enumeration and identification of plastic types using a microscope and FT-IR. The distribution and concentration of microplastics will be studied and compared with the distribution of deep-sea sediments to reveal microplastic hotspots in the seas around Japan. DNA analysis of the biofilm on the MPs will be conducted to determine the microflora on the MPs and their function, and the spread of the plastisphere into the Pacific Ocean by the floating MPs will be studied (see 3.4 for details).

Table 3.5-1. Time and position of the neuston net sampling.

Station No.	Cast No.	Date (UTC)	Start time (UTC)	End time (UTC)	Start Latitude	Start Longitude	End Latitude	End Longitude
8	Cast1	7/2	0:20	0:42	41-00.2803N	142-00.3174E	40-59.6595N	141-59.8295E
	Cast2	7/2	1:01	1:21	41-00.3448N	142-00.1826E	40-59.8943N	141-59.7747E
	Cast3	7/2	1:29	1:49	40-59.8075N	141-59.7720E	40-59.3122N	141-59.3090E
6	Cast1	7/2	6:15	6:35	40-59.8698N	143-01.4199E	40-59.8530N	143-00.5595E
	Cast2	7/2	6:43	7:03	40-59.8339N	143-00.5254E	40-59.7723N	142-59.6515E
	Cast3	7/2	7:10	7:31	40-59.7460N	142-59.5608E	40-59.6350N	142-58.6871E
1	Cast1	7/5	23:15	23:36	40-30.1837N	145-59.7012E	40-29.9653N	145-59.8148E
	Cast2	7/5-7/6	23:44	0:04	40-30.0686N	145-59.8219E	40-29.8768N	145-59.8555E
	Cast3	7/6	0:13	0:34	40-29.9893N	145-59.8401E	40-29.7550N	145-59.7886E
5	Cast1	7/9	23:12	23:32	40-54.3810N	144-01.1496E	40-54.8961N	144-00.3442E

	Cast2	7/9-7/10	23:40	0:00	40-55.0981N	144-00.0348E	40-55.6089N	143-59.2432E
	Cast3	7/10	0:06	0:26	40-55.7631N	143-59.0018E	40-56.2955N	143-58.1781E
3	Cast1	7/10	2:39	2:59	40-48.4987N	144-25.7047E	40-49.2705N	144-25.1047E
	Cast2	7/10	3:07	3:27	40-49.5935N	144-24.8736E	40-50.3951N	144-24.3052E
	Cast3	7/10	3:34	3:54	40-50.6732N	144-24.1190E	40-51.4837N	144-23.5915E

Table 3.5-2. Summary of the surface seawater sampling by buckets and number of microplastics picked up from the neuston net samples for environmental DNA analysis

Station No.	Cast No. of neuston net tow	Surface seawater sampling by buckets (L)		Number of MPs picked up for environmental DNA analysis (pieces)
		A	B	
8	Cast1	4	4	1
	Cast2			1
	Cast3			0
6	Cast1	2	2	1
	Cast2			1
	Cast3			0
1	Cast1	2	2	1
	Cast2			1
	Cast3			0
5	Cast1	2	2	0
	Cast2			0
	Cast3			0
3	Cast1	2	2	1
	Cast2			1
	Cast3			0

3.6. Physiological impacts of plastics on deep-sea megabenthic animals

It is becoming obvious that the abundance of microplastics is increasing in worldwide oceans, raising concerns about their impact on marine ecosystems. As microplastics occupy a similar size range as some planktons, suspended particles, and marine sediments, they are potentially bioavailable to many organisms. Animals living in the deep seas are no exception. However, the distribution of microplastics in deep-sea animals and their physiological effects are poorly understood. We have recently reported that microplastic particles are internalized via the body surface into the gill cells of deep-sea mussels by phagocytosis (Ikuta et al., 2022). Furthermore, polychlorinated biphenyls, well-known persistent chemical pollutants, were detected in deep-sea

chemosynthetic bivalves with limited or no filter feeding, including vesicomid clams and *Bathymodiolus* mussels, suggesting the possibility of contamination by absorbing organic particles such as pollutant-associated microplastics from the body surface (Ikuta et al., 2021; Ikuta et al., 2024). These observations highlighted the need for further detailed investigations to conserve highly endemic and vulnerable deep-sea fauna.

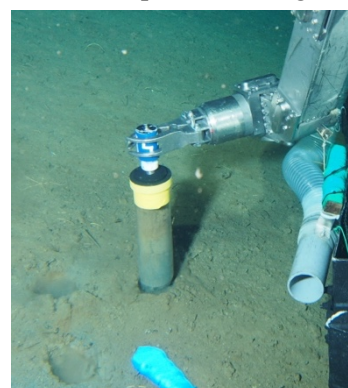
During this cruise, we collected some benthic animals to investigate the distribution of chemical contaminants associated with plastics in these animals and the physiological impacts of plastics on them.

References

- Ikuta, T., Nakajima, R., Tsuchiya, M., Chiba, S., and Fujikura, K. (2021). Interdecadal distribution of persistent organic pollutants in deep-sea chemosynthetic bivalves. *Front. Mar. Sci.* 8, 1735. doi: 10.3389/fmars.2021.751848.
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- Ikuta, T., Tame, A., Takahashi, T., Nomaki, H., and Nakajima, R. (2022). Microplastic particles are phagocytosed in gill cells of deep-sea and coastal mussels. *Front. Mar. Sci.* 9, 1034950. doi: 10.3389/fmars.2022.1034950.

3.7. H-type push core sampling – POPs contamination analysis

Some organic compounds are resistant to environmental degradation and can accumulate in organisms, thereby causing harmful effects. These chemicals are called persistent organic pollutants (POPs) and they have been of great academic and social interest since the late 20th century. We recently reported that polychlorinated biphenyls (PCBs), a long-known POPs, were detected in deep-sea bivalves in Sagami Bay, located near a highly populated region (Ikuta et al., 2021). Furthermore, despite a previous observation of very low levels of marine debris in Myojin Knoll (Nakajima et al., 2021), which is a relatively remote area located far from dense human activity, our recent report showed that PCB contamination in deep-sea mussels collected from this area is comparable to that in *Phreagena* clams collected from Sagami Bay (Ikuta et al., 2021). In our most recent study, PCBs were also detected in the sediments of the *Phreagena* clam habitat in Sagami Bay and were found to accumulate in their ovaries, raising concerns about a potentially severe direct impact on the survival of vulnerable and highly endemic deep-sea chemosynthetic bivalves (Ikuta et al., 2024).



To investigate the contamination of chemosynthetic habitats in this site by POPs recently listed in the Stockholm Convention, in this cruise we have collected bottom sediment samples from these areas using an H-type push corer. On the ship, the outer parts of the sediment cores that had contacted the polycarbonate tube during sampling were trimmed off. The samples were stored in clean glass bottles at -20°C . After the cruise, the amounts of POPs will be analyzed.

References

- Ikuta, T., Nakajima, R., Tsuchiya, M., Chiba, S., and Fujikura, K. (2021). Interdecadal distribution of persistent organic pollutants in deep-sea chemosynthetic bivalves. *Front. Mar. Sci.* 8, 1735. doi: 10.3389/fmars.2021.751848.
- Ikuta, T., Nomaki, H., and Nakajima, R. (2024). Accumulation of polychlorinated biphenyls in

the ovaries of deep-sea chemosynthetic clam *Phreagena okutanii*. *Front. Mar. Sci.* 11. doi: 10.3389/fmars.2024.1358774.

Nakajima, R., Tsuchiya, M., Yabuki, A., Masuda, S., Kitahashi, T., Nagano, Y., et al. (2021). Massive occurrence of benthic plastic debris at the abyssal seafloor beneath the Kuroshio Extension, the North West Pacific. *Mar. Pollut. Bull.* 166, 112188. doi: 10.1016/j.marpolbul.2021.112188.

3.8. Microbes cope with plastic-derived DOM in natural ocean environment

3.8.1. Objectives

During the plastic-marine-debris life cycle, a variety of synthetic chemicals and hydrocarbons are released into the ambient environment as plastic-derived dissolved organic material (P-DOM). As an extra and anthropogenic carbon source, P-DOM could be a potential energy source for heterotrophs that can either promote or inhibit microbial growth, especially in oligotrophic ocean regions, consequently influencing ocean ecosystem health and biogeochemical cycling. However, responses of marine microorganisms to P-DOM are still limitedly known. A deeper understanding of the pertinent traits of marine microbiomes coping with P-DOM would provide critical information to assess ecosystem health in the form of insight into the adaptation of microorganisms to small environmental changes, thanks to their rich genetic pool.

3.8.2. Materials and Methods

In-situ incubation experiments including two treatments and two controls were set up at one nearshore station and one offshore station (Fig. 3.8-1). Briefly, 3 L surface seawater collected at the station was filled into a 5-L clean glass bottle (n=4). Two 5-L clean glass bottles were added 40 ml P-DOM (Tyre) and regarded as positive treatments; Two 5-L clean glass bottles were each added with 40 ml of 0.22- μ m seawater and used as controls. Four bottles were placed in a surface seawater flow-through system on the deck for 72 h. Then the microbes in each bottle were filtered on a Sterivex filter, fixed with *RNAlater* and frozen stored. 20 ml filtrate was acidified and saved for DOC analysis. The remained filtrate was passed through a PPL column for chemical structure analysis.



Fig. 3.8-1. The incubation experiment on deck.

3.8.3. Onboard results

Incubation experiments were performed at St. 8 and St.1, representing the nearshore and offshore sea conditions, respectively. For each incubation experiment, samples for cell counts, eDNA, DOC, and chemical structure analysis were saved before and after the incubation.

3.8.4. Future plan

In the lab, cell counts will be confirmed with the flow cytometer. Shotgun sequencing will be employed to analysis the microbial communities and function potential. DOC will be analyzed by a TOC analyzer, and structures of organic matters will be identified by FTICR-GC-MS.

○ 4. Cruise Log

R/V "YOKOSUKA" YK24-11 Cruise Log		
Date & Time	Description	Weather / Wind / Sea Condition
2024/06/30 Sun.	Noon Position: 38-15.6N,141-55.1E(Off east of Kinkazan)	f / South-5 / 3
8:00	Onboard "YOKOSUKA" at SENDAI SHIOGAMA-KO	
9:00	Let go all shore lines & left SENDAI SHIOGAMA-KO for research area	
10:00-10:30	Carried out education and training for scientists.	
13:10-16:00	SHINKAI 6500 team and Scientists meeting.	
18:00-18:10	Research meeting	
21:45	Arrived at Hachinohe-ko off.	
2024/07/01 Mon.	Noon Position: 40-38.9N,141-36.7E (Off Hachinohe-ko)	bc / West-6 / 4
	Moored at off Hachinohe-ko due to bad weather.	
09:00-10:00	6K briefing.	
13:30	6K Dive meeting.	
18:00-18:10	Research meeting	
2024/07/02 Tue.	Noon Position: 40-58.7N,142-11.2E (Off east of SHIMOKITA HANTO)	b / West-6 / 4
4:30	Arrived at dive point off SHIMOKITA HANTO(St.8)	
5:18	Released XBT at 41-02.6950N, 142-06.1668E	
5:46-7:30	MBES mapping survey .	
9:00	Suspended SHINKAI 6500 submergence due to rough sea.	
9:20-10:49	Carried out Neustone net sampling (St.8)	
15:15-16:31	Carried out Neustone net sampling (St.6)	
16:40	Left research area St.6, then proceeded to research area St.8.	
18:00-18:10	Research meeting	
20:30	Arrived at St.8.	
2024/07/03 Wed.	Noon Position: 41-00.2N,142-00.7E (Off east of SHIMOKITA HANTO)	c / West-6 / 3
9:43	Hoisted up SHINKAI6500	
9:50	Launched SHINKAI6500	
9:57	SHINKAI6500 dove and started her operation #1789	
10:38	SHINKAI6500 landed on the sea bottom (D=860m)	
16:20	SHINKAI6500 left the sea bottom (D=835m)	
15:40	SHINKAI6500 floated	
16:55	Hoisted up SHINKAI6500	
17:04	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.8, then proceeded to research area St.5.	
18:00-18:10	Research meeting	
2024/07/04 Thu.	Noon Position:40-56.0N,144-01.0E (East slope of the Japan trench,submarin canyon2)	o / West-4 / 2
6:20	Released XBT at 40-57.1N, 143-58.4E	
6:40-7:03	MBES mapping survey .	
9:42	Hoisted up SHINKAI6500	
9:49	Launched SHINKAI6500	
9:56	SHINKAI6500 dove and started her operation #1790	
11:56	SHINKAI6500 landed on the sea bottom (D=4,324m)	
15:24	SHINKAI6500 left the sea bottom (D=4,313m)	
17:00	SHINKAI6500 floated	
17:16	Hoisted up SHINKAI6500	
17:25	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.5, then proceeded to research area St.2.	
18:00-18:10	Research meeting	
20:30	Arrived at research area St.2.	
2024/07/05 Fri.	Noon Position:41-15.1N,144-38.7E (East slope of the Japan trench1)	bc / NNW-3 / 2
5:23	Released XBT at 41-17.7N, 144-38.9E	
5:43-6:21	MBES mapping survey .	
8:40	Hoisted up SHINKAI6500	
8:47	Launched SHINKAI6500	
8:54	SHINKAI6500 dove and started her operation #1791	
11:06	SHINKAI6500 landed on the sea bottom (D=4,876m)	
15:22	SHINKAI6500 left the sea bottom (D=4,674m)	
17:03	SHINKAI6500 floated	
17:20	Hoisted up SHINKAI6500	
17:28	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.2, then proceeded to research area St.1.	
18:00-18:10	Research meeting	

2024/07/06 Sat.	Noon Position: 40-30.0N,145-54.2E (Japan Trench)	r/ South-4 / 2
3:30	Arrived at research area (St.1)	
8:15-9:34	Carried out Neustone net sampling (St.1)	
10:47	Released XBT at 40-27.6N, 145-58.8E	
11:26-12:39	MBES mapping survey .	
18:00-18:10	Research meeting	
21:51-22:12	Carried out figure eight turn measurement.	
2024/07/07 Sun.	Noon Position: 40-30.2N,145-54.1E (Japan Trench)	r/ SSW-3 / 2
8:43	Hoisted up SHINKAI6500	
8:49	Launched SHINKAI6500	
8:57	SHINKAI6500 dove and started her operation #1792	
11:16	SHINKAI6500 landed on the sea bottom (D=5,181m)	
15:05	SHINKAI6500 left the sea bottom (D=5,182m)	
17:00	SHINKAI6500 floated	
17:18	Hoisted up SHINKAI6500	
17:26	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.1, then proceeded to research area St.3.	
18:00-18:10	Research meeting	
23:00	Arrived at dive point research area (St.3)	
2024/07/08 Mon.	Noon Position:40-49.8N,144-25.8E (East slope of the Chishima trench1)	bc/ SW-4 / 2
5:24	Released XBT at 40-50.2N, 144-27.6E	
5:42-6:07	MBES mapping survey .	
8:48	Hoisted up SHINKAI6500	
8:54	Launched SHINKAI6500	
9:01	SHINKAI6500 dove and started her operation #1793	
11:51	SHINKAI6500 landed on the sea bottom (D=6,412m)	
14:38	SHINKAI6500 left the sea bottom (D=6,426m)	
17:02	SHINKAI6500 floated	
17:16	Hoisted up SHINKAI6500	
17:24	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.3, then proceeded to research area St.6.	
18:00-18:10	Research meeting	
22:42	Arrived at dive point research area (St.6)	
2024/07/09 Tue.	Noon Position:41-00.2N,143-01.8E (East slope of the Chishima trench1)	o/ SW-3 / 2
5:55	Released XBT at 41-02.3N, 142-56.6E	
6:19-6:48	MBES mapping survey .	
9:38	Hoisted up SHINKAI6500	
9:45	Launched SHINKAI6500	
9:53	SHINKAI6500 dove and started her operation #1794	
10:58	SHINKAI6500 landed on the sea bottom (D=1,876m)	
16:20	SHINKAI6500 left the sea bottom (D=1,890m)	
17:04	SHINKAI6500 floated	
17:19	Hoisted up SHINKAI6500	
17:27	Recovered SHINKAI6500 and finished the operation	
18:00	Left research area St.6, then proceeded to research area St.4.	
18:00-18:10	Research meeting	
21:45	Arrived at dive point research area (St.4)	
2024/07/10 Wed.	Noon Position: 40-49.3N,144-25.1E (West of Kuril Trench)	c/ West-5 / 4
7:00	Left research area St.4, for research area St.4.	
7:30	Arrived at research area (St.5)	
08:11-09:26	Carried out Neustone net sampling (St.5)	
9:40	Left research area St.5, for research area St.3.	
11:35	Arrived at research area (St.3)	
11:38-12:53	Carried out Neustone net sampling (St.3)	
13:00	Left research area St.3, for YOKOSUKA.	
17:05-17:26	Carried out figure eight turn measurement.	
18:00-18:10	Research meeting	
2024/07/11 Thu.	Noon Position: 38-01.5N,141-40.7E(Off the southeast coast of KINKAZAN)	r/ SSW-2 / 2
2024/07/12 Fri.	Noon Position: 34-56.4N,139-41.3E(West of SUNOSAKI)	r/ East-2 / 2
14:28	Let go anchor in 29m of water at YOKOSUKA KO Sec.4.	
2024/07/13 Sat.	Noon Position: 34-56.4N,139-41.3E(West of SUNOSAKI)	
7:00	Heaving anchor,left YOKOSUKA sec.4.	
9:00	Sent out 1st shore line, arrived at YOKOSUKA, then completed voy. No.YK24-11	

● **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.

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