Cruise Summary

1. Cruise Information

● Cruise ID: KH-22-5

● Name of vessel: R/V Hakuho Maru

○ Title of project

● Title of cruise:　Biological oceanographic research cruise for testing observation tools

● Chief Scientist [Affiliation]: Hiroaki Saito, Atmosphere and Ocean Research Institute, the University of Tokyo

● Cruise period: March 6, 2-22 – March 17, 2022

●Ports of departure / call / arrival

● Research area: South of Honshu, region around Amami-Ohshima Island and Suruga Bay

○ Research map

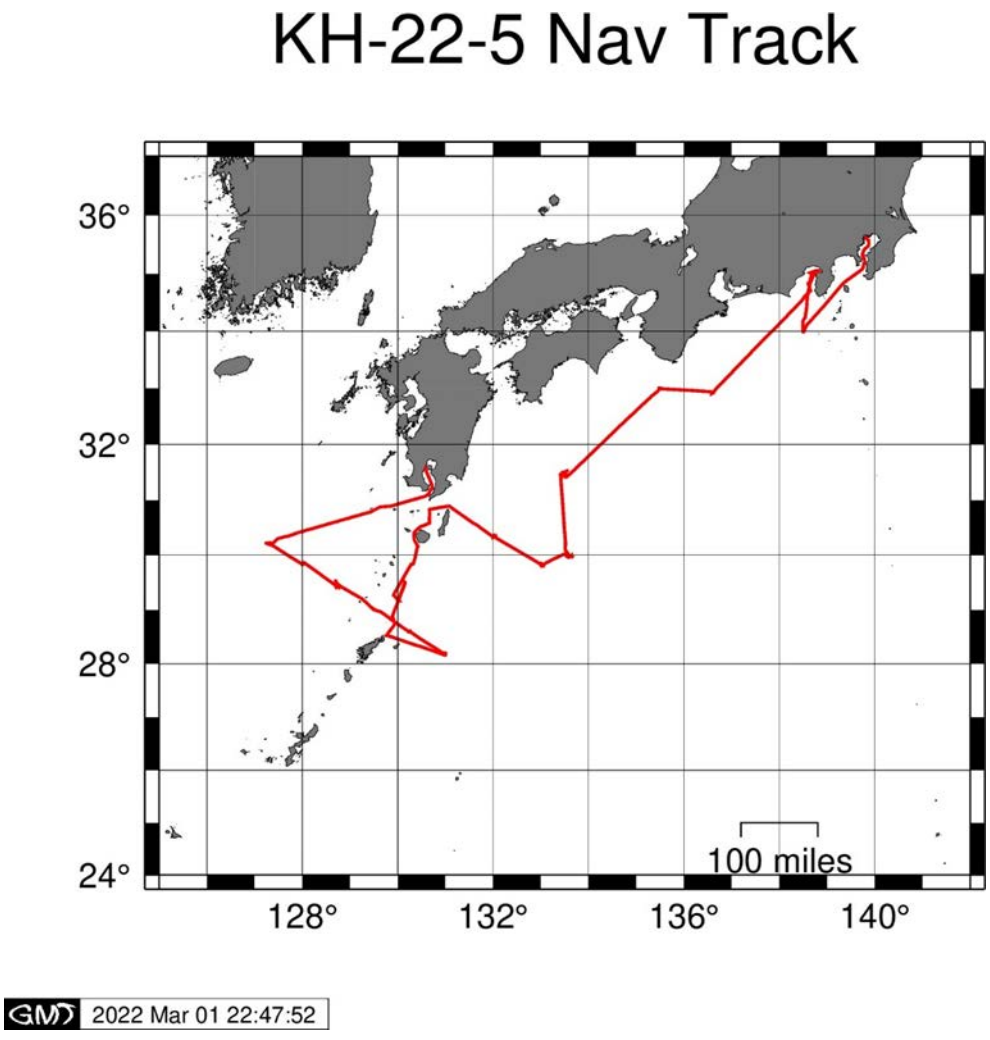

Fig.1 Cruise track

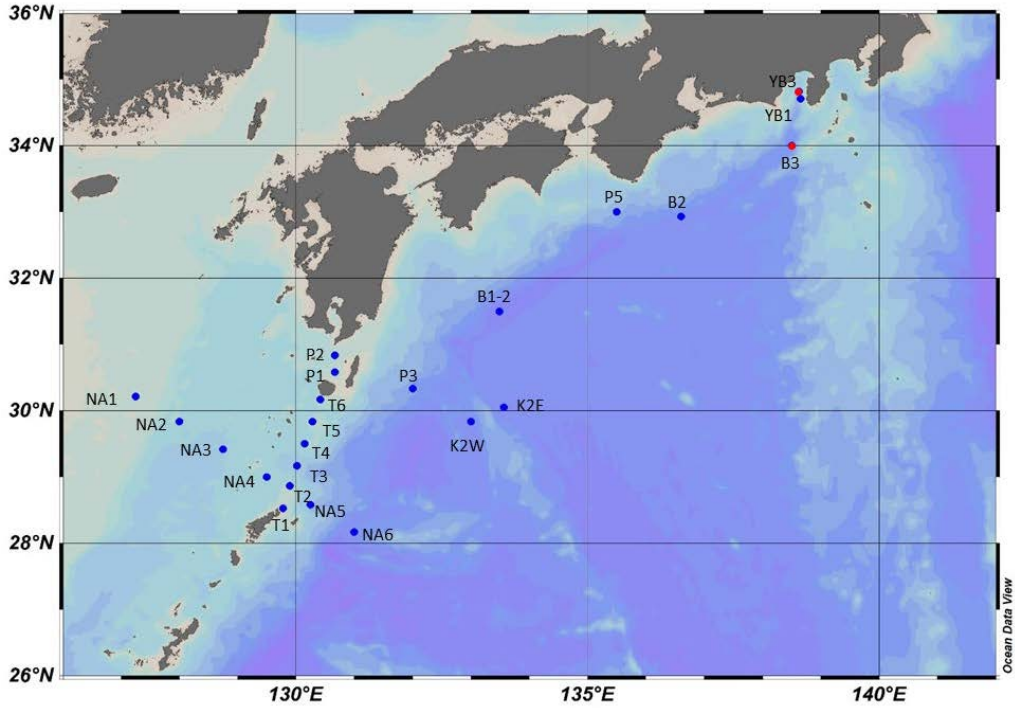

Fig. 2 Map of sampling stations. Red circulus indicate station conducted only beam trawl tow without CTD cast.

2. Overview of Research Activities

● CTD cast and water sampling

Hiroaki Saito, Ryoji Toda, Makoto Takeuchi, Masanari Ashida, Hideo Ishigaki (AORI, UTokyo), Yoshihiko Nakano (Marne Work Japan), Ryosuke Komatsu (MOL Japan)

To examine environmental parameters, measurement of temperature, salinity, in vivo fluorescence of phytoplankton and oxygen were carried out by means of CTD-carousel multisampler system (SBE-911 plus and SBE-32 water sampler Sea Bird Electronics Inc.) down to 1000 m or 1200 m (Stn.NA6, T4, K2W), or near-bottom layer when the bottom depth was shallower than 1000 m (bottom – 10 m). Water samples were also collected to determine concentrations of chlorophyll *a*, oxygen, nutrients and various chemical and biological parameters by each scientist on board (see below for more detail).

● Ocean mixing and carbon cycle in the Kuroshio Current area

Shoko Hirabayashi, Hui Lan (AORI, UTokyo)

This study aims to understand the Kuroshio variability and carbon cycle in the Kuroshio Current area based on high-resolution geochemical analysis. In this cruise, about 7 liters of seawater were collected by

CTD-RMS from 1,200 m to the surface. All tubes, vials and bottles were acid-washed prior to sampling, and thoroughly rinsed with the water to be collected. The seawater samples collected by Niskin bottles will be analyzed for oxygen isotope (δ18O) and dissolved inorganic carbon (DIC) concentration (DIC-conc.), DIC isotopes (DIC-δ13C), dissolved organic carbon (DOC) concentration (DOC-conc.) and isotope (DOC-δ13C), radiocarbon (14C) for both DIC and DOC (DIC-14C, DOC-14C) and Uranium isotopes at the University of Tokyo.
Seawater samples for DIC analysis were added saturated HgCl2 solution and were tightly sealed. The samples for δ18O, DIC-conc, DIC-δ13C, DIC-14C and Uranium isotopes were stocked in the cold storage. Seawater samples for uranium isotopes were filtered using silicone tubing and a peristaltic pump through a 0.45-μm cellulose acetate membrane filter. All δ18O, DIC, Uranium samples were stocked in the cold room. The samples for DOC and POC were filtered using precombusted (450 degrees) GF/F filters immediately in the laboratory. After filtration, all DOC samples were frozen at ～−20 ℃ immediately.

- Ocean DNA for eukaryote studies

Sk Istiaque Ahmed, Zhen Lin, Chuya Shinzato, and Jun Inoue (AORI, UTokyo)

Environmental DNA (eDNA) analysis allows the simultaneous examination of organisms across multiple trophic levels and domains of life, providing critical information about the complex biotic interactions related to environmental conditions. Under our project, Ocean DNA, we are aiming to use amplicon sequencing of environmental DNA to survey eukaryote biodiversity from seawater samples with special reference to fish. During KH22-5, we collected seawater samples using Niskin bottles attached to CTD at four depths in the upper 150m (10, 50, 100 and 150m) at 21 stations (NA1–NA6, T1–T6, P1–P3, K2W, K2E, B1-2, P5, B2, and YB1). CTD profiles were obtained from surface to bottom. Collected water specimens were immediately filtered using Sterivex 0.45 μm HV filters in the laboratory. Those filters were stored in RNAlater solution at minus 25˚C. After this cruise, DNA will be extracted from the filters and metabarcoding analyses will be performed with the other R/V Hakuho Maru cruise (KH-22-1, KH-22-3, and KH-22-4). By comparing species compositions derived from DNA analyses, the fish community and its structure will be examined. This study is conducted as a part of the FSI project “Ocean DNA” of the University of Tokyo.

- Geographic distribution of microorganisms to large marine organisms using eDNA analysis

  Takayoshi Fujiwara, Mako Takada, Masumi Hasegawa and Susumu Yoshizawa (AORI, UTokyo)

DNA extracted from filters after seawater filtration is called environmental DNA (eDNA) and is known to contain DNA from bacteria, plankton to fish. From the analysis of eDNA collected during KH-22-5

research cruise, we aim to clarify the geographic distribution of marine organisms from bacteria to fish. It is not well understood how much the distribution of nekton and planktonic microorganisms is affected by ocean currents. Therefore, this research was conducted in the area around the Kuroshio Current.
At all sampling sites, seawater samples were collected using Niskin water samplers attached to CTD at four depths in the upper 150m (10, 50,
100 and 150m) at 21 stations (NA1–NA6, T1–T6, P1–P3, K2W, K2E, B1-2, P5, B2, and YB1). Collected water specimens were immediately filtered using Sterivex GP 0.22 μm HV filters in the laboratory. The filters were preserved at -20ºC for later analyses. This study is conducted as a part of the FSI project "Ocean DNA" of the University of Tokyo.

● Diversity of zooplankton and micronekton in the Kuroshio Current area

Nakako Tamamushi & Junya Hirai (AORI, UTokyo)

Zooplankton and micronekton are ubiquitous and abundant in the pelagic ocean, playing a significant role in marine food webs and in biogeochemistry. They are also highly diverse and sensitive to environmental changes; therefore, investigating their diversity is important to understand changes in marine environments and ecosystems. During KH-22-5 cruise aboard the R.V. Hakuho-Maru, zooplankton and micronekton samples were obtained to assess both species-level and genetic-level diversity using molecular technique. The following observations were carried out.

VMPS: Bulk zooplankton samples were collected by vertical tows of VMPS 6000D with 100 μm mesh size at the station NA3, NA6, T3, P3, K2W, and B1-2. Samples were collected from three layers (0–200, 200–500, 500–1000 m). All samples were preserved in 99% ethanol for DNA metabarcoding of zooplankton.

NORPAC: NORPAC net with 100 μm mesh size was vertically towed to collect zooplankton at the depth of 0–200 m at the stations NA1, NA3, NA6, T3, T5, P1, P3, K2W, B1-2, P5, and B2. Bulk samples were preserved in 99% ethanol, RNAlater, or 5% formalin. These samples will be used for population genetics, gene expression analysis, and community structure analysis.

ORI: Zooplankton and micronekton were collected by oblique tow of ORI net with 335 μm mesh size (wire out 1,000 m) at the stations NA3, NA6, T3, P3, and K2W. After samplings, fish and shrimp were picked up, and other samples were preserved in 99% ethanol or at -20℃ for DNA barcoding and population genetics.

MOHT: Micronekton were collected at the depth of 0‐200 m by oblique tow of MOHT with 2.0 mm

mesh size at stations NA3, NA6, T3, P3, K2W, and B1-2. After samplings, fish and shrimp were picked up, and other samples were preserved in 99% ethanol or at -20℃ for diet analysis and community analysis of micronekton.

●Trials for the sampling of bathyal and abyssal seafloor faunas

Shigeaki Kojima, Yasunori Kano, Takuya Yahagi, Hiroaki Fukumori, Mizuki Ota, Genki Ishiyama, Takanobu Mogi (AORI, Univ Tokyo), Masanori Okanishi (Misaki Marine Biological Station, Univ Tokyo), and Tsuyoshi Takano (Meguro Parasitological Museum)

Three types of gears, i.e. 4 m ORI-type beam trawl, 3 m Agassiz-type beam trawl and epibenthic sledge, were tested for efficient sampling of macrobenthic faunas from bathyal and abyssal depths. The trials were conducted at the stations K2W, K2N, B1-2, B2, B3, YB1 and YB3 for the 4 m trawl, K2W, K2N and YB3 for the epibenthic sledge, and K2W and B1-2 for the 3m Agassiz trawl. All gears were equipped with (1) an acoustic transponder and (2) a video recorder and LED lights for the real-time 3D positioning of the gear and for post-hoc analysis of the gear attitude and bottom condition, respectively. The 4m ORI trawl was also equipped with (3) a deep-sea pinger to measure distance to the bottom and attitude of the trawl while towing. The exact lines of towing at the stations were decided by creating topographic maps of the sea floor with 10 m contours with the multibeam echosounder of the vessel. The trawls and sledge were towed at 0.5 knot for 0.5 to 1 hour, with the wire out 1.03–1.1 times as long as the depth. A total of 12 tows were made, of which ten were successful: samples were leaked out in a sledge tow at the station K2W due to improper installation of nets; a 4m-trawl haul at B2 yielded only very few specimens as the trawl was towed upside down on the bottom, potentially for a wrong direction of tow with a following current. Sediment samples collected in trawls were sieved with 20 mm, 6 mm, 3 mm and 1 mm meshes, sorted in a cold room (10th Lab) for echinoderms, molluscs, annelids, crustaceans, fish and other animal specimens, which were then preserved in pure ethanol. Samples from the epibenthic sledge was fixed in hot water and preserved in pure ethanol. Two bathyal stations in Suruga Bay yielded much more numerous specimens than the deeper, abyssal stations in Nankai Trough, apparently reflecting difference in actual animal abundance on the sea floors. Data obtained from the acoustic transponder, video recorder and tension meter will further be analyzed and used to establish optimized operation methods and to create an instruction manual for trawl- and sledge-tows in future Hakuho-maru cruises.

● Distribution of microplastics in the Kuroshio region

Yuichiro Nishibe, Rei Yamashita & Guan Lingfeng (AORI, UTokyo)

Microplastic pollution is an emerging issue for the marine environment. Thus, monitoring the distribution and abundance of microplastics is essential to understand the state of plastic pollution and its impact on

marine ecosystem. Here we investigate the distribution, abundance, mass, type and size of plastics in the Kuroshio region including the East China Sea and the western North Pacific. Microplastic samples were collected by surface towing using a neuston net (mouth opening 130 x 75 cm; length 6 m; mesh size 315 μm) at 17 stations. The plastics in the samples will be sorted, counted and analyzed in the land laboratory by using stereomicroscope and a FT-IR. We also collected large volume (100 L) of surface seawater by a stainless bucket to sample the smaller-sized microplastics (<335 μm) at 17 stations. In addition, depth distribution (< 150 m) of microplastics was investigated by collecting large volume of seawater (ca. 100 L) using Niskin bottles at 8 stations. The seawater samples were filtered through 5 μm filter paper onboard to concentrate microplastics and then stored in a refrigerator. The filters will be analyzed to investigate the number, size and polymer type of microplastics by using a μFT-IR.

- The formation mechanism of winter/spring phytoplankton blooms in the Kuroshio Current revealed by dilution experiments

Siyu Jiang, Yubei Wu and Hiroaki Saito (AORI, UTokyo)

The Kuroshio Current is believed to be oligotrophic but supporting spawning and nursery of various fish species around Japan. One explanation to this “Kuroshio Paradox” – high fishery production in oligotrophic environments – is this high production is supported by the winter/spring phytoplankton blooms which is stimulated by the nutrient enrichment from the subsurface water of Kuroshio Current. To figure out the formation mechanism of winter/spring phytoplankton blooms and whether the nutrient-rich subsurface Kuroshio water would induce the surface phytoplankton bloom, the dilution incubation experiments were conducted at four stations around the Kuroshio Current during KH-22-4 and KH-22-5 cruises. The surface water (10 m) was mixed with the subsurface water (20% proportion, 200 m) and incubated for 72 hours. Samples for measuring nutrient concentrations, and for biomass and community composition of phytoplankton and microzooplankton communities were collected every 24 hours. The obtained time series of these parameters are expected to reveal whether the surface phytoplankton bloom occurred and was induced by mixing with the subsurface water. Further, the formation mechanism of bloom, such as the main bloom contributor is also expected to be revealed by times series of growth and mortality rates of various phytoplankton species, and comparing the phytoplankton community composition before and after incubation.