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白鳳丸研究究航海報告

＊ 航海番号　　KH-20-9次研究航海

＊ 航海名称

黒潮域における栄養塩供給のホットスポット：　黒潮パラドックスの解明

Study of the hotspots of nutrient supply in the Kuroshio region: For solving the *Kuroshio Paradox*

＊ 観測海域　　西部北太平洋および東シナ海黒潮域

Kuroshio region in the western North Pacific and East China Sea

＊ 航海期間　　令和2年9月10日（木）～令和2年10月5日（月）

＊ 出港日時・場所　9月10日13時30分　東京港

＊ 入港日時・場所　10月5日11時　東京港

＊ 寄港期間・場所　なし

＊ 研究課題

1. 海洋混合過程の微細観測
2. 海洋物理過程による栄養塩供給機構に関する研究
3. 微生物食物網の構造と動態に関する研究
4. プランクトンおよびマイクロネクトン分布、多様性および生産の地理的変化に関する研究
5. 環境 DNA 手法を用いた海洋生物多様性に関する研究
6. 生物ポンプを駆動する沈降粒子の挙動と分解機構に関する研究
7. マイクロプラスティックの分布に関する研究

＊ 主席研究員（氏名・所属・職名）

齊藤宏明・東京大学大気海洋研究所・教授

＊ 研究内容，主調査者，観測項目

1. 海洋混合過程の微細観測、長井健容、UVMP, RINKO-SUNA および CTD による鉛直微細構造の把握
2. 海洋物理過程による栄養塩供給機構に関する研究、齊藤宏明、採水による栄養塩の超高感度分析と課題１で得られる物理微細構造から栄養塩の供給量、供給機構を把握する

3. 微生物食物網の構造と動態に関する研究、福田秀樹、採水およびマリンスノーキャッチーにより粒状有機物を採集すると共に、LISST による粒状物の形状サイズ把握、バクテリア生産の測定を行う。
4. プランクトンおよびマイクロネクトン分布、多様性および生産の地理的変化に関する研究、高橋一生、マイクロネクトンやプランクトンの分布をネット採集、MOHT 採集により明らかにするとともに、基礎生産、硝酸塩取り込み、窒素固定を測定する。
5. 環境 DNA 手法を用いた海洋生物多様性に関する研究、井上潤、環境 DNA 手法による海域の生物多様性を明らかにする
6. 生物ポンプを駆動する沈降粒子の挙動と分解機構に関する研究、齊藤宏明、セジメントトラップにより沈降粒子を捕捉し、課題３の結果と合わせて沈降粒子の挙動を明らかにする。
7. マイクロプラスティックの分布に関する研究、山下麗、ニューストンネットを用い、マイクロプラスティックを採集し、その分布を明らかにする。

＊ 乗船研究者氏名・所属・職名

齊藤　宏明	東京大学・大気海洋研究所	教授
福田　秀樹	同上	准教授
井上　潤	同上	助教
山下　麗	同上	特任研究員
余 泽庶	同上	大学院学生
海老原 諒子	同上	同上
Yubei, WU	同上	同上
中桐　菜緒	同上	同上
石垣　秀雄	同上	技術専門職員
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佐藤　拓也	東京大学院農学生命科学研究科	大学院学生
長友　佑太朗	同上	同上
藤井　麻緒	同上	同上
山田　洋輔	沖縄科学技術大学	博士研究員
長井　健容	東京海洋大学海洋科学技術研究科	准教授
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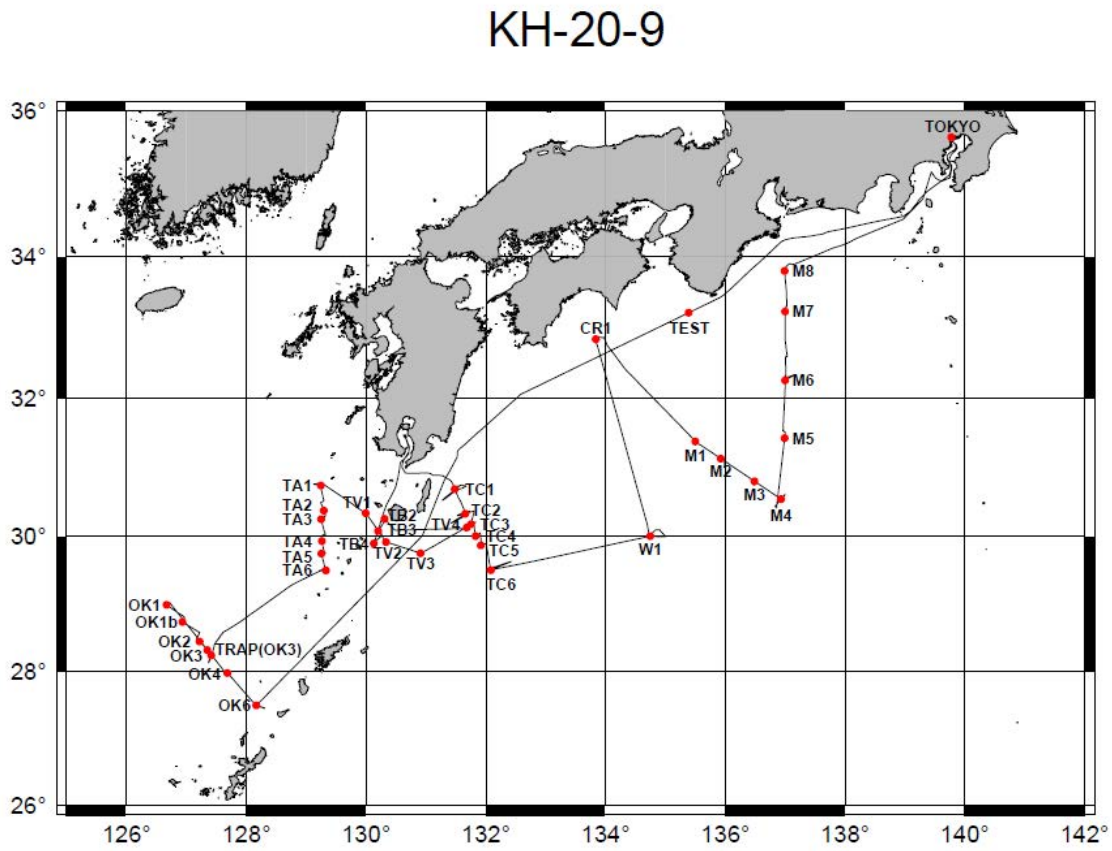
数野　真	同上	同上
横川 真一朗	㈱マリン・ワーク・ジャパン	観測技術員
小松　亮介	㈱MOL マリン	同上

＊ 航跡・測点図



2. 調査概要

本航海では、物理機構による栄養塩供給のホットスポットとその下流域に流軸を横切る観測線を設け、物理、化学、生物に関する学術分野横断観測を行い、各海域に特徴的な物理現象が生態系構造や生物生産に与える影響を把握する。

●CTD cast and water sampling

Hiroaki Saito, Takuya Sato, Hideki Fukuda, Hideo Ishigaki, Makoto Takeuchi, Shinichiro Yokokawa, Rhosuke Komatsu

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 near-bottom layer (bottom – 50 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).

● High-resolution tow-yo profiling of nitrate diffusive flux in the Kuroshio

Takeyoshi Nagai (Tokyo Univ. of Mar. Sci. Tech.)

The Kuroshio has been known as the nutrient stream in its dark subsurface layer. On the other hand, it flows over the shallow topography for example seamounts in the Tokara Strait. How much nutrients can be supplied to the shallower layers, and how far this flux persists are, however, still unclear due to lack of simultaneous observations of turbulence and nutrient concentrations at spatially high resolutions. The nutrient supply in the Tokara Strait can promote the biological productivity in its downstream region. The Kuroshio south of Honshu can also be considered as such downstream regions with respect to the upstream Kuroshio, the Tokara Strait. Since September 2017, the Kuroshio south of Honshu has been taking the Large Meander path. Recent study by the author revealed the elevated surface chlorophyll-a concentration at the trough of the Large Meander. However, it is currently unknown how the elevated chlorophyll-a is emerged at the Large Meander. The objectives of this observation are to resolve nitrate vertical diffusive flux at high lateral resolution in the Kuroshio flowing over the seamounts in the Tokara Strait, and to elucidate the mechanisms responsible for the increase in chlorophyll-a at the trough of the Large Meander.

To obtain the nitrate concentration data and microscale turbulence data quasi-simultaneously, two tow-yo profiling systems were employed in this observation. Both systems use the Underway-CTD winches (Teledyne Oceanscience) at the stern on the starboard side and on the port side. On the starboard side, a Vertical Microstructure Profiler 250 (VMP250, Rockland Scientific), which is equipped with two shear probes, two FP07 thermistors, a CTD (JFE-Advantech), and an accelerometer, was deployed repeatedly. On the port side, a RINKO-Profiler (JFE-Advantech), which equipped with the CTD, fluorescence, turbidity, and dissolved oxygen sensor, was deployed. A Deep-SUNA (SBE) and its battery were attached to the RINKO-Profiler and deployed them altogether. The two tow-yo systems were used alternatively with a ship speed of 2-3.5 knots with respect to the water to obtain microstructure up to 300 m depth, and nitrate and other biogeochemical parameters up to 200 m. The tow-yo observations in the Tokara Strait were carried out from September 19 to September 20, 2020 for about 30 hours along the line from station TV1 through TV4. At the station TV1-4, CTD profiling was obtained. Another tow-yo observations in the Kuroshio Large Meander were conducted from September 30 through October 1 for about 18 hours from station M2-M4. Along the latter tow-yo observation transect line, there were three CTD casts at the stations M2-M4.

The observations in the Tokara Strait shows that intense turbulence occurs all the way from TV1 through TV4 with >O(10^{-7} Wkg^{-1}) of turbulent kinetic energy (TKE) dissipation rates. The large dissipation rates are found especially in the region near the seamounts. The measured nitrate shows distributions roughly align isopycnal increasing with denser water. However, as the profiling proceeds toward the downstream, the nitrate concentrations gradually increase at the same pycnostad in the shallower layers. Chlorophyll fluorescence shows subsurface maximum at 100 m depth in the region close to the station TV1, upstream side. However, when profiling reaches near the seamounts, this

subsurface maximum seems diluted with broader layers of less fluorescence signals compare to that in the TV1. After the profiling arrives in the middle of the station TV3 and 4, chlorophyll fluorescence increases rapidly. This increase is probably attributed to the nitrate injection caused by the observed turbulence at the seamount.

In the tow-yo observations south of Honshu, the microstructure data below the surface boundary layer shows 10-100 times smaller TKE dissipation rates compared to that in the Tokara Strait. However, it shows 10^{-8}-10^{-7} Wkg^{-1} of the dissipation rates of banded turbulent layers. The nitrate again shows distribution roughly align isopycnal with uprising nutricline as the transect observation proceeds, because the tow-yo profiling starts from edge of the Large Meander (or the cyclonic eddy) toward its center of the cold core. On the other hand, near the banded layers of relatively strong turbulence, nitrate shows tongue like interleaving structures with relatively higher chlorophyll fluorescence at 100 m depth. Also, near the center of the cold core of the Large Meander, the chlorophyll fluorescence shows its maximum value at 50 m depth, as the nutricline becomes shallower there.

●An eDNA analysis to estimate geographical distribution pattern of marine animal community

Zeshu Yu 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 eDNA to survey biodiversity from seawater samples during KH20-9. Especially, the Kuroshio Current crosses the Tokara Strait, located between Amami and Tanegashima Islands, and functions as a geographic barrier between the Palearctic and Oriental regions (Watase 1912). So, we are interested in the differences of species compositions between areas across the Tokara Strait.

At all observation stations except for OK4, seawater samples were collected using Niskin bottles attached to CTD at four depth in the upper 150m (10, 50, 100 and 150m). Peristaltic pumps with tubing systems were used to pump the water into filtration capsules, Sterivex filter unites. The filters were preserved at -20ºC for later analyses on land.

● Variability, activity and dynamics of diazotroph community in Kuroshio region

Takuya Sato (GSALS, UTokyo)

This study aims to elucidate the geographical patterns of the nitrogen fixation activity, diazotroph community structure and in-situ dynamics of diazotroph population. Water samples for incubation experiments and DNA samples were obtained using Niskin bottles attached CTD from five relative light intensity depths (100, 25, 10, 1, and 0.1 %) at all intensive stations. To estimate nitrogen fixation, primary production and nitrate uptake, incubation experiments with stable isotope 13C and 15N were conducted. In order to determine diazotroph community structure, water samples for DNA analysis were filtered through 0.22 μm pore size Sterivex-GP filter units. Additionally, the dilution

method (Landry and Hassett 1982) was used to estimate in-situ growth and mortality rate of diazotrophs at the 25 % light depth with on board incubation at eight intensive stations.

● Quantification of radiolarian Acantharia biomass in the Kuroshio Current area

Mao Fujii (GSALS, UTokyo)

Unlike other plankton, Acantharia has a celestite skeleton, which dissolves in a common fixed solution. Therefore, biomass has been underestimated. In this cruise, I obtained their samples from water sampler, plankton net and sediment trap to estimate accurate biomass and distribution with fixatives (Lugol's solution or formaldehyde solution) adding to Sr which prevent the dissolution during the preservation.

Collection of samples for identification and counting of Acantharia will be carried out by means of Niskin bottle sampling, plankton net sampling (NPRPAC and VMPS) and sediment trap. To prevent dissolve of Acantharean skeleton, Sr was used for preservation. 4 % Lugol's solution and $SrSO_4$ was used sample for VMPS, Niskin bottle and NORPAC. 2 % formaldehyde solution and $SrCl_2$ was used for Sediment Trap sample. DNA and DAPI staining samples were also collected by Niskin bottle sampling (4 layer); DNA sample will be analyzed for the distribution of Acantharia at the species level, while DAPI staining sample will be used to estimate the proportion of Acantharia with symbiotic algae.

- Methods, instruments

・Niskin bottle sampling at about all station (4 layer: 10, 30, 50 and 80 m or 8 layer: 10, 30, 50, 80, 100, 125, 150 and 200 m). But DNA sample was collected at only intensive station.

・VMPS sampling (6 layer: 500 – 300, 300 – 200, 200 – 150, 150 – 100, 100 – 50 and 50 – 0 m) at all intensive station.

・NORPAC sampling (0 – 200 m) at all stations except for intensive ones.

・Sediment Trap sampling at OK3 and M4 stations.

●Geographical distribution pattern of micronektnic fish community along the Kuroshio

Yutaro Nagatomo (GSALS, UTokyo)

This study aims to investigate the geographical variation of micronektonic fish community along the Kuroshio from up- to down-stream and to examine its relationship to environmental variables. Micronekton were collected by the Matsuda–Oozeki–Hu Trawl (MOHT, mesh size 1.95 mm) at night from about 500 m depth to surface at all intensive stations. Shallower tows from about 200 m depth to surface were also made at OK6, TC1, TC6, W1, CR and M4. To investigate their potential prey, mesozooplankton were collected at the depth 0 – 200 m by Norpac twin net (mesh size 100 μm) at night. Phytoplankton (POM), seawater samples were obtained from different light depth at 100, 25, 10, 1 and 0.1 % using a bucket and Niskin-X bottles for CN stable isotope analysis to examine their trophic

- Methods, instruments

All of micronekton samples were frozen for latter analysis to determine its biomass, abundance and species composition. Mesozooplankton were preserved with three different methods according to the analytical objectives; 5 % buffered formalin for microscopic observation, 99 % ethanol for DNA analysis and frozen for N and C stable isotopic analysis. POM were collected on pre-combusted GF/F filters and kept frozen for N and C stable isotopic ratio analysis.

●Mesozooplankton community structure, standing stocks and productivity in Kuroshio

Toru Kobari, Yusuke Manako and Shin Kazuno (Kagoshima University)

Oceanographic observations and sample collections were conducted at 5 transects (31 stations) across the Kuroshio current to evaluate spatial variations in mesozooplankton community structure, standing stocks and productivity in the Kuroshio. Samples were collected for mesozooplankton (31samples) with vertical hauls above 200 m depth using a twin-type NORPAC net and for eDNA (35samples) by filtering seawater at discrete 8depths above 200 m using a CTD-CMS. After the cruise (KH20-9), based on the microscopic, biochemical and metagenetic analyses, we evaluated the impacts of advection for coastal community on mesozooplankton standing stocks and productivity in the Kuroshio.

●Investigation Regarding the Distribution of Dinoflagellate in Kuroshio Region

Yubei Wu and Hiroaki Saito (AORI, UTokyo)

Dinoflagellate is dominant microplankton among both coastal and oceanic regions. They were recognized as phototrophic or heterotrophic organisms. Recently, the widespread occurrence of mixotrophic dinoflagellates has been recognized. However, most studies on dinoflagellates are targeted coastal species, and oceanic dinoflagellates are not well studied, especially, the extent of dependences on photosynthesis and hetertrophy is unclear. The question of their ability to adjust the trophic strategy to variable environments remains a mystery. For revealing the mystery, the distribution of dinoflagellate in the Kuroshio region was investigated.

During this expedition, water samples were collected at 10m, 30m, 50m, 100m, and 150m from CTD-carousel multisampler system. The water samples were fixed with 20 mL of 25% glutaraldehyde and 10 mL of acid Lugol’s solution, then preserved under low temperature for performing cell abundance analysis and species identification after the cruise. Water samples for live dinoflagellates specimens were also collected at 30 m. Dinoflagellates were isolated under the Nikon Eclipse TS100 inverted microscope and incubated in 1/10 strength IMK media at 27℃. The dinoflagellate cultures would be used for following culture experiments.

●Reproduction of oncaeid copepod

Nao Nakagiri and Hiroaki Saito

Studies of small copepods (especially oncaeid copepods) had a little attention in the past, because the mesh sizes of traditional plankton nets, 200-335 μm, are too large to capture them. However, such small copepods

do prey important role in food-web dynamics and biological carbon pump in the Kuroshio region. To evaluate the roles of small copepods, it is essential to understand factors controlling the production rate. In this cruise, we examined reproduction of *Oncaea* spp., which regarded as dominant group in small copepods in the Kuroshio region.

Oncaea spp. were collected at each station by NORPAC net and incubation nets (0.45 m diameter, 100 μm mesh size) by vertical tow from 100 m to the sea surface. Samples from NORPAC net were fixed using formalin (final concentration 5%). Adult females of oncaeid copepods obtained by the incubation net were isolated individually under a microscope (LEICA M205) and transferred to containers containing 100 μm filtered seawater, and incubated at *in-situ* temperatures. Microscope observation was performed every 10-24 h for to examine egg production and egg hatching time. After the incubation, adult females and hatched nauplii were and fixed with formalin (final concentration 5%). After the cruise, further analysis will be carried out to investigate relationships between production and egg hatching time of each *Oncaea* species and the ambient environment (nutrient, temperature, etc).

●Vertical flux of biogenic elements

Hiroaki Saito and Hideki Fukuda

Biogenic elements transported by biological pump fuel mesopelagic and bathypelagic ecosystems. In this study, we set Neuer type drifting sediment traps at 3 layers to determine the strength of the biological pump and attenuation rate of sinking flux in the upper mesopelagic zone. The study sites were Stations OK4, CR and M4. Obtained samples were filtered on precombusted GF/F filter for PON/POC, total particulate phosphate analysis and metals, or nuclepore filter (0.8 mm pore size) for mass flux and biogenic silica analyses. For further enumeration of particles such as faecal pellets of meso- and microzooplankton, subsamples were preserved in buffered formalin seawater (2% v/v).

● Investigation of bacteiral community composition and activity on different sinking speed particles

Hideki Fukuda*, Akiko Ebihara (AORI, UTokyo) and Yosuke Yamada (OIST)

Marine aggregate is one of the key components of sinking materials transporting fixed carbon in the surface layer to the ocean interior and the seafloor. Bacteria attached to, grow on and decompose these sinking particles, which regulates particle's density, size, sinking speed and affect carbon and nitrogen fluxes. However, it is still unknown that, 1) what kind of microbial species are attached on the different sinking speed particles?; 2) dose particle composition affect microbial species and activities?; 3) is there any spatial difference of sinking particles and associated microbes in the Kuroshio region?

Activities (observation, sampling, development): In this study, particle and microbial parameters in 3 different sinking speed particles (suspended, slow, fast) were examined in mixed layer, right under the euphotic zone, and +100-400 m deeper than euphotic zone. Different sinking particles were collected

by normal and giant marine snow catchers at OK6, OK3, OK1, TA1, TA3, TA6, TB2, TC3, TC6, W1, CR, M$, M6, and particle and microbial parameters [particle size distribution (PSD), particle organic matter (POM), transparent exopolymer particles (TEP), coomassii stainable particles (CSP), particle elemental composition, bacterial abundance and production, microbial community composition] were measured for different sinking particle fractions. Also, depth profiles of PSD, POM, TEP, CSP, bacterial parameters (abundance, surface properties and production), microbial community composition were measured from 10 to 1000 m except for PSD (0-200 m), production (0-300 m at MV and M lines) and microbial community composition (10 or 40 m, 200 m) at each station for references.

-Collection of different sinking speed particles (normal and giant marine snow catchers; OSIL, USA)

-Depth profiles of PSD [in situ particle sizing instrument (LISST-100X and LISST-Holo, Sequoia Scientific, USA)]

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We can answer above three questions after sample analyses and validations. All the data obtained during KH-20-9 cruise will be submitted to Data Management Group (DMG) of JAMSTEC.

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●Monitoring of microplastics in the Kuroshio Current

Rei Yamashita (AORI, UTokyo)

Floating marine debris, particularly plastic, is widely distributed in the world's oceans. The abundance of floating plastic was reported to be high in subtropical and transitional waters in the North Pacific Ocean. The Kuroshio Current is hypothesized to aid in the transport of these plastics all over the North Pacific Ocean. We investigate the distribution, abundance, mass, type and size of plastics in the Kuroshio Current area and compare this cruise results with our previous study (Yamashita and Tanimura, 2007). Samples were 33 (all stations, excluding stn.TB4) collected by a surface tow using a neuston net (mouth opening 50 x 50 cm; side length 3 m; mesh size 330 μm). Each sample was collected for 10 min at a ship's speed of 2 kt. Plastics might be especially abundant around the Kuroshio Current flows.

Due to collecting for smaller size plastics (<330 μm), the 100 L surface seawater samples (stns. TC6, CR, W1) were filtered through 5 μm filter paper (mixed cellulose ester membrane filter paper; 293 mm Ø; ADVANTEC) on board the vessel. Filters will be identified and counted using a μFTIR microscope (Thermo OMNIC Nicolet iN10) in the laboratory.

●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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