

**KH-24-3 R/V Hakuho-Maru**

# **Cruise Report**



*Photo by AORI Takeuchi*

**26 Aug. – 22 Sep. 2024**  
**Penang - Fremantle**





**R/V Hakuho-Maru KH-24-3 Cruise**

**Integrated observational research for physical  
oceanography, biogeochemistry and biological  
oceanography in the eastern Indian Ocean**

Departure: August 26, 2024 (Penang, Malaysia)

Arrival: September 22, 2024 (Fremantle, Australia)

## Introduction

Researchers specializing in physical oceanography, geochemistry, biogeochemistry, and biological oceanography collaborated to collect and analyze data and conducted the following observations with the aim of clarifying the physical structure, biogeochemical cycles including natural and anthropogenic trace elements and isotopes, and the structure and dynamics of marine ecosystems.

1. Observation of the water column physical structure, oxygen, and phytoplankton fluorescence by means of CTD observations
2. Water sampling for chemical and biological samples using the CTD water sampling system and bucket sampling
3. Collection of micronekton using MOHT nets
4. Collection of zooplankton using ORI net, VMPS net, multi-net, NORPAC net and incubation net
5. Collection of microplastics using Neuston nets
6. Observation of seawater optical properties using underwater radiometer
7. Observation of current direction and velocity using ADCP
8. Collection of seawater through the experimental surface water sampling system
9. Continuous measurement of photosynthetically active radiation (PAR) using a PAR sensor
10. Continuous observation of atmospheric aerosols
11. Collection of sea surface microlayers
12. Photoshooting by means of a drone

Due to stormy sea condition, stations at 10°N, 10°S and 15°S were cancelled.

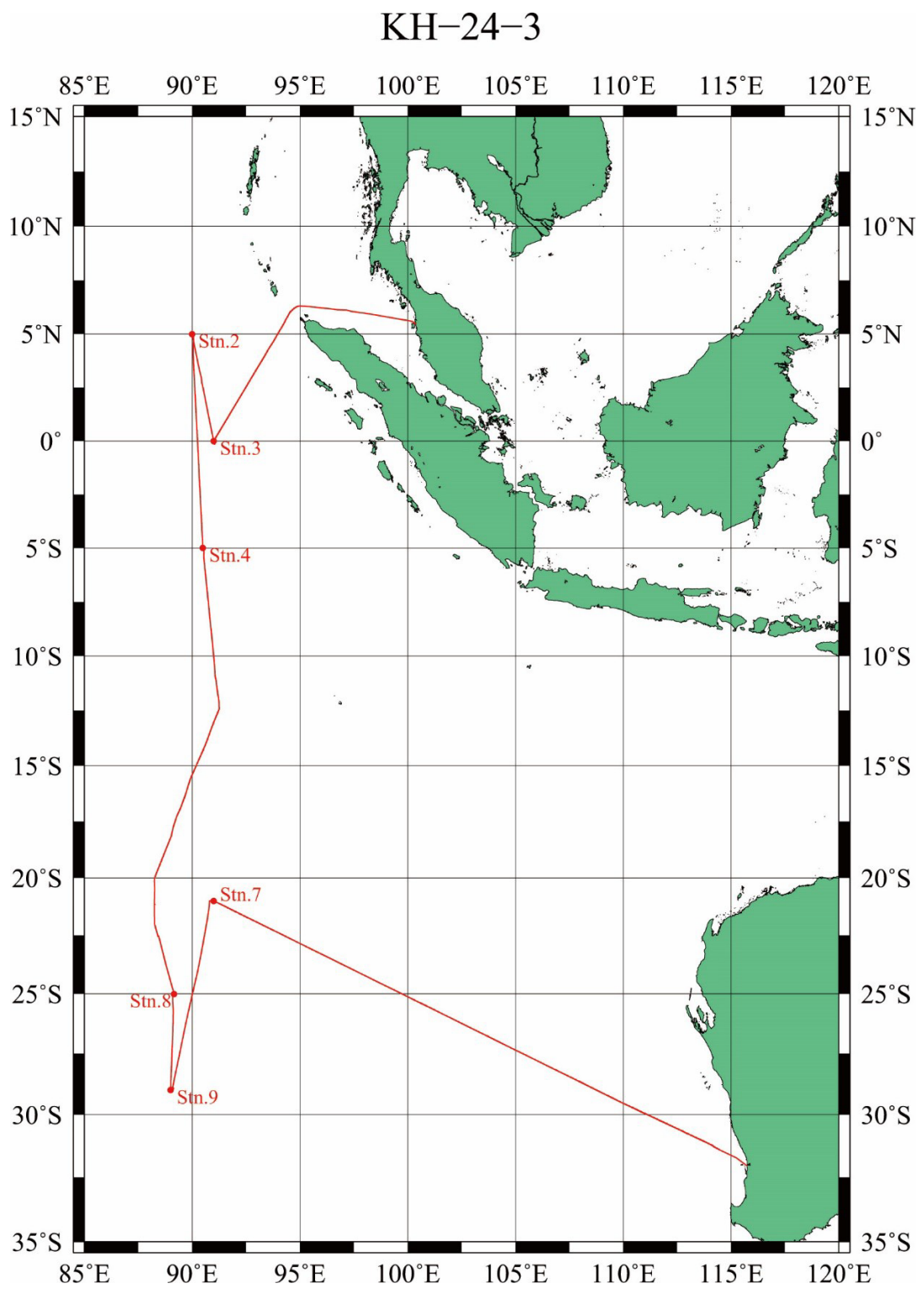
Table. Location of sampling stations

| Station | Lat(°N) | Long(°E) |
|---------|---------|----------|
| 2       | 5°00'   | 90°00'   |
| 3       | 0°00'   | 91°00'   |
| 4       | -5°00'  | 90°30'   |
| 7       | -21°00' | 91°00'   |
| 8       | -25°00' | 89°10'   |
| 9       | -29°00' | 89°00'   |

## List of Scientists on board KH-23-4

|                  |                     |                                         |
|------------------|---------------------|-----------------------------------------|
| Hiroaki SAITO    | Professor           | The University of Tokyo                 |
| Hajime OBATA     | Professor           | The University of Tokyo                 |
| Hiroshi OGAWA    | Professor           | The University of Tokyo                 |
| Koji HAMASAKI    | Lecturer            | The University of Tokyo                 |
| Junya HIRAI      | Technical Staff     | The University of Tokyo                 |
| Hideo ISHIGAKI   | Technical Staff     | The University of Tokyo                 |
| Makoto TAKEUCHI  | Professor           | The University of Tokyo                 |
| Chiho FUNAKI     | Graduate Student    | The University of Tokyo                 |
| Jiakang LIU      | Graduate Student    | The University of Tokyo                 |
| Yutaka OKAMOTO   | Graduate Student    | The University of Tokyo                 |
| Natsumi ONO      | Graduate Student    | The University of Tokyo                 |
| Nakako TAMAMUSHI | Graduate Student    | The University of Tokyo                 |
| Manatsu UEOKA    | Graduate student    | The University of Tokyo                 |
| Youhei YAMASHITA | Associate Professor | Hokkaido University                     |
| Haruka KUBO      | Graduate Student    | Hokkaido University                     |
| Kazuhiro NORISUE | Associate Professor | Niigata University                      |
| Arata YANAGISAWA | Graduate Student    | Niigata University                      |
| Shuai XIN        | Graduate student    | Toyama University                       |
| Kuo Hong WONG    | Assistant Professor | Kanazawa University                     |
| Akihiro TAKAMURA | Graduate Student    | Kanazawa University                     |
| Yoko IWAMOTO     | Associate Professor | Hiroshima University                    |
| Cheng Yi TEY     | Graduate student    | Hiroshima University                    |
| Hisashi ENDO     | Associate Professor | Kyoto University                        |
| Siyu JIANG       | Researcher          | Kyoto University                        |
| Takuya SATO      | Researcher          | Kyoto University                        |
| Mitsuhide SATO   | Assistant Professor | Nagasaki University                     |
| Takako MASUDA    | Senior Scientist    | Fisheries Research and Education Agency |
| Nobuharu KOMAI   | Marine Technician   | Marine Work Japan                       |
| Mako NAKAO       | Marine Technician   | MOLMEC                                  |

Cruise Track



## Physical oceanographic property

Hiroaki Saito<sup>1</sup>, Hajime Obata<sup>1</sup>, Hideo Ishigaki<sup>1</sup>, Makoto Takeuchi<sup>1</sup>, Nobuharu Komai<sup>2</sup>,  
Mako Nakao<sup>3</sup>, and participants of KH-23-4.

1. Atmosphere and Ocean Research Institute, The University of Tokyo

2. Marine Work Japan

3. MOLMEC

Correspondence: Hiroaki Saito

### Introduction

Water circulation in the Eastern Indian Ocean is totally different from the Pacific Ocean and Atlantic Ocean due to seasonal monsoon which reverse the boundary currents (Schott et al., 2009). The dynamic change in current system and seasonal change in fresh water supply as well as the intrusion of intermediate and deep waters formed in the Southern Ocean influence biogeochemical cycles and food-web structure and dynamics. However, our understanding of the physical oceanographic property and its impact to biogeochemical cycle and ecosystem dynamics is limited especially in summer monsoon period. We carried out full-depth (surface to 20 m above the sea bottom) CTDO observation to examine the physical properties of the water column in the Eastern Indian Ocean. The obtained data is essential information to understand biogeochemical and ecological research carried out by scientists on board RV Hakuho-maru KH-24-3.

### Results

Vertical physical oceanographic structure of the study area is divided into two groups, norther stations (Sts 2,3,4) and southern stations (St.7,8,9) although sharing the Central Deep Water (CDW) at deeper layer than 27.7  $\sigma\theta$ .

In northern stations, surface temperature was high ( $>27^{\circ}\text{C}$ ) and salinity was low ( $<34.5$ ). Thick low oxygen layer ( $<2\text{ mL L}^{-1}$ ) was formed at mesopelagic layer at 25.0-25.7  $\sigma\theta$ . At St.2,  $\text{O}_2$  concentration was lower than  $0.5\text{ mL L}^{-1}$  between 200-440 m. In the surface layer, low saline water ( $<34.5$ ) occupied at the surface layer and formed strong pycnocline. At St.4, thermocline was quite strong around 67 m, and the steepest thermocline was  $1^{\circ}\text{C m}^{-1}$ . This suggests that lateral transport of the surface water. Source of the surface water is an issue to be solved through further analysis of physical oceanographic data. At Sts 2 and 3, salinity profiles deeper than 26.8  $\sigma\theta$  ( $\sim 350\text{ m}$ ) were similar each other, declining with depth from 35.0 to 34.7. Besides,  $\text{O}_2$  concentration at

St.2 was lower than St.3 at upper  $27.4 \sigma\theta$  ( $\sim 1000$  m), suggesting high organic matter flux and remineralization in the mesopelagic layer at St.2. At St. 4, salinity in the mesopelagic and upper bathypelagic layers ( $26.8$ - $27.6 \sigma\theta$ ) was lower than Sts 2 and 3. Besides,  $O_2$  profile at  $26.9$ - $27.3 \sigma\theta$  at Sts 3 and 4 were similar each other with 2<sup>nd</sup>  $O_2$  minimum around  $27.2 \sigma\theta$ . These suggested complex mixing process at the mesopelagic layer. Further studies with chemical properties are to be done after the cruise.

In the southern stations,  $O_2$  concentration was higher than  $4 \text{ mL L}^{-1}$  in the mesopelagic layer where high salinity ( $>35.5$ ) Subtropical Subsurface Water (SSW) distributed.  $O_2$  minimum was observed at  $27.2$ - $27.4 \sigma\theta$  in which Indian Deep Water (IDW) distributed. In the surface layer, water column was well mixed and deep seasonal thermocline was formed. At St. 8, surface mixed layer reached 180 m depth.

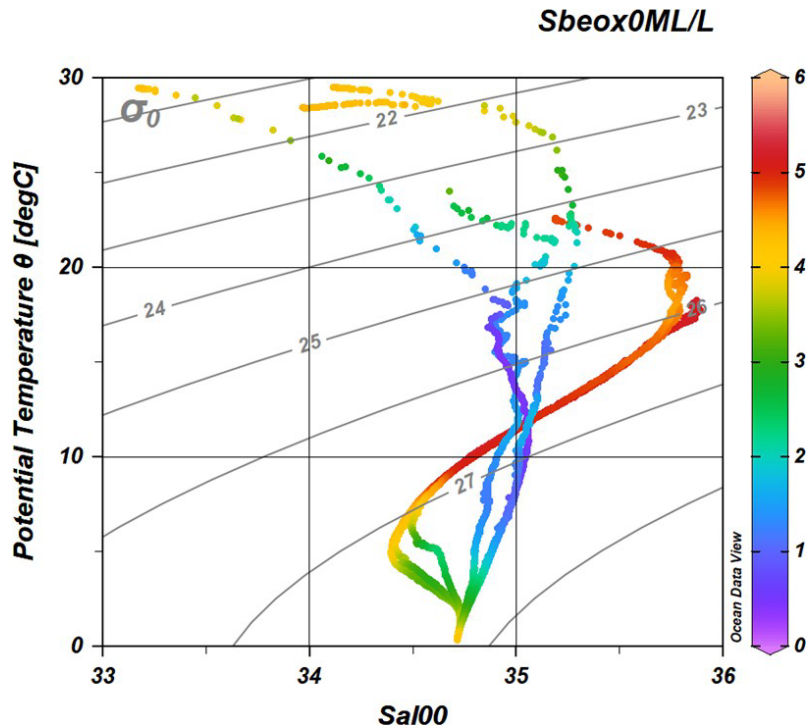


Fig.1 TS diagram. Color bar indicate  $O_2$  concentration ( $\text{mL L}^{-1}$ ).

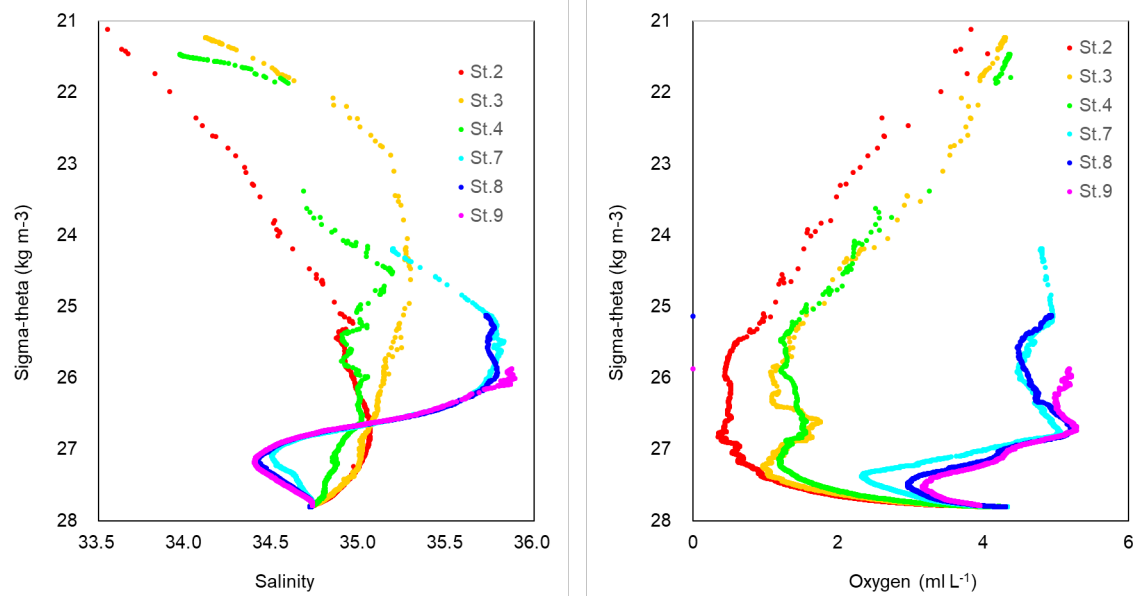


Fig. 2. Vertical profiles of salinity (left) and oxygen (right).

Discrete water samples were collected to calibrate  $O_2$  sensor attached at a CTD system.  $O_2$  concentration of the water samples were measured on board by standard titration method, and obtained calibration equation:

$$O_2 \text{ (mL L}^{-1}\text{)} = 1.0400 \cdot [O_2 \text{ Sensor}] + 0.0382, r^2 = 0.9982$$

In this cruise report, uncorrected data of  $O_2$  was used to depict the figures.

## Optical measurement of sea water

Hiroaki Saito<sup>1\*</sup>, Takuya Sato<sup>2</sup>

1. Atmosphere and Ocean Research Institute, The University of Tokyo
2. Institute for Chemical Research, Kyoto University

\*: Correspondence: Hiroaki Saito

Optical property of seawater was examined by means of underwater radiometer PRR800 (Biospherical Inc.). PRR800 is designed to measure underwater downwelling irradiance, upwelling radiance and depth through free-falling of the profiler. The relative light depth to the sea surface irradiance was calculated using a spectral radiometer PRR810 which was set on the deck. Table 1 shows conditional relative light depth at each station. Further data analysis will be carried out after the cruise. At station 3, the relative light depth was calculated by only data from PRR800 since data from PRR810 was not collected.

Table 1. Relative light depth to sea surface irradiance (m). Numbers in parenthesis are obtained by extrapolation using attenuation coefficient and relative irradiance to the surface at 1.5-2.5% light depth.

| Station | St.2 | St.3 | St.4 | St.8  | St.9 | St.7  |
|---------|------|------|------|-------|------|-------|
| 25%     | 21   | 21   | 23   | 25    | 25   | 26    |
| 10%     | 38   | 40   | 42   | 49    | 44   | 52    |
| 1%      | 72   | 73   | 89   | (114) | 95   | (117) |

# Distributions and their speciation of trace metals in the eastern Indian Ocean

Hajime Obata<sup>1</sup>, Manatsu Ueoka<sup>1</sup> and Naoya Kanna

1. Marine Inorganic Chemistry Division, Atmosphere Ocean Research Institute,  
The University of Tokyo

Correspondence: Hajime Obata

## *1) Distributions of trace metals in the eastern Indian Ocean*

### 1-1) Objective

Trace metals, such as Fe, Co, Mn, Zn and Cu, are now thought to be essential for phytoplankton growth in the open oceans. However, full-depth distributions of trace metals have not been investigated intensively in the eastern Indian Ocean. To understand the marine biogeochemical cycles of trace metal, we need to investigate the distributions of trace metals in these areas, especially in spring. In this study, we will study the full-depth distributions of dissolved trace metals (Fe, Co, Mn, Zn, Cu and Cd etc.) in the eastern Indian Ocean.

### 1-2) Samples

Seawater samples for vertical profiles were collected using Teflon-coated X-type Niskin bottles mounted on a CTD/Carousel array. Filtered samples were obtained through a cleaned 0.2 µm filter cartridge (Acropak, Pall) connected to sampler directly with pressured air. Filtered samples (250 mL of LDPE bottle) and unfiltered (125 mL of LDPE bottle) are acidified to pH<1.8 with ultra pure HCl (Tamapure AA-100) and stored.

### CTD sampling

Station: Stn. 2, 3, 4, 7, 8, 9

Depth (m): 10, 30, 60, 100, 150, 200, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000, B-20, SCM

### 1-3) Analytical methods

All the trace metals will be determined by using chelating resin preconcentration and ICP mass spectrometry.

## *2) Trace metal speciation in the eastern Indian Ocean*

### 2-1) Introduction

Trace metals, such as Fe, Co and Cu, are essential micronutrients for phytoplankton in the ocean. At low concentration levels, trace metals can limit the growth of marine

phytoplankton in culture. Additionally, speciation is also considered to be an important factor of the biological availability of trace metals. However, little is known about the organic complexation of trace metals in seawaters of the eastern Indian Ocean. In this study, we will investigate trace metal speciation in the eastern Indian Ocean by using cathodic stripping voltammetry (CSV).

## 2-2) Sample

Seawater samples were collected using Teflon-coated X-type Niskin bottles mounted on a CTD/Carousel array. Filtered samples were obtained through a cleaned 0.2 µm filter cartridge (Acropak, Pall) connected to sampler directly with pressured air. Filtered samples (500mL of FLPE bottle) are frozen at -18°C and stored.

## CTD sampling

Station: Stn. 3, 4, 7, 8, 9

Depth (m): 10, 30, 60, 100, 150, 200, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000, B-20, SCM

## 2-3) Methods

On the land-based laboratory, ligand concentrations and conditional stability constants for Co and Cu will be obtained from a titration using CSV (Saito and Moffett, 2001; Campos and van den Berg, 1994; Whitby et al., 2018; Wong et al., 2018).

# **Distributions of dissolved Pb isotopes and Te species, and particulate trace elements in the eastern Indian Ocean**

Kazuhiro Norisuye<sup>1</sup>, Arata Yanagisawa<sup>2</sup>, Tohru Fukazawa<sup>3</sup>, Hajime Obata<sup>4</sup>

1. Faculty of Science, Niigata University

2. Graduate School of Environmental Science and Technology, Niigata University

3. Marine Ecology Research Institute

4. Atmosphere and Ocean Research Institute, The University of Tokyo

## **Introduction**

Lead (Pb) and tellurium (Te) are particle-reactive trace elements in the ocean and their oceanic distributions are mainly controlled by particle scavenging/exchange as well as water mass advection. Judging from their high crustal enrichment factors in the atmospheric aerosols and high particle reactivity (short oceanic residence time) in the ocean, impact of their atmospheric input on their distributions and cycles in the surface ocean are expected to be significant (anthropogenic source is predominant for Pb while less information is documented for Te so far). The eastern Indian Ocean is unique in reversal monsoon/surface water currents and interaction between lower atmosphere and upper surface ocean (e.g., Indian Ocean dipole), which makes study on these elements in this oceanic region intriguing.

Te has two different oxidation states of Te(IV) and Te(VI), and Te(IV)/Te(VI) redox process may be key to understand the biogeochemical cycle and input of this element. Pb has four stable isotopes of <sup>204</sup>Pb, <sup>206</sup>Pb, <sup>207</sup>Pb and <sup>208</sup>Pb, and their isotopic ratios are useful in characterizing anthropogenic/natural sources of this element and investigating removal/exchange process in the water column.

The purpose of this study is to investigate these trace elements and their isotopes in the eastern Indian Ocean. Dataset obtained by us at some similar locations during the previous KH-18-6 cruise (inter monsoon season compared to summer monsoon period of the present KH-24-3 cruise) are available to investigate how the different opportunity/season can be involved with change in their distributions. In addition, we investigate particulate trace elements since particulate matter is an important carrier for many trace elements to the deep ocean. In this cruise, two different pore size of 0.2 and 0.8  $\mu\text{m}$  is adopted to investigate size fraction of trace elements.

**1. *Vertical distribution of dissolved Te species and Pb isotopes.***

Water samples were collected at depths of 10, 30, 60, 100, 150, 200, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000 m and 20 m above the seafloor (bottom minus 20 m) at Stns. 3, 2, 4, 8, 9 and 7 in this order along ~90°E meridian. The water samples were filtered through an AcroPak cartridge filter (Pall Life Sciences) by the pressure of compressed air and transferred from X type Niskin bottles to polyethylene bottles. The filtered samples were acidified with 20% HCl (Tampure AA-100, Tama chemicals). They will be purified on land laboratory and run on appropriate mass spectrometry (sector field ICPMS for Te, and multiple collector ICPMS for Pb isotopes).

**2. *Size fraction of suspended particulate trace elements***

At Stn. 3, each 8 L sample was collected at depths of 10, 30, 60, 100, 150, 200, 300, 500, 1000, 2000, 3000, 4000 and bottom minus 20 m. Particulate matter was obtained by passing the seawater samples through supor membrane filters (i.d. 47 mm) using an aspiration device. We used filters of two different pore size of 0.2 and 0.8  $\mu\text{m}$  to investigate size fraction of trace elements. The filter samples were stored at  $-20^{\circ}\text{C}$  at No.10 lab. They will be digested using our digestion system, and analyzed for several particulate trace elements, such as Al, P, Ti, Mn, Fe, Co, Cu, Zn, Cd, Mo, Pb and Bi.

### **3. Distributions of particulate and dissolved thiols, and amino acids in the eastern Indian Sea**

Kuo Hong Wong<sup>1</sup>, Takamura Akihiro<sup>1</sup>, and Hajime Obata<sup>2</sup>

<sup>1</sup>Institute of Science and Engineering, Kanazawa University

<sup>2</sup>Atmosphere and Ocean Research Institute, The University of Tokyo, Japan

Correspondence: Kuo Hong Wong

#### **1) Objective**

Thiols are organic metal-binding ligands produced by various marine phytoplankton in response to stress from metal toxicity. Hence, we attempt to test the effectiveness of thiols as biomarkers of metal toxicity in the ocean. Some phytoplankton may be more susceptible to metal stress than others, thus producing larger amounts of thiols. The production of thiols requires glutamic acid, which is a bio-essential amino acid. Globally, there are very few thiol datasets in the open ocean, and none is available in the Indian Ocean. In this study, we will elucidate the distributions of particulate and dissolved thiols, and glutamic acid in the eastern Indian Ocean.

#### **2) Samples**

Seawater samples were collected using Teflon-coated X-type Niskin bottles mounted on a CTD/Carousel array in the upper 1000 m at two stations, and in the upper 200 m at the other 4 stations. Samples used for the analysis of particulate thiols composition were collected unfiltered into 5 L acid-cleaned cubic containers, respectively. Immediately after sampling, 5 L of seawater samples were filtered using 0.7 µm GF/F filters. Filters for particulate thiols analysis were placed in 1 mL of a solution containing 6.3 mM diethylenetriamine-pentaacetic acid (DTPA) and 0.1% v/v trifluoroacetic acid. Filters were stored in dark at -20°C. Seawater samples for dissolved thiols and glutamic acid analysis were filtered directly from the Niskin bottles using a 0.2 µm capsule filter under pressure with filtered air. Filtered samples (Dissolved thiols: 100 mL in brown high density polyethylene bottles, amino acids: 50 mL in brown glass vials) were stored at -20°C until analysis.

#### **CTD sampling**

- Particulate thiols: Stn. 2, 3, 4, 7, 8, 9
- Dissolved thiols: Stn. 2, 3, 4, 7, 8, 9
- Amino acid: Stn. 2, 3, 4, 7, 8, 9

Depth (m): 10, 30, 60, 100, 150, 200, chlorophyll maximum (at all stations)  
400, 500, 750, 1000 (at St. 2 and 8 only)

#### **1-3) Analytical methods**

Particulate and dissolved thiols will be measured using high performance liquid chromatography (HPLC) coupled with fluorescence detection after derivatization using monobromobimane (Minocha et al., 2008; Tang et al., 2003). Amino acids including glutamic acid will be measured using HPLC coupled with fluorescence detection after derivatization using o-Phthaldialdehyde (Lindroth and Mopper, 1979).

# Distributions of Chl-a, REEs and Nutrients in Seawater and Atmosphere

Xin Shuai

University of Toyama

## 1. Introduction

To trace the sources of aerosols and estimate their contribution to the Southeastern Indian Ocean, understanding the material transportation between ocean and atmosphere.

## 2. Materials and methods

### a. Aerosol

Two air samplers (KIMOTO 120SL Tokyo Direc Corporation AN-200) are installed on the ship's upper deck to collect aerosols on filters. Filters are replaced every 2-3 days. To prevent sample contamination from ship emissions, a Wind Selector (Wind Monitor-MA, Model 05106 + Marine Wind Tracker, Model 06206: R. M. Young Company) is used to sample only when headwinds (within 180 deg; -90 to +90 deg relative to the bow) with wind speeds of 2 m/s or higher are present. Samples are stored frozen condition and then transported to a land-based laboratory (both in Indonesia and Japan) for further analysis.

### b. Surface seawater (Chl-a, REEs, Nutrients)

These samples were taken every 0.5 degrees from the tap, about 6 m depth.

- Samples for Chl-a are collected on a filter (Whatman GF/F Filter  $\phi$ 25 mm, cellulose filter pore size; 10  $\mu$ m, 2  $\mu$ m, 0.2  $\mu$ m) using a coupled manifold and circulating aspirator (WJ-20; SHIBATA) for filtration and then washed with 7 mL DMF (N, For the measurement of Chl-a, a fluorometer (10 AU; Turner Designs, Inc.) is used; samples for HPLC and biomarkers are also collected on the filter (Whatman GF/F Filter Samples for Cell Count were collected on a filter (cellulose filter  $\phi$ 25 mm pore size; 0.45  $\mu$ m) at reduced filtration pressure and stored in the dark, shielded from light.
- REE samples (~500 mL) are filtered with 0.2  $\mu$ m cellulose acetate membrane filters within 24 hours, and then the filtrate is immediately acidified to pH < 1.8 with ultrapure HCl

(TAMAPURE AA-100, Tama Chemicals) in the clean bench on board. The samples are then stored at room temperature and brought into a land-based laboratory (both in Indonesia and Japan) for further analysis.

- Nutrients samples were directly frozen after sampling.

### **3. Analysis**

#### **a. Aerosols**

In a clean laboratory, ceramic scissors divide filters into six equal parts. Two parts are used for measuring major chemical components ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) and nutrient salt measurement ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{2-}$ ), and three parts are used for rare earth element measurement. The extraction method for both groups of samples involves extracting in 40ml of Milli-Q water, shaking well in a settling tube, leaving at room temperature for about 1 hour, and followed by a 15-minute extraction using an ultrasonic vibrator (USD-4R; ASONE). The extracted solution is filtered through a  $0.2\mu\text{m}$  pore size disk filter for measurement.

Major chemical components are measured using ICP-AES and ion chromatography (Cation, optima 7300DV; PerkinElmer Co., Ltd.; Anion, 883 Basic IC plus; Metrohm AG), nutrient salts are measured using a flow-through automated chemical analyzer (AutoAnalyzer3; SEAL Analytical, Inc.), and rare earth elements is measured using a mass spectrometer (ICAP-Q; Thermo Fisher Scientific).

#### **b. Surface Seawater**

- Chl-a is collected by fluorometer (10-AU; Turner Designs, Inc.), dyes by HPLC (equipment in preparation) and biomarker samples by gas chromatography with hydrogen flame ionization detector (Agilent 7890A GC; 50 m HP-1 capillary column, 0.32 mm i.d., 0.17  $\mu\text{m}$  film thickness; Agilent Technologies, Inc.) and Cell Count will be measured using a scanning electron microscope (JEOL Ltd.; JSM-6700F).
- REE concentrations will be measured using Agilent 7700 ICP-MS with a high-sensitivity sample introduction system (APEX-IR).
- Nutrients samples will be defrozen and measured by Quattro Auto Analyzer in the laboratory of the University of Toyama.

# **Spatial distributions and chemical characteristics of organic matter in the eastern Indian Ocean**

Hiroshi Ogawa<sup>1</sup>, Youhei Yamashita<sup>2</sup>, Haruka Kubo<sup>3</sup>

1. Atmosphere and Ocean Research Institute, The University of Tokyo

2. Faculty of Environmental Earth Science, Hokkaido University

3. Graduate School of Environmental Science

Correspondence: Hiroshi Ogawa

## **General Objectives**

Biogeochemical cycles of biophilic elements such as carbon, nitrogen and phosphorus in the ocean is closely related to both the homeostasis and the change of the global environments. These elements are recycling between inorganic and organic matter mainly through biological production and degradation. We have a relatively large data stock of the elements in the inorganic form, i.e. CO<sub>2</sub>, dissolved inorganic carbon (DIC) and nutrients such as NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup>. However there is a limited store of data of organic elements including both dissolved organic matter (DOM) and particulate organic matter (POM) compared with DIC and nutrients. Furthermore, little studies on OM have been conducted in the Indian Ocean compared with Atlantic Ocean and Pacific Ocean.

Main focus of our research is to get a data set of spatial distributions of some parameters of DOM and POM along the meridian section around 90° east from the equatorial to the south subtropical regions in the eastern Indian Ocean. These results could be compared with those obtained from our previous researches in the Pacific Ocean (e.g., KH-11-10, KH-12-3, KH-13-7, KH-14-3, KH-17-4, and KH-19-6 cruises) and former research in the Indian Ocean (KH-18-6 cruise), which can be a great help for our deep understanding the dynamics and chemical characteristics of OM in the Indian Ocean. Detailed descriptions of each study are as follows.

## ***Spatial distributions of bulk dissolved organic matter (DOM) and particulate organic matter (POM)***

Seawater samples for DOM analyses were collected from all the depth layers in the routine casts of CTD/CMS sampling at each station. For filtration of seawater, an in-line filter unit with a GF/F filter was installed in a drain-cock of Nsikin bottle. The filtrates were sealed in glass ampoules and they are kept frozen until analyses on land. Dissolved organic carbon (DOC) and nitrogen (DON) measurements will be conducted by high temperature combustion (HTC) method. Dissolved amino acids in combined (DCAA) and

free (DFAA) form will be measured by ultra-high performance liquid chromatography (UHPLC) method.

The samples for particulate organic carbon (POC) and nitrogen (PON) analyses were collected from the fixed 6 layers (10, 30, 100, 150, 200 m, SCM) at each station. Suspended particles were collected onto a Whatman GF/F filter from 12 L of seawaters. Amount of organic carbon and nitrogen will be determined after going back to land facility.

H. Ogawa

### ***Radiocarbon distributions of dissolved organic carbon***

Radiocarbon,  $\Delta^{14}\text{C}$ , is incorporated into virtually all carbon reservoirs on earth and is a powerful tracer of natural processes. With the development of Accelerator Mass Spectrometry (AMS), which requires much smaller sample size and analysis times, increasing numbers of measurements have been provided new insight into sources and cycling of discrete carbon pools for the oceanographic community. Nonetheless, there is still a residual question about how to make sense of the fact that dissolved organic carbon (DOC) have differences in their circulation pattern observed between in the Atlantic and the Pacific Ocean. Furthermore, there is an only quite limited study on  $\Delta^{14}\text{C}$  of DOC in the Indian Ocean.

Seawater samples for the measurement of radiocarbon content were collected with Niskin bottles from 5-6 depths (10, 100, 1,000, 2,000, 3,000 m, and bottom-20 m) at Stn. 3, 7, and 9. Prior to the cruise, sample bottles were cleaned with 10% HCl and MQ water, and then combusted at 550 °C for 5 hr.  $\Delta^{14}\text{C}$  of DOC samples were gravity-filtered through a 47-mm-diameter Whatman GF/F glass fiber filter, which had been pre-combusted at 450 °C for 3 hr. The filtrate was collected in 1 L glass bottles with Teflon-lined caps using  $^{14}\text{C}$ -clean techniques and kept at -20 °C in the dark until analysis at the single stage AMS system in Atmosphere and Ocean Research Institute, the University of Tokyo.

H. Ogawa

### ***Full-depth spatial distribution of CDOM***

In order to understand a full-depth spatial distribution of chromophoric dissolved organic matter (CDOM), we directly attached two CDOM fluorescence sensors (Wetlabs and Seapoints) to CTD sensors, and CDOM fluorescence was monitored along with the CTD observations. Seawater samples obtained from routine casts were filtered with GF/F filter and poured into a pre-combusted glass vial with teflon-lined cap after triple rinsing, and then, kept frozen (-20 °C) on board. CDOM fluorescence will be determined in a laboratory on land to correct the data obtained by CDOM sensors. In addition,

excitation emission matrix (EEM) fluorescence and UV-Vis absorbance will be determined to characterize quality of CDOM.

Y. Yamashita, H. Kubo

### ***Spatial distribution of DBC***

To determine abundance of dissolved black carbon (DBC), water samples were collected from 5-6 depths (10, 100, 1000, 2000, 3000 m, and bottom-20 m) at Stn. 3, 7, and 9. 9 L of samples were filtered using a pre-combusted GF/F filter (Whatman). A pre-concentration of DBC by solid phase extraction (SPE) were performed onboard with SPE cartridge (1 g, Bond Elut PPL, Agilent Technologies). DBC concentration will be analyzed by the benzenepolycarboxylic acids (BPCAs) method with HPLC. As the source of DBC, aerosol samples were collected with high volume aerosol samplers located at the upper deck. The samples were collected from 28 August to 18 September, 2024. Each sample was collected for about 3 days.

Y. Yamashita, H. Kubo

### ***Changes in $\delta^{13}$ of DOC with photodegradation***

To determine changes in  $\delta^{13}$  values of DOC with photodegradation, water samples were collected from 1000 and 3000 m at Stn 2, 100, 500, and 5000 m at Stn 4, 750 m at Stn 7, 200 and 2000 m at Stn 8, and 300 m at Stn 9. 36 L of samples were filtered using a pre-combusted GF/F filter (Whatman). A pre-concentration of DBC by solid phase extraction (SPE) were performed onboard with SPE cartridge (1 g, Bond Elut PPL, Agilent Technologies). The concentrated samples will be irradiated by natural sunlight at Hokkaido University, and changes in  $\delta^{13}$  values of DOC along with irradiation will be determined.

Y. Yamashita, H. Kubo

### **Acknowledgements**

The aerosol samples were collected in collaboration with Dr. Iwamoto (Hiroshima University).

# **Microbial diversity and functions in seawater, sea surface microlayer and sea spray aerosols in the Eastern Indian Ocean**

Koji Hamasaki<sup>1</sup>, Yutaka Okamoto<sup>1</sup> and Yoko Iwamoto<sup>2</sup>,

<sup>1</sup>Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>2</sup>Graduate School of Integrated Sciences for Life, Hiroshima University

Correspondence: Koji Hamasaki

## **Introduction**

Prokaryotic communities play vital roles in marine biogeochemical cycles. Especially, actively growing cells require more amount of organic matter and nutrients for their growth and are more vulnerable to predatory grazing by protists and viral lysis compared to inactive ones. Thus, the investigation of the diversity and functions of actively growing cells contributes to understanding marine biogeochemical cycles. During this cruise, we explored prokaryotic diversity and functions in the water column in the Eastern Indian Ocean. To analyze actively growing cells, bromodeoxyuridine (BrdU), a halogenated nucleoside and thymidine analogue, is used in this study.

The sea surface microlayer (SML) is a thin surface film located at the interface between the sea surface and the atmosphere; typically the top 1mm of surface seawater. The SML accumulates various chemical and biological materials because of its surface tension, which leads to higher organic matter concentrations such as chlorophylls and TEPs and also higher abundance and productivity of microbes than those in the underlying water (UW). Organic matter and microbes originating from the SML are major sources of bioaerosols in the open ocean and determines the physicochemical characteristics of sea spray aerosols (SSA) such as cloud condensation nuclei (CCN) activity and ice nuclei (IN) activity. Although the microbial activity changes physicochemical characteristics of SML and subsequently the characteristics of SSA, knowledge on microbes presenting in the SML and SSA is limited. In the present cruise, we collected samples to explore prokaryotic diversity and functions in the SML, UW and SSA in order to understand a role of microbes in air-sea interactive processes.

## **Materials and Methods**

*Seawater sampling for microbial analyses*

Water samples (10 L) were collected at 10 depths from 0 m to Bottom-20m (10m, 100m SCM, 200m, 400m, 1000m, D0min, 2000m, B-20) at St.2, St.3, St.4, St.7, St.8 and St.9 (Routine “Shallow”, “Middle” and “Deep” CTD casts). On the time of deploying Shallow CTD cast, surface seawater samples (0m) were collected with the use of a plastic bucket. In addition to CTD Niskin-bottle sampling, seawater samples (~5m) were also collected from the outlet of onboard seawater pumping system at St.5' (10°S, 91°E), St.6' (15°S, 90°12'E) and St.7' (20°S, 88°E) during the transition from St.4 to St.8. After the sampling, water samples were serially filtered onto 3.0-µm pore-size polycarbonate filters (47mm Nuclepore) and 0.22-µm cartridge filters (Sterivex, Millipore), and the filters were subsequently stored at -80 °C until further processing in the lab.

In order to analyze actively growing bacteria and measure bacterial production, seawater (10/20 L) was additionally collected from 4 depths (0, 100, 1000 and B-20m) and subjected to the addition of bromodeoxyuridine (BrdU) at a final concentration of 20 nM. After the addition of BrdU, the sample bottles were incubated in a dark condition for 20-30 hrs at *in situ* temperature ( $\pm 2$  °C). After the incubation, water samples were serially filtered onto 3.0-µm pore-size polycarbonate filters (47mm Nuclepore) and 0.22-µm cartridge filters (Sterivex, Millipore), and the filters were subsequently stored at -80 °C until further processing in the lab. Also, an aliquot of water (10 ml duplicate) was subsampled and stored at -80 °C after the addition of TdR solution (10 µM final).

Another set of seawater samples (300ml) were filtered onto 0.4-µm pore size polycarbonate filters (25mm Nuclepore). Filters were stained by 0.2-µm prefiltered Alcian Blue (final conc 0.02%) and Coomassie Brilliant Blue (final conc 0.04%) stains, respectively. Each stained filters will be used to measure TEP (Transparent Exopolymer Particles) and CSP (Coomassie Stained Particles), which can quantify polysaccharide-rich, gel-like particles and transparent proteinaceous particles in sea water respectively. Furthermore, as subsamples, 1.5ml seawater samples from each depth were fixed by Glutaraldehyde (2% in final conc.) for counting bacterial abundance by flow cytometry.

#### *Sea surface microlayer sampling for chemical and microbial analyses*

The sampling was performed at St.3 and St.9 on a Zodiac boat launched from Hakuho-maru. Sea surface microlayer samples were collected with the use of the drum sampler at about half miles away from the mother ship. Rotating the drum at 8 rpm for c.a. 1-3 hours resulted in the collection of 1-10 L sea-surface microlayer (SML) waters. Underlying water (UW) (10 L) was also collected from the 50 cm below the surface at an opposite side of the boat using HYROHT glass bottle water sampler (Shibata). Exact location of the sampling was recorded by

GPS with several meters accuracy. Wind speed and air temperature were measured by using a handheld anemometer. Water temperature and salinity were measured by using a handheld sensor.

After coming back to the mother ship, collected water samples of SML and UW were aliquoted for further analyses including measurements of chl a, nutrients, suspended particles (TEP and CSP), bacterial abundance by using FCM and bacterial production as well as AGB community analysis by using BrdU. Also, the water samples were used for bubble bursting experiments as described in the following section. The rest of water was filtered onto 3.0- $\mu\text{m}$  PC and 0.22- $\mu\text{m}$  Sterivex filters for microbial diversity and community structure analyses, and the filters were subsequently stored at  $-80\text{ }^{\circ}\text{C}$  until further processing in the lab.

#### *Bubble bursting experiment*

Bubble bursting experiments were conducted using SML and UW samples collected at 2 stations (St.3 and St.9). Furthermore, they were conducted using seawater collected from the outlet of onboard seawater pumping system at St.5' ( $10^{\circ}\text{S}$ ,  $91^{\circ}\text{E}$ ), St.6' ( $15^{\circ}\text{S}$ ,  $90^{\circ}12'\text{E}$ ) and St.7'

( $20^{\circ}\text{S}$ ,  $88^{\circ}\text{E}$ ) during the transition from St.4 to St.8. A glass-made scrubbing bottle filled with 700 to 1000 ml of either SML or UW sample was used to generate bubble bursting aerosols (BBA). Compressed dry air ( $2.0\text{ L min}^{-1}$ ) was bubbled through the glass filter positioned below the air-water interface. The generated BBA were collected on 47 mm quartz fiber (for chemical analysis) and PTFE filter (0.45 $\mu\text{m}$  pore size, Advantec) (for microbial analysis) filters set in polycarbonate filter holders (NL-I-01, NILU Products AS) at the flow rate of  $3\text{ L min}^{-1}$  for 6 to 12 hours. One hour bubbling of sterilized artificial seawater was performed after the bubbling times of SML and UW in order to flush remaining contaminants out from the lines of air flow.

#### *Aerosol sampling for microbial analyses*

The high-volume air sampler (model AS900, Kimoto Electric Co. Ltd.) with a virtual impactor separating coarse and fine particles (50% cut-off diameters of  $2.5\text{ }\mu\text{m}$ ) was placed on the upper deck to collect ambient aerosol samples at a flow rate of  $1100\text{--}1200\text{ L min}^{-1}$ . A Teflon filter (ADVANTEC, PF040, 90 mm) was replaced every day. After collections, the filter was cut into two parts with an ethanol-cleaned scissor. Both coarse and fine particles were collected on a central part and fine particles were collected on an outer part. The separated filters were stored at  $-80\text{ }^{\circ}\text{C}$  until further microbial analysis in the lab. Also, a small piece of each filter was subsampled, fixed with 2 % glutaraldehyde (diluted in equal volumes of 70% ethanol and MiliQ water) and stored at  $-80\text{ }^{\circ}\text{C}$  for counting prokaryote cells.

**Expected Results**

Filter samples from the SML, UW and aerosols will be subjected to DNA extraction, PCR amplification of 16S rRNA gene and sequencing, which provides the data on bacterial and archaeal diversity and community structures of each sample and suggests their transition among these fractions. KH23-3 research cruise data proved that bacterial compositions were clearly different between SML and SSA samples and bacterial selection may exist in the process of aerosol emission. We expect that KH24-3 data can support this result. The bubble bursting experiment compliments these results by showing direct connection between the SML and sea spray aerosols. Also, the chemical analysis of aerosol samples is expected to show the relationship between abundance and community structures of microbes and the abundance and composition of organic matters in the SML and aerosols. This would be the report on showing the dynamic nature of microbes during the process of SML and SSA formation in the open ocean.

# **Ecology of marine microbes and viruses and their roles in the biogeochemical cycles in the Indian Ocean**

Hisashi Endo, Siyu Jiang, and Takuya Sato

Institute for Chemical Research, Kyoto University

Correspondence: Hisashi Endo

## **1. Introduction**

Marine plankton assemblages are composed of various species, including protozoa, phytoplankton, and bacteria. Their composition and physiological state are tightly coupled to the ecosystem and biogeochemical functions. Recently, we established a novel method to evaluate the lytic cell death of plankton based on the rRNA metabarcoding of both the cellular and extracellular (cell-free) fractions using a high-throughput sequencing (HTS) technique. Additionally, recent advances in library preparation techniques enable us to obtain gene expression profiles of plankton communities from a few liters of seawater. In this study, we combine these state-of-the-art techniques to elucidate the lytic mortality of marine plankton and its effects on physiological status and biogeochemical cycles.

## **2. Distribution and activity of protists and their viruses**

To elucidate the vertical distribution of lytic cell mortality, cellular and cell-free rRNA samples were collected from 6 depths (10 m, SCM, 1% light depth, 200 m, 500 m, and 1,000 m) at the 6 sample stations (Stn. 2, 3, 4, 7, 8, and 9) using Niskin bottles or from a depth of research seawater at 3 sites (Stn. 5', 6', and 7'). Samples for flow cytometry, POC, microscopy, excitation emission matrix (EEM), and metagenome/metatranscriptome analyses were also taken from 2–6 depths at the same station. For DNA and RNA samples, seawater (10 L) were pre-filtered through 144  $\mu\text{m}$  Teflon mesh to remove large particles. Seawater (400 mL) was filtered through the 47 mm diameter 0.2  $\mu\text{m}$  pore size polycarbonate filter (Isopore) with a gentle vacuum ( $< 0.013$  MPa) to separate into cell-associated and cell-free rRNA fractions, then stored at  $-80^{\circ}\text{C}$  until analysis. For metagenome/metatranscriptome samples, seawater (10 L) was filtered onto 0.2  $\mu\text{m}$  polycarbonate filters and stored at  $-80^{\circ}\text{C}$  until analysis. For flow cytometry samples, seawater (2 mL) were preserved with paraformaldehyde (0.2% final concentration) and stored at  $-80^{\circ}\text{C}$  until analysis. Seawater samples for microscopic analysis (500 mL) were fixed with Lugol's iodine solution (1% final concentration) and then stored at  $4^{\circ}\text{C}$  until analysis. Throughout the cruise period expect EEZ, photosynthetically active radiation

(PAR) was monitored with a PAR sensor (METER).

## **2. Production and degradation of cell-free rRNA**

To evaluate in-situ cell lysis of the protist community, incubation experiments were carried out. Ambient seawater pre-filtered through 144  $\mu\text{m}$  Teflon mesh were collected from 4 depths (10 m, 1% light depth, 200 m, and 500 m) using Niskin bottles. Prior to the incubation, purified ribosomes from *Escherichia coli* strain B (New England Biosciences, cat. P0763S) were added to the seawater to measure the degradation rate of free ribosomes. Incubation was conducted for 24 hours on-deck or in a laboratory incubator at in-situ temperature and light conditions. Cellular and cell-free rRNA samples were collected as described above at the first and the end of the incubations, then stored at  $-80^{\circ}\text{C}$  until analysis on land.

# Activity and community of diazotrophs and phage-diazotroph relationship in the eastern Indian Ocean

Takuya Sato\*

Institute for Chemical Research, Kyoto University

Correspondence: Takuya Sato

## Introduction

Nitrogen-fixing microorganisms (diazotrophs) play an important role in controlling marine productivity by converting nitrogen gas into biologically available form, ammonium. Recent studies have shown that more diverse diazotrophs significantly contribute to the nitrogen fixation in wider regions than previous thoughts. However, our knowledge about the variation of their activity and ecological role is still scarce. This study aims to elucidate the spatial patterns of the nitrogen fixation activity and diazotroph community structure in the eastern Indian Ocean. Additionally, in order to understand the phage-diazotroph relationship, the phage community was also investigated.

## 1. Nitrogen fixation, primary production, and nitrate assimilation

To estimate nitrogen fixation and its contribution to primary and new productions, incubation experiments for nitrogen fixation, primary production, and nitrate assimilation were conducted. Water samples were collected from 3 depths (10 m, 10% and 1% relative light depth) or only from the surface pumping system. Nitrogen fixation was estimated by using the  $^{15}\text{N}$  gas dissolution method (Mohr et al. 2010) together with primary production by uptake of  $^{13}\text{C}$ -sodium bicarbonate at all stations. After the addition of N and C tracers to light-level adjusted 4 L polycarbonate bottles, incubations were conducted for 24 hours using an on-deck incubator flowing surface seawater. Nitrate assimilation was estimated by the Michaelis-Menten kinetics approach to avoid the overestimation.  $^{15}\text{N}$ -labeled nitrate was added to light-level adjusted 2 L polycarbonate bottles with final concentrations of 10, 20, 100, 1000 nM. The incubations were conducted for 6 hours during daytime and terminated by gentle vacuum filtration of the seawater samples through a pre-combusted glass fiber filter. The filters were then frozen for later analysis on land.

## **2. Diazotrophs and phage community structure**

In order to determine the diazotroph community structure and active diazotrophs, water samples for DNA and RNA analysis for the diazotroph community were collected from 10 m and 1% light depth at every station. The approximate 10 L seawater samples for the DNA and RNA were filtered onto 0.22  $\mu\text{m}$  pore size sterivex filters. The filter units were flash frozen and stored at  $-80^{\circ}\text{C}$  until the analysis on land. Also, in order to collect the virion fraction, virions in 10 L filtered ( $< 0.22 \mu\text{m}$ ) water were concentrated using the  $\text{FeCl}_3$  flocculation method (John et al. 2011). The samples were kept in the dark at  $4^{\circ}\text{C}$  until DNA extraction. All extracted DNA and RNA samples will be subjected to metagenomic or metatranscriptome analysis on land.

# Meridional and vertical distributions of Chlorophyll *a*

Siyu Jiang<sup>1</sup>, Jiakang Liu<sup>2</sup>, Akihiro Takamura<sup>3</sup>, Chiho Funaki<sup>4</sup>, Hisashi Endo<sup>1</sup>

<sup>1</sup> Institute for Chemical Research, Kyoto University

<sup>2</sup> Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>3</sup> Graduate School of Natural Science and Technology, Kanazawa University

<sup>4</sup> Graduate School of Agriculture and Life Science, The University of Tokyo

## 1. Introduction

To understand the meridional and vertical distributions of phytoplankton biomass across the cruise, the chlorophyll *a* (Chl *a*) concentrations were measured onboard by a fluorometer.

## 2. Materials and methods

Seawater samples (277 mL) were collected directly from Niskin bottles. Especially, a bucket was used to collect seawater from the sea surface (0 m). Seawater samples were filtered through 25-mm-diameter Whatman GF/F filters (GE Healthcare Life Sciences, Pittsburgh, PA, USA) under low vacuum ( $< 0.02$  MPa). The filters were immediately immersed in 5 mL N,N-dimethylformamide. Chl *a* was extracted at  $-20$  °C in dark for at least 24 hours and measured onboard by a fluorometer (10-AU-005; Turner Designs, San Jose, CA, USA) following a non-acidification method.

## 3. Results

The vertical distributions of Chl *a* at each station were shown in Figure 1. Stations 2 and 3 had a similar vertical distribution, that is, higher Chl *a* concentrations ( $> 0.1$  mg m<sup>-3</sup>) at both surface (10 m) and subsurface chlorophyll maximum (SCM). In contrast, the other four stations showed the lower surface concentrations ( $< 0.1$  mg m<sup>-3</sup>), and deeper SCM with the concentrations lower than 0.3 mg m<sup>-3</sup>. Especially, Station 7 showed a relatively typical Chl *a* profile in oligotrophic waters, with a very low surface concentration (only 0.055 mg m<sup>-3</sup>) and a deep SCM at around 150 m.

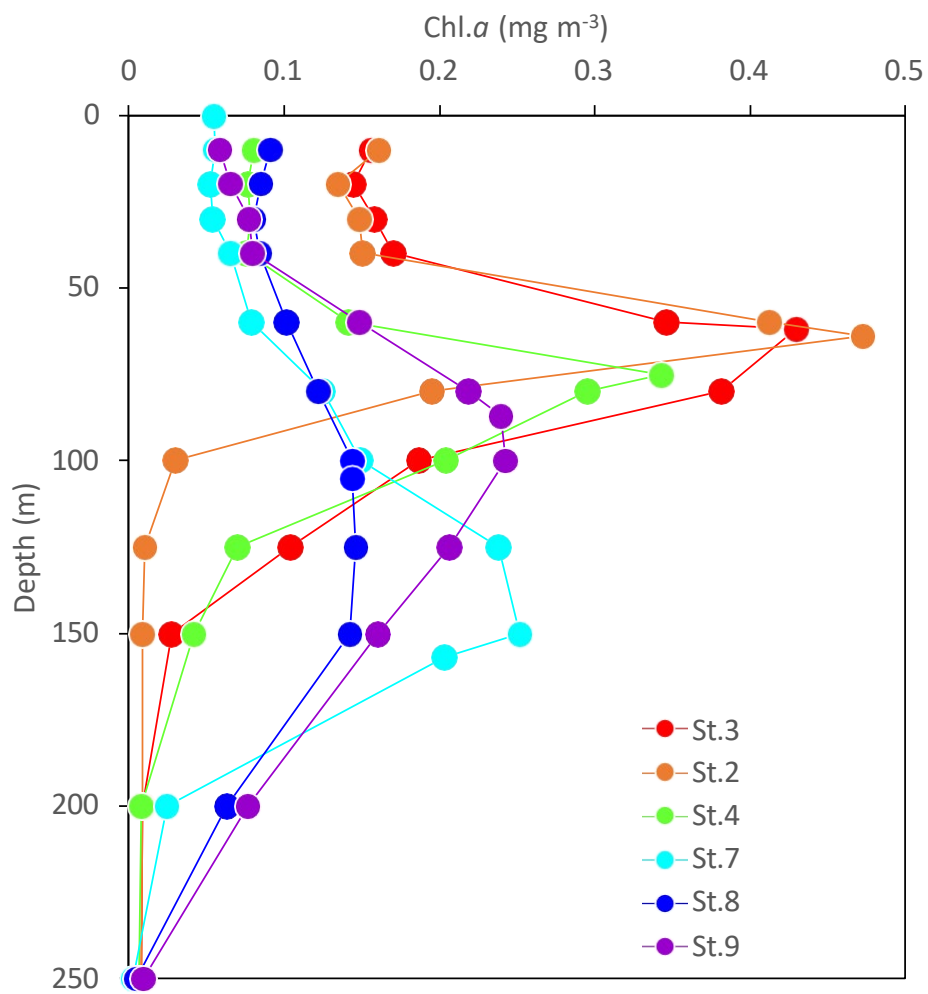


Figure 1. Vertical distributions of Chl *a*

# **Formation mechanism of surface phytoplankton blooms in the Eastern Indian Ocean revealed by subsurface seawater enrichment experiments**

Siyu Jiang<sup>1\*</sup>, Jiakang Liu<sup>2</sup>, Hisashi Endo<sup>1</sup> and Hiroaki Saito<sup>2</sup>

<sup>1</sup> Institute for Chemical Research, Kyoto University

<sup>2</sup> Atmosphere and Ocean Research Institute, The University of Tokyo

\*Correspondence: Siyu Jiang

## **1. Introduction**

The Eastern Indian Ocean (EIO) is considered to be oligotrophic and exhibit low chlorophyll *a* (Chl *a*) concentrations. However, high Chl *a* levels (surface phytoplankton blooms) were occasionally reported and had been attributed to physical events that enriched nutrients from the subsurface seawater and supported the surface phytoplankton growth (Jiang et al., 2022). However, the detailed mechanism of such phytoplankton bloom remains less studied. For instance, is this kind of bloom always occur with vertical mixing? Or, it occurs in certain regions or under certain conditions? If it occurs, how does it initiate, develop and decay? How is the magnitude? What is the dominant phytoplankton? To answer these questions, the subsurface seawater enrichment incubation experiments were conducted at four stations across the EIO.

## **2. Materials and methods**

The subsurface seawater enrichment experiments were conducted at the 10 m layer at four stations throughout the cruise, by using a refined dilution experiment (Landry and Hassett, 1982). All carboys, tubes and capsule filters were washed with 10% HCl and rinsed with distilled water and then seawater for 3–4 times before use.

At each station, eight pre-washed polycarbonate carboys (13.3 L capacity, labelled with A~H) were use for incubation (Figure 4.22.1). Firstly, four of them (A, B, E, F) were filled with the subsurface seawater (~ 250 m) to 20% of capacity. Then, the carboys labelled with A~D were filled with particle-free seawater (prepared by filtering

natural seawater (10 m) through an acid-rinsed 0.2- $\mu$ m filter capsule (Pall Corporation, New York, NY, USA)) to 80% of capacity. Finally, carboys labelled with A~F were filled with natural seawater (10 m) to the capacity. In addition, two carboys labelled with G and H were filled with 100% natural seawater (10 m) as controls. Samples for analyzing nutrients, Chl *a*, picophytoplankton community, phytoplankton pigments, DNA/RNA and microzooplankton community were collected from natural seawater as initial samples.

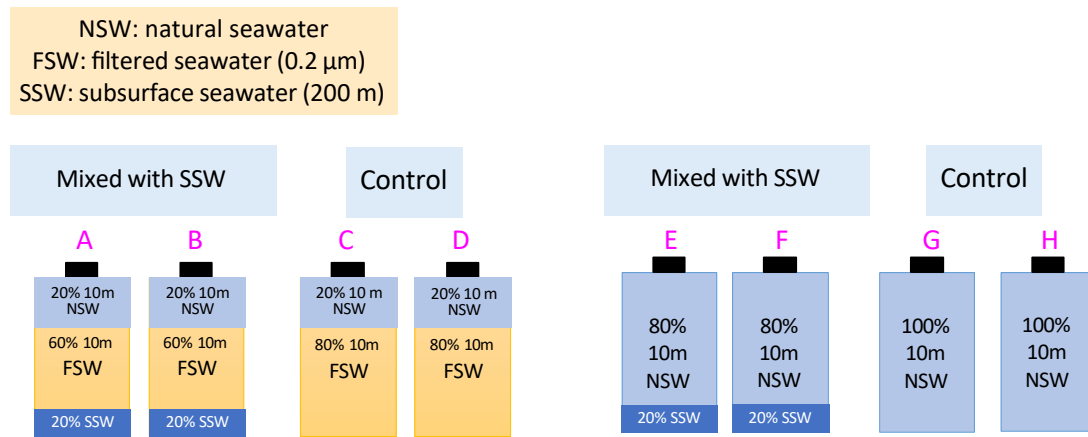


Figure 1. Schematic diagram of the incubation experiment.

All carboys were gently mixed and those labelled with A~F were sampled for nutrients. Then, these carboys were tightly capped and incubated for totally 72 h in an on-deck incubator with running surface seawater. For simulating the ambient light, the incubator was covered by a neutral-density filter to 50% of the surface light irradiance.

After incubating for 24 h and 48 h, all carboys were gently mixed and sampled for analyzing nutrients, Chl *a*, picophytoplankton, and phytoplankton pigments with a 3-port polypropylene filling/venting closure (Thermo Scientific). One of the ports was used to transfer water for sampling. The other two ports were used for venting and were connected to two 0.2- $\mu$ m PTFE filters to avoid any contaminations from air. In addition,

0.2 L water was sampled from carboys E~H for examining microzooplankton community. After sampling, all carboys were tightly capped, gently mixed and put back in the on-deck incubator. At the end of incubation (72 h), all carboys were gently mixed and sampled for nutrients, Chl *a*, picophytoplankton community, phytoplankton pigments, microzooplankton community as well as DNA/RNA.

The detailed sampling method is described as following:

To examine the concentrations of inorganic nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{Si(OH)}_4$ ), water samples were collected in 12 mL acrylic tubes and stored in a gas-tight aluminum bag in a deep freezer at  $-80^\circ\text{C}$  and analyzed on land using a BL-Tec autoanalyzer (QuAAtro, SEAL Analytical Inc., Mequon, WI, USA).

For measuring Chl *a* concentrations, seawater samples ( $\sim 0.6$  L) were filtered through 25-mm-diameter Whatman GF/F filters (GE Healthcare Life Sciences, Pittsburgh, PA, USA) under low vacuum ( $< 0.02$  MPa). The filters were immediately immersed in 5 mL N,N-dimethylformamide. Chl *a* was extracted at  $-20^\circ\text{C}$  in dark for at least 24 hours and measured onboard by a fluorometer (10-AU-005; Turner Designs, San Jose, CA, USA) following a non-acidification method.

To reveal the picophytoplankton community composition, seawater (4 mL) for flow cytometry (FCM) analysis was fixed immediately with buffered paraformaldehyde (final concentration of 0.5%), placed at room temperature for 15 min, and stored in a  $-80^\circ\text{C}$  freezer until analyzed.

For analyzing concentrations of phytoplankton specific pigments, seawater ( $\sim 2$  L) for high-performance liquid chromatography (HPLC) analysis was filtered on 25-mm- diameter Whatman GF/F filters under low vacuum ( $< 0.02$  MPa). The filters were immediately frozen and stored at  $-80^\circ\text{C}$ .

Seawater for microzooplankton analysis (0.2~0.5 L) was fixed by Lugol's iodine solution (final concentration 3%) in dark polyethylene bottles and preserved at

4~6 °C until analysis.

For collecting DNA/RNA samples, seawater (~ 7 L) was filtered on 0.2 µm pore size Supor® PES filter membranes (47 mm, Pall Corp., Port Washington, NY, USA) with gentle vacuum (< 0.013 MPa). Filters are frozen in cryogenic vials immediately in a deep freezer at -80 °C until analysis.

The obtained parameters in this 72-hours incubation experiments 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 data of growth and mortality rates of various phytoplankton, and changes in phytoplankton community composition before and after incubation.

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# **Distribution and community composition of dinoflagellates in the eastern Indian Ocean**

Jiakang Liu<sup>1</sup>, Siyu Jiang<sup>2</sup> and Hiroaki Saito<sup>1</sup>

Atmosphere Ocean Research Institute, the University of Tokyo

Institute for Chemical Research, Kyoto University

Correspondence: Jiakang Liu

## Introduction

Dinoflagellates are major components of marine ecosystems and play an important role in food web dynamics. At the same time, their distribution is influenced by environmental condition. There is large environmental difference in the Eastern Indian Ocean, target are of this study. The sea surface water temperatures is higher in the Bay of Bengal and equator while low in the south. Salinity is generally high but can vary due to rainfall and river inflows. Additionally, the climate is diverse, with monsoon-driven storms and tropical cyclones affecting sea conditions and coastal ecosystems during certain seasons. It is expected different species occur from north to the south of the meridional transect in this study. Dinoflagellates have three types of trophic strategy, which are autotrophy, heterotrophy and mixotrophy. The different trophic strategy is one of key characteristics to adapt various environments. We still don't know the biogeography, diversity and ecological roles of dinoflagellates in the oceanic ecosystems, especially in the Indian Ocean. In this study, we investigated the community composition of dinoflagellates and factors influencing the distribution of dinoflagellates in eastern Indian Ocean.

## Materials and Methods

At each station, water samples are collected from 4 layers (surface layer (10 m), upper euphotic zone (40 m), lower euphotic zone (60, 80, or 100 m), below the euphotic zone (150 m). Water samples were transferred from Niskin bottles attached to CTD/Carousel system through silicon tube to two 2.3-liter PC bottle s.

The seawater in each bottle was filtered through 0.8  $\mu$ m Supor-800 with gentle vacuum ( $< 0.013$  MPa) as DNA samples (Cheung et al., 2017, 2020). After filtration, the filters were put into cryogenic vials and frozen immediately in a deep freezer at  $-80^{\circ}$  C until analysis.

After filtration, all filtering equipment cups were rinsed with ethanol and distilled water for next use. To minimize contamination, we used the same bottles for each sampling layer and rinsed well as described above. We also collected surface seawater (6m) through an experimental seawater supplying system at two stations at 15oS and 20oS. For the water sampling, different sampling bottles from CTD sampling and filtration devices were used to

avoid contamination.

Table 1. Water sampling station and depth.

| Station   | 2   | 3   | 4   | 15S:090E | 20S:088E | 7   | 8   | 9   |
|-----------|-----|-----|-----|----------|----------|-----|-----|-----|
| Depth (m) | 10  | 10  | 10  | 6        | 6        | 10  | 10  | 10  |
|           | 40  | 40  | 40  |          |          | 40  | 40  | 40  |
|           | 60  | 100 | 80  |          |          | 80  | 80  | 80  |
|           | 150 | 150 | 150 |          |          | 150 | 150 | 150 |

# **Fine-scale vertical profiles of microbial communities around subsurface chlorophyll maxima (SCM)**

Mitsuhide Sato

Graduate School of Integrated Science and Technology, Nagasaki University  
Correspondence: Mitsuko Sato

## **Introduction**

Pico- and nanophytoplankton are central primary producers in the oligotrophic open ocean. They can easily respond to nutrient inputs from atmospheric deposition or other processes due to their efficient nutrient acquisition and high growth rate. Its chlorophyll biomass often exhibits a distinct subsurface peak around the depth of 1% relative light intensity to the surface, called the subsurface chlorophyll maximum (SCM). The SCM sometimes coincides with a plankton carbon biomass peak, but sometimes results from low light adaptation of the phytoplankton, which shows no apparent carbon biomass peak. In addition, different phytoplankton populations exhibit different depths of subsurface abundance peaks, reflecting their photophysiological characteristics. This leads to acute vertical variations in microbial community structures around the SCM. However, in conventional sampling protocols, seawater samples are collected at approximately 10 m intervals around the SCM at minimum, typically 25 m intervals, which often fails to capture the drastic vertical variations around the SCM. In the present study, I performed fine-scale sampling to reproduce the vertical profiles of microbial communities around the SCM from discrete measurements.

## **Materials and Methods**

Flow cytometric samples were obtained from different depths between 0 and 300 m of routine (Middle and Shallow) casts and fixed with 1% glutaraldehyde for analysis on land. In addition to routine sampling, high-resolution sampling around the SCM was conducted at all the sampling stations. The depth of the SCM was determined from the fluorescence profile obtained during the downward cast. During the subsequent upward cast, seawater samples were collected from 10 depths around the SCM at approximately 3 m intervals as the cable was slowly reeled up.

All the samples will be analyzed in a land laboratory for enumeration of *Prochlorococcus*, *Synechococcus*, nano-sized cyanobacteria, eukaryotic phytoplankton, and heterotrophic prokaryotes and nutrient measurement by the conventional method. In addition to flow cytometric enumeration, photosynthetic parameters of phytoplankton communities, including quantum efficiency of photosynthetic system II, half-saturation irradiance, and electron transport rate were obtained from fine-scale sampling around the SCM. All parameters were measured on board using a LabSTAF benchtop fluorometer borrowed from Dr. MASUDA, Takako. The results obtained on board will be analyzed in detail on shore.

# Ecological study on mixotrophic microplankton in the eastern Indian Ocean

Chiho Funaki<sup>1\*</sup>, Kazutaka takahashi<sup>1</sup>

<sup>1</sup> Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences,  
The University of Tokyo

*\*On-board*

Correspondence: Chiho Funaki

## Introduction

Mixotrophic microplankton whose size is 20-200µm has attracted much attention recently due to its unique functional role in the marine food web. Its common occurrence suggests that the marine food web is more complex than previously thought as it can affect the ecological efficiency, prey-predator relationship, and biogeochemical cycle within the ecosystems. Therefore, we aimed to determine the spatiotemporal distribution pattern of mixotrophic microplankton to infer their roles in the food webs in the Indian ocean.

## Materials and Methods

### *Distribution and composition of micro plankton*

Water samples (4 L) were collected from 0, 10, 60 m, and SCM using Niskin-X bottles on a CTD-CMS and a plastic bucket. The water samples were concentrated to <250 mL by reverse filtration with 10 µm mesh and preserved with 4 % acid Lugol's solution. Samples will be analyzed using an inverted microscope after being settled by gravity in the lab. Microplankton will be identified, and the composition in terms of number and biomass will be determined.

### *Growth of microplankton*

Water samples (4.5 L) were collected from 10 m depth. Samples were filtered by 200 µm mesh to remove predators and incubated for 24h in a water bath at the sea surface temperature and under 30% of sea surface light intensity. Initial and incubated samples in triplicate were preserved with 4 % acid Lugol's solution. Chlorophyll-*a* fluorescence of each sample was also measured. 250 mL of samples which were filtered onto GF/F filters were soaked in DMF, and the fluorescence in the extraction were measured by a fluorometer. Preserved samples will be analyzed in the lab. The number of microplankton will be counted, and the growth rate of each microplankton will be calculated from the difference in number between initial and incubation bottles.

### *Mixotrophy of microplankton*

Water samples (4 L) collected from 0, 10, 60 m, and SCM were concentrated to less than 50 mL by reverse filtration with 10 µm mesh and were fixed in 1% glutaraldehyde and stained by DAPI. Fixed samples were filtered onto 8 µm pore-size black membrane filters and stored at -80 °C until further analysis in the lab.

### *Dilution experiment*

Dilution experiments were conducted to measure microplankton growth and grazing rates. Water samples were collected from 10 m and predators were removed using 200  $\mu\text{m}$  mesh. Particle-free water was also collected from 10 m by filtering seawater through 0.2  $\mu\text{m}$  filter capsules. Water samples were transferred to the polycarbonate bottles (2.4 L) to the measured volume of four dilution treatments (dilution factors of 0.25, 0.5, 0.75, and 1), and the bottles were filled with particle-free water. Then, a solution of inorganic nutrients (1 mL) was added to the final concentrations of 0.5  $\mu\text{mol L}^{-1}$   $\text{NH}_4\text{Cl}$  and 0.03  $\mu\text{mol L}^{-1}$   $\text{KH}_2\text{PO}_4$  to avoid nutrient limitation on phytoplankton growth. All samples were incubated in duplicate for 24h in a water bath at the sea surface temperature and under 30% of sea surface light intensity. After incubation, 250 mL were filtered onto GF/F filters to measure chlorophyll *a* fluorescence and 2L of samples were preserved with 4 % acid Lugol's solution. Preserved samples will be analyzed in the lab using an inverted microscope after being settled by gravity. The number of microplankton will be counted, and the growth rate of each microplankton will be calculated.

### **Expected Results**

The vertical distribution and community composition of microplankton will be determined through the analysis of Lugol's samples. The analysis of filter samples will show the contribution of mixotroph to the community. Growth rates and grazing rates, derived from the onboard incubation experiments will bring valuable insight about the ecological role of mixotrophic microplankton.

# Visualization and quantification of primary producers in Indian Ocean

Takako Masuda<sup>1\*</sup>, Kazutaka Takahashi<sup>2</sup>

<sup>1</sup>Fisheries Resources Institute, Japan Fisheries Research and Education Agency

<sup>2</sup> Graduate School of Agriculture and Life Science, The University of Tokyo

*\*On-board*

Correspondence: Takako Masuda

## Introduction

Primary production by phytoplankton forms the basis of aquatic food webs. In most studies primary productivity is estimated by bulk measurements, specifically by mass spectrometry analysis. The bulk measurements provide the quantity of primary production, but it cannot tell which organisms are the primary producers. To identify the primary producer in the Indian Ocean, we will visualize and quantify cellular C and N uptake at bulk and single-cell level. Further, we will evaluate primary productivity by variable fluorescence approaches.

## Materials and Methods

### *Experimental setup and sample collection for C and N uptake.*

We carried out <sup>13</sup>C and <sup>15</sup>N uptake experiments using natural phytoplankton assemblages collected at stations 2, 3, 4, 7, 8, and 9 from 10 m depth using Niskin-X bottles on a CTD-CMS or at station 5', 6', 7' from "water for research" pumped up from the bottom of the ship (about 6m depth). Water samples were distributed into 2.3-liter or 1.2-liter PC bottles, which were rinsed overnight in a neutral detergent, followed by 0.3 N HCl, and rinsed with Milli-Q water. At all stations, we performed 6 treatments: Initial (without enrichment), Control (NaH<sup>13</sup>CO<sub>3</sub><sup>-</sup>), NO<sub>3</sub><sup>-</sup> (Na<sup>15</sup>NO<sub>3</sub>+NaH<sup>13</sup>CO<sub>3</sub><sup>-</sup>), NO<sub>3</sub><sup>-</sup>+Fe (Na<sup>15</sup>NO<sub>3</sub>+FeCl+EDTA+NaH<sup>13</sup>CO<sub>3</sub><sup>-</sup>), NH<sub>4</sub><sup>+</sup> (<sup>15</sup>NH<sub>4</sub>Cl+NaH<sup>13</sup>CO<sub>3</sub><sup>-</sup>), Glu (<sup>15</sup>NH<sub>4</sub>Cl+<sup>13</sup>C-glucose). The concentrations of enrichment were 100 nM for NO<sub>3</sub><sup>-</sup> or <sup>15</sup>NH<sub>4</sub><sup>+</sup>, 200 μM for NaH<sup>13</sup>CO<sub>3</sub><sup>-</sup> or 2μM for <sup>13</sup>C-glucose and 1nM for Fe and EDTA.

Triplicate 2.3-liter bottles were incubated for 6 hours for bulk mass spectrometry analysis, in addition, one 1.2-liter bottle was incubated for single cell analysis using NanoSIMS and variable fluorescence analysis. Samples for mass spectrometry analysis were collected onto 25 mm  $\varnothing$  pre-combusted GF/F filter, and the samples for NanoSIMS were collected onto 47 mm  $\varnothing$  0.2 μm polycarbonate filters (Nuclepore). Samples for mass spectrometry analysis were kept stored at -80°C, and NanoSIMS were fixed with glutaraldehyde (1% final concentration), flash-frozen in liquid nitrogen and stored at -80°C until analysis on land. To estimate the efficiency of photosynthesis, variable chlorophyll fluorescence was measured to estimate electron transport rate using LabSTAF (Schuback et al., 2021).

### *Phytoplankton photosynthesis.*

To assess phytoplankton photosynthesis along the transect of the cruise, samples from "water for research" pumped up from the bottom of the ship (approximately 6-m depth) was continuously measured by single-turnover variable chlorophyll fluorescence approach using LabSTAF (Schuback et al., 2021).

**Expected Results**

Mass spectrometry analysis will allow us to estimate nitrate and ammonium, and bicarbonate assimilation rates as well as particulate nitrogen (PN) and particulate organic carbon (POC). Single cell using NanoSIMS analysis will allow us to identify key primary producer in the observed stations. This would be the first report on showing the key primary producer in the Indian Ocean. Variable chlorophyll fluorescence analysis together with  $^{13}\text{C}$  uptake results will allow us to estimate primary productivity as well as electron requirement for C uptake.

## **Diversity of zooplankton and micronekton in the eastern Indian Ocean**

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-24-3 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. We also collected microplastic and neuston samples using a neuston net. 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 2, 3, 4, 7, 8, and 9. 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.

**Multinet:** Bulk zooplankton samples were collected by oblique tows of Multinet with 100 µm mesh size at the station 2, 3, 4, 7, and 9. Samples were collected from five layers (0–200, 200–500, 500–1000, 1000–1500 m, and 1500–2000 m). All samples were preserved in 99% ethanol for meta-analysis of zooplankton communities.

**NORPAC:** NORPAC net with 100 µm mesh size was vertically towed to collect zooplankton at the depth of 0–200 m at the stations 2, 3, 4, 7, 8 and 9. 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 2, 3, 4, 7, 8, and 9. After samplings, large fish and shrimp were picked up, and other samples were preserved in 99% ethanol or at -20°C for DNA barcoding and population genetics.

**MOHT:** Micronekton were collected at the depth of 0–500 m by oblique tow of MOHT with 1.95 mm mesh size at stations 2, 3, 4, 7, 8 and 9. After samplings, large fish and shrimp were picked up, and other samples were split and preserved in 99% ethanol, 5% formalin, or at -20°C for diet analysis and community analysis of micronekton.

**Neuston net:** Neuston and microplastic at the sea surface were collected by Neuston net with 335 µm mesh size at stations 2, 3, 4, 7, 8 and 9. Samplings were carried out for 20 minutes with ship speed of 2.0 knot. Samples were preserved in 5% formalin for counting amounts of microplastic.

# Ecological study on planktonic gastropod in the eastern Indian Ocean

Natsumi Ono<sup>1\*</sup>, Kazutaka Takahashi<sup>1</sup>

<sup>1</sup> Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences,  
The University of Tokyo

*\*On-board*

Correspondence: Natsumi Ono

## Introduction

Planktonic gastropods including neogastropods, pteropods, and some nudibranchs are distributed throughout world's ocean. They exhibit a variety of feeding strategies, from suspension feeding to carnivory, and play significant roles in marine food webs and biogeochemical cycles. In particular, some pteropods belonging to Thecosomata, have unique feeding adaptations using mucous webs to capture a wide size range of organic particles, including pico- and nanoplankton, indicating that they are key species in the food webs of oligotrophic waters. This study aims to reveal the distribution, species composition, and feeding habit of planktonic gastropod in the eastern Indian Ocean.

## Materials and Methods

### *Distribution and composition of planktonic gastropod*

Samples were collected by a vertical tow from 0 m to 200 m using NORPAC-twin net with a mesh size of 0.10 mm. After the collection, the samples were immediately preserved with formalin for the laboratory analysis using microscope to determine the community composition. Other samples were fixed by ethanol for genetical analysis about species identification as well as gut contents. Additionally, to investigate vertical distribution, samples were collected from seven layers at 0-50 m, 50-100 m, 100-150 m, 150-200 m, 200-300 m, 300-400 m, 400-500 m depth using VMPS6000D. These samples were preserved with formalin and analyzed to assess vertical distribution patterns.

### *Feeding habits (Fecal pellet collection)*

Planktonic gastropods were collected from 0 m to 100 m using Nansen closing net. The collected planktonic gastropods were incubated for 24h at the sea surface temperature in filtered seawater. After the incubation, planktonic gastropods were identified, and their fecal pellets were collected. The collected fecal pellets were preserved with ethanol, while the planktonic gastropods were stored at -20°C. The fecal pellets will be analyzed using metabarcoding techniques in the lab. The frozen planktonic gastropods will be served to determine stable isotopic ratio to infer their trophic level.

## Expected Results

Formalin samples will reveal the horizontal and vertical distribution of planktonic gastropod. In addition, the fecal pellets and frozen samples will show valuable insight about the role of planktonic gastropod in the food webs in the oligotrophic open ocean.

# Atmospheric nutrients deposition to the Eastern Indian Ocean and its impact on marine ecosystem

Yoko Iwamoto<sup>1\*</sup>, Cheng Yi Tey<sup>1\*</sup>, Kazuhiko Takeda<sup>1</sup>

<sup>1</sup>Graduate School of Integrated Sciences for Life, Hiroshima University

*\*On-board*

Correspondence: Yoko Iwamoto

## Introduction

Nutrient inputs from the atmosphere are particularly important in supporting primary production in the open ocean and may affect the carbon cycle in the Earth's surface through the biological pump. In South Asia, where economic growth is remarkable, the amount of atmospheric emission of anthropogenic nitrogen is increasing. This could change the amount and geographical pattern of atmospheric nitrogen deposition into the Indian Ocean, affecting the marine ecosystem. Furthermore, the Indian monsoon system can shape strong spatiotemporal patterns of atmospheric nutrient deposition. However, atmospheric observation over the Indian Ocean has still been limited. Atmospheric observation in KH-18-6 in 2018 over the Eastern Indian Ocean successfully observed clear north-to-south gradient in nitrogen deposition during the early winter monsoon. In the present KH-24-3 cruise, sampling of aerosol and rainwater samples were performed on the cruise track to evaluate the ocean fertilization effect of atmospheric nutrients deposition over the Eastern Indian Ocean during late summer.

## Methods

### *Aerosol sampling for chemical analyses*

Size-fractionated aerosols (PM<sub>2.5</sub> and PM<sub>>2.5</sub>) were collected on Teflon filters by a high-volume sampler (AS-9, Kimoto Electric Co. Ltd.) at 24 hours intervals at a flow rate of about 14 m<sup>3</sup> h<sup>-1</sup> on the upper deck. After collections, the filter samples were stored at -18 °C, prior to chemical analysis (for ionic species, trace metal and phosphorus) at home laboratory. To avoid contamination from ship exhaust, the aerosol sampler was automatically controlled by a wind sector to operate only when the relative wind direction ranged from -90° to 90° of the bow and relative wind speed was larger than 1 m s<sup>-1</sup>. A total of 24 samples were collected.

### *Aerosol sampling for individual particle analysis*

Aerosol particles were collected on carbon-coated nitrocellulose (collodion) films for 5 minutes at a flow rate of 1.0 L min<sup>-1</sup>, by using cascade impactors. The samples were stored under the dry conditions at room temperature. Single particle analysis using a transmission electron microscopy will be performed at home laboratory. A total of 21 samples were collected.

#### *Rainwater sampling for chemical analysis*

Rainwater samples were collected in plastic bottles using a self-build rainwater sampler on the compass deck. After the collection, pH of rainwater was measured immediately. The samples were separated into 50 ml sterilized tubes and stored at  $-18^{\circ}\text{C}$ . Ionic species in the rainwater will be analyzed at home laboratory. A total of 6 samples were collected.

#### *Measurement of aerosol number size distributions*

To obtain the number size distribution of aerosol particles ( $0.3$  to  $5\text{ }\mu\text{m}$ ), ambient air was sampled continuously through an inlet (placed at the upper deck) via silicon tubing and then introduced to an optical particle counter (OPC) (KC-01E, RION Co. Ltd.). The size bins of the OPC were diameter of  $>0.3$ ,  $>0.5$ ,  $>1.0$ ,  $>2.0$ ,  $>5.0\text{ }\mu\text{m}$ . The OPC was installed in a wooden box placed at the bow part of the upper deck.

#### *Measurement of $\text{O}_3$ concentrations*

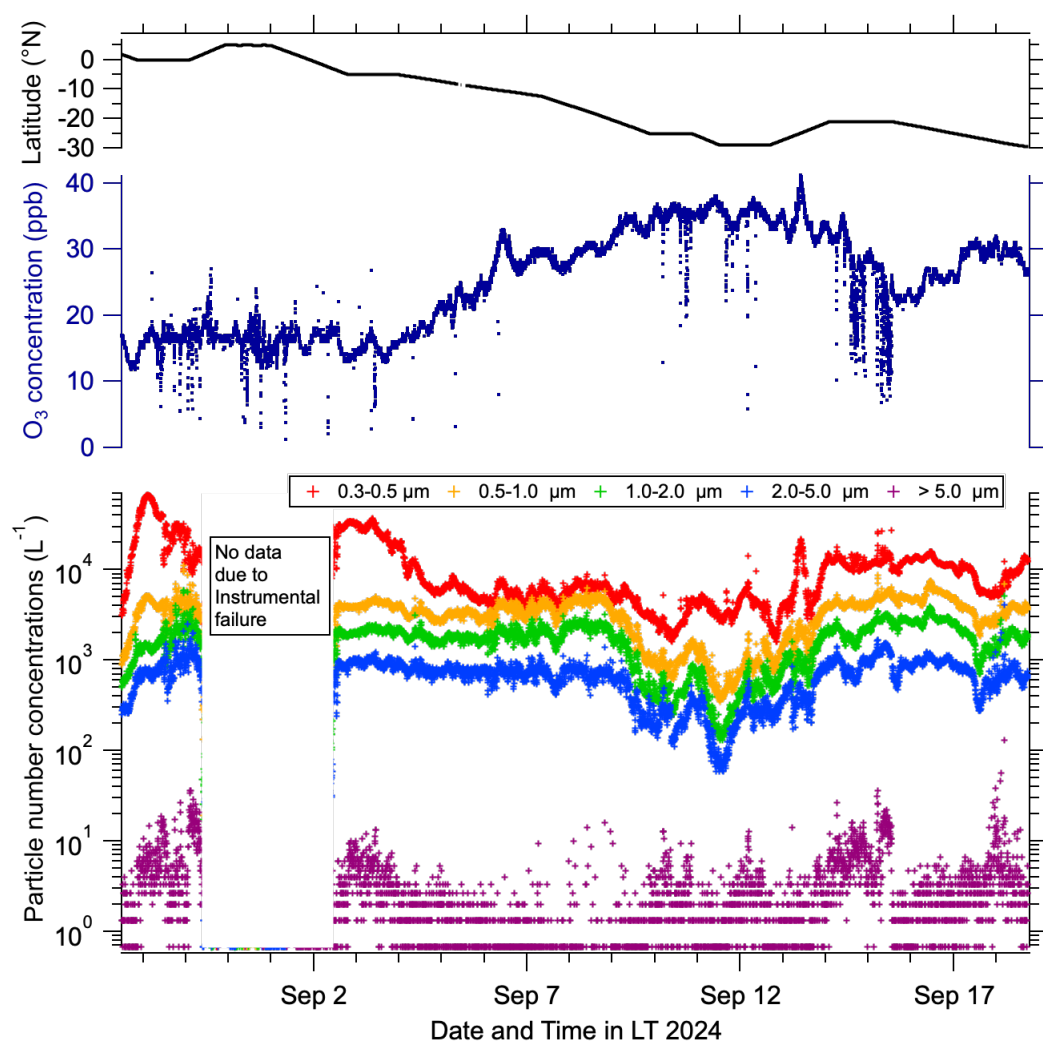
Ozone ( $\text{O}_3$ ) concentrations in the atmosphere were continuously measured from 28th August to 18th September 2024. Ambient air was sampled through an inlet placed at the upper deck via Teflon® tubing and introduced to Ozone Monitor (Model-1150; Dylec Inc.), which installed in the 1st laboratory.

#### *Seawater sampling for inorganic iodine*

It is known that iodine in the atmosphere is oceanic origin and involved in loss of tropospheric ozone. For the vertical profiles of halogen species including iodine, the water samples were collected using Niskin bottles at stations 2, 3, 4, 8 and 9. Surface seawater samples from the faucet on board were also collected. All the seawater samples were collected in 125-mL HDPE bottles. The bottles were stored at  $4^{\circ}\text{C}$  on board. Halogen species will be analyzed by an ion chromatography system at home laboratory.

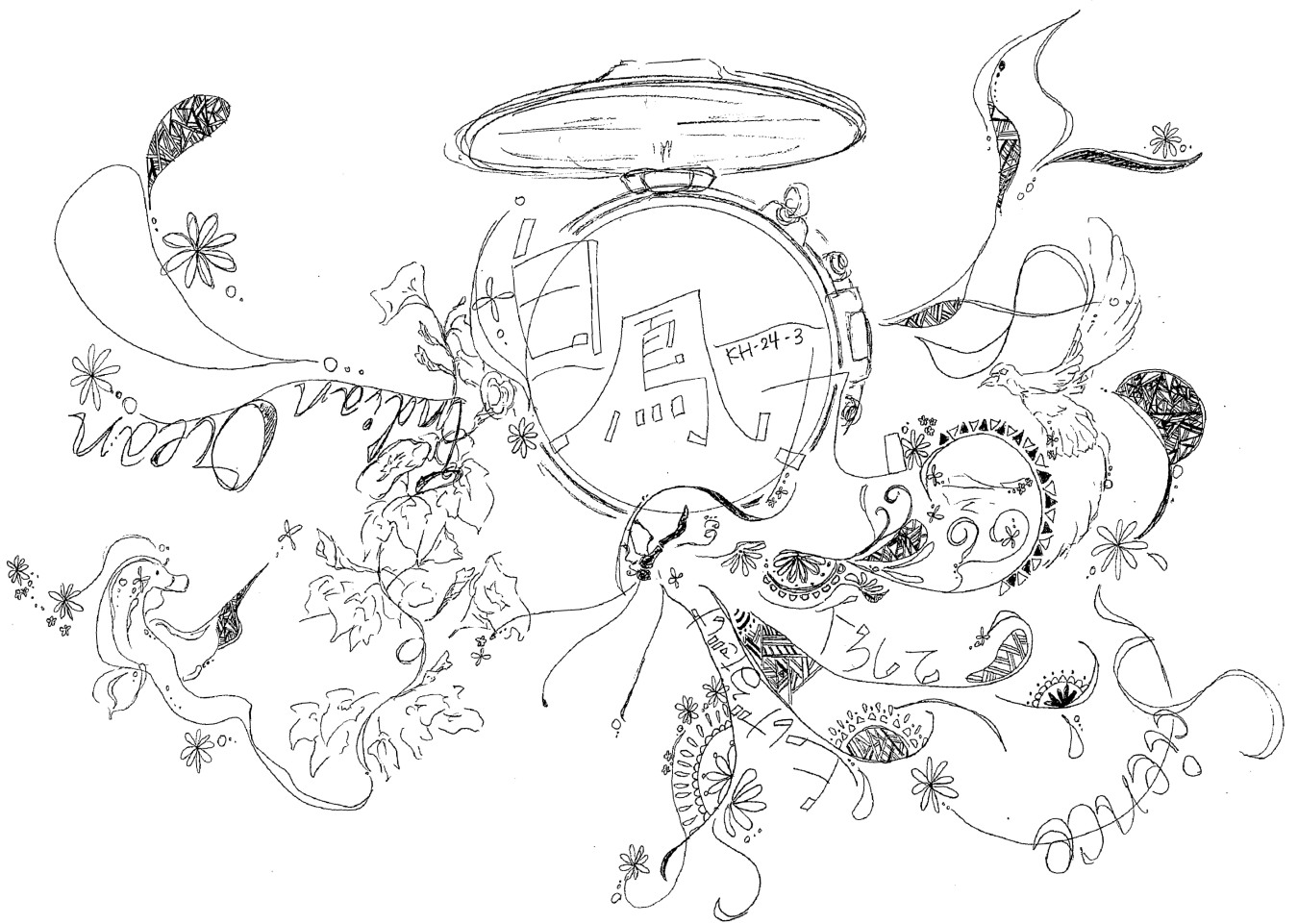
#### **Preliminary results**

Figure 1 shows temporal variations of atmospheric ozone and particle concentrations. Ozone concentrations ranged from 10 to 20 ppb near the equator, and gradually increased to 30 to 40 ppb at higher latitudes. This is consistent with the global distribution of tropospheric ozone concentrations observed by satellites. Five-days backward trajectories calculated by NOAA HYSPLIT trajectory models showed clear difference of air mass transport pathway by ocean area; air masses influenced by the Indonesian islands reached the ocean area of  $5^{\circ}\text{N}$  to  $5^{\circ}\text{S}$  (28 August to 2 September), and pristine maritime air masses reached the ocean area of  $8$  to  $25^{\circ}\text{S}$  (5 to 13 September). From 14 September onwards, air masses influenced by the Australian continent also reached the observation area. Particle concentrations generally varied according to the origin of the air mass.



**Fig.1** Temporal variation of latitude (top), atmospheric O<sub>3</sub> (middle) and particle concentrations (bottom). The occasional drops in O<sub>3</sub> concentration were due to the influence of ship emission, which will be removed by screening after the cruise.





## 白鳳丸研究航海報告

\* 航海番号 KH-24-3次研究航海

\* 航海名称 東部インド洋における海洋物理・生物地球化学・生態系の統合的観測研究  
Integrated observational research for physical oceanography,  
biogeochemistry and biological oceanography in the eastern Indian  
Ocean

\* 観測海域 東部インド洋  
Eastern Indian Ocean

\* 航海期間 令和6年8月26日（月）～令和6年9月22日（日）

\* 出港日時・場所 8月26日14時00分 ペナン港（マレーシア）

\* 入港日時・場所 9月22日13時 フリーマントル港

\* 寄港期間・場所 なし

### \* 研究課題

1. CTD による海洋物理環境観測およびクリーン採水による化学・生物試料の採取
2. MOHT ネット、ORI ネット、VMPS ネット、マルチネット、NORPAC ネット、飼育ネットによる動物プランクトン・マイクロネクトンの採集
3. ニューストンネットによるマイクロプラスチックの採集
4. 水中照度計による海水光学特性観測
5. 大気エアロゾルの連続観測
6. 海表面マイクロレイヤー採集
7. 空中ドローンによる撮影海洋混合過程の微細観測

### \* 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. CTD による海洋物理環境観測およびクリーン採水による化学・生物試料の採取  
齊藤宏明
2. MOHT ネット、ORI ネット、VMPS ネット、マルチネット、NORPAC ネット、飼育ネットによ

る動物プランクトン・マイクロネクトンの採集 平井淳也

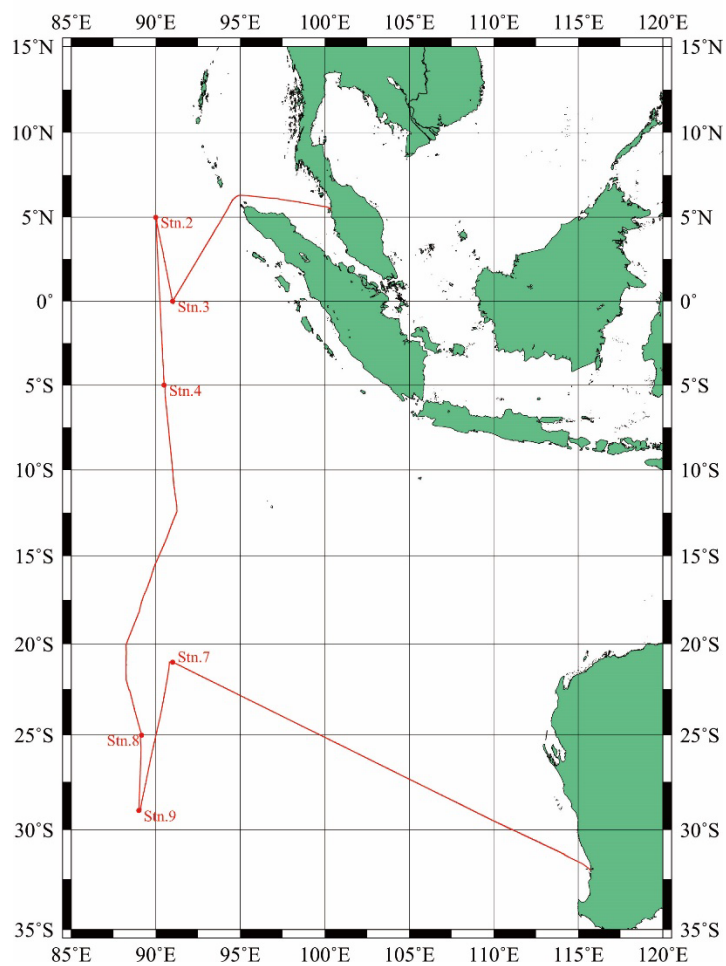
3. ニューストンネットによるマイクロプラスチックの採集 平井淳也
4. 水中放射計による海水光学特性の観察 齊藤宏明
5. 大気エアロゾルの連続観測 岩本洋子
6. 海面ミクロ層の採集 浜崎恒二
7. ドローンによる写真撮影 竹内誠

\* 乗船研究者氏名・所属・職名

|                |            |        |
|----------------|------------|--------|
| 齊藤 宏明          | 東京大学       | 教授     |
| 小畑 元           | 東京大学       | 教授     |
| 小川 浩史          | 東京大学       | 教授     |
| 濱崎 恒二          | 東京大学       | 教授     |
| 平井 淳也          | 東京大学       | 講師     |
| LIU, Jiakang   | 東京大学       | 大学院学生  |
| 上岡 万夏          | 東京大学       | 大学院学生  |
| 玉蟲 奈佳子         | 東京大学       | 大学院学生  |
| 岡本 諭賢          | 東京大学       | 大学院学生  |
| 船木 千帆          | 東京大学       | 大学院学生  |
| 小野 夏実          | 東京大学       | 大学院学生  |
| 石垣 秀雄          | 東京大学       | 技術専門職員 |
| 竹内 誠           | 東京大学       | 技術専門職員 |
| 山下 洋平          | 北海道大学      | 准教授    |
| 久保 悠           | 北海道大学      | 大学院学生  |
| 則末 和宏          | 新潟大学       | 准教授    |
| 柳澤 輝汰          | 新潟大学       | 大学院学生  |
| WONG, Kuo Hong | 金沢大学       | 助教     |
| 高村 晃拓          | 金沢大学       | 大学院学生  |
| XIN, Shuai     | 富山大学       | 大学院学生  |
| 遠藤 寿           | 京都大学       | 准教授    |
| 佐藤 拓哉          | 京都大学       | 特定研究員  |
| JIANG, Siyu    | 京都大学       | 博士研究員  |
| 岩本 洋子          | 広島大学       | 准教授    |
| TEY Cheng Yi   | 広島大学       | 大学院学生  |
| 佐藤 光秀          | 長崎大学       | 助教     |
| 増田 貴子          | 水産研究・教育機構  | 主任研究員  |
| 駒井 信晴          | マリンワークジャパン | 観測技術員  |
| 中尾 眞子          | MOLMEC     | 観測技術員  |

\* 航跡・測点図

KH-24-3



## **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 Cooperative Research Cruise office.

E-mail: [kyodoriyo@aori.u-tokyo.ac.jp](mailto:kyodoriyo@aori.u-tokyo.ac.jp)