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Cruise Report
KH-19-6 Leg 1-3

16 Oct. - 16 Dec. 2019
Honolulu- Valparaiso- Punta Arenas

Cruise Information

● Cruise ID: KH-19-6 Leg1 - Leg3

● Name of vessel: R.V. Hakuho Maru

● Title of cruise: Interdisciplinary research on oceanography and earth science in Weddle Sea and southern Pacific -Around the world cruise for anniversary of 30 years of R.V. Hakuho Maru-

● Chief Scientist: Koji Hamasaki and Atsushi Tsuda (Atmosphere and Ocean Research Institute, University of Tokyo)

● Cruise period: 16 Oct. - 16 Dec. 2019

● Ports of departure / call / arrival: Honolulu (U.S.A)- Valparaiso (Chile)- Punta Arenas (Chile)

● Research area: Eastern Pacific

● Contact: Atsushi Tsuda

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Introduction

The R.V. Hakuho-Maru was bone in 1989 in Shimonoseki. Then, 2019 is 30-years anniversary for her. Prof. Ikehara of Kochi Univ. proposed an anniversary cruise by all disciplines of oceanography, and I (A.Tsuda) and Prof. Ohshima of Hokkaido Univ. agreed with his idea and have been discussed our cruise plan in detail. Finally, we made an around -the-World cruise focusing on the eastern part of the South Pacific and the Southern Ocean. The leg 2 and 3 mainly focusing on biogeochemical cycles, including carbon, nitrogen, trace metals, phytoplankton/zooplankton ecology and biological diversities in the eastern South Pacific. The biogeochemical cycling and ecosystem of the eastern South Pacific Ocean also change with the physical oceanographic regime. Moreover, the eastern South Pacific is one of the least influence of aeolian dust (iron) and anthropogenic disturbance. Therefore, it must be important to compare western North Pacific where is located at the most populated region of the world, the ecosystems are also influenced by various anthropogenic perturbations such as pollutants, plastic deblis, overfishing, marine heat wave, etc. The eastern South Pacific is, however, seriously understudied region relative to the other regions of Pacific Ocean and the Atlantic Ocean, and our knowledge on the biological and biogeochemical processes are limited. Understanding the mechanisms of biogeochemical cycle and ecosystem responses to natural and anthropogenic perturbations is an emergent issue since increasing human activity is degrading the quality and quantity of marine ecosystem services, on which our society is dependent. In order to tackle with the emergent issue, it is essential to take an interdisciplinary approach, under which physical oceanographers, biogeochemists, biological oceanographers, and atmospheric chemists work together to unveil physical-chemical-biological interactions in the eastern South Pacific. If you are interested in our data, please contact PIs.

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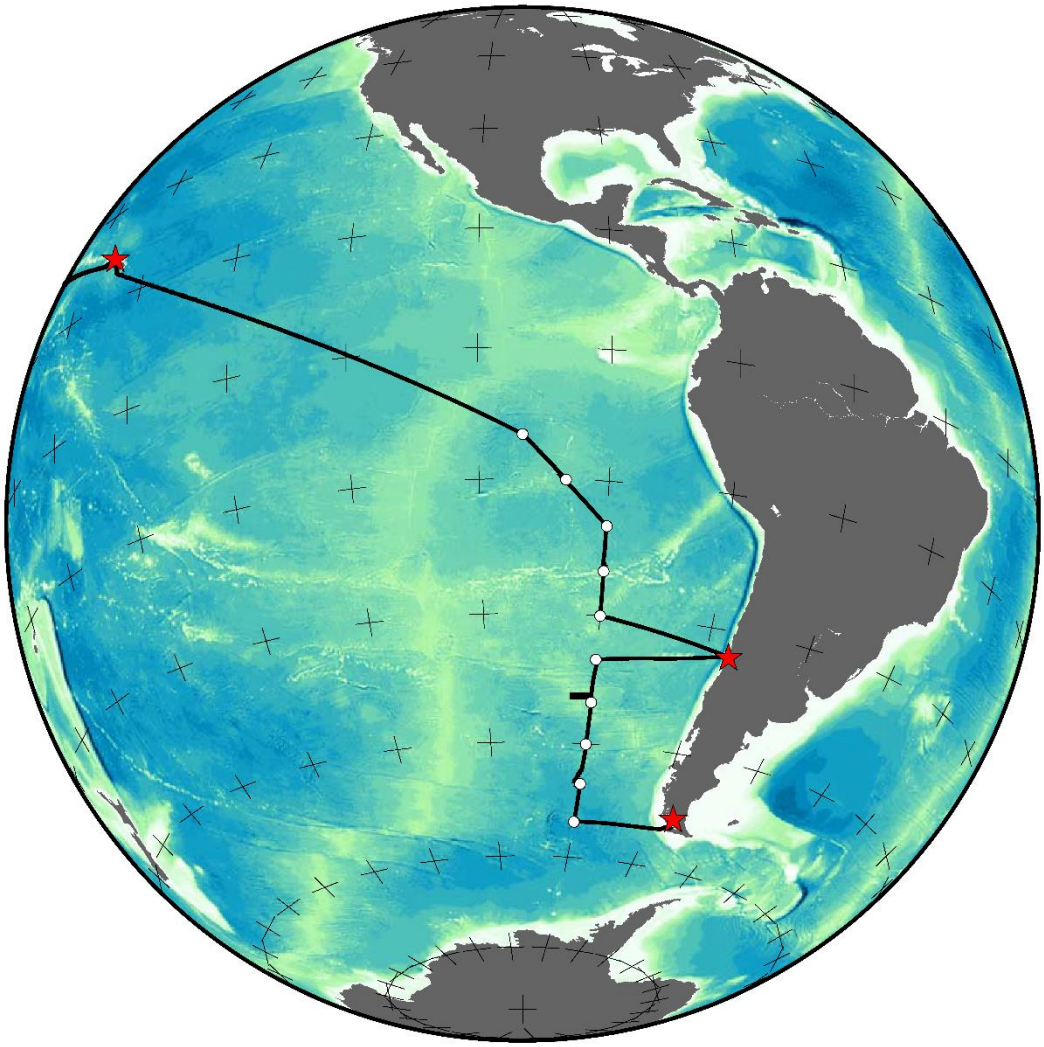
Cruise Track



Optical measurement of sea water

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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.

Fluorometric determination of Chlorophyll *a*

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To estimate phytoplankton biomass, chlorophyll a (chl-a) concentrations were measured by fluorometric analysis. Seawater samples were collected by clean Niskin bottles (5, 10, 20, 30, 50, 75, 100, 125, 150, 200, 250 m) and a plastic bucket (0 m). Water samples were filtered (<0.015 MPa) through 25mm-diameter Whatman GF/F filter. Phytoplankton pigments retained on the filters were immediately extracted in glass tubes with 6 ml of N,N-dimethylformamide. The tubes were stored in freezer to extract chl-a at least for 24 hours. Fluorescences of each sample were measured by Turner Design fluorometer (10-AU-005), which was calibrated against a pure chl-a. We applied the fluorometric determination of "Non-acidification method" (Welschmeyer, 1994).

Latitudinal distribution of nanomolar nutrients in the eastern South Pacific Ocean

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Tropical and subtropical oceans are characterized by low concentrations of surface nutrients, because of upward nutrient supplyrestrictedby a strong stratification. This oligotrophic seascape had traditionally been thought of as a fairly homogeneous habitat. However, recent studies using highly sensitive analytical methods have revealed that surface nutrients show dynamic geographic variations at nanomolar levels (Hashihama, 2013 Oceanogr. Japan). Among various nutrient fields in the oligotrophic habitat, there exist unique domains with drastic variation of phosphate. Surface phosphate is extremely depleted to <10 nM in the western subtropical North Pacific, compared to the central subtropical North Pacific and the western and central subtropical South Pacific (Hashihama et al., 2009 GRL).

Spatial variations of nanomolar nutrients are thought to influence biological production in various oligotrophic habitats. However, knowledge of nanomolarnutrient regime in the eastern South Pacific Oceanis limited.Furthermore, since concentrations of inorganic nutrients are extremely low in the oligotrophic water, labile fraction of dissolved organic nutrients such as urea may be important for biological utilization as alternatives of inorganic nutrients.In this cruise, we examined spatial distributions of nanomolar inorganic and organic nutrients along a meridian transect of the eastern South Pacific Oceanin order to reveal nanomolar nutrient regime there.

Spatial distributions and chemical characteristics of organic matter in the eastern South Pacific Ocean

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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_2, dissolved inorganic carbon (DIC) and nutrients such as NO_3^- and PO_4^{3-}. 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 eastern South Pacific Ocean compared with other Pacific regions.

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°west from the north (10°south latitude) to the south (55°south latitude) in the eastern South Pacific Ocean. These results could be compared with those obtained from our previous researches in the other Pacific regions, such as KH-05-2, HH-08-2, KH-11-10, KH-12-3, KH-13-7, KH-14-3 and KH-17-4 cruises, which can be a great help for our deep understanding the dynamics and chemical characteristics of OM in whole the Pacific Ocean.

Dynamics of particulate biogenic elements in the eastern South Pacific Ocean

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Nutrient concentration in the euphotic zone is highly variable with latitude in the eastern South Pacific Ocean. In the oligotrophic regions, DON and DOP play significant contribution of ecosystem dynamics as well as DIN and DIP, or dissolve inorganic nutrients, and the role has been investigated. The relative contribution of the particulate forms (i.e., organisms and detritus) is estimated to be larger in the oligotrophic region than one in the eutrophic regions. However, the role of particulate forms including zooplankton is not fully studied. In order to understand the role of particulate forms of biogenic elements (N, P, Si, C) in the biogeochemical cycles, following observations were carried out.

Vertical flux of biogenic elements

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Biogenic elements transported by biological pump fuel mesopelagic and bathypelagic ecosystems. In this study, we set Neuer type drifting sediment traps (8 tubes per trap) 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 2, 4, 6 and 8. In order to determine the export flux from euphotic zone (EZ, 1% light depth), the upper sediment trap was set near the euphotic zone (EZ, 1% light depth), and the setting depth of each station was 90, 100, 100, 90 m, respectively. To determine the vertical attenuation of the flux, second trap was set 100 m below the EZ and the third trap at 500 m. Obtained samples were filtered on pre-combusted GF/F filter for PON/POC and total particulate phosphate analysis. Attenuation rates of C, N, P flux during sinking (i.e., 100m sinking from EZ, upper-mesopelagic zone of EZ-500m), and the difference in the attenuation rats will be investigated with examining the relationship with biological/environmental parameters such as chl.a concentration, primary production, new production, nutrient concentrations, zooplankton biomass and species composition. For further enumeration of particles such as fecal pellets of meso- and microzooplankton, subsamples were preserved in buffered formalin seawater (3% v/v). In order to examine the shape, size and other apparent characteristics, such as color, of sinking particles, we set “gel trap” in one of the tubes. The sticky surface of the gel makes it possible to keep the shape of particles without formation of aggregate at the bottom of the tube. The sinking particles on the gel were photographed for further analysis of the external characteristics of sinking particles.

Trace Metals and Nd isotopes

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Distributions of trace metals in the eastern South Pacific

Trace metals, such as Fe, Cu, Co, Mn, and Zn 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 South Pacific Ocean yet. To understand the marine biogeochemical cycles of trace metals, we need to investigate their distributions in these areas. In this study, we will study the full-depth distributions of dissolved trace metals (Cu, Co, Mn, Zn and Cd etc.) in the eastern South Pacific.

Trace metal speciation in the eastern South Pacific

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. Considering the uptake of trace metals by phytoplankton, speciation is 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 South Pacific. In this study, we will investigate trace metal speciation in the eastern South Pacific by using cathodic stripping voltammetry (CSV).

Distributions of Nd isotopic composition and REE concentrations in the eastern South Pacific

Nd isotopic composition ($^{143}Nd/^{144}Nd$), one of the useful isotopic tracers in geochemistry, is frequently utilized in the field of marine chemistry. Water masses indicate specific Nd isotope ratios reflecting the geology of Nd source area. Since the mean residence time of Nd is relatively shorter than deep water circulation, the less homogenized isotopic composition of Nd ($^{143}Nd/^{144}Nd$) is expected to be a strong tracer for water masses, as well as relative abundance of rare earth elements (REE) concentration (normalized REE pattern). In this cruise, we will determine the distribution of Nd ICs and REE concentrations in the eastern South Pacific.

Stoichiometry of iron and nutrient supply from the sub-Antarctic mode water and limitation factor for phytoplankton growth in the eastern South Pacific

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1. Iron distribution in the western North Pacific

Determining the distribution of Fe in the global ocean including the processes involved in oceanic cycles is important for understanding the biological production of

ocean and its impact on the biogeochemical cycle and climate. Various Fe sources occur in the ocean such as atmospheric dust, river input, re-suspension of sediment on the shelf, glacial/sea ice melt, hydrothermal activity. To grasp all of the factor affecting the Fe distribution, sources and cycles in the eastern South Pacific, extensive transect observations of the Total dissolvable and dissolved Fe were carried out. Especially, to compare the North Pacific biogeochemical system (Nishioka and Obata, 2017), we focused on the stoichiometry of iron and nutrient supply from the sub-Antarctic mode water and limitation factor for phytoplankton growth in the eastern South Pacific.

Samples for Total dissolvable Fe and dissolved Fe analysis were collected from Stn.1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 by using acid-cleaned Teflon-coated 12-liter Niskin-X bottles. The Niskin-X bottles were placed in a clean-air booth and the sample seawater was filtered through an AcroPak 200 Capsule filter unit having 0.8/0.2 micro-meter pore-size Supor Membrane (Pall) attached directly to the spigot with silicon tubing under a pressure of compressed clean air. Filtered seawater was collected in 125-ml LDPE bottles after rinsing 3 times. Ultrafiltration for measuring soluble Fe in the dissolved phase (soluble < 1000 kDa) (Nishioka et al., 2001) were also conducted at Stn. 3 and 8 to reveal the physical Fe form in seawater. Sample for measuring Fe(III) solubility were also collected at Stn. 3 and 8.

All filtrates and unfiltered samples collected in 125-ml polyethylene bottle were then added distilled HCl and stored. Then the concentrations of Fe (III) in the buffered samples will be determined with an automatic Fe (III) analyzer (Kimoto Electric Co. Ltd.) using chelating resin (MAF-8HQ) concentration and chemiluminescence detection (Obata et al., 1993) at onshore laboratory.

2. Incubation experiment for investigating limiting factor for phytoplankton growth

In order to understand relationship between the stoichiometry of upward Fe and nutrient flux (Fe, N, P, Si) and the influence of co-limiting factor for surface phytoplankton growth along the transect, shipboard culture experiments which regulating Fe and nutrients availability were conducted with subsurface water (～300 m) and surface Chl.a maximum water at Stn.3, 6 and 9. The samples in 250-ml PC bottles were cultured in temperature and light control incubator during 4-7days, and sample for measuring size-fractionated Chl.a, pigments and nutrients were collected from the cultured bottles on Day 0, 1, 3, 5, 7 (till Day 5 for Stn.9 experiment). Chl.a concentration from cultured bottles were measured onboard. The other parameter (nutrients and pigments) samples will be analyzed onshore laboratory.

Chemical speciation of dissolved Fe and vitamin B_{12} in the eastern South Pacific

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Iron (Fe) can control phytoplankton stocks in the approximately 30% of world ocean including high-nutrient low-chlorophyll regions (e.g., Moore et al., 2013). The thermodynamically favored oxidation state of Fe, Fe(III), is strongly hydrolyzed and its removal is mainly constrained by complexation with natural organic ligands such as humic substances and polysaccharide. These organic ligands appear to regulate not only Fe solubility and distribution, but also its bioavailability. In the surface water, Fe(III) can be reduced to Fe(II) by photochemical reaction, which is more soluble and kinetically labile. Fe(II) is unstable in the oxygenated seawater, it has been suggested that Fe(II) decay differs depending on temperature, pH and organic matters. Recent studies by the GEOTRACES project have gradually revealed distribution and speciation of dissolved Fe in the world ocean (http://www.geotraces.org/dp/idp2017), although organic ligands distribution and its behavior in the eastern South Pacific remain unclear.

Vitamin B_{12}, a cobalt-containing ring-contracted tetrapyrrole compound, also plays significant role in cell metabolism such as driving citric acid cycle. It has been suggested that about half of eukaryotic phytoplankton require exogenous vitamin B_{12}, so that vitamin B_{12} could influence on phytoplankton composition in the ocean. Despite the biogeochemical importance of vitamin B_{12} in the ocean, there have not been enough data about vitamin B_{12} distribution and turnover in the oceans to paint a clear picture because of its extremely low concentration in seawater (~10^{-12} M) (e.g., Suffridge et al., 2017).

Distributions of Bi, Te, Pb isotopes and suspended particulate matter in the Eastern South Pacific Ocean

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Bismuth (Bi), tellurium (Te), and lead (Pb) are particle-reactive trace elements in the ocean and their oceanic distributions are mainly controlled by particle scavenging and sinking. Bi in the deep water shows remarkably simple exponential decay with depth in the vast area of the subarctic North Pacific, implying that the biogeochemical cycle of Bi is very simple in the North Pacific. Te is highly concentrated in ferromanganese crusts in the seafloor with an enrichment factor of Te in Fe-Mn crusts relative to mean abundance in earth's crust exceeding ~10^4, suggesting the involvement of manganese oxide particles on the removal of Te. In addition, two different oxidation states of Te(IV) and Te(VI) with different chemical properties occur in the deep water and the transformation between Te(IV) and Te(VI) may be key to understand the biogeochemical cycle of this element. Pb has four stable isotopes of ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb, and their isotopic ratios are useful in characterizing Pb sources in the ocean and investigating removal process in the deep water column. In this cruise, we will focus on these trace elements and isotopes to have a better understanding of the biogeochemical cycles of these elements, especially with respect to involvement with suspended particulate matter (SPM) in the Eastern South Pacific Ocean.

Diversity and ecological function of viruses in the South Pacific Ocean

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Marine viruses are the most abundant life forms in the ocean, and they possibly infect all marine organisms. A major cause of mortality of marine bacteria and phytoplankton is caused by viral infections, providing a large impact on food web

structures and geochemical cycles in the ocean. Marine viruses associated with microbial communities are generically diverse, and molecular method using high-throughput sequencing revealed diversity and biogeography of marine viruses in the global oceans. Viral pathogens, which cause a significant loses in aquaculture, have been also well studied in fishery important organisms including fish and shrimp. Regardless of ecological importance of marine viruses, studies are mainly limited in microbial and industrially-important organisms. In particular, diversity and ecological role of viruses associated with marine zooplankton are still poorly understood The following observations were carried out during KH-19-6 leg 2 to investigate diversity and ecological function of viruses in the South Pacific Ocean.

Microbial Diversity and Functions in the Eastern South Pacific

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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 explore prokaryotic diversity and functions in the water column of the Eastern South Pacific, which is one of the most undersampled areas all over the world. To analyze actively growing cells, bromodeoxyuridine (BrdU), a halogenated nucleoside and thymidine analogue, is used.

Seawater (5 or 10 L) were collected at depths from 0 to Bottom-50 m (0, 10, surface chlorophyll maximum, 100, 200, 400, 1000, oxygen minimum, 2000, Bottom-50 m at all stations (from Stn. 1–10) using a bucket and Niskin-X bottles on a CTD-CMS. After sampled, seawater was serially filtered on 3.0-μm polycarbonate filters (Nuclepore, Whatman) and 0.22-μm cartridge filters (Sterivex, Millipore), and the filters were stored

at −80°C until further analysis. In addition, 1.5 mL of seawater for the flowcytometric counts of prokaryotic cells was fixed with 2% glutaraldehyde at 4°C for several hours, flash-frozen in liquid nitrogen, and stored at −80°C until further analysis.

For the analysis of actively growing cells, seawater (10 or 20 L) was collected at depths from 0 to Bottom-50 m (0, 100, 1000, and Bottom-50 m) at 4 stations (Stn. 2, 4, 6, and 8). After sampled, BrdU (20 nM final conc.) was added to seawater, and seawater was incubated for 12 or 24 hours at the *in-situ* water temperature (± 2°C) under the dark condition. After incubated, seawater was filtered and stored as described above.

Microbial Diversity and Functions in the Sea Surface Microlayer and Marine Aerosols of the Eastern South Pacific

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The sea surface microlayer (SML) is a thin (< 1 mm) surface film located at the interface between the atmosphere and the ocean. Various chemical and biological materials accumulate in the SML due to its surface tension, which leads to higher concentration of organic matter such as transparent exopolymer particles (TEPs) and higher abundance of microorganisms such as phytoplankton and bacteria compared to the underlying water (ULW). Organic matter and microorganisms originating from the SML are a major source of bioaerosols in the open ocean and determines the physicochemical properties of sea spray aerosols (SSAs) such as cloud condensation nuclei (CCN) and ice nuclei (IN) activities. Although microbial activity changes the physicochemical properties of the SML and subsequently SSAs, information on microorganisms in the SML and marine aerosols is limited. During the present cruise, we explore prokaryotic diversity and functions in the SML, ULW, and SSAs to understand the roles of microorganisms in the air-sea processes.

SML samplings and analyses

SML samples were collected at Stn. 5 and Stn. 8 using a custom-built rotating drum sampler on a Zodiac boat about 500 m away from the R/V Hakuho-maru. The drum was rotated at 8 rpm for about 3 hours to collect 5−10 L of SML samples. ULW samples (10

L) were collected at 0.5 m depth on the opposite side of the Zodiac boat using a Hyroht glass bottle sampler (Shibata). Sampling location was recorded by GPS with several meters accuracy. Surface water temperature and salinity were measured using a handheld sensor (YSI Model 30), and air temperature and wind speed were measured using a handheld anemometer (WS-03SD).

Samples were used for the analyses of nutrients, chlorophyll *a*, prokaryotic abundance (flowcytometry), and prokaryotic diversity and functions. Samples for prokaryotic diversity and functions were serially filtered on 3.0-μm polycarbonate filters (Nuclepore, Whatman) and 0.22-μm cartridge filters (Sterivex, Millipore), and the filters were stored at −80°C until further analysis.

Biological Consumption of Carbon Monoxide (CO) and Dynamics of CO-oxidizing Bacteria in the Eastern South Pacific

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Carbon monoxide (CO), an indirect greenhouse gas, is supersaturated in surface waters with respect to its atmospheric concentration, and the ocean generally acts as a source of atmospheric CO. CO in surface waters is mainly produced by the photochemical degradation of chromophoric dissolved organic matter (CDOM), consumed aerobically by CO-oxidizing bacteria, and also lost by emission to the atmosphere. Although approximately 80% of CO produced in the water column is consumed by CO-oxidizing bacteria, information on the abundance, community structure, and diversity of marine CO-oxidizing bacteria is limited.

Tens to hundreds of μm of the sea-side thin film is known as the sea surface microlayer (SML). The SML is defined as less than 1,000-μm thick uppermost layer of the ocean water column. The SML is located at the interface between the atmosphere and the ocean and plays critical roles in global biogeochemical cycles and climate change through the regulation of the air-sea exchange of relatively insoluble, climate-related gases such as CO. The SML forms physically, chemically, and biologically distinct environments compared to the subsurface water (SSW) usually at 0.5–1 m depth.

Although the SML shows significant enrichment of bacteria and thus active biochemical processes by bacteria is suggested in the SML, which potentially changes sea-air CO flux, information on the dynamics of CO-oxidizing bacteria in the SML is limited.

During the present cruise, we collected seawater in the water column and in the SML to clarify the community structure and diversity of CO-oxidizing bacteria and the relationship between biological CO consumption and the abundance/expression of a CO-oxidizing gene.

Isolation of Bacteriophages which Infect *Verrucomicrobia*

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Marine viruses are the most abundant components in the ocean. Marine bacteriophages infect or lyse specific host and control bacterial abundance and diversity. Bacteriophages affect biogeochemical cycles; thus, marine viruses are important to understand ecological cycles.

Verrucomicrobia is a phylum of gram-negative bacteria and ubiquitous in soil. Recently, *Verrucomicrobia* appears ubiquitous also in marine environments and constituted average 2% of the total bacteria in the water column. The phylum of *Verrucomicrobia* contributes to degrading polysaccharides which are major components of biomass in aquatic ecosystems. Thus, *Verrucomicrobia* may play important roles in carbon and organic matter cycles.

To our knowledge, the only one lytic phage infects *Verrucomicrobia*, and little is known about *verrucophages* and their effect on bacterial population. During this cruise, we collected seawater to attempt the isolation of *verrucophages* and investigate their effect.

Microscale bacterial diversity

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Bacteria are now recognized as key players in marine biogeochemical processes. However, the findings are based on the context of macro- and mesoscale oceanographic features. The diversity and functions of bacteria (about one micrometer cell size) should be defined by processes and interactions occurring on a much smaller scale, it is needed to focus on bacterial dynamics at microscale, not large scale. Investigating on their diversity and functions at this microscale would be important to better understanding of marine biogeochemical processes.

In this cruise, we collected on-board pumping seawater samples at a certain interval of latitude and longitude to explore bacterial diversity at microscale in order to understand a role of bacteria in marine biogeochemical processes. Additionally, we performed a microcosm experiment to investigate the microscale dynamics of bacteria during a phytoplankton bloom at some stations.

South Pacific Ocean Viromes from High Latitudes

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Viruses are the most abundant biological entities in the oceans, being able to modify the microbial community composition, the host metabolic activity, and altering the evolutionary trajectory of hosts through lateral gene transfer events. In the oceans, the marine microbial food web is a major player in the recycling of carbon and nutrients and in the regulation of energy transfer to higher trophic levels. Viruses through a process known as the “viral shunt” play an essential role in fueling the microbial loop through cell lysis of bacteria and phytoplankton. Then, viral-mediated release of fixed carbon and nitrogen associated with dissolved organic matter represents a major source of DOM for bacterial consumption increasing the level of CO2 respiration of the the entire ecosystem.

It is proposed that viruses in the oceans follow a seed-bank model, with high local-

diversity and global distribution. In this model, viruses (seeds) are passively transported by ocean currents, and then viral communities are structured by local conditions and host availability, as supported by recent global surveys. However, most of these global surveys such as the Tara Oceans Viromes and the Pacific Ocean Viromes, have neglected the high latitudes of the South Pacific Ocean (SPO). Consequently, the genomic characterization of viral communities from high latitudes (35-55° South) and their host are an interesting opportunity to expand the catalog of oceans viral diversity and test biogeographic hypothesis.

The effect of ultraviolet radiation on shaping the planktonic community in the Pacific open waters

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Ultraviolet radiation is generally considered to be harmful to living organisms; many studies have been done focusing on the negative influence of increasing UV dose on living organisms, such as causing high mortality and low productivity through laboratory or *in situ* incubation experiment particularly in relation to the rapid global depletion of stratospheric ozone layer in late 1980s. These studies revealed that many planktonic organisms accumulate photoprotective compounds such as carotenoids and mycosporine-like amino acids (MAAs) possibly in order to lower the damage from UV radiation. Further researches also indicated that these compounds were only produced by phytoplankton and transfer to higher trophic level through food chain. In recent field studies, UV radiation is also considered as an important driver of diel vertical migration in high transparent waters. These findings are strongly suggest that planktonic community in epipelagic environment have various strategies against to UV radiation. In other words, it is very likely the UV radiation is important selective pressure to shape the planktonic community structure, but the related knowledge are extremely limited in the marine environment. Therefore in this study we aimed to investigate the effect of UV radiation on structuring marine planktonic community in open waters of Pacific Ocean. The research area extended from North Pacific (Leg.1) to South Pacific (Leg. 2 and 3), in which the primary productivity, phytoplankton composition ultimately

determining underwater UV attenuation are widely variate and thus data could be compared to have a better understanding the role of UV as a selective pressure to construct the planktonic community.

Spatio-temporal distribution pattern of diazotrophs in the Pacific Ocean

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Biological nitrogen fixation by diazotrophs is an important nitrogen source for the epipelagic ecosystem in the oligotrophic open waters. As the growth of the diazotroph is known to be limited by the phosphorus and iron, the spatial and temporal distribution of the diazotrophs is also expected to be affected by the availability of these nutrition while the details are not investigated very well. Therefore, we aimed to investigate the distribution pattern of diazotrophs in various spatial and temporal scales in the subtripical and tropical Pacific Ocean. In this study we employed molecular analysis technics to reveal the effect of the physiological state of diazotrophs on the distribution pattern.

Latitudinal variability of diazotroph community structure and nitrogen fixation in the Southeast Pacific Ocean

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Nitrogen fixing microorganism (diazotroph) plays an important role in controlling marine productivity by converting nitrogen gas into biologically available form, ammonia. Recent studies based on sequencing *nif* H as a proxy for diazotroph community have shown that more diverse diazotrophs have significantly contribute to the nitrogen fixation in wider regions than previous thought. However, our knowledge about spatial variation of their activity and ecological role is still scarce. Therefore, this study aims to elucidate the latitudinal patterns of the nitrogen fixation activity and diazotroph community structure in the southeast Pacific Ocean. Additionally, in order to understand

the ecological importance of recently found unicellular diazotroph, we investigated their net growth rates and microzooplankton grazing rates on them.

Vertical distribution of protistan zooplankton Rhizaria

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Rhizaria is one of the main eukaryotic super-kingdoms comprising of diverse marine single-celled zooplankton such as foraminifera, radiolaria, and phaeodaria. Recent studies on rhizarian biomass revealed the high abundance of this group which has been overlooked so far, and made emphasis on the importance of their contribution in biogeochemical cycles. However, despite their importance, knowledge of their distribution, diversity, and ecology is quite limited. Therefore, elucidating the fundamental information of this group is essential for further understanding of their role in biogeochemical cycles as well as in marine ecosystem.

Here, we carried out vertical sampling of rhizarian zooplankton for examining their vertical distribution and standing stock. To understand their molecular diversity of this group, DNA analysis will also be conducted using the specimens from the samples.

Samples were collected by Vertical Multiple Plankton Sampler (VMPS6000D, Tsurumi Seiki Co., Ltd.) from 1000-500 m, 500-200m, 200-150m, 150-100m, 100-50m, 50-20m, 20-0m. Samples were collected at all stations during Leg. 2 and Leg. 3. All samples were preserved in 99% ethanol at 4℃.

Assessment of planktonic foraminiferal photosymbiosis

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Recent studies of macroevolutionary history of planktonic foraminifera made emphasis on the importance of photosymbiotic ecology as a key driver for increasing biodiversity of this taxon. Therefore, disclosing the magnitude and the diversity of photosymbiosis in planktonic foraminifera, and elucidating their phylogenetic relationships are important to understand the ecological strategies of planktonic foraminifera. However, previous studies of photosymbiosis in planktonic foraminifera were mainly focused on species that are easy to culture, and exhaustive investigation of photosymbiosis for the whole species of planktonic foraminifera has not been conducted. One way to identify photosymbionts is to detect photosynthetic activity of the algae within the cell of foraminifera (*in hospite*). Here, we used one of the non-invasive assessments of photosynthesis, active fluorometry, to elucidate photosymbiotic nature among modern planktonic foraminifera.

Collection of planktonic foraminifera:

Planktonic foraminifera were collected by Vertical Multiple Plankton Sampler (VMPS6000D, Tsurumi Seiki Co., Ltd.) from 1000-500m, 500-200 m, 200-150 m, 150-100 m, 100-50 m, 50-20 m, 20-0 m. Samples were collected at all stations during Leg. 2. After collection, living foraminifera were sorted and isolated under a stereoscopic microscope. Isolated specimens were transferred to culture dishes (Multidish, Nunclon) filled with filtered seawater (0.22 μm-filtration), and maintained at room temperature until fluorometric measurement. Selected foraminiferal specimens were analyzed with active fluorometry.

Symbiont detection using active fluorometry:

When in photosymbiotic state, algae can photosynthesize actively inside the host cell. Therefore, detecting the signal of potential photosynthetic activity can be used to differentiate symbiotic and non-symbiotic species. Fast repetition rate fluorometry (FRRf) (DF-14, Kimoto Electric Co., Ltd.) was used to assess the presence of symbionts and the photophysiology of the symbionts if any. To obtain the fluorescence induction curve in photosystem II (PSII), the instrument generates a series of blue flashlets (a wavelength of 470 nm with a 25-nm bandwidth, excitation light intensity of 30 mmol m^{-2} s^{-1}) at a repetition rate of 500 kHz in saturation phase, and 20kHz in relaxation phase. The variable fluorescence [F_v (maximum fluorescence F_m – minimum fluorescence F_0)] was used to assess the presence of active symbionts. The potential photochemical efficiency (F_v/F_m), effective absorption cross-section of PSII (σ_{PSII}) and minimum turnover time of electron at Q_A (τ_{QA}) are the parameters used to evaluate their photophysiological fitness. After the measurement, specimens were picked onto micropaleontological slides and air-dried. The slides were preserved at –20°C for onshore DNA analysis.

Depth distribution of photosymbiotic partner of planktonic foraminifera

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For photosymbiotic planktonic foraminifera, acquisition of symbionts from surrounding seawater at very early stage of ontogeny is essential to survive. However, where in the water column the acquisition of the symbionts happens is unknown, which makes difficult to understand the life history of planktonic foraminifera. The presence of the specific symbiont (symbionts in free-living status) should limit the habitable depth of juvenile foraminifera. Therefore, the vertical distribution of the free-living symbionts can provide important information to restrict the acquisition depth of symbionts, i.e., roughly the reproduction depth of photosymbiotic planktonic foraminifera.

Samples were collected at all stations during Leg. 2. The sampling depth of seawater was 200 m, 75 m, 30 m, 10 m, and SCM. For St. 1, 2, and 3, since the SCM depth was close to the other assigned depth, the sampling depth was changed to 200m, 125 m, 75 m, 30 m, and 10 m. 4.6L of seawater samples were filtrated with 0.8 μm polycarbonate filter (Nuclepore Track-Etched Membranes, Whatman). The filters were dried and then stored at –20°C.

Diversity and ecology of zooplankton in the South Pacific Ocean

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Zooplankton 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-19-6 cruise aboard the R.V. Hakuho-Maru, zooplankton samples were obtained to assess both species-level and genetic-level diversity of zooplankton using molecular technique. The following observations were carried out on this cruise for each research topic.

Geographical distribution pattern of mesozooplankton and micronektonic fish community in the eastern South Pacific Ocean

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Micronekton are relatively small but actively swimming organisms ranging 2– 20 cm with intermediate swimming ability between plankton and the larger nekton and represent a critical intermediate trophic link between mesozooplankton, and the higher trophic levels in the ocean. In particular, micronektonic fish composed of mainly mesopelagic fish like myctophid is an important component in the open-ocean food web because of its abundance and biomass, but there is little knowledge about their community structure in the South Pacific Ocean. This study aims to investigate the geographical variation of micronektonic fish community along 90°W in the South Pacific ocean in order to reveal the relationship between the physical, chemical and biological environment and their community structure. In this study we also collected samples for CN stable isotopic analysis to examine the food web structure from primary producer to organisms at higher trophic levels.

Micronektonic fish were collected at night from about 500 m depth to surface at all stations except for St.6 with oblique tow of the Matsuda–Oozeki–Hu Trawl (MOHT, mesh size: 1.95 mm). Collected samples were immediately frozen on board. Wet weight and standard length of collected micronektonic fish will be measured, and identified into species using morphological features and DNA analysis will be also made.

To investigate potential prey composition of micronektonic fish, mesozooplankton were collected at the depth 0-200 m by Norpac twin net (mesh size 100 μm) at all stations. The collected samples were divided by a Folsom's splitter; One of collected samples was preserved in 5% buffered formalin and the other was preserved in 4% Lugol for microscopic observation and another was one was preserved in 99% ethanol for DNA analysis and the other was size-fractionated (>2.0, 2.0 –1.0, 1.0 – 0.5, 0.5 – 0.2 mm) and frozen at -20ºC for N and C stable isotopic analysis.

To reveal the food chain structure in the planktonic/micronektonic community, phytoplankton (POM) were also collected at all stations except for St.6 for N and C stable

isotope analysis. Seawater samples were obtained from different light depth at 100, 10, 1, and 0.1% to collect POM using an acid-cleaned bucket for the surface water and Niskin-X bottles. 4.5 or 9 L of water samples are pre-filtered by a 0.2 mm nylon mesh to remove larger particles, and particles in the filtrates are collected on precombusted GF/F filters. The filters were then frozen at -20 ºC on board for later analysis on land. In addition, surface plankton larger than 0.2 mm and particles smaller than 0.2 mm were collected using seawater pumped from the bottom of the ship along transect during the cruising between stations. We also collected water samples fixed with acid-lugol solution for heterotrophic/mixotrophic microplankton observation at 7 layers from surface to 200 m. The samples were also added strontium sulfide to prevent the dissolution of the skeleton of acantharians which were scarcely quantified due to its fragility of the skeleton.

Community structure, Nitrogen and Carbon stable isotope ratios of mesozooplankton in South Eastern Pacific

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Carbon and nitrogen stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of organisms are controlled by not only biological factors, such as catabolism and assimilation, but also physical environmental conditions that influence the isotope ratios of primary producers (phytoplankton). Mesozooplankton, including meroplankton (larvae of benthic animals), is one of the key trophic levels in the marine ecosystem. To examine how different water properties (*i.e.*, nutrients and temperature) affect in marine food webs, and especially to verify the change to nitrogen isotope ratio due to anoxic water mass, we collected mesozooplankton samples at Eastern Eqatorial Pacific and along a trasect 20.0 - 55S,

90.0W in October 29 – December 16, 2019, during the cruise of the KH-19-6 leg.2 and 3 of the *R/V Hakuho-maru*.

Microplastics in the Southeast Pacific Ocean

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The distribution of microplastic in the open ocean of the southern hemisphere is largely undocumented. Substantial numbers of studies on floating microplastics have been reported in the Northern Hemisphere including North Pacific, yet very few data are available in the South Pacific. In the present study, we conducted microplastic surveys off the coast of Chile to fill gaps in the Southeast Pacific.

Neuston net sampling for surface microplastic analysis

Floating microplastic samples were collected at Stns 1 to 10 using a neuston net with a rectangular mouth opening of 75 cm height and 100 cm width, equipped a 333 μm mesh opening net with a collecting bottle at the cod end. At each station, the net was towed three times for 20 min each along the surface of the starboard side. At the stn 10, each tow was carried out for 10 min due to a logistical reason. The trawl speed ranged between 1 and 2 knots. A flow meter was installed at the net mouth to estimate the volume of water filtered during each tow. The collected samples were fixed with 5% formalin and stored at room temperature until analysis.

Multidiscipline analyses based on sediment cores

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We collected sediment corers to analyze magnetostatic bacteria, macrobenthos, and sedimentology of abyssal depths in the southeastern Pacific. Core samples were obtained using a multiple corer, which collects eight sediment cores per cast. Cores were stored in different conditions for further analyses (preserved as collected, sliced or sieved). Photographs of split cores are shown in Fig. 3. Some basalts and manganese nodules were detected in the remaining of sieved sediment samples (Fig. 4). Various analyses will be conducted after the cruise (e.g., analyses on macrobenthos, magnetostatic bacteria, microplastic, paleoceanography and sedimentology).

KH-19-6 Leg1 Leg 2, Leg 3 cruises
Underway Geophysics

Masakazu Fujii (NIPR)
Kosuke Inoue (AORI, UTokyo)

1. Instruments

Following instruments equipped with the R/V *Hakuho-maru* were utilized for shipboard geophysical observation in the KH-19-6 cruise; a multi-narrow beam echo sounder (MBES); proton precession magnetometer (PPM), shipboard three-component magnetometers (STCM), shipboard gravimeter, and sub-bottom profiler (SBP).

The seafloor bathymetry was observed using the MBES (SeaBeam3020, L3 Communications ELAC Nautik). Its operating frequency and maximum number of beams is 20 kHz and 301, respectively. The SeaBeam3020 system is consist of acoustic arrays of transmitters and receivers on the bottom of the ship and is controlled by the software "Hydrostar" installed on a Windows computer at the Lab. 1 (Fig. 1a). Auto swath width, auto intervals, and equi-beam-angle modes were applied in this cruise. The seawater sound velocity structure was determined by vertical profiles of several CTD cast observations, in which seawater conductivity (equivalent to salinity), temperature, and depth were measured.

The total and vector magnetic fields are measured using the PPM (PR-745, Kawasaki Geol. Eng. Co). The PPM measurements were performed every 20 sec when the proton sensor (Fig. 1b) was towed 330 m away from the ship. Measured magnetic data are merged with longitude, latitude, and time data from the ship GPS and recorded on a laptop computer at the Lab 3. (Fig. 1c).

The vector magnetic fields were measured using two sets of STCMs; the STCM-

AORI (SBM-89, GAUSS), and STCM-KOBE (SFG-1211, Tierra Technica). Both measurements are performed continuously with sampling frequency of 8 Hz during the cruise. The three fluxgate sensors orthogonally aligned to each other were rigidly fixed in the compass deck (Fig. 1d). Measured data are merged with attitude data (heading, pitch, and roll) and are recorded in a laptop computer set at the Lab. 1. Attitude data were obtained by a gyro of the OCTANS installed in the Lab. 9 for the STCM-AORI, and by a ring-laser gyro (RLG, Nihon Koku-densi Kogyo) installed in the Lab. 1 for the STCM-KOBE.

Gravity field data were obtained from the shipboard gravimeter (D-004, LaCoste & Romberg, ZLS Co.). The system time drift is 3 mGal or less per month, static repeatability is 0.2 mGal, and measurement range is 7000 mgal. The gravimeter is operated using the software UltraSys installed in a computer at the Lab. 9. Gravity fields are measured every 10 seconds. The control system basically consists of remote and host computers. The former performs all real-time activity associated with controlling the platform and gravity meter as well as maintaining the system clock. The latter receives the data, computes the cross-coupling correction, and performs the final filtering before archiving the data. The instrument drift is corrected using on shore gravity field data measured by a portable gravimeter (CG-5 AUTOGRAV, SCHINTREX).

The sub-seafloor structure was observed using the SBP (Bathy2010, SyQwest Inc.). Operating acoustic wave is 3.5 or 12 kHz chirp. This system is consist of acoustic sensor on the bottom of the ship and is controlled by the software installed on a Windows computer at the Lab. 3 .

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