R/V Yokosuka Cruise Report

YK16-E01

Hydrothermal vent survey at CIR 8-17°S
with AUV Urashima

Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)
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4. Notice on Using

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1. Cruise Information

- Cruise ID: YK16-E01
- Name of vessel: R/V Yokosuka
- Title of the cruise: Hydrothermal vent survey at CIR 8-17oS with AUV Urashima
- Title of proposal:
  [1] Survey for hydrothermal fluid vents at Central Indian Ridge 8-17oS by AUV Urashima
  [2] N₂ fixation and nutrient dynamics at Indian Ocean
- Cruise period: Jan 11 2016 - Jan 31 2016
- Ports of departure / call / arrival: Port Louis (Mauritius) - Port Louis (Mauritius)
- Research area: Central Indian Ridge 8-17oS
Research map & Ship track
2. People Onboard

2.1 Researchers
Principal Scientist Onboard (PSO) & Representative of the proposals
KA WAGUCCI, Shinsuke [JAMSTEC, D-SUGAR]
Science party
KITADA, Kazuya [JAMSTEC, SRRP]
MAKABE, Akiko [JAMSTEC, SIP project team]
NUNOURA, Takuro [R&D Marine Biosciences]
GROSSMANN, Mary [OIST]
CHEN, Chong [JAMSTEC, D-SUGAR]
TAKAHASHI, Ayu [JAMSTEC, SIP project team]
Technical Assistant
MINAMIZAWA, Satomi [NME]

2.2 AUV Urashima
Submersible Op. Manager
AKIHISA ISHIKAWA
Operation team
1st Submersible Staff YOSHITAKA SASAKI
2nd Submersible Staff FUMITAKA SAITO
2nd Submersible Staff TAKUMA ONISHI
2nd Submersible Staff YUDAI TAYAMA
2nd Submersible Staff RYO SAIGO
3rd Submersible Staff NAOTO MINAMINO

2.3 R/V Yokosuka
Captain
YOSHIYUKI NAKAMURA
Crews
Chief Officer YASUHIKO SAMMORI
2nd Officer TAKESHI EGASHIRA
3rd Officer RYO YAMAGUCHI
Chief Engineer EIJI SAKAGUCHI
1st Engineer TAKASHI OTA
2nd Engineer KENICHI SHIRAKATA
3rd Engineer YOICHI YASUE
Chief Electronic op. MASAMOTO TAKAHASHI
2nd Elect. Op. MICHYASU KATAGIRI
3rd Elect. Op. YOSHIKAZU KURAMOTO
Boatswain HATSUO ODA
Quarter Master YASUO KONNO
Quarter Master DAIJUKE YANAGITANI
Quarter Master TAKUYA MIYASHITA
Quarter Master HIROTAKA SHIGETA
Sailor KOSEI KAWAMURA
Sailor SHINYA KOJIMA
No.1 Oiler JUNJI MORI
Oiler TATSUOMI CHINO
Oiler RYO SATO
Assistant Oiler RYO MATSUUCHI
Assistant Oiler HIROMU FUKUDA
Chief Steward SUETO SASAKI
Steward SHINSUKE TANAKA
Steward TSUYOSHI NAGATOMO
Steward YOSHITAKA YAMAMOTO
Steward SHINOBU OHYU
3. Science

3.1 General purpose of the cruise

[1] To locate undiscovered hydrothermal vent by AUV Urashima dives (U234-242) with acoustic observation (MBES, SSS, and ADCP) and physico-chemical sensors (turbidity, ORP, and methane).


3.2 Cruise log

See Table in Appendix

3.3 List of observation equipment

<table>
<thead>
<tr>
<th>[1a] AUV Urashima</th>
<th>JAMSTEC</th>
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<tr>
<td>[1b] ORP sensor</td>
<td>ORP-14HI, Kimoto Inc. (developed by Prof. Kei Okamura)</td>
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<td>[1c] Turbidity sensor (x2)</td>
<td>ATU6-CMP, JFE Advantech</td>
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<td>[1d] AUV Magnetometer</td>
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<tr>
<td>[1e] Methane sensor</td>
<td>METS (S/N 1351), FRANATECH</td>
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<td>[1f] Zoo Plankton Sampler</td>
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<td>[2a] RINKO Profiler</td>
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<td>[2b] 12L Niskin-X bottle</td>
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<td>[2c] Plankton Net</td>
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<td>[2d] Plastic Bucket</td>
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<td>[2e] On-deck Incubator</td>
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3.4 Sampling and processing

3.4.1 AUV Urashima dives

3.4.1.1 Hydrothermal plume survey [All]

Introduction

The Central Indian Ridge (CIR) is a major Mid-Ocean Ridge with a full spreading rate of ~40 mm/year, which is intermediate when compared with those of the fast-spreading East Pacific Rise (EPR) and the slow-spreading Mid-Atlantic Ridge (MAR). While lots of deep-sea vent fields with high-temperature hydrothermal fluids have been discovered and investigated in the EPR and MAR (Baker & German 2004; Beaulieu et al. 2013; 2015), only four fields have been identified in the CIR.

The first example of hydrothermal vent in the CIR, the Kairei field, has been studied intensively since its discovery in 2000 (Gamo et al. 1996; 2001). Following the discovery of the Edmond
field in 2001 (van Dover et al. 2001), the third and fourth vent fields at the CIR, the Solitaire and Dodo fields, were suggested by water column chemistry in 2006 (Kawagucci et al. 2008) and discovered in 2009 by the dives of the Human-Occupied Vehicle (HOV) Shinkai6500 during the R/V Yokosuka YK09–13 cruise (Nakamura et al. 2012).

A recent paper (Son et al., 2014) reported long-range (sparse) hydrothermal plume survey in CIR 8-17oS by 118 vertical CTD-MAPR hydrocasts, and 35 of 118 hydrocasts detected water column anomaly in NTU, ORP, and/or methane concentrations. The survey pointed to occurrence of several active hydrothermal fields. However, discovery of the vent by ROV/HOV dives is still difficult due to spatial gap between such sparse observation (several kilometers interval) and visibility of ROV/HOV dives (<20 m view). AUV dive has an ability to fill the gap by acquiring precise bathymetry and spatial (particularly horizontal) distribution of physico-chemical anomalies in water column (Yoerger et al. 2007). AUV Urashima dives in this cruise aim to locate the suggested but undiscovered vents in CIR 8-17oS. In addition to monitoring physico-chemical characteristics of water column by sensors for turbidity, ORP, and methane, acoustic water-column anomaly exhibited on multi-beam eco sounder and side scan sonar were also utilized to identify hydrothermal plume.

**Dive results**

Five dives (U236, 237, 238, 240, 241) were carried out at 14o20’S region. At U236 dive, AUV Urashima was reached at the deep, touched onto the seafloor, and returned as emergent situation to the surface. During U237 dive, strong turbidity, temperature increase (in Urashima’s CTD), and other signals were obtained at eastern ridge of the CIR spreading center, northern part of which connects to non-transform offset. Grid cruising during U238-241 revealed spatial distribution of the water column anomalies that indicated probable vent location at eastern slope of the ridge (or small basin behind the ridge). Precise bathymetry at around the probable vent was acquired during U237, U238, and U241 with 400 kHz MBES whereas 200 kHz MBES was installed and tested at U 239-240 dives for future use.

U234 dive was the first dive of the cruise, but AUV Urashima cannot keep her position in water possibly due to heavy weight. U235 dive was carried out at 11oS region, where strongest anomaly had been shown in previous CTD-MAPR investigation, but did not detect any signals of hydrothermal activity. U239 dive was carried out far from the MOR for 200 kHz MBES test.

3.4.1.2 Seafloor geophysics [Kazuya Kitada & Ayu Takahashi]

A total of eight AUV “Urashima” dives (Dives #234 - #241) were completed at the hydrothermal fields along the Central Indian Ridge. During these dives, we have successfully obtained high-resolution bathymetric data, side scan sonar image, sub-bottom profile, and magnetic field. Magnetic field data were collected by three fluxgate magnetometers mounted in
the payload space of AUV Urashima to reveal the characteristics of crustal magnetic structures. One was attached to the top space and the others were attached to the port and starboard sides. For three dives (Dives #234, #236 and #241), 8-figure turns were conducted during descending to bottom in order to correct the effect of the vehicle’s permanent and induced magnetic fields.

### 3.4.1.3 Deep-sea planktons [Chong Chen & Mary Grossmann]

**Introduction**

The Zooplankton Sampler (ZPS hereafter; McLane Research Laboratories, Inc.) is an autonomous plankton sampler designed for *in situ* collection of zooplanktons underwater over an extended period of time. It filters seawater through a 100μm mesh which comes in a belt of 50 sample strips max. On cruise YK16-E01, the sampler unit (including the sample belt and the pump) as well as the battery (36V, McLane Research Laboratories, Inc.) were removed from the original frame to be mounted on AUV URASHIMA in attempt to sample larvae of hydrothermal vent animals to understand their dispersal and connectivity (Fig. 1).

**Installation**

Two ZPSs were on-board R/V YOKOSUKA. The URASHIMA team constructed a platform for the ZPS sampling unit, in the front payload chamber. The unit is attached to the platform using four screws and a metal extension to the base of unit, also made by the URASHIMA team. The battery was placed on bottom of the URASHIMA hull.
As running the pump was expected to cause significant interference with the magnetometer, the ZPS pump was set to run at the minimal settings (Fig. 2). It was unfortunately not possible to disable the pump completely through the CrossCut software. Instead of pumping, the water flow was generated by the force of URASHIMA cruising forward. A plastic pipe was attached between the front water inlet of URASHIMA and the ZPS to bring water to the sampling mesh (Fig. 3).

**Operation Procedure**

Prior to the dive operation, a fresh sampling belt was set to the ZPS sampling unit which is then handed over to the URASHIMA team. The ZPS seals a sample by rolling a piece of mesh over the sampling belt mesh, and the end of each sample is detected via a magnetic strip attached to the end of every sample strip. Special caution is therefore required here to make sure the sampling belt and the mesh is not caught in the cover, or else the sample will not seal. The battery is tested to make sure there is sufficient power remaining for a whole dive (over 36.0V was used as a threshold during the present cruise) prior to handing over to the URASHIMA team. The handover occurred the day before a dive operation.

After the team installed the ZPS on URASHIMA, the battery unit was connected to the sampling unit. The deployment settings were done by connecting a Windows XP computer to the

![Fig. 3: Left, water inlet for the ZPS (lower); Right, pipe from water inlet to ZPS.](image3)

![Fig. 4: Deployment settings for the ZPS during YK16-E01](image4)
battery unit (as the computer of ZPS is actually inside the battery hull) using the comms cable. Through the CrossCut software, the deployment time was set (during this cruise sampling was set to begin one hour after URASHIMA was in the water). Sampling frequency was set to every 15 minutes, and a total of 45 sampling events were programmed (Fig. 4). It is important at this time to test that the sample belt turns well and stops automatically after one sample through the manual operation menu – “move sample belt one positon”.

After recovery of URASHIMA following a successful dive, the ZPS was first connected to a computer to see how much battery voltage was remaining. If there were sufficient voltage for another full dive, the battery was not replaced. The battery unit is extremely heavy and so was replaced only whenever necessary. At this time, the deployment data for the completed dive can be offloaded on to the computer. When first connecting the ZPS to a computer post-dive, the system will ask if you would like to complete automatic belt take up, please make sure to select “No” here. The sampling unit is then removed from URASHIMA and moved to the wet lab. If a back-up ZPS sampling unit was ready, this was handed over to the URASHIMA team for immediate installation for the next dive. Settings for the next dive can either be done in the evening before the dive day, or in the morning of the dive day.

In the wet lab, the sampling unit is washed with freshwater. Then the cover was removed to access the sample belt, which was carefully moved to the desk and placed in middle of two dissecting microscopes. The sample belt was carefully unrolled, passing the sealing mesh under one microscope and the sample belt under another. Two scientists carefully examined both the mesh and sample belt at the same time for any particles and planktons sampled. When a specimen was located, it was preserved in 99% ethanol in a 1.5ml eppendorf tube, noting down the sampling time, date, and URASHIMA dive number. Specimens from the same sampling time were preserved in the same tube.

When sampling from the belt has been completed, the belt and mesh were submerged under freshwater in a bucket overnight to dissolve any remaining salt. The belt was re-installed in the sampling unit the following morning, ready for the next deployment.

Results
The ZPS completed eight successful dives during YK16-E01 (Dives #234-241), and some marine particles and planktons were sampled from the deep water, proving that it is possible to capture plankton samples using the ZPS on-board AUV URASHIMA. This is the first cruise where this has been attempted. However, the sample quantity was low, likely due to the low water flow through the sampling mesh. There are two possible future solutions to this issue: 1. To actively use the pump,
provided that it does not interfere with other devices installed on URASHIMA, or 2. To significantly increase the water inlet surface area, by attaching a large funnel in front of URASHIMA or elsewhere. Otherwise, only one notable failure occurred during this cruise, where after URASHIMA dive #239 the sampling belt was found broken inside the sampling unit. After investigation, it was revealed that this was due to the rubber used to construct the sampling belt was hardened due to aging, and could not withstand the pulling force. As the sample belt used on this cruise was purchased many years ago, to prevent this happening on future cruises, new sampling belts should be purchased prior to each cruise.

Shore-based Sample Analyses
Specimens taken by the ZPS will be transported to JAMSTEC Yokosuka Headquarters for morphological identification as well as genetic sequencing (H. Watanabe & C. Chen). This will allow determination of whether or not hydrothermal vent larvae has been collected. If any larvae of vent animals are identified their sampling location can be pinpointed using URASHIMA’s dive track, as the sampling period is time-stamped.

3.4.2 Surface Sampling (SS)
3.4.2.1 Set up and operation [All]
Sampling performed using a 200 m rope, fixed to the winch cable and passed onto a davit at the rear of the deck. A simple Plankton Net (RIGO Co.: 30 cm diameter, 75cm length) equipped with a 100 µm nylon mesh was attached to the end of the rope with a 10 kg chain (as sinker). A RINKO CTDO profiler with another 5 kg sinker was attached at the bottom of the chain (Figure and Photo).

After the rope end was 10 m down to the seafloor from the deck, a 12L Niskin-X type bottle was mounted on the rope. The CTD-Net-Niskin system was towed vertically to a depth of about 150 m, at a winch speed of about 0.8 m/s. The system was stopped for 1 minute at 150-m depth, and then pulled up. At approximate chlorophyll maximum depth, monitored by the RINKO profiler, messenger(s) was thrown to the Niskin bottle. Two or 3 tows were performed at each sampling period. After the CTD-Net-Niskin system operation, bucket casts were carried out for surface water sampling.
3.4.2.2 Nutrient biogeochemistry [Akiko Makabe]

Natural abundance of nutrients, dissolved and particle organic matter, and volatiles

Seawater for measurement of nutrient abundance were subsampled into 10-mL tubes (3-8 tubes per sampler) after 3 times rinse by sample water and stored at -80°C. Nutrient abundance will be analyzed by Dr. Taketoshi Kodama.

Seawater for measurement of dissolved and particle organic matter (DOM & POM) were subsampled into 250-mL polycarbonate bottles (3 times rinse with sample seawater before filling). In onboard laboratory, the subsampled seawater was filtered with a pre-combusted 25-mm GF/F filter, and the filtrate was introduced into a polyethylene bottle. POM abundance and their carbon and nitrogen isotope ratios will be determined by elemental analyzer connected with an isotope ratio mass spectrometer (EA-IRMS) at JAMSTEC. Nitrogen-bearing DOM (DON) and its nitrogen isotope ratio will be determined by combination between POR, denitrification, and CF-IRMS.

Activity measurements of nitrogen fixation

Seawater for measurement of N\textsubscript{2} fixation rate were subsampled into 250-mL polycarbonate bottles (3 times rinse with sample seawater before filling) and then sealed without bubbles and head space. In onboard laboratory, 10 mL of \textsuperscript{15}N\textsubscript{2}-enriched or air-saturated seawater were introduced into the sealed polycarbonate bottles via a needle by gas-tight syringe along with removal of original sample water via another needle to another syringe. The \textsuperscript{15}N\textsubscript{2}-enriched and air-saturated seawater were prepared from surface seawater, first filtered through 0.2 \textmu m membrane and then degassed through a degassing membrane. The 90 mL of degassed filtrate was then introduced into a PTFE gas tight bag, and 2 mL of \textsuperscript{15}N\textsubscript{2} gas or air was added. The bag was shaken vigorously for 1 min by hands and then shaken moderately by a shaker until using (at least one hour).

The polycarbonate bottles were put in the incubators on funnel deck of R/V Yokosuka plumbed with surface seawater and shaded to 100, 10, and 1 % of full sunlight. Duration of the incubations was 24 or 48 hours. The incubated water was filtered with a pre-combusted 25-mm GF/F filter, and the filtrate was introduced into a polyethylene bottle. The filters and filtrates were stored frozen until onshore analyses. By analyzing \textsuperscript{15}N abundance of the filter (PON) and filtrate (DON & DIN) with an isotope ratio mass spectrometer (IRMS) at JAMSTEC, the N\textsubscript{2} fixation rates in two size fractionation will be estimated.

Activity measurements of nitrification

Seawater for measurement of nitrification rate were subsampled into 125-mL glass vials (2 times rinse
with sample seawater before filling) without bubbles and head space and capped with butyl rubber stopper and aluminum seal. The $^{15}$N-enriched ammonium, urea, or glutamine were added to the vials as the final concentration was 50 nM. As experimental control, the inhibitors, erythromycin or allylthiourea, for bacteria activity or ammonia oxidation process by bacteria, respectively, were injected to some vials.

The surface samples, taken by bucket, were put in the incubator on funnel deck of R/V Yokosuka plumbed with surface seawater and shaded to 1 % of full sunlight. Subsurface samples, taken by Niskin sampler, were put into a dark incubator with the temperature at the sampling depth. Duration of the incubation was about 12 hours for all the nitrification samples. The incubated waters for quantifying $^{15}$N enrichment in nitrite and nitrate were filtered with 0.45 m membrane filter and stored frozen until onshore analysis. The incubated waters for quantifying $^{15}$N enrichment in nitrous oxide were poisoned by adding 0.2 mL of saturated HgCl$_2$ solution and stored at room temperature. The nitrification rate will be estimated by analysis with the IRMS at JAMSTEC.

### 3.4.2.3 Microbes and virus [Takuro Nunoura]

Prokaryotes play a major role in marine biogeochemical fluxes. Biogeochemical transformation rates and functional diversity of microbes are representative major topics in marine microbial ecology. The objectives of this study were to determine the abundance and diversity of prokaryotes and viruses.

#### Methods

**Microbial abundance**

Samples for microbial abundances (prokaryotes, eukaryotes and viruses) were collected in every sampling. Samples were fixed with glutaraldehyde (final concentration 0.5%) or mixed with Glycerol-EDTA, and frozen at -80°C. The abundance of microbes and viruses will be measured by a flow cytometry in JAMSTEC after nucleic acid staining with SYBR-Green I.

**Microbial diversity**

Microbial cells in water samples were filtrated on cellulose acetate filter (0.2µm) and stored at -80°C. Environmental DNA or RNA will be extracted from the filtrated cells and used for 16S/18S rRNA gene tag sequencing using Ion PGM or MiSeq, clone analysis for functional genes (e.g. *amoA* and *nifH*), quantitative PCR for genes for 16S rRNA and *amoA*, metagenomics and/or metatranscriptomics.

**Viral diversity**

Viral particles were obtained by a chemical flocculation method (John et al. 2012) and filtrated on polycarbonate filter (0.45µm). The filters were stored at 5°C. Viral particles will be resuspended in
3.4.2.4 Zoo planktons [Mary Grossmann]

**Introduction**

As in many plankton groups, the phylogeny of pelagic cnidarians is based almost entirely on morphological characteristics. Due to difficulties in preserving gelatinous organisms, it is not generally possible to collect both morphological and genetic information from the same organism, unless detailed taxonomic work is performed on board before preservation. For these reasons, there is little or no genetic validation of the current cnidarian phylogenies.

For pelagic cnidarians, many species were described in the early 20th century, during the great oceanographic expeditions. Some have never been recorded since, due to poor drawings or a faulty original description, or perhaps simply due to regional distributions of certain species. Atlantic jellyfish have been quite intensively studied, and there have been great recent advances in studies of Pacific and Southern Ocean diversity. However, the Indian Ocean remains little studied, although it is the type locality of a great number of species, collected during the *Discovery II* campaigns.

On cruise YK16-E01, we collected plankton samples and sorted and identified pelagic cnidarians so as to validate previous morphological information and acquire genetic information for species from the Indian Ocean so as to compare these results with previous data from the Atlantic and Pacific Oceans.

**Method**

At the end of each CTD-Net-Niskin tow, the net was recovered, washed down with seawater and the plankton transferred to a 450 mL glass jar (Fig. 2). The sample jars were moved to the 20°C wet lab container, and cnidarians were manually sorted out, identified and photographed. Identified samples were then either preserved in 5% seawater-buffered formaldehyde solution to serve as morphological references, or were preserved in 99.9% Ethanol for future DNA extraction. After the isolation of the
animals of interest, the rest of the bulk plankton sample was preserved in 5% seawater-buffered formaldehyde solution for future plankton community studies.

Results
A total of 19 plankton samples were collected, from 8 sampling stations. About 40 cnidarian taxa were sorted out of the samples for further morphological and genetic analyses.

Shore-based Sample Analyses
Specimens sorted out of the bulk samples will be transported to OIST for detailed morphological studies as well as genetic sequencing. This will allow determination of whether or not the Indian Ocean populations are more closely related to the Pacific or Atlantic groups. It will also help confirm the validity of some doubtful Indian Ocean species.

The bulk plankton samples will be imaged using a plankton scanner, and the plankton counted and identified to main taxonomic groups. This information will help understand the spatial variation of surface plankton in the tropical Indian Ocean, and also provide background information for the studies of nutrient and phytoplankton concentrations carried out concurrently during YK16-E01.
4. Notice on Using

Notice on using: Insert the following notice to users regarding the data and samples obtained.

This cruise report is a preliminary documentation as of the end of the cruise. This report may not be corrected even if changes on contents (i.e. taxonomic classifications) may be found after its publication. This report may also be changed without notice. Data on this cruise report may be raw or unprocessed. If you are going to use or refer to the data written on this report, please ask the Chief Scientist for latest information. Users of data or results on this cruise report are requested to submit their results to the Data Management Group of JAMSTEC.
## Appendix Table: CTD, Niskin water sampling, plankton net sampling, water sampling by bucket, and AUV dive List

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<th>Longitude</th>
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*Weather Notation*

- **b** Blue sky (Cloud 0-2)
- **bc** Fine but Cloudy (Cloud 3-7)
- **c** Cloudy (Cloud 8-10)
- **o** Overcast (Cloud 10)
- **r** Rain

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Trial dive for MBES(200kHz)

MBES(200kHz) was low signal level