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# PRELIMINARY REPORT

# FOR YOKOSUKA

# Cruise no. YK 13-10

## Horizon deep, Tonga Trench

## South Pacific Ocean

## Oct 6 – Oct 21, 2013

Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)

# Contents

# 1. Cruise information

Cruise number:　YK 13-10

Ship name: R/V Yokosuka and Shinkai 6500

*Title of the cruise*: QUELLE Quest4 -Tonga Trench-

Chief Scientist: KITAZATO, Hiroshi (JAMSTEC)

Representative of Science Party: KITAZATO, Hiroshi, Michibayashi Katsu

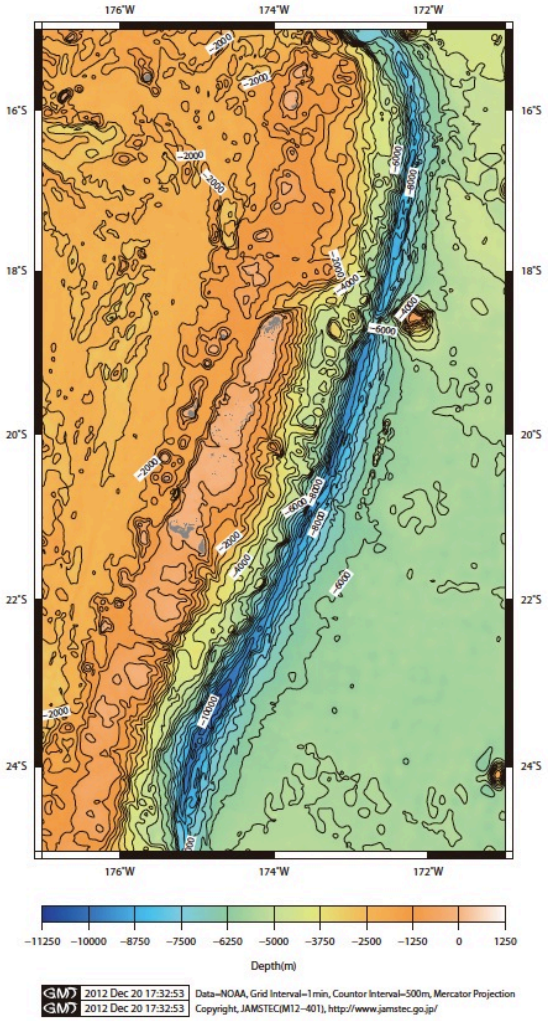
(Shizuoka University)

Cruise Period: 6th of October, 2013 (Sunday) – 21st of October, 2013 (Monday)

Port call: Suva, Fiji ~ Nuku'alofa, Tonga

Research area: Tonga Trench, South Pacific Ocean

Research Map:



# 2. Onboard Researchers, crews and Shinkai 6500 operation team members

**1.Research group**

| | |
|---|---|
| Hiroshi Kitazato | Japan Agency for Marine-Earth Science and Technology |
| Katsuyoshi Michibayashi | Shizuoka University |
| Takashi Toyofuku | Japan Agency for Marine-Earth Science and Technology |
| Akinori Yabuki | Japan Agency for Marine-Earth Science and Technology |
| Osamu Ishizuka | Japan Agency for Marine-Earth Science and Technology |
| Atsushi Okamoto | Tohoku University |
| Ronnie N. Glud | University Southern Denmarl |
| Frank Wenzhoefer | Max Planck Institute for Marine Microbiology |
| Ashley Rowden | National Institute of Water and Atmospheric Research |
| Sione Nonu | Ministry of Lands,Environment, Climate Change & Natural Resources |
| Suehiro Nitta | Japan Agency for Marine-Earth Science and Technology |
| Yuko Nonaka | Japan Agency for Marine-Earth Science and Technology |
| Toshimasa Nasu | Nippon Marine Enterprise |

**2.Operation team of the SHINKAI 6500**

| | |
|---|---|
| Submersible Op. Manager | Toshiaki Sakurai |
| Depty Submersible Op. Manager | Kazuhiro Chiba |
| Depty Submersible Op. Manager | Yoshitaka Sasaki |
| 1nd Submersible Staff | Kazuki Iijima |
| 1nd Submersible Staff | Shinobu Omika |
| 1nd Submersible Staff | Mitsuhiro Ueki |
| 1nd Submersible Staff | Keita Matsumoto |
| 2nd Submersible Staff | Hitomi Ikeda |
| 2nd Submersible Staff | Hirofumi Ueki |
| 2nd Submersible Staff | Keigo Suzuki |
| 2nd Submersible Staff | Akihisa Ishikawa |
| 2nd Submersible Staff | Masaya Katagiri |

**3.Captain and crew of the R/V Yokosuka**

| | |
|---|---|
| Captain | Shinya Ryono |
| Chief Officer | Naoto Kimura |
| 2nd Officer | Tetsuo Shirayama |
| 3rd Officer | Hiroharu Omae |
| Chief Engineer | Eiji Sakaguchi |
| 1st Engineer | Kazunori Noguchi |
| 2nd Engineer | Kenichi Shirakata |
| 3rd Engineer | Kota Kataoka |
| Chief Radio officer | Masamoto Takahashi |
| 1st Electronic Operator | Hiroki Ishiwata |
| 2nd Electronic Operator | Ryosuke Komatsu |
| Boat Swain | Yoshiaki Kawamura |
| Quarter Master | Kazumi Ogasawara |
| Quarter Master | Jiro Hanazawa |
| Quarter Master | Daizuke Yanagitani |
| Sailor | Kazuho Ikeda |
| Sailor | Yoshihiro Ogawa |
| Sailor | Kenta Nasu |
| No.1 Oiler | Kazuaki Nakai |
| Oiler | Shinya Sugi |
| Oiler | Masayuki Fujiwara |
| Oiler | Tatsuomi Chino |
| Oiler | Toshinori Matsui |
| Chief Steward | Sueto Sasaki |
| Steward | Shinsuke Tanaka |
| Steward | Masanao Kunita |
| Steward | Kazuma Sonoda |
| Steward | Shiho Shimizu |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# 3. Research cruise

## 1) Aim of YK13-10 Cruise at *Tonga Trench*

The Tonga Trench is located in the western South Pacific and is 10,850 meters deep at its deepest point, known as the Horizon Deep. This is the deepest area in the world except for the Challenger Deep in the Mariana Trench. The main objective is to describe the biological communities of the hadal ecosystem and comparing them with other sites such as Mariana, Japan, and Kermadec Trenches. For instance, a hadal amphipod *Hirondellea dubia* is known to be a dominant species in such areas. We will examine the genetic divergence and phylogenetic relationship of this hadal amphipod in order to determine connectivity between trenches. In addition we will collect data on biogeochemical cycles, such as carbon and nitrogen, which are essential to understand functional relationships and processes within the hadal ecosystem.

There are a number of general aspects that will be investigated during the survey:

**A- Discovery of hadal communities in the Tonga Trench**

- Mega-, macro-, and meio-benthic organisms on the seafloor and in sediment
- Bacterial flora in sediment and seawater

**B- Genetic divergences and connectivities between trenches**

- DNA barcoding of hadal amphipod *Hirondellea* spp. for identifying phylogenetic position and gene flows

**C- Carbon and nitrogen fluxes**

- Deploy profiling lander system
- Deep-sea benthic ecology and ecosystem function
- Determine the food web structure in hadal ecosystem

**D – Geology and Geochemistry of Tonga Arc**

- Understanding the tectonic evolution of the Tonga Arc through submarine geological survey, in particular the description of peridotite sequences
- Understanding serpentinite-related chemosynthetic community composition, and production mechanisms of chemical components including $CH_4$ and sulfide
- Understanding the geological evolution of the Tonga Arc

**E- Applied Biology, evaluation of useful microorganisms**

- Screening for useful agents and metabolisms from isolated microorganisms
- Isolation of culturable microorganisms

- Investigation of enzymes in microorganisms

## 2) Periods and areas

Lander Observations and Dive cruise was held on Tonga Trench during Oct 6 through Oct 21, 2013. In total five Shinkai6K's dives and six lander (three deployments were held 9 sites.

## 3) Coordinators

Drs. Hiroshi Kitazato (executive director of Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology) and Katsuyoshi Michibayashi (Professor of Shizuoka University) are chief scientists. In total, 11 international scientists, Japan, New Zealand, Denmark and Germany took part in the cruise with 1 Tonga observer, two promotional administrative staffs.

## 4) Description of Dives

At each site, we plan to carry out a) observations of rock and sediment characters and benthic biological communities and b) measurements of environmental variables with CTDO systems. Benthic animals are recorded high definition tv camera systems and still cameras. Some interesting rocks, organisms and others are captured by manipulators and slurp gun sampler. Precise observations of SWI by landers will be made with H-type push cores with 82mm inner diameter. Both faunal and DNA analyses plans to conduct using biological samples for understanding biodiversity changes of Tonga Trench. General oceanographic observations will also be carried out routinely during the cruise.

**Expected survey schedule *in the Tonga Arc~Trench***

Research strategy:

There will be a total of 12 working days during the cruise. 4 dives will occur at the inner slope of Tonga Trench, by Dr. Michibayashi; 5 working days at Horizon Deep for deploying the free-fall camera sampling system and profiling lander, and then one dive and 2 profiling lander casts at 6000m on the abyssal plain beyond the Tonga Trench.

Survey steps for each area:

Step 1: Acquisition of detailed topographic data using a multi-beam echo-sounder (MBES) and definition of diving sites.

Step 2: Transect dives on the inner slope of Tonga Arc in selected depth zones. During each dive, a geological survey will be done for outcrop observations, sampling of rocks and biota, and measurements of environmental parameters with CTD, with DO and other sensors.

Step 3: In the region ofthe second deepest point of the world, Horizon Deep, we plan to deploy the free-fall camera sampling system and profiling landers at 2 sites (6000 m and 10,000 m).

## 5) Instruments

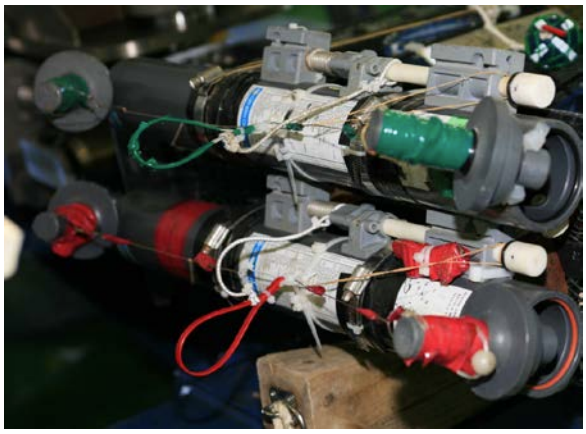
Suction sampler (Slurp gun): Benthic animals and rocks sampling

（dimension: 210×210×580mm. weight-in-air: ca. 16.5kg）

Push sediment corer (H type): Sediment sampling

(dimension: diameter 82mm × length 500mm, weight-in-air: ca. 3kg)

Niskin bottle water sampler(φ89×851 mm, weight-in-air 3.2 kg)



## 6) Log of daily scientific activities

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# 4. Seabeam Maps

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# 5. Dive records

Dive #1369

Dive #1370

Dive #1371

# Dive #1369

Track chart

Event mark list

(Removed for public distribution.)

# Dive #1370

Track chart

Event mark list

(Removed for public distribution.)

# Dive #1371

Track chart

Event mark list

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# 6. Scientific observation
# and
# Research proposals

## General results of the Cruise

Hiroshi Kitazato and Katsuyoshi Michibayashi

YK13-10 cruise made research on the Tonga Trench area, in particular to the Horizon Deep, the 2nd deepest point of the world. The objectives of the survey are to: (i) describe the environmental characteristics of the "hadal zone", including depths of greater than 10,000 meters, and sample the organisms living in this environment, and (ii) find out exactly what is going on there, unravel the correlation between organisms and their habitats, and learn how the trench environment was created.

This is the first exploration with a manned research submersible in the Tonga Trench. For the first time, sediment was sampled and video footage was taken by using a sediment sampling lander system with a video camera attachment. Also for the first time, the oxygen profile was measured in the upper layer of the seabed, using a separate sediment profiling lander. This was the second time, however, that video footage, sediment samples, and oxygen profiles have been taken at water depths greater than 10,000 meters by JAMSTEC in an oceanic trench.

We embarked from Suva, Fiji for the Tonga Trench area on October 6 and called at here, Nuku'alofa, Tonga, on October 21. Our research team consists of 14 members, 10 Japanese, one from New Zealand, one from Germany, one from Denmark and one from Tonga.

During the cruise, 6 lander deployments and 3 Shinkai 6500 dives are made at Tonga Trench area. Throughout these surveys, we recorded many deep-sea video images, collected organisms, waters, sediments and rocks, and measured in situ oxygen profiles.

Summary of research achievements is as follows.

(1) A detailed bathymetric chart of an area around the Horizon Deep of the Tonga Trench was drawn. Geological features in the fastest-moving subduction zone were successfully revealed.

(2) At depths of 10,805 meters and 6,250 meters at the Horizon Deep of the Tonga Trench, we achieved successfully: (i) sampling of organisms living on, in or

near the seabed, (ii) sampling of undisturbed sediment in the upper layer of the seabed, (iii) sampling of bottom water, (iv) measurement of oxygen profile, and (v) video recording of the seafloor with a high-definition camera. This video recording and the observation of seabed sediments are the very first to be made at the deepest point of the Tonga Trench. JAMSTEC plans to compare the observation data with the data obtained at the Challenger Deep.

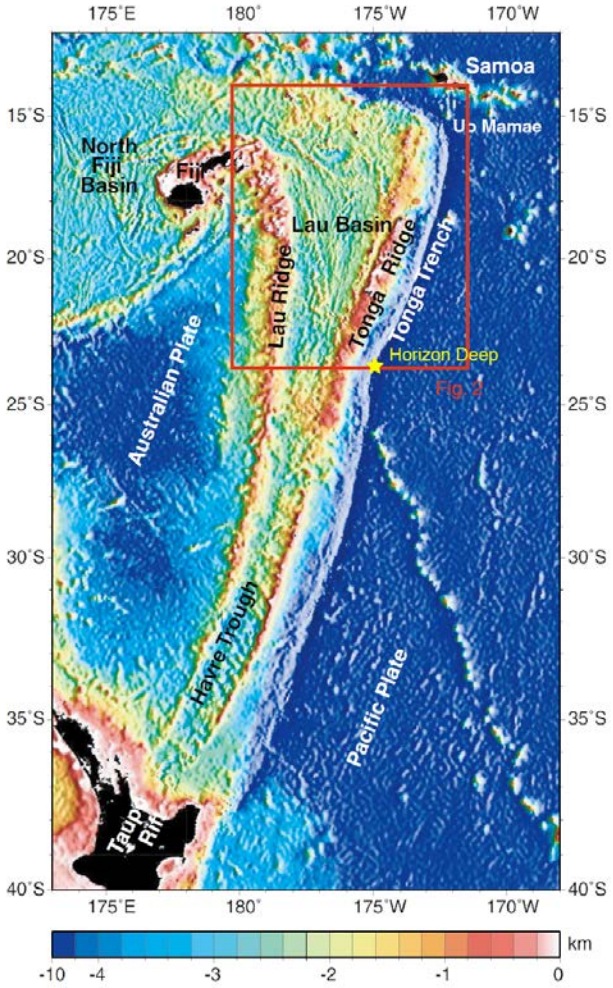
(3) A supergiant (>20 centimeters long) hadal amphipod, *Alicella gigantea*, was sampled. This was first time this supergiant amphipod has been sampled in the Tonga Trench. This amphipod has only been previously sampled in the South Pacific Ocean at the the Kermadec Trench. In addition, other amphipod species (e.g. *Hirondellea dubia*) and other hadal organisms were observed and sampled.

(4) Marine geological survey was carried out on the landward (western) slope of the Tonga Trench. Rock and core samples were successfully collected, which will provide a clue to geological features and genesis of the Tonga Trench and Arc.

## 1) Geology

Katsuyoshi Michibayashi

The Tonga Trench is one of the deepest trenches in the world and is a typical arc–trench system where the trench is parallel to the arc. The inner trench wall has a steep slope and the depth of its escarpment extends from 6,000 to >10,000 m below sea level, with its deepest part being 10,866 m in Horizon Deep (Macleod, 1994) (Fig.1).




The Tonga intra-oceanic arc is recognized as a type example of an extension-dominated, non-accretionary convergent margin (e.g., Tappin *et al.*, 1994), characterized by active extensional tectonism throughout the forearc and landward trench slopes. The forearc from 14°S to 26°S may be subdivided latitudinally into three major blocks, based on morphology, structure, and sediment geometry (Tappin, 1994): the northern block (north of ~18°30'S), the central block (~18°30' to 22°S) and the southern block (~22° to 26°S). Changes in the morphology of the forearc as convergence changes from normal in the south to highly-oblique in the north have been documented by Wright et al. (2000).

Fig. 1 Bathymetry map of Tonga-Kermadic Trench

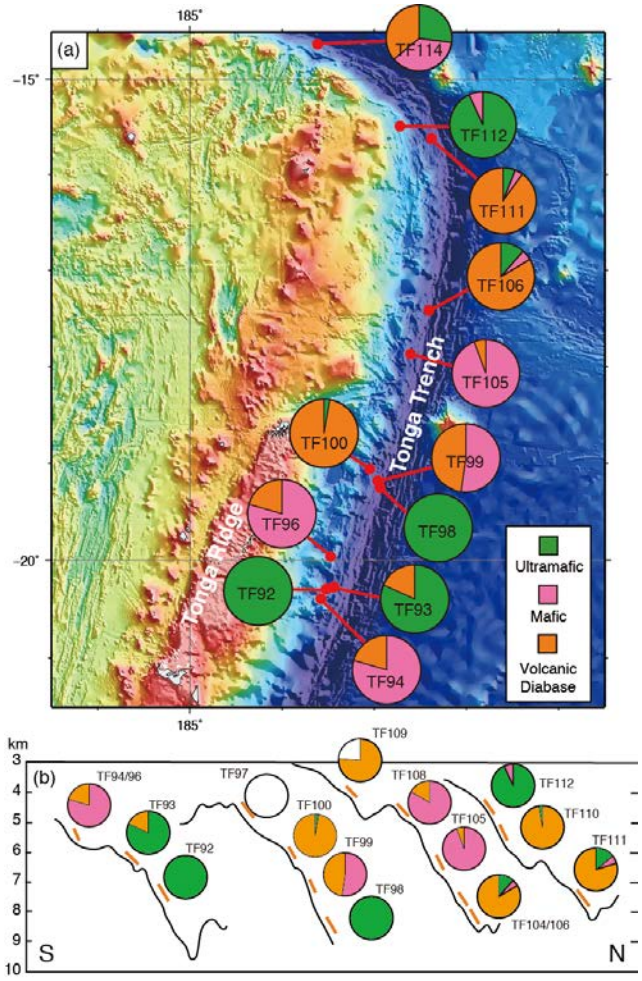



Fig. 2 Bathymetry map around the south to northern terminus Tonga Trench. (a) Dredge sites and circle graphs of lithology distribution collected by

GPS measurements indicate that the instantaneous plate convergence across the northern Tonga trench is 24 cm/y, which is the fastest recorded convergence velocity on Earth (Bevis *et al*., 1995). In addition, the instantaneous plate spreading across the northern Lau Basin is 15 cm/y (Bevis *et al*., 1995). Schellart *et al*. (2006) also noted that this region is experiencing slab rollback and back-arc extension.

Numerous dredge surveys of the Tonga Trench were carried out in the early 1960s, revealing that the trench lacks an accretionary prism and is dominated by mafic and ultramafic igneous rocks (e.g., Fisher & Engel, 1969). The crustal sections may represent primitive island arc crust or older oceanic crust that has been modified by arc volcanism (Bloomer & Fisher, 1987).

A range of rock types were dredged at several localities on the landward slopes of the Tonga Trench during Boomerang Leg 8 aboard the R/V *Melville* (Fig. 2). The scientific literatures of this cruise have been summarized in Meffre *et al*. (2012). Ultramafic rocks were recovered from the deepest sites (7000–9000 m), whereas gabbros and diabases were more common at shallower sites, and volcanic rocks were mainly recovered from sites shallower than 5000 m (Bloomer *et al*., 1996). Most notably, pristine peridotites are found at the deepest part of the landward trench slope.

The Tonga Trench preserves a record of geologically important processes that

link subduction and mantle flow, as well as extension and crustal generation. Clift *et al*. (1998) and Wright *et al*. (2000) discussed the tectonics and topography of the Tonga Trench and fore arc. Recently, Meffre *et al*. (2012) presented whole-rock geochemistry, and $^{40}Ar/^{39}Ar$ and U–Pb dating results on rocks dredged from the Tonga forearc. Most of the ages young northwards, suggesting the occurrence of successive domains with distinct geological histories.

The YK13-10 cruise is the first time in scientific history to study Tonga Trench by submersible. The major objectives of the YK13-10 cruise are:

1) To investigate and collect rock samples along the land-ward slope by Shinkai6500 in order to identify geological structure and lithological distribution
2) To investigate the degree of serpentinization in order to understand fluid-rock interaction on the deep sea floor
3) To investigate the limit of the life in relation to serpentinization on the deep sea floor.

**Shore-based Studies**

A comprehensive work plan for the rock samples was developed by the shipboard scientific party. This work will include major element analyses, trace element analyses, geochronology studies, mineral analyses, petrographic characterization, and radiogenic isotope characterization. The work will be completed at Shizuoka University, the Geological Survey of Japan/AIST, JAMSTEC, Tohoku University and other possible collaborators.

**Other shore-based studies include:**

Bathymetric data will be merged with existing data and synthesized at GSJ and Shizuoka University Volcanic and geologic synthesis will be done at Shizuoka, GSJ, JAMSTEC and Tohoku, etc. Links between arc mantle-crustal structure and characteristics of magma will be extensively investigated with shore-based collaborators.

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## 2) Ultra Deep lander system

Ronnnie N Glud & Frank Wenzhoefer

The instrument consist of 4 main components; ballast system, flotation unit, battery and most importantly the measuring unit.

The instrument is autonomous and once released from the ship it sinks to the sea-floor by a speed of approx. 45-50 m $min^{-1}$. Once settled at the seabed the central measuring unit carry out a pre-programmed measuring routine, before ballast is released and the now positively buoyant system ascend by a speed of approx. 45 m $min^{-1}$.

The central measuring unit holds an array of microsensors (Fig 1). On this cruise 8 $O_2$ microelectrodes, 2 resistivity and 1 temperature sensor were mounted at each of the 4 deployments. The thin sensors are lowered into the sediment with a pre-programmed step size (on this cruise 0.25 mm) using a high resolution elevator system. At each step they record a signal proportional to the $O_2$ content of the sediment. The resistivity sensors provide information on the sediment porosity and document the relative position of the sediment-water interphase. Total profiling distance was set to 20-26 cm and after reaching the maximum depth, the sensor array is moved back up to the start position. The entire measuring unit is then moved horizontally and a new vertical measuring routine is initiated. This procedure is repeated as many times as possible at the given deployment time (at this cruise 3-5 times). The ultra deep lander is modified from a previous version that successfully has been deployed from *RV Natushima* many times (Glud et al 2009a, 20009b) and at Challenger Deep (Glud et al 2013).

Sensors were calibrated against bottom water samples with known $O_2$ concentrations an on-board determination of the “zero”-current of the sensors. The bottom water samples were recovered by Niskin bottles mounted on the “camera-lander” and the $O_2$ concentrations were determined by Winkler titrations.

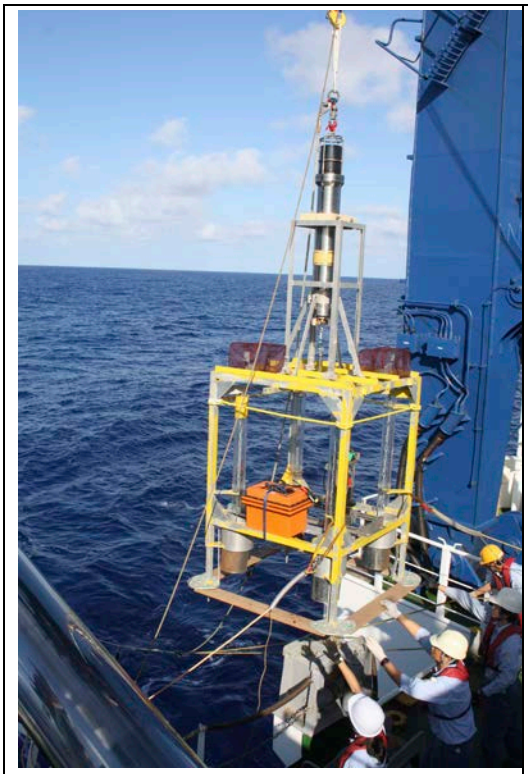

*Fig. 1: Ultra-deep lander being deployed; and the array of microelectrodes applied*

## Research

**Background**

The amount of organic material that escapes mineralization and is retained in the sediment record is the single most important factor determining the $O_2$ levels of the global ocean and our planet. Today we have a reasonable good understanding of the processes that are responsible for mineralization of organic material and which factors regulate the efficiency by which organic material is degraded in the seabed. However, to obtain firm quantitative measures we need to conduct direct measurements in the respective areas of our ocean. The current global data base of high-quality measurements of benthic carbon mineralization rate is around 400 spread over the global ocean (Glud 2008). This limited database is mainly concentrated to a few regions of the global ocean and there exist large "white", unexplored areas of the

seabed – including the deep trenches. Trenches only account for less than 2% of the global seabed area, but could via sediment focusing act as traps for organic material. Consequently the mineralization efficiency at these great depth could play a crucial role for the global carbon (and $O_2$) household. *We hypothesize that trenches play an un-proportional large role for retention and turn-over of organic material in the deep-sea*

The benthic mineralization is mainly driven by vast numbers of Bacteria and Archaea. Currently even the most basic information on abundance and distribution of microbes in trench sediments are missing. One of the main mortality factors for microbes in deep-sea sediments are deadly virus infections (Danovaro et al 2008) and bacterial cell walls appear to be a major component of relict marine organic material (Lomstein et al 2009) . Currently there exists no knowledge about the role of viruses for regulating the mortality or the diversity of microbes in trench sediments, or in deep sea sediments in general.

We want to quantify the carbon mineralization efficiency at Horizon deep (the second deepest site on Earth) and compare it to conditions at the neighbouring abyssal plain – further we wish to quantify the distribution and diversity of prokaryotes and virus in these sediments. We have previously carried out similar investigations in The Japanese Trench and the Mariana Trench. The result will add to our growing database on microbial carbon mineralization in hadal settings and allow for comparison between hadal environments experiencing different regimes of vertical carbon export.

**Strategy**

We measured a number of $O_2$ microprofiles at Horizon deep and at the abyssal plain using the Ultra-deep lander system. From these profiles we can calculate how much $O_2$ is consumed in the sediment and also where it is consumed within the oxic zone. This provides indications on which processes are responsible for the benthic $O_2$ consumption. The $O_2$ consumption rate provides an integrated measure of the total benthic carbon mineralization rate.

Recovered sediment cores were sectioned and the respective slices will be used for dating by the $^{210}$Pb procedure. Combined with information on the distribution of organic material (and the C/N ratio) this will allow us to quantify the sediment accumulation rates and the retention of carbon and nitrogen in the sediment record. Thereby the mineralization efficiency can be determined at the investigated sites. Important back-ground information on sediment density and porosity that are required for the calculations will also be determined. Further, complementary information on sediment accumulation (and mixing) will be obtained from depth profiles of phototrophic pigments and $^{234}$Th in the sediment.

The vertical distribution of Bacteria (+ Archea) and virus will be determined by an optimized extraction, staining and counting procedure (Middelboe and Glud 2006, Siem-Joergensen et al 2008). Some samples will also be used to investigate the community composition after amplification by use of different molecular techniques. Two sites were investigated and will be compared; the Horizon Deep at a depth of ~10.800m and a site west-ward of the central basin at ~6.200 m water depth. In total we had 4 lander deployments and retrieved sediment cores from three sites, all is summarized in Table 1.

**Table 1:** Ultra-Lander deployments and recovered sediment samples taken

| Station | Position | | Depth (m) | Gear | Sample |
|---|---|---|---|---|---|

| | Latitude | Longitude | | | |
|---|---|---|---|---|---|
| 1-1 | 23° 36.6771’ S | 174° 16.8787’ W | 6256 | Ultra-Lander | $O_2$, resistivity, temperature profiles |
| 1-2 | 23° 36.6344’ S | 174° 16.8133’ W | 6253 | Ultra-Lander | $O_2$, resistivity, temperature profiles |
| 2-1 | 23° 16.4294’ S | 174° 44.9826’ W | 10817 | Camera-Lander | Core 3: $^{210}Pb$, $^{234}Th$, Porosity, Bacteria and Virus abundance, Chl a/Pigments, $C_{org}$, bottom water oxygen content |
| 2-2 | 23°16.5148’ S | 174° 44.8747’ W | 10807 | Ultra-Lander | $O_2$, resistivity, temperature profiles |
| 2-3 | 23° 16.5085’ S | 174° 45.1347’ W | 10807 | Camera-Lander | Core 3: $^{210}Pb$, $^{234}Th$, Porosity, Bacteria and Virus abundance, Chl a/Pigments, $C_{org}$, PCR<br>Niskin bottle: Bottom water oxygen content |
| 2-4 | 23° 16.5436’ S | 174° 45.2294’ W | 10805 | Ultra-Lander | $O_2$, resistivity, temperature profiles |
| Dive #1370 | 23° 36.7727’ S | 174° 17.3064’ W | 6254 | Shinkai6500 | Niskin bottle: Bottom water oxygen content<br>PC 3: PCR, Porosity, Bacteria and Virus abundance, Chl a/Pigments<br>PC 8: $^{210}Pb$, $^{234}Th$, Bacteria and Virus abundance<br>PC 7: PCR, Porosity<br>PC 6: $^{210}Pb$, $^{234}Th$, Chl a/Pigments |

## Preliminary data

During this cruise we had mechanical problems with the horizontal and vertical sledge; this meant that we did not get so many profiles as anticipated. Furthermore the "shallow" stations were covered by many small to medium sized stones which broke many of the electrodes while measuring the first set of profiles. In total we obtained 10 and 9 in situ profiles at the abyssal stations and in the Horizon Deep, respectively. Detailed analysis of the $O_2$ microprofils data will conducted back home, but we have carried out some preliminary calculations. Not all profiles have been analysed, but it is already clear that - in accordance with our hypothesis – the sediments of Horizon deep consume more $O_2$ (i.e. higher microbial activity) as compared to sediments of the neighbouring abyssal plain. This is exemplified in Figure 2. Both profiles have constant $O_2$ levels in the overlying water phase and reaching the sediment surface (depth of -4 cm) $O_2$ levels begin to decline, but the attenuation is much faster at the Horizon deep where anoxia is reached at a relative depth of ~20 cm. At the shallower site the electrode broke at a relative depth of ~-17.5 cm, but O2 had declined much less that in the corresponding profile from the Horizon Deep. Preliminary calculations on the profiles indicate that the $O_2$ consumption at the Horizon deep is 6 times higher than at the abyssal station.

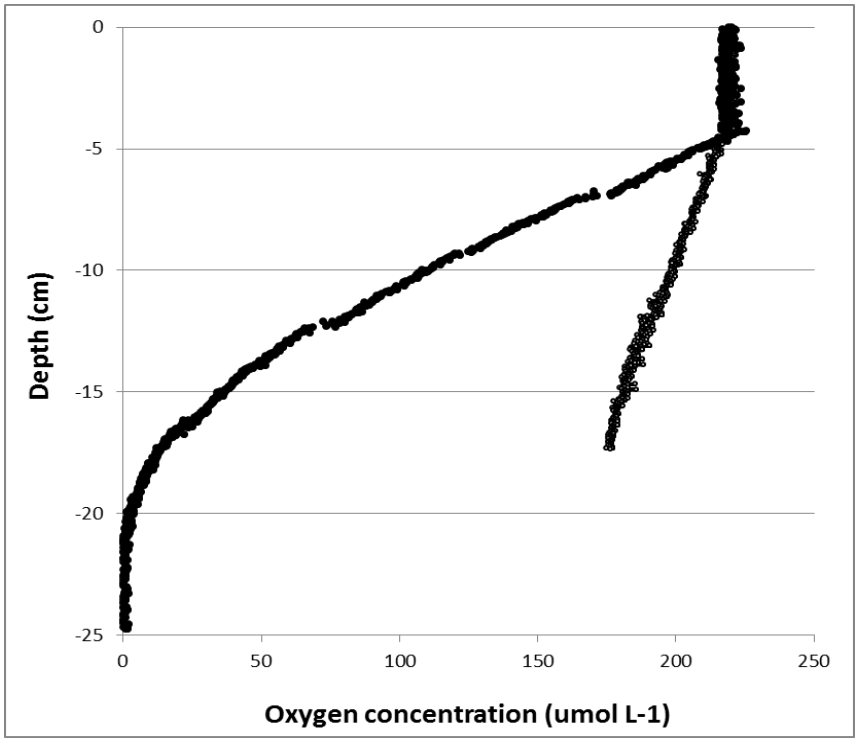


*Fig 2. Preliminary oxygen microprofiles as measured at Horizon deep (closed symbols – 10.805m) and at the abyssal plain (open symbols – 6.253m), respectively (see text for explanation).*

In total we obtained four sediment cores from "6200m" site, and 2 cores from the Horizon deep. These sediment samples await analysis in our respective laboratories. However, the preliminary results revealing the potential importance of trenches for benthic carbon preservation call for more extensive investigations of the trench system.

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## 3) Deep-sea camera system and Winkler titration

Kazumasa Oguri, Takashi Toyofuku, Ronnie N Glud, Frank Wenzhoefer and Hiroshi Kitazato

To record sediment surface and organisms and to collect sediments, deep-sea camera system was developed (figure 3-1; Murashima et al., 2009). This instrument consists of nineteen deep-sea floats and main aluminum frame mounted transponder/releaser, HDTV camera (figure 3-2), light, battery, CTD, three short core samplers and ballast weight. In this cruise, the camera system was used to collect sediments, sea floor observations, collection of organisms with baited trap, and depth, temperature and salinity measurements in water column. When the camera system was released from the ship, the CTD was started to record pressure, conductivity and temperature. The camera was started to record by the power supply from internal timer circuit. Sediments were collected in the core tubes mounted on the tripod legs during the landing (figure 3-3). The transponder released the ballast weight when receiving the release command from the ship. The position of the camera system was always monitored on board to receiving the acoustic signal from the transponder. Table 3-1 shows the setting of the camera system in each deployment. Figure 3-4 shows the landing sites.

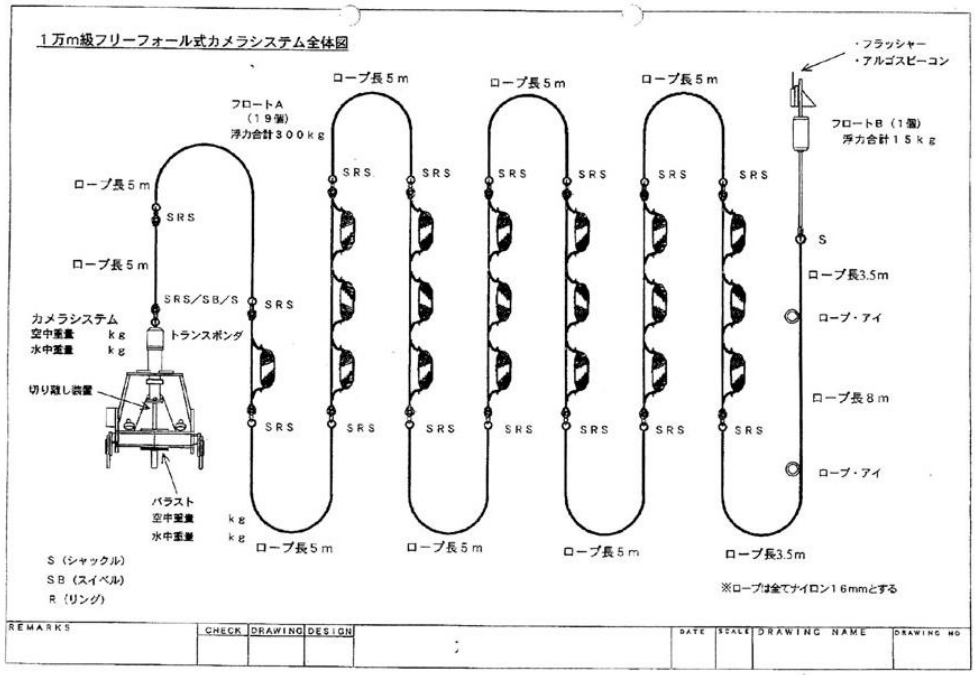
Reference

Murashima, T. Nakajoh, H. Takami, H. Yamauchi, N. Miura, A. Ishizuka, T. (2009) 11,000m class free fall mooring system. Proceedings of OCEANS 2009-EUROPE, 2009. OCEANS '09, p1-5.

Table 3-1: Setting of the deep-sea camera system in each deployment.

| | Date (UTC) | Deployed from (UTC) | Recovered at (UTC) |
|---|---|---|---|
| Deployment 2-1 | 2013/10/10 | 5:37 | 1:05 |
| Deployment 2-3 | 2013/10/12 | 2:25 | 3:31 |
| | Landed at (UTC) | Left at (UTC) | Max. CTD depth (m) |
| Deployment 2-1 | 5:37 | 8:53 | 10817 |

| | | | |
|---|---|---|---|
| Deployment 2-3 | 5:27 | 13:33 | 10811 |




※重量

| | | |
|---|---|---|
| カメラシステム本体 | 空中 283.7kg　水中 156.7kg（清水） | 153.5kg（海水：比重 1.025） |
| 投棄バラスト | 空中 214kg　水中 186.5kg（清水） | 185.9kg（海水：比重 1.025） |
| 係留系 | 空中 586.5kg 水中-257.9kg（清水） | -279.0kg（海水：比重 1.025） |

Figure 3-1: Schematic overview of deep-sea camera system.

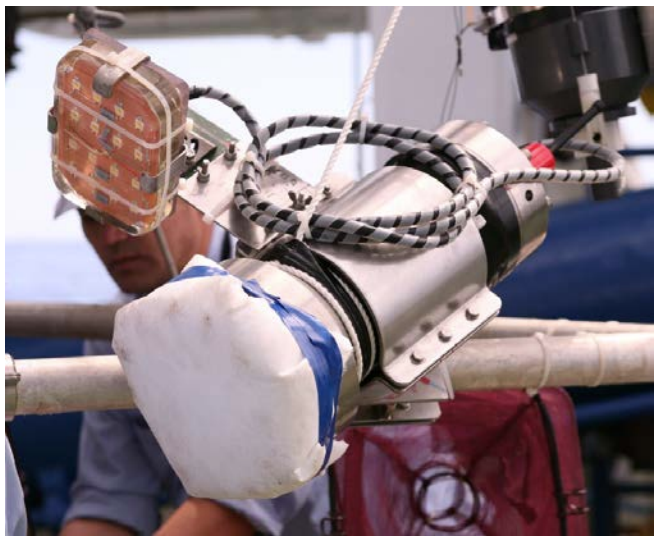


Figure 3-2: Deep-sea camera system. Figure 3-3: HDTV camera equipment used for the video recording.

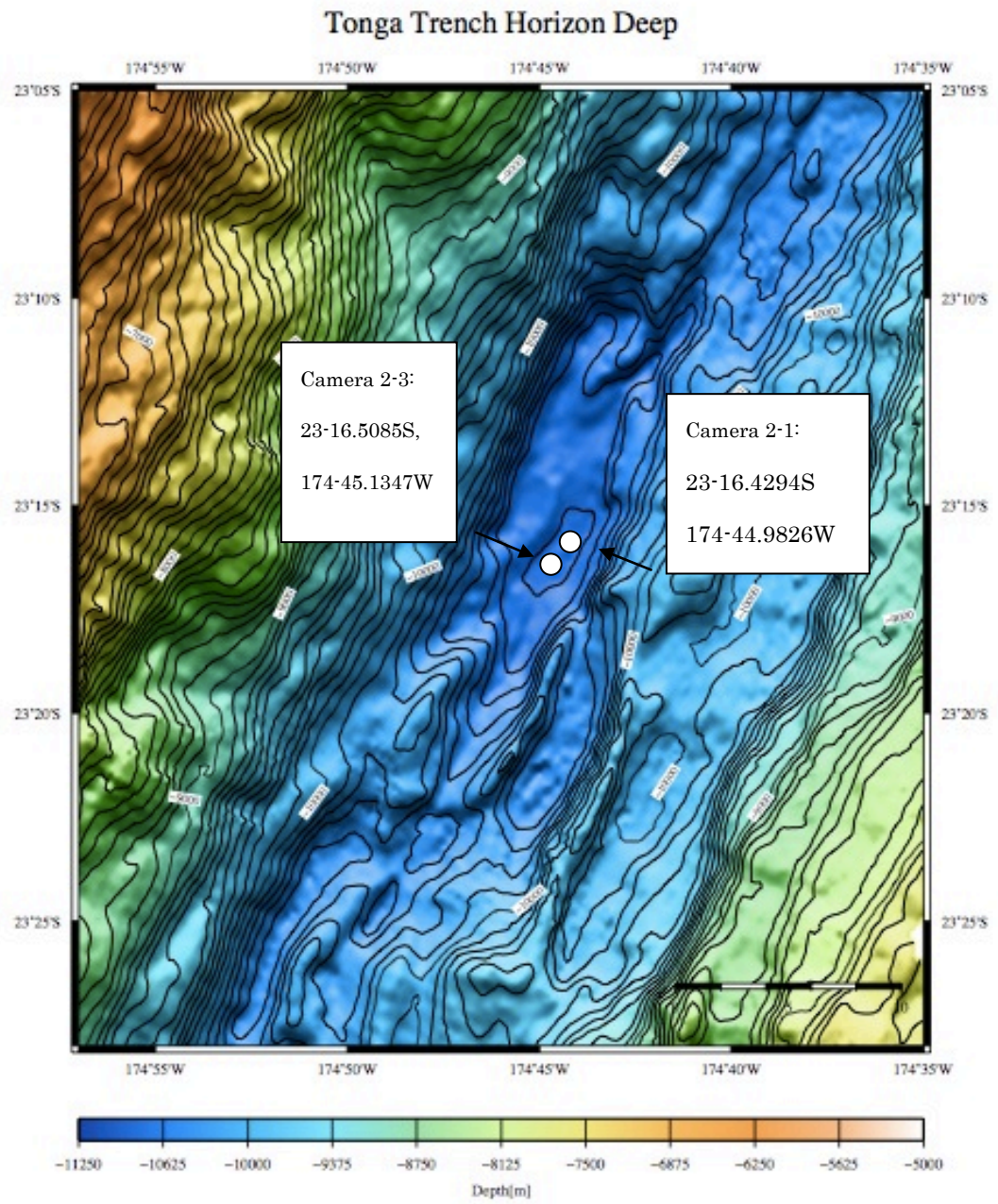



Figure 3-4: Landed points of the deep-sea camera system.

**CTD profiles**

Depth, temperature and salinity of water column were measured by SBE 49 CTD system mounted on the camera system. The CTD recorded pressure, conductivity and temperature from release to the recovery of the camera system. The depth was obtained to convert the pressure. Profiles of temperature and salinity are shown in figures 5.

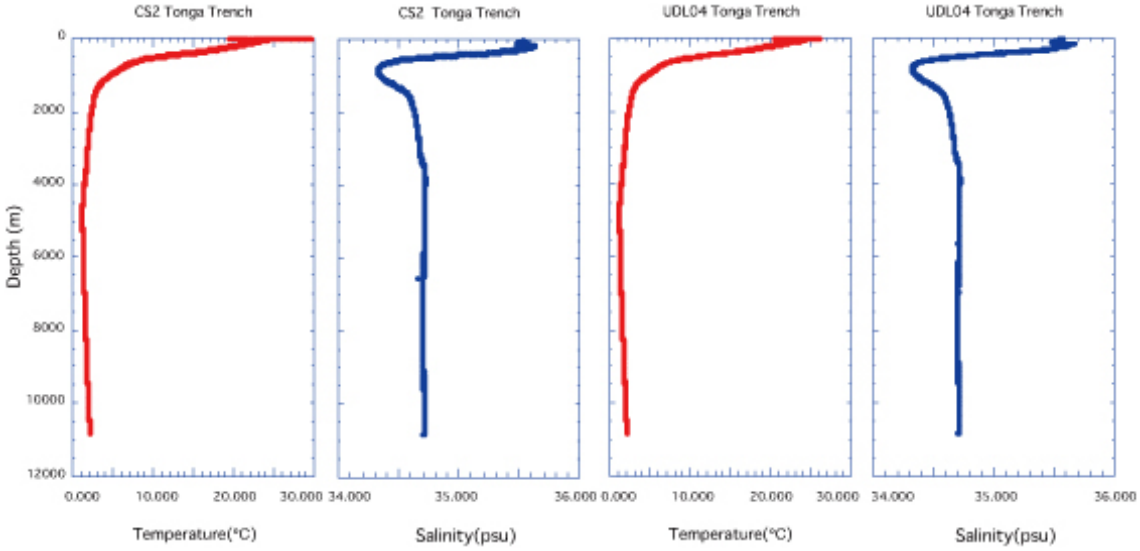



Figure 5: Profiles of temperature and salinity at Camera 2 deployment and Ultra deep lander 4.

**Oxygen concentration (Winkler titration)**

In order to know dissolved oxygen (DO) concentration of bottom water, water sampling was performed by Niskin bottle sampler attached to an ultra deep lander. As well, overlying water of sediment cores collected by camera system was used for the determination of the oxygen concentration. 100ml of water was collected from Niskin samplers or sediment core tubes to DO bottles by Tigon tubing. Oxygen in water was fixed by 1 ml of $MnCl_2$ and 1 ml of KOH-KI solutions. Then, oxygen concentration was determined by Winkler titration following the procedure described by Japan Meteorological Agency (1980). In the calculation, both $MnCl_2$ and KOH-KI solutions used for the fixation was supposed to be air-saturated. The results of oxygen concentration are shown in the Table 5-2.

Table 5-2: Results of oxygen concentration measurements by Winkler titration.

| 2013/10/11 | | | water depth |
|---|---|---|---|
| Camera-Lander 1 | | St. 2-1 | 10800 m |
| | Thiosulfate (ml) | | Oxygen (μM) |
| Bottle 20 | 0.790 | | 215.7 |
| | 0.800 | | 218.4 |
| | 0.796 | | 217.3 |
| | | | |
| Bottle 29 | 0.804 | | 219.5 |
| | 0.810 | | 221.1 |
| | 0.811 | | 221.4 |
| | | | |
| Bottle 21 | 0.808 | | 220.6 |
| | 0.817 | | 223.0 |
| | 0.810 | | 221.1 |
| | | | |
| | | Average | **219.8** |
| | | StD | 2.3 |

| 2013/10/13 | | | water depth |
|---|---|---|---|
| Camera-Lander 2 | | St. 2-3 | 10811 m |
| | Thiosulfate (ml) | | Oxygen (μM) |
| Bottle 20 | 0.827 | | 225.8 |
| | 0.826 | | 225.5 |
| | 0.824 | | 224.9 |
| | | | |
| | | | |
| Bottle 29 | 0.819 | | 223.6 |
| | 0.824 | | 224.9 |
| | 0.818 | | 223.3 |
| | | | |
| Bottle 21 | 0.831 | | 226.9 |
| | 0.822 | | 224.4 |
| | 0.821 | | 224.1 |
| | 0.827 | | 225.8 |
| | | | |
| | | Average | **224.9** |
| | | StD | 1.1 |

| 2013/10/15 | | | water depth |
|---|---|---|---|
| Shinkai6500 | | dive #1370 | 6245m |
| | Thiosulfate (ml) | | Oxygen (μM) |
| Bottle 21 | 0.810 | | 221.1 |
| | 0.795 | | 217.0 |
| | 0.798 | | 217.8 |
| | | | |
| | 0.791 | | 215.9 |
| | | | |
| Bottle 29 | 0.809 | | 220.8 |
| | 0.799 | | 218.1 |
| | 0.795 | | 217.0 |
| | 0.795 | | 217.0 |
| | | | |
| Bottle 21 | 0.796 | | 217.3 |
| | 0.805 | | 219.8 |
| | 0.796 | | 217.3 |
| | 0.800 | | 218.4 |
| | | | |
| | | Average | **218.1** |
| | | StD | 1.6 |

**Captured video**

Sediment surface and organisms were recorded by HDTV camera of the camera system. The camera was set to record sediment surface. One LED light was set to the direction for the recording images. The recording time was shown in the table 6. Typical images of the sea floor and organisms in each deployment are shown in the figure 6.

Table 6. Date and time of video recording.

| | Date (UTC) | Recorded from (UTC) | Recorded to (UTC) | Max. CTD depth (m) |
|---|---|---|---|---|
| Deployment 1 | 2013/10/10 | 9:00 | 12:00 | 10817 |
| Deployment 2 | 2013/10/12 | 12:00 | 15:00 | 10811 |

Figure 6. Video captured images recorded at 9:00 on 10/10 during the deployment 1 at 10817m depth. Amphipods swimming above sea bottom were observed.

Figure 6 (continued). Video captured images recorded at 12:00 on 10/12 during the deployment 2 at 10811m depth. Weak ripple structures were seen on the sea bottom. Amphipods and holothuria were observed.

## 4) Metazoan investigations

Ashley Rowden

Hadal amphipod assemblage and population structure

*Background and Research Objective*

Amphipods (particularly lysianassoid amphipods) are the dominant scavenging fauna at hadal depths and are known to rapidly intercept and consume carrion falls at the deepest trench depths. The variation in assemblage structure at among- and within-trench levels suggests that amphipod communities have been isolated over geological timescales and each has evolved different ecological features and different key species (see introduction of Jamieson et al. 2011). However, sampling effort remains small, and each new investigation has discovered species new to science, and our understanding of connectivity among trenches using molecular techniques is in its infancy. Which factors determine the biogeographic distribution and depth limits of hadal organisms remains an open question, but one which can be addressed by continuing to extend the sampling of the most conspicuous component of the trench fauna.

The objectives of the present research are to: (1) sample and identify scavenging amphipod species in the hadal environment of the Tonga Trench; (2) describe the scavenging amphipod assemblage structure of the Tonga Trench (including previous data from the trench – Blankenship et al. 2006) and compare with the structure of scavenging amphipod assemblages described from elsewhere in the South Pacific Ocean, in particular the Kermadec and Peru-Chile Trenches (e.g. Jamieson et al. 2011, Fujii et al. in press); and (3) examine the genetic connectivity of amphipod populations among other hadal environments in the South Pacific, in particular the Kermadec Trench (e.g. Jamieson et al. 2013).

*Methods*

Scavenging amphipods were primarily collected by means of baited traps attached to two free-fall landers deployed at two locations in the Horizon Deep, at depths of ~6200 m and ~10,800 m. On the sediment sampling-camera lander, traps were placed at the base of the lander (i.e. at the seabed) and on the frame at a height of 1.5 m above the seabed. Two traps were placed on the frame of the sediment profile lander at 1.8 m

above the seabed. No traps were placed at the base of the sediment profile lander in order to avoid the risk that amphipods attracted to the bait at the seafloor might collide with the delicate profile electrodes as they entered the sediment. The overall dimensions of these traps were 25 x 25 x 36 cm. They were covered with a 3 mm mesh and had two 6 cm diameter openings for amphipods to enter the trap (Figure 1). The traps were baited with pieces of raw fish (mainly mackerel) placed in fine mesh bags attached to one of the inside walls of the traps. Traps were able to catch scavenging amphipods attracted to the bait during the time the landers were deployed at each site; this length of time varied between lander deployments (Table 1) but was typically 6 hours.

Baited traps were also deployed at the seabed during one dive by Shinkai 6500 to the ~6200 m site. Here two of the traps described above (attached together) and one larger trap (overall dimension 45 x 21 x 55 cm; 11 mm mesh; 5 and 8 cm openings) were placed on the seafloor at the beginning of the dive and recovered immediately before the submersible returned to the surface (i.e. 3 hours, Table 1). On the same dive one amphipod was also collected using the submersible's suction sampler.

None of the traps had closure mechanisms and so amphipods could theoretically escape and enter the traps during the descent and ascent of the landers/submersible. It is considered likely, given the size and arrangement of trap openings and the wash experienced by the traps at the sea surface during recovery of the landers/submersible, that some amphipods would have been lost during sampling.

On recovery of the traps, any amphipods caught were carefully removed from the traps using forceps or gloved hands, and fixed and preserved in 100% ethanol. The number of amphipod specimens caught per trap was estimated. Positive identification of only two species was possible during the voyage. Further taxonomic identification and complete enumeration will occur at the NIWA laboratory. Also at the NIWA laboratory, tissue will be removed from some amphipods for molecular analysis to take place at the University of Aberdeen and/or Woods Hole Oceanographic Institution laboratories.

*Preliminary results*

Preliminary examination of the material taken at the ~6200 m site suggest that at least 4 species of scavenging amphipod were sampled. Most notable among these species is the supergiant amphipod *Alicella gigantea* (Figure 2). This is the first time this species

has been recorded from the Tonga Trench. Although *Alicella gigantea* has an apparently cosmopolitan distribution it has only been recorded from the South Pacific once before (in the Kermadec Trench, Jamieson et al. 2013). Of the other amphipods present in the samples taken at the shallower hadal site, species of the genus *Eurythenes* and *Scopelocheirus* were both present, and could be the same as those sampled previously from the Tonga and Kermadec Trenches (Blankenship et al. 2006, Jamieson et al. 2011).

At the deepest hadal site, the deepest ever sampled in the Tonga Trench at 10, 811 m, there appears to be only one species present, and in relatively high abundance. That is, *Hirondellea dubia* – which has been previously recorded down to 10,787 m in the Tonga Trench by Blankenship et al. (2006).

**Table 1: Sample details for scavenging amphipods**

| *Site/Dive No.* | *Depth (m)* | *Gear* | *Sample Time (hh:mm)* | *Total no. of species (estimate)* | *total no. of individuals (estimate)* |
|---|---|---|---|---|---|
| 1-1 | 6256 | Baited traps on lander | 2:46 | 4 | 90+ |
| 1-2 | 6253 | Baited traps on lander | 11:16 | 1 | 120+ |
| 2-1 | 10,817 | Baited traps on lander | 15:16 | 1 | 230+ |
| 2-2 | 10,807 | Baited traps on lander | 7:02 | 1 | 60+ |
| 2-3 | 10,807 | Baited traps on lander | 8:06 | 1 | 600+ |
| 2-4 | 10,805 | Baited traps on lander | 9:43 | 1 | 630+ |
| 1370 | 6255 | Baited traps deployed by submersible | 3:00 | 2 | 3 |

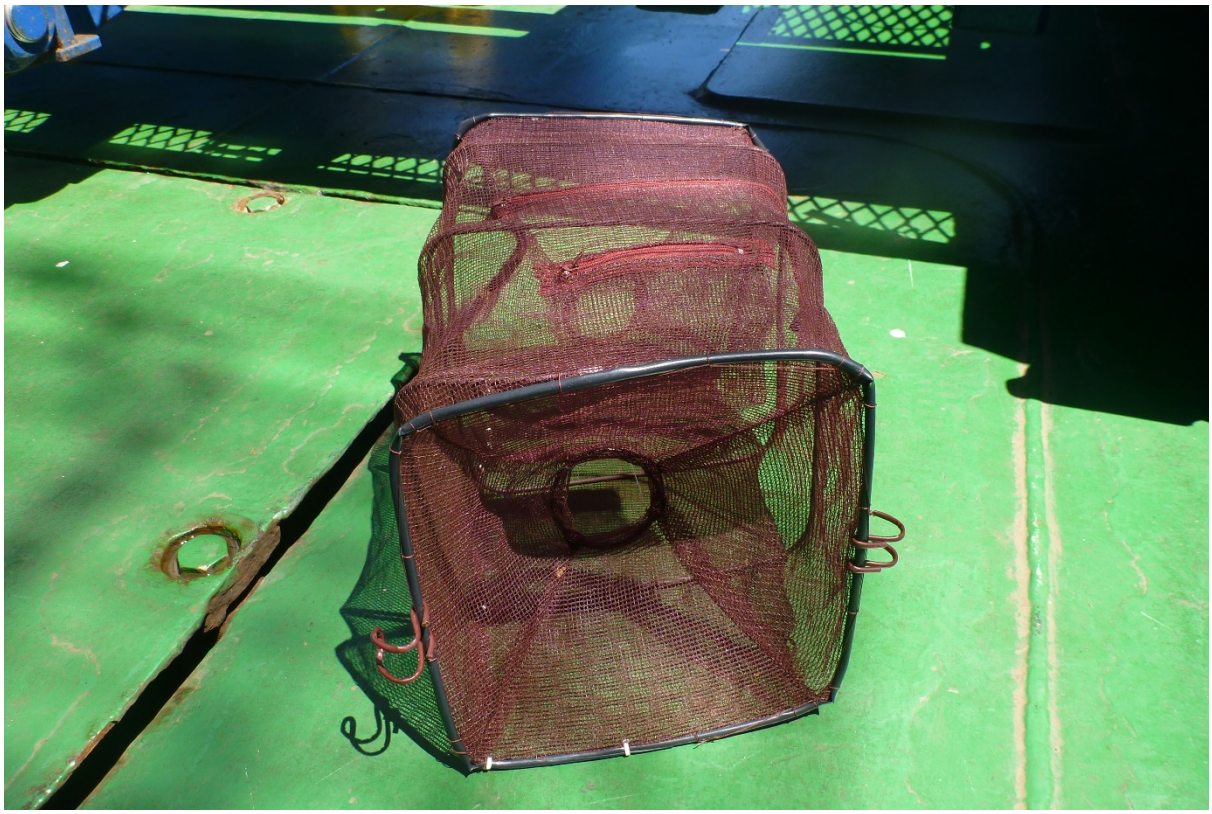


Figure 1: Trap used with bait to catch amphipods from the Tonga Trench

Figure 2: Specimen of the supergiant amphipod *Alicella gigantea* caught by baited trap in the Tonga Trench

**Hadal nematode assemblage structure**

*Background and Research Objective*

Nematodes, like other taxonomic groups, have been found to decrease in species richness and abundance with water depth, primarily it is thought in relation to decreasing food quantity and/or quality with depth. Nematode species richness and abundances have been found to be particularly low at the deepest parts of trenches, with only one notable exception to this trend being observed in the eutrophic hadal environment of the Peru-Chile Trench (see introduction in Danovaro et al. 2002). However, quantitative information on nematodes from hadal sediments is limited, and the relationship between the productivity of the surface water above trenches (or other deep-sea environments) and nematode species richness/abundance is far from certain and requires further examination (Leduc et al. 2012). Ideally, nematode assemblages from the deepest parts of trenches beneath oligotrophic regions of the South Pacific Ocean should be sampled in order to allow more direct comparisons with data from trenches beneath eutrophic areas in the same region.

The objectives of the present research are to: (1) sample and identify hadal nematode species in the Tonga Trench; (2) describe the nematode assemblage structure of the Tonga Trench and compare with the structure of nematode assemblages described from elsewhere in the South Pacific Ocean, in particular the Peru-Chile Trench (e.g. Danovaro et al. 2002) and in the future from the Kermadec Trench (as part of the HADES-K trench project).

*Methods*

Samples for nematodes were recovered from sediment cores taken by the sediment sampling-camera lander deployed at a depth of ~10,800 m, and by the submersible Shinkai 6500 at the shallower hadal site at the edge of the Horizon Deep, at ~6200 m (Table 2). Two sediment cores per lander deployment and four cores from the submersible dive were sub-sampled using a cut-off syringe (internal diameter 26 mm) to a depth of 10 cm, after the supernatant water had been siphoned off. Sediment was extruded from the sub-cores, sectioned (0-1, 1-2, 2-3, 3-4, 4-5, 5-10 cm) and the

sections fixed and preserved (10% buffered formalin) in separate containers. The sediment remaining, after sub-cores had been removed for nematodes, foraminifera and protists, was also collected and stored in formalin (0-10 cm) or frozen (>10 cm).

*Preliminary Results*

No initial processing of the sediment samples for nematodes was undertaken on the voyage because the appropriate mesh size sieve was unavailable. Thus samples will be processed and nematodes identified later at the NIWA laboratory, and no preliminary results can be recorded here.

**Table 2: Sample details for nematodes**

| *Site/Dive No.* | *Depth (m)* | *Gear* | *No. of sub-cores* |
|---|---|---|---|
| 2-1 | 10,817 | Cores on lander | 2 |
| 2-3 | 10,807 | Cores on lander | 2 |
| 1370 | 6,255 | Cores placed by submersible | 4 |

**Hadal megafauna assemblage structure**

*Background and Research Objective*

There have been very few quantitative surveys of hadal megafauna, particularly from the deepest depths of the world's trenches. Records of hadal fauna have been mostly collected by remote gears (i.e. trawls) or static landers which collect very limited information about the density of megafauna on seafloor. Surveys using towed cameras, ROVs or submersibles are better able to collect such information using video and still photography. Such data is of particular importance for understanding the relationship between habitat and faunal species richness and abundance, and for understanding hadal ecosystem function when coupled with environmental measurements of oxygen consumption and carbon mixing. No data for benthic megafauna using the likes of submersibles has yet been collected from trenches in the South West Pacific Ocean.

The objectives of the present research are to: (1) sample and identify megafauna species in the Tonga Trench; (2) describe the megafauna assemblage structure of the Tonga Trench (including previous data from the trench – e.g. Jamieson et al. 2008) and

compare with the structure of megafauna assemblages described from elsewhere in the South Pacific Ocean, in particular the Kermadec Trench (e.g. Jamieson et al. 2008, Jamieson et al. 2009, Jamieson et al. 2011; and also in comparison to future data collected as part of the HADES-K trench project).

*Methods*

Benthic megafauna were recorded on video and by still photography along transects conducted by the submersible Shinkai 6500 at sites in the Tonga Trench between 5900 and 6500 m depths. Megafauna were also recorded by video taken during the static deployments of the sediment sampling-camera lander at sites in the Horizon Deep at ~6200 m and ~10,800 m. All observation periods were ~3 hours duration (Table 3).

Only very preliminary examinations of these video and still images was made during the voyage. Detailed observation and recording of the megafauna and habitat will be undertaken later at the NIWA laboratory when submersible-based records can be directly matched to submersible position and depth, and lander-based records can be correlated with deployment time. The behaviour of the fauna recorded by the lander camera will also be examined.

*Preliminary Results*

Megafauna was generally very sparse in the Tonga Trench. The most common group of organisms observed were elasipod holothurians, and their feeding traces were also visible on the video. Also observed was at least two decapod species (possibly *Benthesicymus crenatus* and a red caridean shrimp), seapens, anemones, amphipods, and seastars. Specimens of the holothurians, anemones and a brisingid seastar were captured for identification by the suction sampler of the submersible.

**Table 3: Sample details for megafauna**

| *Site/Dive No.* | *Depth (m)* | *Gear* | *Sample Duration* |
|---|---|---|---|
| 2-3 | 10,807 | Video camera on lander | 3:00 |
| 1369 | 6498 - 6050 | Video camera and stills images of submersible | 3:03 |
| 2-4 | 10,805 | Video camera on lander | 3:00 |
| 1370 | 6253 - 6255 | Video camera and stills images of submersible | 3:00 |
| 1371 | 6465 - 5917 | Video camera and stills images of submersible | 2:09 |

**Future analyses**

In the future, analyses will be conducted in accordance with the stated objectives of each of the research themes described above. Collaboration between NIWA scientists and scientists that took part in this research cruise is expected, e.g. it will be important for analyses of the amphipod and nematode data to include information about bacteria and phaeopigment content of the sediment collected by Ronnie Glud and Frank Wenzhoefer (as their analysis of carbon mineralisation may require information about the relative abundance of amphipods and nematodes), as it is with scientists involved in past (Alan Jamieson et al, HADEEP) and future trench projects (Tim Shank et al, HADES-K).

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## 5) Protist Investigation

Akinori Yabuki and Frederic Sinniger

### Introduction

Understanding the precise diversity of eukaryotes is among the most important and fundamental biological subjects. While many microbial eukaryotes have been found and described in the past, their precise diversity seems not to be fully understood. The PCR surveys conducted with the environmental DNA revealed that many microbial eukaryotes still remain to be found in a wide range of natural environments and some of them are distinctly novel in the tree of life (e.g., Not et al. 2007, Kim et al. 2010). The diversity of deep-sea microbial eukaryotes has been occasionally analyzed and it was also shown that a number of possible novel microbial eukaryotes are living there (e.g., López-García et al. 2001; Sogin et al. 2006). However, many of them were just shown as the results of the PCR conducted with the environmental DNA and they still remain to be identified. Furthermore, such studies were performed just in several selective deep-sea environments. The studies to identify and describe deep-sea microbial eukaryotes in various deep-sea environments are expected to go on.

The Horizon deep that is located in the Tonga trench is the second deepest point in the world and the diversity of microbial eukaryotes living there has not been studied. Although it is oligotrophic environment in the Horizon deep, the existences of several multi cellular eukaryotes (e.g., sea cucumbers and amphipods) and prokaryotes have been confirmed. Hence, the microbial eukaryotes also supposed to live there and some of them are possibly novel.

### Strategy

*On R/V Yokosuka*

The sediment core samples that were collected from 10,800 m depth (on board ID: YK13-10 Camera Lander St. 2-1 Core 1 Frame 11, YK13-10 Camera Lander St. 2-1 Core 3 Frame 16) were used as the initial samples and inoculated into the flasks filled with the media. The amphipods collected by the bait trap (at 6,500 m [on board ID: YK13-10 Ultra Deep Lander St. 1-1 Bait-trap2] and 10,800 m depth [on board ID: YK13-10 Camera-cording Lander St. 2-1 Bait-trap3]) were also used as the initial

inoculation samples, since some microbial eukaryotes survive attaching crustaceans (e.g., Silverman et al. 2004; Skovgaard and Daugbjerg 2008). Five kinds of media (ESM, KLB, RDHL I, RDHL X and $CO_2$ treated RDHL X) were used for initial incubation. The inoculated samples are incubated at 4°C.

*On land (including perspective)*

The incubated samples are constantly observed and microbial eukaryotes will be isolated, when the cells of microbial eukaryotes that are possibly novel come out. The cell isolation will be performed with micro capillary and isolated cells will be moved to pure media. After the cultures are established, the experiments to understand the morphological/ultrastructural characteristics and phylogenetic position will be conducted. Based on the acquired information derived from those experiments, the taxonomic assignment on deep-sea microbial eukaryotes will be performed.

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## 6) Foraminifera in the Horizon deep of Tonga Trench

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**Introduction**

What kinds of organisms do dwell in the second deepest point of the world ocean? This is a simple question. In 2005, we published a quick report in terms of species composition of foraminifera that dwell in the Challenger Deep (Todo et al., 2005). The fauna was composed of only 13 species. Soft-shelled foraminifera occupy 99% of the fauna. The lineage of soft-shelled foraminifera are thought to be traced back to the pre-Cambrian Era (Pawlowski et al., 2003). The other hand, deep trenches are isolated "Island" by shallow depth. The unique biodiversity and ecosystem would be developed among each trench (Gooday et al, 2008a,b). Foraminiferal assemblages should be compared among trenches to make sure this hypothesis.

**Method of study**

Surface undisturbed core samples are planned to use for foraminiferal faunal assemblage research. Syringe subcores are inserted into each core. Each subcore will be sliced every 0.5 cm intervals from core top to 3 cm and every 1 cm intervals for 3 to 5 cm. Sliced sediments are fixed by 5% seawater formalin 0.5% rose Bengal solution. Just before sorting, we plan to wash fractions with 32 μm opening mesh. Then, every stained individuals will be sorted at species level under binocular stereo-microscope.

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## 7. Notice on using

This cruise report is a preliminary documentation as of the end of the cruise. It may not be corrected even if changes on content (i.e. taxonomic classification) are found after publication. It may also be changed without notice. Data on the cruise report may be raw or not processed. Please ask the Chief Scientist for the latest information before using.

Users of data or results of this cruise are requested to submit their results to Data Integration aned Analysis group (DIAG), JAMSTEC.