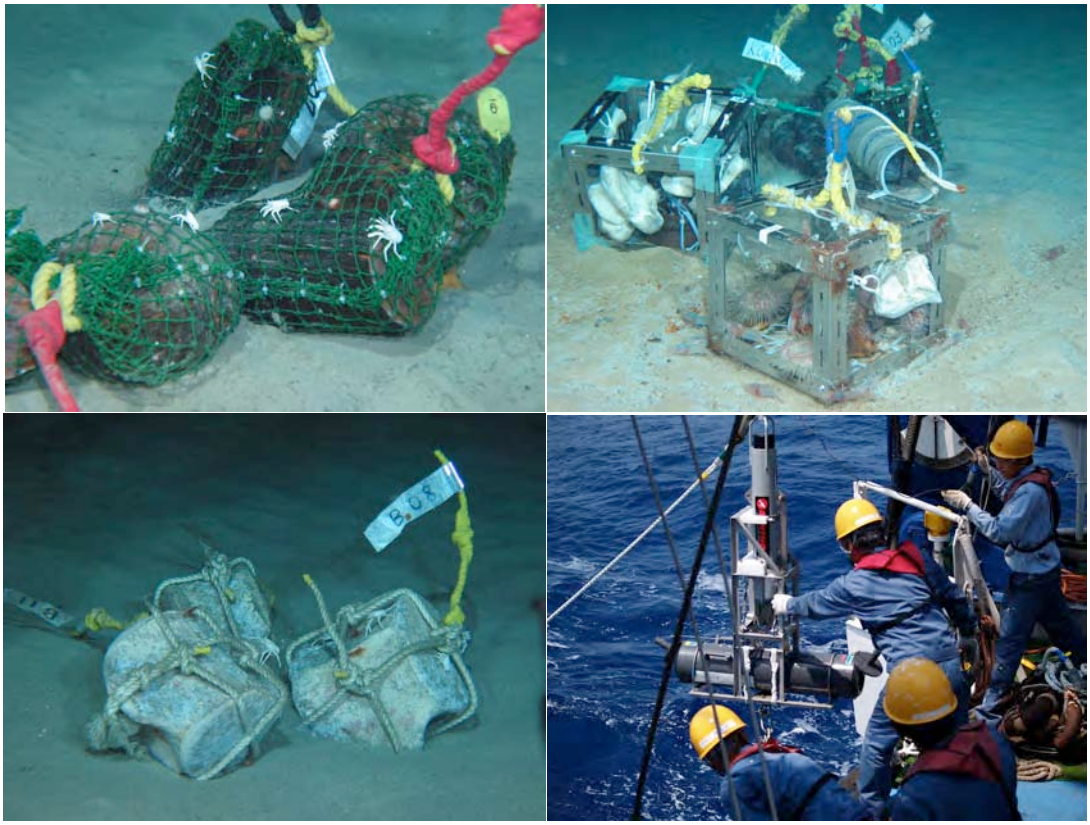
doi : 10.17596/0003341

Onboard Report

of

The Hyper-Dolphin/Natsushima Cruise in the Nansei Shoto Trench

(NT10-07 leg 1)



April 20 – May 1, 2010

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

Contents of NT10-07 cruise report

Abstract

This cruise is part of a project that began in June 2008 (cruise NT08-12). The main goal is to investigate how colonization and degradation of biogenic substrates (mammal bones and wood) on the seafloor may vary with depth. This will allow us to better evaluate substrates persistence time interval, and their role in the evolution of chemosynthesis-based fauna. Two complementary projects are conducted in parallel at the same investigation sites. In the first project (led by Y. Fujiwara, JAMSTEC), biodiversity and degradation rates are investigated on mammal bones (initially whale vertebrae, later completed with cow and pig bones) and wood logs (Keyaki) deployed at different depths. The second project (led by Y. Shirayama, Kyoto University) looks at the food-web structure and succession of the communities colonizing different species of plants.

The study area is located in the Nansei Shoto Trench, east of the Okinawa Trough in the Western Pacific. Substrates were deployed in 2008 and 2009, at 6 sites along a depth gradient ranging from 275 m depth to 5000 m depth.

During the NT10-07 leg 1 cruise, we conducted 8 Hyper-Dolphin dives during which we observed substrates at 3 sites (275 m, 500 m and 1000 m). Some of them were recovered. Sediment, water, plankton and benthos sampling as well as *in situ* chemical measurements of the sulphide release by the substrates were also conducted at each site. In addition, we deployed fresh substrates in order to renew those retrieved or lost.

The substrates collected during the cruise NT10-07 leg 1 cruise showed that degradation rates of vegetal biogenic substrates were negatively correlated with depth. Substrates at shallower sites decayed much more quickly as a result of a much faster colonization. However, variation in species composition and density was observed depending on the nature of the substrate. For bones, no such trend was identified. Indeed, shallowest bones were found intact whereas those from deeper areas were colonized.

In order to evaluate whether larval stages have the ability to migrate upwards, we deployed some substrates in the water column at 1000 meters depth. The results of this experiment will be compared with that of substrates deployed at similar depth on the bottom.

要旨

本研究の目的は異なる深度に設置した生物基質（動物の骨や木材）にどのような生物群集が形成されるかを深度間，基質間で比較することであり，2008年に実施したNT08-12航海からの継続である．本研究によって，生物基質がどのぐらいの期間海底で生物群集を維持するのかを把握するとともに，また化学合成生物群集の進化における生物基質の役割を評価できる．この航海では二つの実験が同時進行している．一つは動物の骨（鯨骨，牛骨，豚骨）を基質とする生物群集の研究であり，生物多様性や基質の分解速度の深度別比較を主体とする．もう一つは植物（丸太，ココナッツなど）を基質とする生物群集の研究であり，こちらは主に形成された生物群集の食物網や遷移を明らかにすることを目的にしている．

海域は南西諸島海溝で，2008年と2009年に水深275mから5000mの間で6つの異なる水深に基質を設置した．

NT10-07レグ1航海では3サイト（275, 500, 1000mサイト）で「ハイパードルフィン」による潜航を8回実施した．過去に設置したいくつかの基質を回収するとともに堆積物，周辺海水，プランクトンおよびベントスを採集した．また各地点で硫化物濃度などの化学計測を行った．また新たにいくつかの基質を海底設置した．回収した植物基質の分解速度は概ね深度に依存しており，浅海域ほど分解が早かった．生物群集の構成と生物密度の変動は使用した基質の特性に依存していた．一方，鯨骨には類似した傾向はなく，最も浅海に設置した鯨骨は生物による分解が少なく，ほぼ無傷に近い状態であった．

また動物遺骸に蝟集する生物の分散過程を明らかにするために，水深1000m付近に新たなコロニゼーションデバイスを設置した．

1. Cruise information

Cruise number: NT10-07 Leg1
Ship name: R/V Natsushima
Submersible: ROV Hyper-Dolphin
Title of the cruise: Colonization patterns of biogenic substrates at different depths.
Chief Scientist: Florence PRADILLON (Jamstec)
Proposal numbers and titles:
S10-86 : Influence of depth on colonization of biogenic substrates by deep-sea fauna (F. Pradillon)
S10-74: Investigation of the food web structure of deep-sea benthic community associated with sunken wood (Y. Shirayama)
Cruise period: April 20, 2010 – May 1, 2010
Port call: April 20 Departure from Naha
April 30 Arrival at Kagoshima
Research Area: Nansei Shoto Trench, Okinawa, within the area located between the following coordinates: 23°45.0'N - 125°40.0'E and 25°00.0'N - 126°30.0'E. Depth: 275m – 2997m. See Figure 1.
Cruise schedule:

04/20 Departure from Naha port
04/21 XBT
HD#1112 & HD#1113
04/22 HD#1114 & HD#1115
04/23 Dives cancelled due to weather conditions
04/24 Dives cancelled due to weather conditions
04/25 XBT
HD#1116
XBT
04/26 MBES survey in the 2000 meters depth area
Deployment of a mooring line by free-fall
Slant range to estimate the mooring position
04/27 XBT
HD#1117 & HD#1118
04/28 HD#1119
04/29 Transit
04/30 Arrival at Kagoshima port

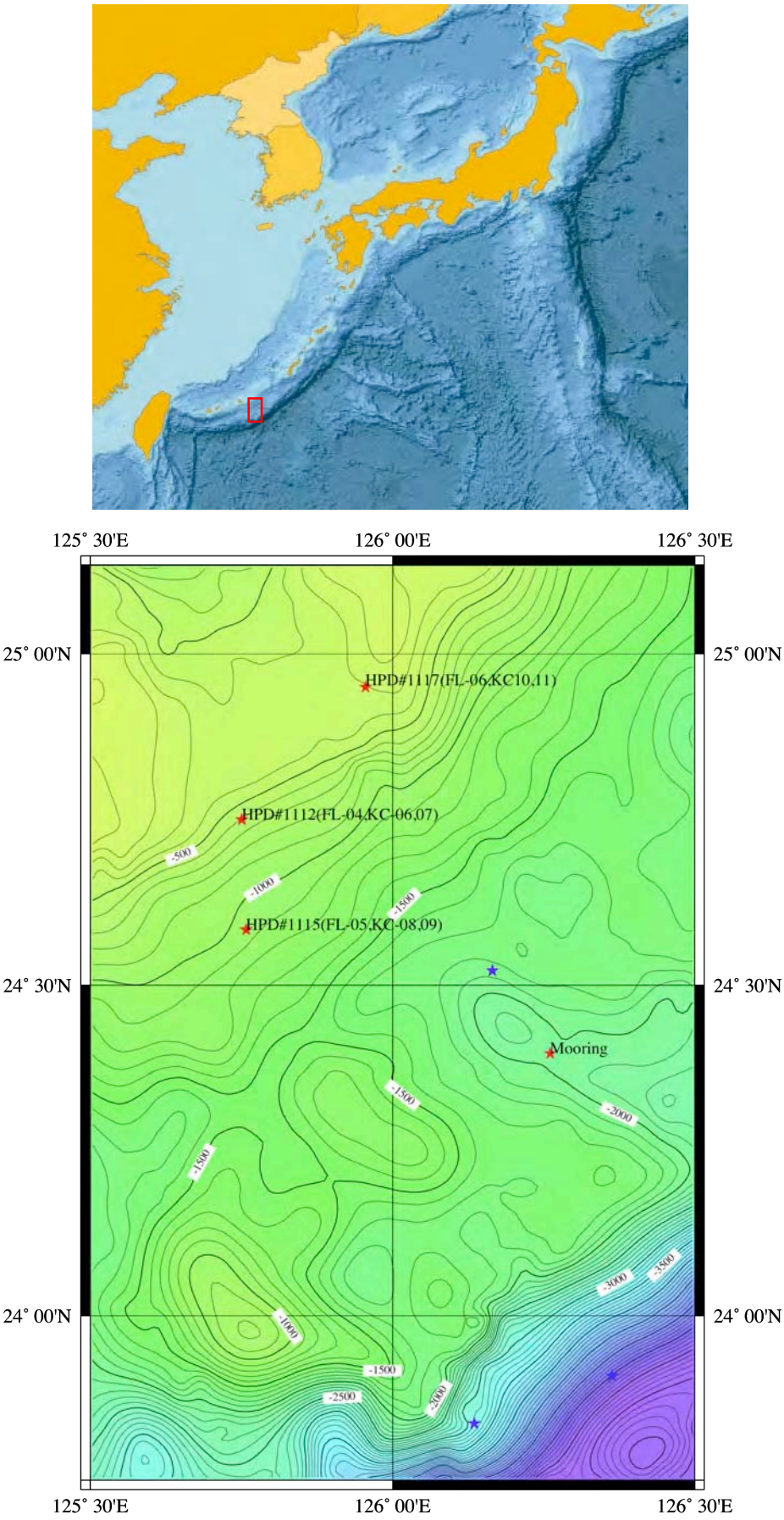


Fig.1-1. Survey area of NT10-07-leg 1. Stars indicate all deployment sites ; those in red were visited during NT10-07-leg 1.

2. Research group

Chief Scientist	PRADILLON Florence (JAMSTEC)
Vice Chief Scientist	FUJIWARA Yoshihiro (JAMSTEC)
Scientists	KAWATO Masaru (JAMSTEC)
	SHINOZAKI Ayuta (JAMSTEC)
	UMEZU Yuichi (JAMSTEC)
	MIYAZAKI Masayuki (JAMSTEC)
	YAMAMOTO Tomoko (Kagoshima University)
	KOMIYAMA Ryota (Okayama University)
	SUZUKI Masahiro (Ishinomaki Senshu University)
	GALAND Pierre (CNRS - Banyuls Observatory)
	MIMORI Ryosuke (Tokyo Sea Life Park)
	ISETO Toru (JAMSTEC)
	NEMOTO Suguru (Enoshima Aquarium)
	NISHIMOTO Atsushi (Kyoto University)
	FUJIMOTO Shinta (Kyoto University)
Marine technician	AOKI Misumi (Nippon Marine Enterprises, Ltd.)

3. Proposals

3.1. Influence of depth on colonization of biogenic substrates by deep-sea fauna

Florence PRADILLON, Yoshihiro FUJIWARA, Masaru KAWATO, Atsushi NAGAHORI, Ayuta SHINOZAKI, Yuichi UMEZU (Jamstec)

In the deep-sea, large biogenic materials sinking on the seafloor from surface waters (carcasses of marine mammals, wood or plant remains) dramatically enhance food input locally, and trigger the development of dense and specific communities. These materials are either directly consumed by metazoans, or degraded by heterotrophic bacteria that generate reduced compounds (hydrogen sulfide from sulfate reduction). In turn, reduced compounds fuel autotrophic bacteria. Many metazoans colonizing biogenic substrates exhibit associations with either heterotrophic or autotrophic bacteria. These species usually have close relatives among the species colonizing other chemosynthesis-based ecosystems such as hydrothermal vents or cold seeps. Biogenic materials may thus have been stepping stones for the colonization of deep chemosynthesis-based ecosystems.

The significance of these biogenic materials as stepping-stones for dispersal of chemosynthetic organisms depends on their abundance on the seafloor, which, in turn depends on their individual persistence time-interval. Physical parameters of the environment (temperature, pressure, oxygen) and surrounding communities may both affect colonization processes and rates of degradation of these organic materials, and the evolution of the communities developing on them.

Usually, organisms colonizing biogenic materials are specialists that require such substrates to complete their life-cycle. They are often similar at high taxonomic level, but

distinct species have been recorded at different depths or in different geographic location. These differences probably arise from a complex interplay between dispersal capabilities, larval physiological tolerance, as well as species-species interactions after recruitment, including interactions with non-specialist species from the background.

In this project, we aim at investigating the effect of depth on colonization processes and degradation rates of biogenic substrates, in order to better understand how chemosynthesis-based communities may colonize new substrates, persist over time, and spread towards new habitat. The proposed study is part of a larger project that began in June 2008 with the deployment of various biogenic substrates (some of them shown within Fig. 3-1-1, others being deployed by other teams at the same sites) at different depths during NT08-12, and again later during NT09-10 (Table 3-1-1).

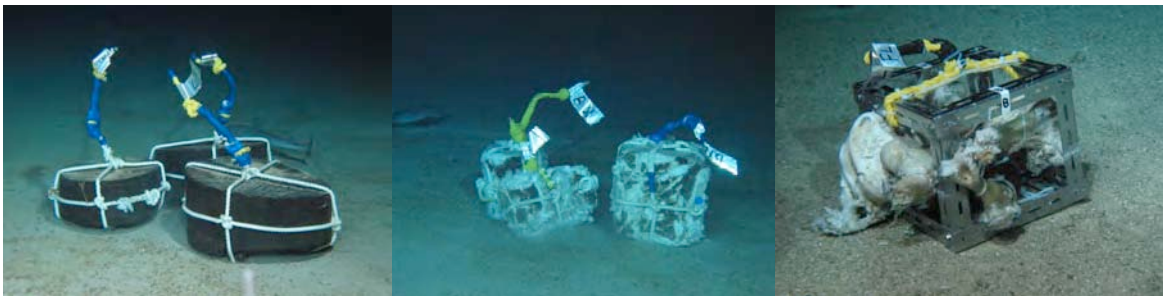


Fig. 3-1-1. From left to right : Keyaki wood logs, whale vertebrae, stainless steal frame with cow and pig bones.

Depth	Latitude	Longitude	Deployment Cruise
275m	24°57.187′N	125°57.288′E	NT08-12 & NT09-10
495m	24°44.995′N	125°45.985′E	NT08-12
510m	24°45.003′N	125°45.016′E	NT09-10 (free fall)
1001m	24°35.008′N	125°45.509′E	NT08-12
1988m	24°31.297′N	126°09.912′E	NT08-12
2997m	23°50.142′N	126°08.084′E	NT08-12
4970m	23°54.522′N	126°21.75′E	NT08-12 (free fall)

Table 3-1-1. Deployment sites of biogenic substrates.

One of the best-studied biogenic substrate around Japan is the carcasses of large whales. Several symbiotic species colonize whale bones and may significantly contribute to their degradation. *Osedax* species (Rouse et al 2004) are polychaete worms that harbor heterotrophic bacterial symbionts in a root organ. With this root, they dig into the bones and digest lipids and collagens. The mytilid mussels *Adipicola* harbor chemoautotrophic bacteria that use the hydrogen sulfide produced by the bacterial degradation of the bones.

Osedax has been found on every freshly sunken whale carcasses studied so far. Seventeen species of *Osedax* have been observed world-wide, and additional new ones were identified around Japan (Fig. 3-1-2). Their distribution suggests that depth, rather than geographic distance is a barrier to dispersal and colonization of new carcasses. The number of *Osedax* species potentially able to colonize and degrade bones is very likely to vary with depth. This might be of importance to understand how bone substrates may persist differently over time on the bottom, and ultimately how chemosynthesis-based ecosystem species may disperse, persist and evolve.

Fig. 3-1-2. *Osedax* "yellow palps" from Sagami Bay, 925 m depth.

Laboratory studies on *Osedax* larvae suggest that they mainly disperse over short distances near the sea floor. However, it cannot be excluded that *Osedax* might have a dual reproductive strategy and may be able to produce long dispersing larvae when local conditions become unsuitable. During this cruise, we will use biogenic substrates, and more specifically bones deployed at different depth to study the depth distribution of *Osedax* species, and to test the vertical migration potential of their larval stages.

More specifically, our objectives are:

(1) **Retrieve some of the biogenic substrates already deployed** in 2008 and 2009 in order to continue the time series of substrate observation.

(2) **Deploy an array of new substrates** (frames supporting cow and pig bones) at the same depths where the other substrates are already immersed. With these new deployments we aim at specifically investigating the depth distribution range of *Osedax* species.

(3) **Deploy substrates in the water column** using a mooring line in order to investigate vertical migration potential of larval stages.

(4) **Carry out *in situ* chemical measurements** of the hydrogen sulfide enrichment at the surface of the substrates, in order to characterize its relation with symbiotic species (conducted by Nadine Le Bris & Pierre Galand, Banyuls Oceanological Observatory, France).

3-2. Investigation of the food web structure of deep-sea benthic community associated with sunken-wood.

Yoshihisa SHIRAYAMA, Atsushi NISHIMOTO (Kyoto University)

What's sunken wood community?

Previous studies

High biomass assemblages were reported around organic falls on the deep-sea floor, which were associated with not only whale carcasses but also plants. Sunken woods had been known to harbor biological assemblages with high biodiversity. In 1970's,

several studies had been done mainly for the description of new species and community composition. However, such studies tended to decrease after 1970's. After Distel (2000) showed the evolutionary stepping stone hypothesis about Mytilidae, sunken wood communities attracted attention again from the point of view of chemosynthesis.

Differences between whale- and wood-fall

There are two different food sources around organic falls. One is the substrate itself and the other is the chemosynthetic bacteria originating under anoxic decomposition. Food sources in each organic falls are different because of their contents. The whale carcasses are mainly composed with protein and lipid, whereas woods are made of cellulose and lignin. Thus, the former is a more suitable food source for most animals than the latter. The chemosynthetic condition is also distinctly different between the two. The concentration of hydrogen sulfide around the former is much higher than that around the latter, and well-developed chemosynthetic ecosystems were reported from whale bones.

The necessity of the study regarding the animal community associated with sunken wood.

The concentration of hydrogen sulfide around sunken wood is lower than that around whale carcasses, but sunken wood probably maintain a stable hydrogen sulfide condition. So sunken woods may contribute as evolutionary stepping stone at earlier stage than whale carcasses. Although some organisms such as *Adipicola* sp. attract attention, studies of sunken wood, especially at community level, are very few. At first we need to accumulate the basic information about sunken wood community as fast as possible. Then, in this study, we try to understand the organization and succession of the community.

Problems and solution.

It is very difficult to understand the sunken wood community using natural samples because we cannot get the background information of samples, such as wood species, duration time after reaching to the sea floor and the water depth where each animal invaded. To overcome these problems, deployment experiments using such wood where its background is known are needed.

3-3. Related projects

Some additional projects related to the main objectives of the proposals were addressed during this cruise. They are the following:

- Succession of whale-fall community (Tomoko YAMAMOTO, Kagoshima University)
- Taxonomy and enzymatic study of microorganisms from organic substrates (Masayuki MIYAZAKI, Jamstec)
- Diversity of marine microbial communities on large organic falls (Pierre GALAND,

CNRS-Observatoire Océanologique de Banyuls-sur-Mer, France)

- Geochemical and isotopic analysis to understand whale fall ecosystem food-webs and functioning (Ryota KOMIYAMA, Shou SHIMAMURA, Toshiro YAMANAKA, Okayama University)

- Development, growth and survival of mussels attached to bones and sunken woods (Kenji OKOSHI, Masahiro SUZUKI, Ryohei YAMAGUCHI, Ishinomaki Senshu University).

- Colonization processes in chemosynthetic ecosystems (Sylvie GAUDRON, University Paris VI)

- Survey on the deep-sea meiofauna with emphasis on tardigrades (Shinta FUJIMOTO, Kyoto University)

4. Results

4-1. Hyper-Dolphin dives

Dive list

Dive# date	Main purpose of the dive	Site	Positioning system	Landing(JST) Left bottom(JST)	Latitude(N)	Longitude(E)	depth (m)
1112	Recover biogenic substrates deployed in 2009 at 502 m. Deploy new substrates.	Nansei Shoto Trench	WGS-84	8:45	24°45.005'N	125°45.039'E	492
4/21/10				12:02	24°44.999'N	125°45.996'E	499
1113	Recover biogenic substrates deployed in 2008 at 498 m.	Nansei Shoto Trench	WGS-84	14:10	24°44.993'N	125°45.001'E	494
4/21/10				15:54	24°44.990'N	125°44.965'E	498
1114	Recover biogenic substrates deployed in 2008 at 498 m.	Nansei Shoto Trench	WGS-84	8:49	24°45.008'N	125°45.004'E	499
4/22/10				9:57	24°45.001'N	125°45.044'E	501
1115	Recover biogenic substrates deployed in 2008 at 1004 m. Deploy new substrates.	Nansei Shoto Trench	WGS-84	14:03	24°35.011'N	125°45.525'E	1000
4/22/10				15:46	24°35.019'N	125°45.495'E	1004
1116	Recover biogenic substrates deployed in 2008 at 1004 m.	Nansei Shoto Trench	WGS-84	14:32	24°35.048'N	125°45.488'E	997
4/25/10				15:58	24°35.011'N	125°45.500'E	1004
1117	Recover biogenic substrates deployed in 2008 & 2009 at 278 m. Deploy new substrates.	Nansei Shoto Trench	WGS-84	8:30	24°57.164'N	125°57.295'E	274
4/27/10				9:25	24°57.188'N	125°57.293'E	276
1118	Recover biogenic substrates deployed in 2008 & 2009 at 278 m.	Nansei Shoto Trench	WGS-84	11:06	24°57.151'N	125°57.303'E	274
4/27/10				12:20	24°57.188'N	125°57.293'E	277
1119	Recover biogenic substrates deployed in 2009 at 502 m.	Nansei Shoto Trench	WGS-84	8:22	24°44.978'N	125°45.046'E	495
4/28/10				8:33	24°45.003'N	125°45.044'E	500

Preliminary Results of the ROV Hyper Dolphin Dive #1112

Date: April 21, 2010

Site: Nansei Shoto Trench (500m free fall deployment site 2009 NT09-10)

Landing*:* Time: 08:45, Lat: 24˚45.005'N, Long: 125˚45.039'E, Depth: 492 m (WGS84)

Leaving*:* Time: 12:02, Lat: 24˚45.003'N, Long: 125˚45.046'E, Depth: 499 m (WGS84)

Chief observer: Florence PRADILLON (JAMSTEC)

Purpose: Search and recovery of bones and cedar wood pieces deployed by free fall, deployment of new substrates, and *in situ* chemical measurement using SPOT.

Dive summary: After we found the 2009 free fall deployment site, we collected a frame with cow bones (FL03) and a cedar wood piece (KC03). Whale vertebrae were not found, probably removed by sharks despite being heavily weighted. Before collecting the substrates we conducted in situ H_2S and pH measurements at their surface. We collected sediment cores, benthic fauna, plankton and water. We also deployed 1 new frame with cow and pig bones (FL04) and 2 new cedar wood pieces (KC 06, 07).

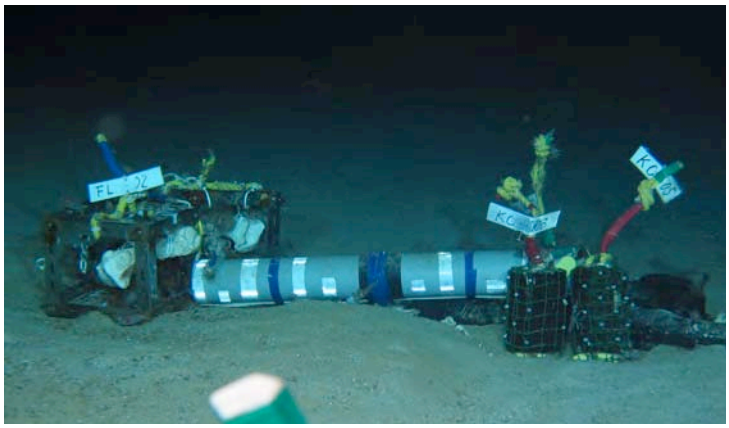


Fig. 4-1-1. Free fall deployment of cow bones within frames and cedar wood pieces on the seafloor at 500 m depth.

Preliminary Results of the ROV Hyper Dolphin Dive #1113

Date: April 21, 2010

Site: Nansei Shoto Trench (500 m deployment 2008, NT08-12)

Landing*:* Time: 14:10, Lat: 24˚44.993'N, Long: 125˚45.001'E, Depth: 494 m (WGS84)

Leaving*:* Time: 15:54, Lat: 24˚44.990'N, Long: 125˚44.965'E, Depth: 498 m (WGS84)

Chief observer: Yoshihiro FUJIWARA (JAMSTEC)

Purpose: Retrieval of CHEMECOLIs and chemical measurement using SPOT.

Dive summary: Chemical measurement was conducted around three CHEMECOLIs (C10, 11, 12) that were deployed during NT08-12 at a depth of 500 meters. All three CHEMECOLIs were retrieved separately in three sample boxes.

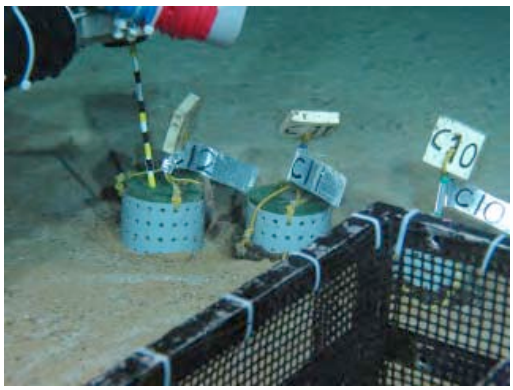


Fig. 4-1-2. Chemical measurement using SPOT on CHEMECOLIs.

Preliminary Results of the ROV Hyper Dolphin Dive #1114

Date: April 22, 2010

Site: Nansei Shoto Trench (500 m deployment 2008, NT08-12)

Landing*:* Time: 08:49, Lat: 24˚45.008'N, Long: 125˚45.004'E, Depth: 499 m

Leaving*:* Time: 09:57, Lat: 24˚45.001'N, Long: 125˚45.044'E, Depth: 501m

Chief observer: Tomoko YAMAMOTO (Kagoshima University)

Purpose: Recovery of biogenic substrates at a depth of 495 m.

Dive summary: One terrestrial plant (TP04), one wood log (L03) and a ROV-Homer were recovered at the depth of 495 m in the Nansei Shoto Trench. Sediment was sampled using MBARI cores and sterile cores. Water was also sampled. The frame with bones deployed at 510 m depth yesterday was found to be fine.

Fig. 4-1-3. TP and Wood Log deployed during NT08-12 were recovered, seen here two years after deployment, at 495m.

Preliminary Results of the ROV Hyper Dolphin Dive #1115

Date: 22, April 2010

Site: Nansei Shoto Trench

Landing Time: 14:03, Lat: 24°35.011N, Long: 125°45.525E, Depth: 1,000m

Leaving Time: 15:46, Lat: 24°35.019N, Long: 125°45.495E, Depth: 1,004m

Chief observer: Atsushi NISHIMOTO (Kyoto university)

Purpose: Recovery of biogenic substrates deployed for 2 years at 1000 m in the Nansei Shoto Trench, and deployment of new substrates.

Dive summary: We did find all deployments including whale bones at the 1,000m site although they were lost at the two shallower depths in this experimental system. One whale vertebrae (B13) with Osedax was recovered. The 3 sets of TP looked relatively fresh although bacterial mats were observed on the cross section of the oak and at the surface of coconuts. Only some specimens of Galatheidae gen. and and Echinoidea were obsevred. We recovered TP08. 2 Cedar pieces were deployed (KC08, 09).

Fig. 4-1-4. Left: whale vertebrae. Right: TP.

Preliminary Results of the ROV Hyper Dolphin Dive #1116

Date: April 25, 2010

Site: Nansei Shoto Trench (1000m site)

Landing*:* Time: 14:32, Lat: 24˚35.048'N, Long: 125˚45.488'E, Depth: 997 m (WGS84)

Leaving*:* Time: 15:58, Lat: 24˚35.011'N, Long: 125˚45.500'E, Depth: 1004 m (WGS84)

Chief observer: Masayuki MIYAZAKI (JAMSTEC)

Purpose: Recovery of a wood log at a depth of 1004 m.

Dive summary: Recovery of wood log (L04) that was deployed during NT08-12 at a depth of 1000 meters, in the square gray sampling box. In addition, sediment was collected from the place of the whale bone recovered at dive #1115.

Fig. 4-1-5. Plankton sampling using suction sampler on the wood log (L-04).

Preliminary Results of the ROV Hyper Dolphin Dive #1117

Date: April 27, 2010

Site: Nansei Shoto Trench (275m site)

Landing*:* Time: 08:30, Lat: 24˚57.187'N, Long: 125˚57.288'E, Depth: 276 m (WGS84)

Leaving*:* Time: 09:26, Lat: 24˚57.187'N, Long: 125˚57.288'E, Depth: 276 m (WGS84)

Chief observer: Pierre GALAND (CNRS)

Purpose: Deploy new frame and recover frame, wood log, TP and cedar. Do chemical measurement using SPOT. Take core samples from sediments below retrieval sites of log and TP. Collect water, plankton over log and bone frame. Collect animals if time allows.

Dive summary: Short dive due to the arrival of a fishing boat on the site. SPOT was cancelled due to lack of time. Frame was deployed, Log recovered in a bag but stayed on sea floor, 1 frame recovered in the large sample box (FL01), 1 TP recovered in the medium sample box (TP02). Plankton sampling on Log and Frame. Sterile core and Mbari Core taken from below log but very little sediment in Mbari because of sandy bottom. 2 cedars (KC10, 11) and one frame (FL06) were deployed.

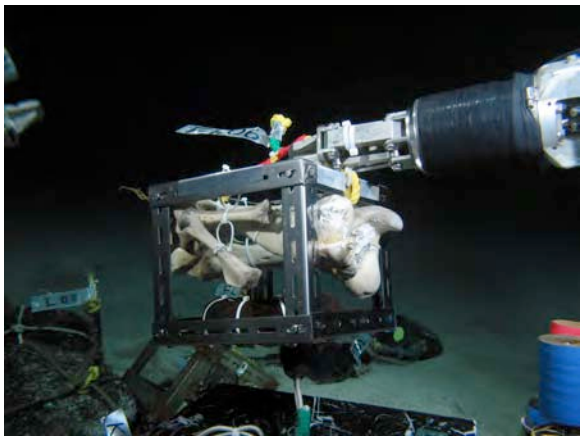


Fig. 4-1-6. Deployment of Frame FL06.

<u>Preliminary Results of the ROV Hyper Dolphin Dive #1118</u>

Date: April 27, 2010

Site: Nansei Shoto Trench (275m site)

Landing*:* Time: 11:06, Lat: 24˚57.187'N, Long: 125˚57.288'E, Depth: 276 m (WGS84)

Leaving*:* Time: 12:20, Lat: 24˚57.187'N, Long: 125˚57.288'E, Depth: 276 m (WGS84)

Chief observer: Pierre GALAND (CNRS)

Purpose: To complete Dive #1117 that was terminated early. Recover Log01 and KC01. Take core bellow the TP recovery site. Take pictures of the site and substrates.

Dive summary: Log L01 and KC01 were recovered in boxes. Sterile core and Mbari Core were taken from below the TP site but sandy bottom gave mixed cores. Captures of small shrimps, observation around a small "reef", pumping along the reef and recovery of reef stone.

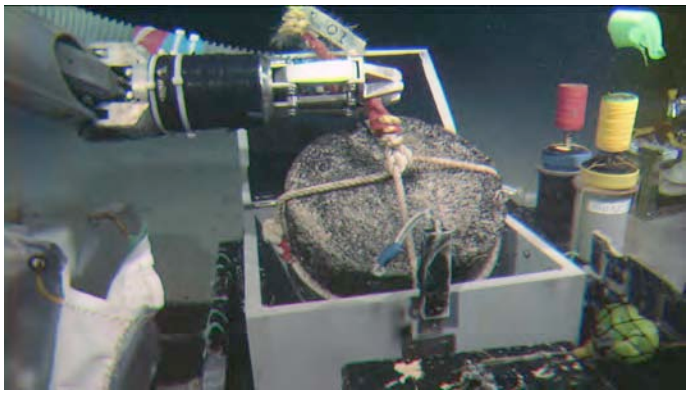


Fig. 4-1-7. Log 01 lifted out from the bag and in the grey box.

Preliminary Results of the ROV Hyper Dolphin Dive #1119

Date: April 28, 2010

Site: Nansei Shoto Trench (500m free fall deployment site 2009 NT09-10)

Landing*:* Time: 8:22, Lat: 24˚44.978'N, Long: 125˚45.046'E, Depth: 495 m (WGS84)

Leaving*:* Time: 8:33, Lat: 24˚45.003'N, Long: 125˚45.044'E, Depth: 500 m (WGS84)

Chief observer: Suguru NEMOTO (Enoshima)

Purpose: Recovery of a frame with bones deployed 9 months earlier.

Dive summary: Due to weather conditions, we spent only 10 minutes on the bottom to collect water (Niskin bottles), recover a frame with bones (FL02), and sample benthos.

4-2. Mooring deployment

In order to assess whether larvae have the potential to migrate vertically from the bottom, we deployed bones in the water column using a newly designed mooring system equipped with a "baited trap".

A SeaBat survey was conducted prior to the deployment of the mooring in order to identify the deployment area. The chosen area was sufficiently flat and wide to ensure that the mooring would land at the desired depth after free fall deployment from the R/V Natsushima, despite drift. The mooring system was deployed on April 26th and the trap was moored at about 1070 m depth.

4-3. Onboard Results

4-3-1. Influence of depth on colonization of biogenic substrates by deep-sea fauna

Substrates were collected and deployed from the three shallowest sites (4 shallowest deployment sites in Table 2-1). Due to weather constraints, deeper sites at 2000m and 3000m were not visited during this cruise.

Bones fauna

Whale vertebrae deployed in 2008 at 275 and 500 meters depth disappeared, whereas all other substrates remained at their position. In 2009 (NT09-10), a new set of 3 vertebrae was deployed by free-fall at 500 meters depth along with other substrates (cow and pig bones mounted in stainless steal frames, cedar wood pieces). All these substrates were strongly attached to a 50 kg sinker. When we found the deployment during this cruise, all substrates were still there, except the 3 vertebrae. The rope used to attach them had been cut off. The loss at 275 and 500 was attributed to sharks that we observed arriving shortly after the deployment of fresh substrates.

At 1000 meters depth, which was visited for the first time since the initial deployment in 2008, however, we found the whale vertebrae. One of them was retrieved. Large *Osedax* specimens were observed both *in situ*, and later onboard. *Osedax* were mostly growing on the two epiphysal discs still attached on both sides of the vertebra.

On board, the vertebra was kept within a tank with chilled (3ºC) filtered seawater with low oxygen concentration. 3 morphological types of *Osedax* were observed: a large type similar to *O. frankpressi*, and 2 other types, smaller and without pinnules on their palps. Genetic barcoding analysis will be conducted on land.

Other species were found but they were not abundant: a few amphipods, capitellid polychaetes and some small anemone.

In 2009 we deployed cow and pig bones (femurs) mounted on stainless steal frames. These colonization frames were more specifically intended to test Osedax colonization at different depth. We also wanted to test whether framed bones (thus less accessible)

would be less attractive to sharks. During this cruise, we could find the cow and pig bones deployed in 2009.

At 275 m depth, the bones remained uncolonized and were mostly intact at recovery. On the contrary, the bones deployed at 495 m were heavily degraded, and colonized by Osedax sp. and numerous small mytillid bivalves. Barcoding analysis will be used to identify these species.

New frames with cow and pig bones were redeployed during the cruise at 275m, 500m, and 1000m depth.

Sunken-wood fauna

One wood log (keyaki) was collected from each of three sites at depths of 275, 500 and 1,000 m, where they were deployed in 2008. The wood log collected at the depth of 275 m was markedly decomposed. The log was tender and easy to break even by hand. Many dead shells of wood-boring bivalves and mytilid mussels were found in the log, which were dominant species in a log collected in 2009. Live specimens of a smaller wood-boring bivalve were localized primarily in surface areas of the log including bark. Sipunculans were most dominant and were found throughout the whole area.

Biodiversity was highest on the wood log collected at the depth of 500 m. Wood eaters (mainly wood-boring bivalve) and symbiont-harboring mussels (representative of chemoautotrophy-dependent species) were both dominant, which is similar to that from the log collected at the depth of 275 m in 2009. Several species of gastropods were found on the surfaces.

The log collected at the depth of 1000 m was relatively fresh and wood-boring bivalves were dominant but located only in the upper layer of the log. No invertebrates harboring chemoautotrophic symbionts were collected.

4-3-2. Investigation of the food web structure of deep-sea benthic community associated with sunken-wood.

In this cruise, we recovered TP (a set of oak and coconut) from 275m, 500m and 1000m sites, and cedar from 275m and 500m sites.

We newly deployed a couple of cedars at 275m, 500m and 1,000m sites.

We did not do any sorting where material was broken into pieces on the ship because we will do CT scans to measure the invasion rate by wood boring bivalves into these plant materials. We consider it as one of the important index for describing the succession of sunken wood. So here, we will describe only the outside appearance.

Oak

At 275m, the color of cross section changed from brown to black, which indicated some degradation by bacteria. The siphon of wood boring bivalves emerging from cracks in the wood was observed.

At 500 m, we also observed that the color of cross section changed to black and invasion by wood boring bivalves appeared much more intense.

At 1000 m, onboard observation of the surface of the oak did not reveal the presence of wood boring bivalves in this sample. Further investigation of the inside of this sample will be conducted at our laboratory. Heterotrophic bacteria formed mat and covered large area of cross section, but the color of cross section was brown, which was completely different from other two samples, and it looked very fresh.

Cedar

At 275m, many wood boring bivalves invaded the cedar, and there was some area where was intensively invaded by them and their fecal pellets were arrested around there. They bore into this sample not only from sap wood but also from heart wood. We need to investigate also about inside by CT scan.

At 500m, many wood boring bivalves invaded the cedar. Some tunnels were empty, their inhabitants being already dead probably. In addition to those bivalves, we observed another wood borer -found onto no other samples during this cruise-, *Limnoria* sp., which was typical group in shallow water. Because of the invasion by *Limnoria* sp., there was complex room in this sample and many animals, such as *Idas* sp. and Echinoidea, used those tunnels for their shelter.

Coconuts

At 275 m depth, we observed that few Teredinidae bored into mesocarp part of coconuts, and also from this sample, we found few marks which indicated the existence of the remains of *Xylophaga* sp.. The part of mesocarp seems not to be suitable substrate for them. The endocarp was not exposed and probably no animals invaded inside of coconuts.

At 500 m depth, we could not find any opening of wood boring bivalves from this sample. Exocarp was worn down probably due to the floor current and the surface of this sample became nappy. Meso- and endo-carp were cracked and bacterial mats formed around the exit of split because of the oozed coconuts water, but most of coconuts water was still kept inside of endocarp.

At 1000 m depth, calyx was still attached to the fruit and this coconut was kept relatively fresh. Although bacterial mat covered surface of this sample, we could not find any opening of wood boring bivalves and crack. There must be no invasion into endocarp because there was no crack in this sample.

4-4. Future studies

4-4-1. Influence of depth on colonization of biogenic substrates by deep-sea fauna

The substrates recovered during this cruise were either directly dissected on board, or kept with their live communities within tanks. Future studies will include the

identification of the colonists using morphological and molecular techniques. We expect that a number of these colonists will be new to science since this is the first attempt to sample fauna that feed on biogenic substrates at these depths and in this region.

We will also investigate symbiotic relationships with bacteria in the case of chemosynthesis-dependent species that host bacterial symbionts. Phylogenetic relationships with closely related species will also be investigated, and biogeographic trends will be analysed.

This project started 2 years ago and we planed to retrieve these substrates at different time intervals, in order to analyze and compare their degradation rate over time and according to depth, as well as the changes in the communities associated to these substrates. During this cruise, in addition to retrieve some of the substrates deployed on the seafloor for 1 or 2 years, we renewed our bone deployments with 3 new frames installed on the seafloor at 275 m, 500 m, and 1000 m. Additional bones were deployed above the seafloor. In the next years, we will continue the sequential recovery of all these substrates.

4-4-2. Investigation of the food web structure of deep-sea benthic community associated with sunken-wood.

Future sampling and deployment

We have completed the new deployment of a set of cedars at the 1,000 m site during the NT10-07 cruise, so we want to recover one of them next year. At 275 m and 500 m, we want to recover cedars next year because the succession on this substrate is very fast and we need to recover them at short interval. And about 2,000 and 3,000m sites, due to the bad condition we could not recover them again, so we want to recover TP and newly deploy a set of cedar next year.

In addition to upper experiment, we want to newly deploy several wood species at the same time and compare between them. It's because the seasonal pattern of wood boring bivalve's settlement should affect the following community succession. Although now we deployed wood trunk itself, in order to realize upper experiment, we need to narrow down the working hypothesis and make compact deployment system.

Further analysis

- Imaging of inside structure

The invasion rate is very important factor for the evaluation of succession speed so we will use CT scan for imaging. And we also want to clarify the way of usage of tunnels by other animals.

- Food web

For samples whose mass is enough to measure carbon and nitrogen stable isotope ratios, we will measure them to clarify their food source and compare the food web

between samples. If there will be some need for using other techniques in order to clarify the food web around the sunken wood, we want to adopt other techniques actively.

- Taxonomy

There will be many non-described species around sunken wood community, so we will describe new species in corporation with taxonomist.

5. Acknowledgments

We are very grateful to Susami Satoshi, Captain of the R/V Natsushima, and his crew, as well as to Mitsufuji Kazuya, Commander of the ROV Hyper-Dolphin, and his team, for their collaboration and their valuable help during this the whole cruise. We also deeply appreciated the contribution of Kasaya Takafumi in the design and construction of the mooring system, and Aoki Misumi who helped us during the whole process of the mooring experiment, from the first sketches to the deployment on the seafloor.

6. Notice

This cruise report is a preliminary document provided at the end of the cruise. It may not be corrected even if changes in 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 the latest information.

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