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Natsushima Cruise Report

NT12-09

Kagoshima Bay and off Noma-Misaki

Evolutionary and developmental biology on siboglinid polychaetes,

Lamellibrachia satsuma and *Osedax japonicus*

Apr.11,2012-Apr.16,2012

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

Contents

1. Cruise Information

● Cruise ID: NT12-09

● Name of vessel: R/V Natsushima

● Title of the cruise: ROV Hyper Dolphin research dive, deep sea research, FY2012

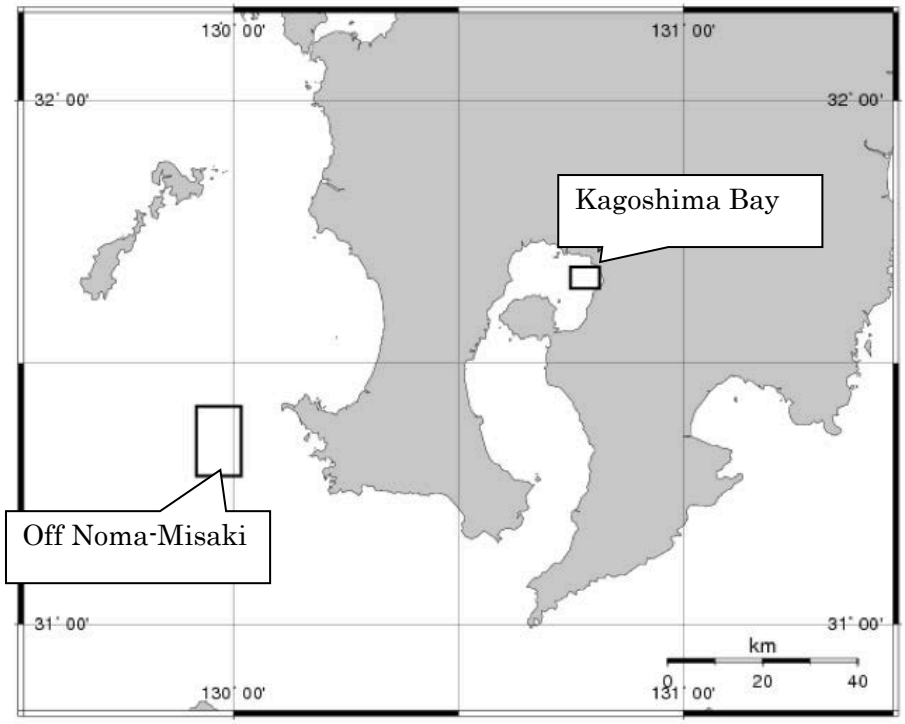
● Title of proposal: Evolutionary and developmental biology on siboglinid polychaetes, *Lamellibrachia satsuma* and *Osedax japonicus*

● Cruise period: Apr.11,2012-Apr.16,2012

● Ports of call: Kagoshima - Yura

● Research area: Kagoshima Bay and off Noma-Misaki

● Research Map



2. Researchers

2-1. Research group

Chief scientist [Affiliation]: Norio Miyamoto [JAMSTEC]

Representative of the science party [Affiliation]: Norio Miyamoto [JAMSTEC]

Participants list

Norio Miyamoto	Institute of Biogeosciences, JAMSTEC
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Taishi Tsubouchi	Institute of Biogeosciences, JAMSTEC
Ayuta Shinozaki	Institute of Biogeosciences, JAMSTEC
Atsushi Nagahori	Institute of Biogeosciences, JAMSTEC
Sang-Jin Kim	Korea Ocean Research & Development Institute
Yoichi Yusa	Nara Women's University
Natsumi Yasuda	Nara Women's University
Hiroshi Miyake	School of Marine Biosciences Kitasato University
Mitsuru Jinbo	School of Marine Biosciences Kitasato University
Yumiko Tada	School of Marine Biosciences Kitasato University
Kazuma Yoshii	School of Marine Biosciences Kitasato University
Yuji Onishi	Okayama University
Akira Sasaki	KAGOSHIMA CITY AQUARIUM
Kazuki Nishida	KAGOSHIMA CITY AQUARIUM
Masashi Ito	Nippon Marine Enterprises, LTD.

2-2. *Operation team of the ROV Hyper-Dolphin*

Submersible Operation Manager	Yoshinari Ono
1st Submersible Technical Officer	Homare Wakamatu
2nd Submersible Technical Officer	Katsushi Chiba
2nd Submersible Technical Officer	Shigeru Kikuya
2nd Submersible Technical Officer	Atsushi Takenouchi
2nd Submersible Technical Officer	Ryo Saigo
2nd Submersible Technical Officer	Teppei Kido

2-3. *Captain and crew of the R/V Natsushima*

Captain	Yoshiyuki Nakamura
Chief Officer	Naoto Kimura
2nd Officer	Isao Maeda
3rd Officer	Akira Suzuki
Chief Engineer	Hiroyoshi Kikkawa

1st Engineer	Kimio Matsukawa
2nd Engineer	Takahiro Mori
3rd Engineer	Hozumi Kuratomi
Chief Electronic Operator	Tokinori Nasu
2nd Electronic Operator	Yosuke Komaki
3rd Electronic Operator	Ryosuke Komatu
Boat Swain	Tadahiko Toguchi
Able Seaman	Yasuo Konno
Able Seaman	Nobuyuki Ichikawa
Able Seaman	Nao Ishizuka
Sailor	Kazuho Ikeda
Sailor	Hideo Ito
Sailor	Yasunobu Kawabe
No.1 Oiler	Kiyoshi Yahata
Oiler	Masanori Ueda
Oiler	Ryota Suzuki
Assistant Oiler	Ryo Sato
Assistant Oiler	Shin Torao
Chief Steward	Teruyuki Yoshikawa
Steward	Shinsuke Tanaka
Steward	Masanao Kunida
Steward	Hideki Kubota
Steward	Kei Ito

3. Proposals

Evolutionary and Developmental biology of siboglinid polychaetes

Norio Miyamoto, Ayuta Shinozaki, Atsushi Nagahori and Yoshihiro Fujiwara (JAMSTEC)

Vestimentiferan tubeworms and bone-eating *Osedax* are deep-sea invertebrates living at hydrothermal vents, hydrocarbon seeps and whole bones. They have quite unique body plans lacking a digestive system. Most importantly, they lack segmented body plans, except for the opisthosoma, which is the most posterior end of the tubeworm. In addition, they harbour bacterial symbionts in the trophosome. Since morphological features of tubeworms have no affinity for existent phyla, they had classified into the independent phylum Vestimentifera. Recent molecular phylogenies, however, have strongly suggested that vestimentiferans and *Osedax* are modified polychaetes and assigned into the family siboglinidae together with franulates and moniliferans. Although vestimentiferans and *Osedax* have been downgraded as a family of annelids, their enigmatic body plans have been fascinated evolutionary biologists. However, our knowledge on the body plan of these worms and its origin are limited. This is largely because of poor accessibility to specimens to examine.

The purposes of the present study are to elucidate the evolutionary origin of the vestimentiferan and *Osedax* body plans and to reveal the cellular characteristics of symbiosis of the worms. The aim of this cruise is collecting the vestimentiferan *Lemellibrachia satsuma* and *Osedax japonicus*. Colonies of *L. satsuma* was found in the Kagoshima Bay. We collect animals from the colonies and also collect whole bone on which *L. satsuma* attaches. Colonies of *O. japonicus* was observed on a whole bone deployed at off Noma-Misaki in the previous cruise. We cut the whole bone and collect several pieces of the bone.

Collected animals are kept in aquaria in a laboratory. We will observe the development of *L. satsuma* and *O. japonicus*, and fixed their embryos and larvae. We investigate the expression patterns of genes essential for the segmentation, endoderm development, and so on, to elucidate development of the worms. Then we will compare the results with that of the other polychaetes and discuss on the evolution of the vestimentiferans and *Osedax*.

Taishi Tsubouchi (JAMSTEC)

It’s my view that environmental microorganism is one of the most powerful key players for preservation and/or alteration of ecosystems. From a standpoint of inhabitation, it’s inferable that environmental microorganism and their habitat are influenced one another all of the time, however no example of report have been yet which evaluate the correlation numerically. So I propose I’ll try to analyze dynamic changes of microbial community structure using multivariate statistical technique, which occurs by the application of a stimulus like “whale bone”.

Rearing and sex determination of *Osedax japonicus*.

Yoichi Yusa and Natsumi Yasuda (Nara Women's University)

Osedax japonicus, a bone-eating worm, is a member of a characteristic group of deep-sea annelids (family Siboglinidae). As in other species of this genus, *Osedax japonicus* is found only on bones of vertebrates (mostly whales). This is the only known *Osedax* to have gelatinous cocoons in which embryos grow, making it suitable to follow development and rearing of the embryos.So, rearing has been tried in this species (as well as in several other *Osedax* species) but the rearing of individuals of the next generation has been successful only once, at JAMSTEC. The first purpose of this study was to develop a rearing system of this species. The second purpose was to study the mode of sex determination of this species. All *Osedax* species have separate sexes with large females and dwarf males. Although environmental sex determination is suggested in *Osedax*, there has been no good evidence for it. Since many polychaetes have genetic sex determination, it is important to study the mode of sex determination of *O. japonicus*.

Estimation of tube production rate and development of easy rearing method for vestimentiferan tube worm, *Lamellibrachia satsuma*.

Hiroshi Miyake, Yumiko Tada (Kitasato University) and Aya Fukami (Urawa Business High School)

Our group has been developing the easy rearing tank for vestimentiferen tube worm *Lamellibrachia satsuma*. We would like to observe the growth of tube of tube worm in the raring system. To estimate tube production, tube worms will be introduced into transparent tube and then kept in the tank which regulate H_2S and CO_2 concentration.

Purification and distribution of a tubeworm *Lamellibrachia satsuma*

Mitsuru Jimbo, Kazuma Yoshii (School of Marine Biosciences, Kitasato University)

Animals usually acquire nutrients by predation, but some animals acquire them from symbionts. To keep these relationships, the animals need to have some factors for symbiosis. One candidate for the symbiosis is a lectin, which is a sugar binding protein, and involved in immune system. The lectin involvement for symbiosis was suggested in legume, corals, nematodes, and so on. Deep sea invertebrates such as tubeworms and bivalves, also symbioses with chemosynthetic bacteria. It is expected that the lectins involve to symbiosis of deep sea organisms. We found a lectin from the hemolymph of a tubeworm *Lamellibrachia satsuma.* The lectin agglutinated symbiotic bacteria, suggesting that the lectin binds to chemosynthetic bacteria to have some effects to them. However, the amino acid sequence and their distribution in the tissue were not known. Our objective is cDNA cloning for *L. satsuma* lectin, and determination of the tissue distribution of the lectin.

Yuji Onishi (Okayama University)

To research the nutrient source of organisms and understand chemical flux around whale bone, I will conduct the isotope analysis on the samples of organisms collected during this NT12-09

cruise. By comparing the analytical result of the samples of organisms from whale bones, I consider nutrient source of animals inhabited around whale bone.

Sang-Jin Kim (Korea Ocean Research & Development Institute)
To understand the role of endosymbiont in tubeworm (*Lamellibrachia satsuma*) the samples will be collected from the Kagoshima bay. The DNA and RNA samples will be extracted from the endosymbiotic microorganisms in the trophosome and from the animal-body tissue, respectively, and the libraries will be constructed. Both libraries will be sequenced by NGS method and the data will be analyzed to elucidate the role of endosymbiotic microorganisms and the interaction between endosymbiotic microorganisms and tubeworm.

Akira Sasaki and Kazuki Nishida (Kagoshima City Aquarium)
The vestimentiferan tubeworm *Lamellibrachia satsuma* and the macrobrachium shrimp *Periclimenes thermohydrophilus* are known from the "Tagiri" vent in the Kagoshima-Bay. In order to understand their ecology and to display them in the Kagoshima City Aquarium, we try to collect living animals.

4. Observations and Results

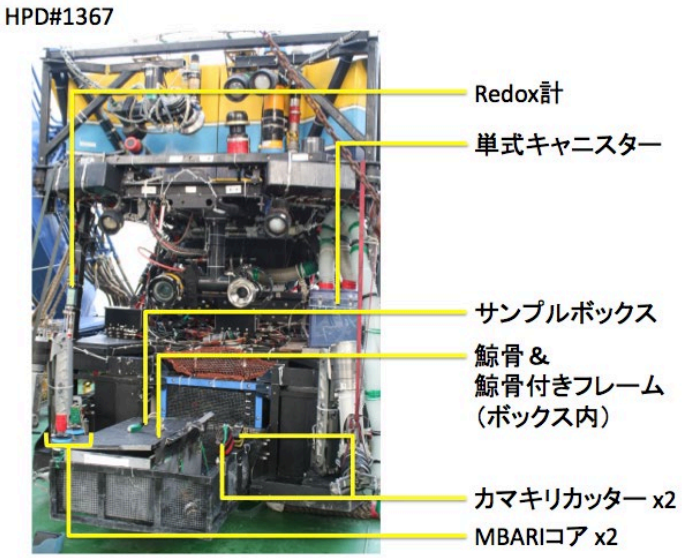
4-1. Dive results

Preliminary results of the ROV Hyper Dolphin Dive #1367

***Date*:** April 12, 2012
***Site*:** Kagoshima Bay
***Depth*:** 97-105m
***Landing* (Lat., Long., Time, Depth):** 31º39.719'N, 130º48.069'E, 8:28, 105m
***Leaving* (Lat., Long., Time, Depth):** 31º39.752'N, 130º48.055'E, 10:37, 102m
***Chief observer*:** Norio Miyamoto, JAMSTEC

***Purpose*:**
1) Retrieval of whale bones
2) Collecting *Lamellibrachia satsuma* and animals living around its colony
3) MBARI corer sampling
4) Deployment of whale bones

***Payload equipment*:**
Long box, slurp gun, MBARI corer (2), whale vertebra, frame with whale bones, Redox meter



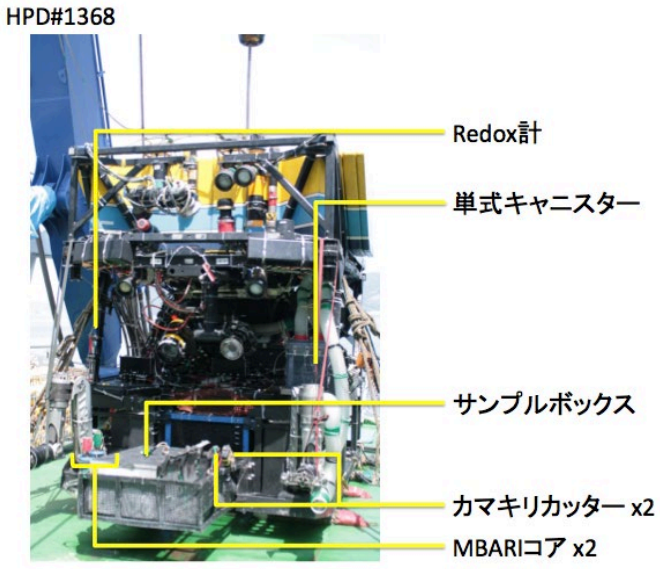
Dive summary
Soon after landing, we found many active vents (event #2). We deployed a whale vertebra and a frame with several pieces of whale bones on the muddy sea floor northwest of the (event #2). The vehicle ran toward northeast to find the previously deployed substrates. We, however, could not find them and gave up to retrieve them in the dive. We collected *Lamellibrachia* from a colony, and collected animals living in the colony by slurp gun. Then, We left the bottom.
3-5. Payload list with photographs

Preliminary results of the ROV Hyper Dolphin Dive #1368

***Date*:** April 12, 2012
***Site*:** Kagoshima Bay
***Depth*:** 101-102m
***Landing* (Lat., Long., Time, Depth):** 31º39.738'N, 130º48.053'E, 12:50, 102m
***Leaving* (Lat., Long., Time, Depth):** 31º39.751'N, 130º48.055'E, 14:38, 101m
***Chief observer*:** Norio Miyamoto, JAMSTEC

***Purpose*:**
1) Retrieval of whale bones
2) Collecting *Lamellibrachia satsuma* and animals living around its colony
3) MBARI corer sampling

***Payload equipment*:**
Long box, slurp gun, MBARI corer (2), Redox meter



Dive summary
After landing, the vehicle ran around the event #11 in order to find the deployed whale bones, which could not find the previous dive. About 1 hour running , we find the whale bones. After the observation of the substrates, we collected two nets containing several pieces of whale bone (H855-1 and H855-2) and a whale vertebra (H454-6). Next, we collected sediment by a MBARI corer from where the whale vertebra was. We collected sediment from normal sea floor about 5m away from the 1st corer sample. Finally we collected animals living in a *Lamellibrachia* colony by slurp gun. Then, We left the bottom.

Preliminary results of the ROV Hyper Dolphin Dive #1369

***Date*:** April 13, 2012

***Site*:** Off Noma-Misaki

***Depth*:** 225-226m

***Landing* (Lat., Long., Time, Depth):** 31º20.993'N, 129º59.131'E, 8:47, 225m

***Leaving* (Lat., Long., Time, Depth):** 31º20.998'N, 129º59.156'E, 13:14, 226m

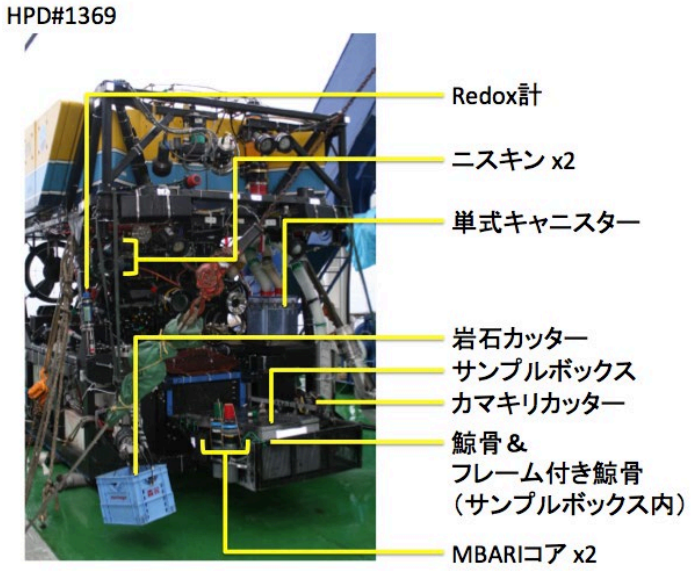
***Chief observer*:** Norio Miyamoto, JAMSTEC

***Purpose*:**

1) Deployment of a whale vertebra and pieces of whale bones attached to a frame
2) Retrieval of whale bones
3) Collecting animals around the bones
4) MBARI corer sampling
5) NISKIN water sampling

***Payload equipment*:**

Long box, whale vertebra, flame, slurp gun, MBARI corer (2), NISKIN (2) Redox meter



Dive summary

Soon after landing, we found the whale fall #6 deployed in 2002. A whale vertebra (H 1369-1) and small pieces of whale bones attached to a frame (H 1369-2) were deployed near a concrete sinker. Next we cut the maxillary bone of the whale by a hydraulic cutter and collected 5 pieces of the bone. Surface fauna of the whale bone was collected by the slurp gun. Sediment and water were collected near the whale #6 and 5m away from the whale by MBARI corers and NISKIN samplers, respectively. Because of the high turbidity, we gave up to collect organisms, then left the bottom.

Preliminary results of the ROV Hyper Dolphin Dive #1370

***Date*:** April 13, 2012

***Site*:** Off Noma-Misaki

***Depth*:** 249-251m

***Landing* (Lat., Long., Time, Depth):** 31º18.515'N, 129º59.372'E, 15:42, 249m

***Leaving* (Lat., Long., Time, Depth):** 31º18.514'N, 129º59.377'E, 16:25, 251m

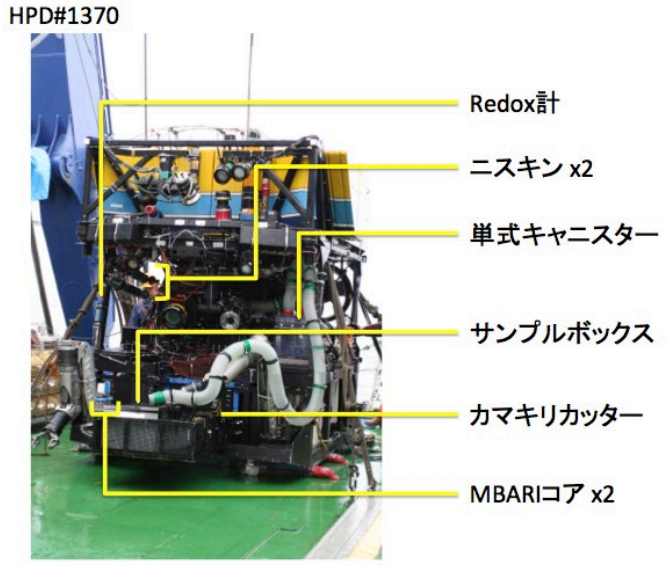
***Chief observer*:** Yoshihiro FUJIWARA, JAMSTEC

***Purpose*:**

1) Observation of #12 whale
2) Benthos sampling around the carcass
3) MBARI corer sampling
4) NISKIN water sampling

***Payload equipment*:**

Long box, slurp gun, MBARI corer (2), NISKIN water sampler (2), Redox meter



Dive summary

The ROV landed 10 m away from the #12 whale carcass. The visibility was extremely low provably due to the spring bloom (Fig. 1). Therefore, no observation of the carcass was conducted. Sediment sampling using MBARI corers was failed because of defective setup of the corers. Water sampling was conducted 4m away from the carcass and in the water column at a depth of 30m where fluffy materials were abundant in the water column (Fig. 2).

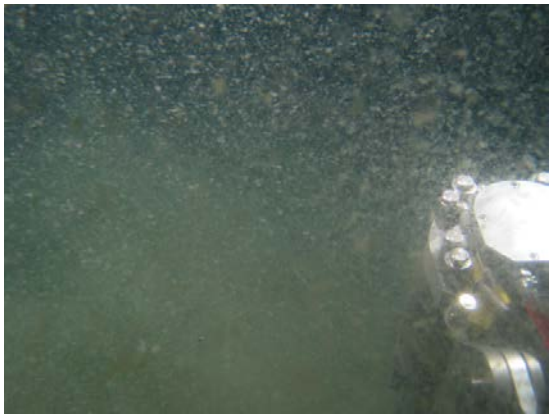


Fig. 1. Low visibility at deep-sea bottom.

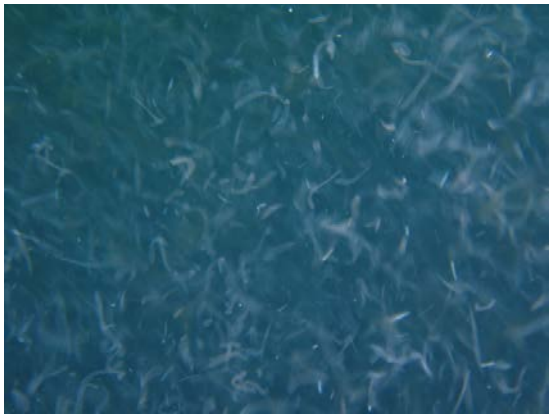


Fig. 2. High density of fluffy materials at a depth of 30m

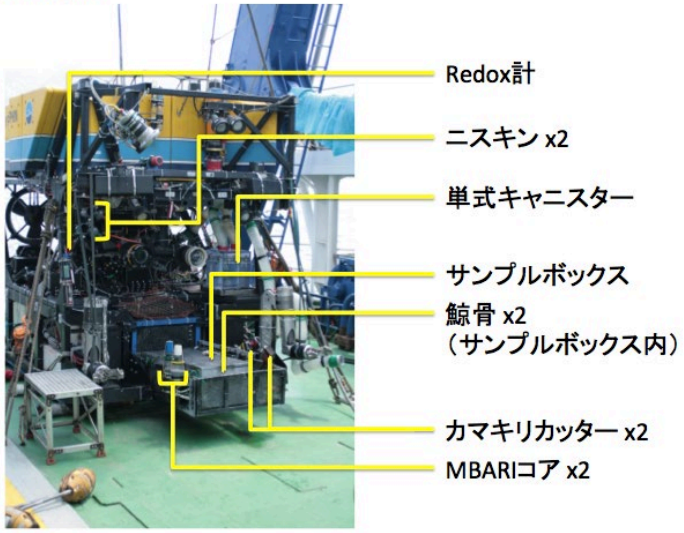
Preliminary results of the ROV Hyper Dolphin Dive #1371

***Date*:** April 14, 2012
***Site*:** Kagoshima Bay
***Depth*:** 102 m
***Landing* (Lat., Long., Time, Depth):** 31º39.728'N, 130º48.057'E, 14:11, 102 m
***Leaving* (Lat., Long., Time, Depth):** 31º39.745'N, 130º48.056'E, 15:06, 102 m
***Chief observer*:** Mitsuru Jimbo, Kitasato University

***Purpose*:**
1) Deployment of whale bones
2) Collecting *Lamellibrachia satsuma* and animals living around its colony
3) MBARI corer sampling
4) NISKIN water sampling

***Payload equipment*:**
Long box, slurp gun, MBARI corer (2), whale vertebra, frame with whale bones, Redox meter, NISKIN water sampler

HPD#1371



Dive summary

The vehicle was landed near the vent (event #2). The vehicle navigate toward event #10. In the way to event #10, the whale vertebra (1371-1) and a frame with whale bones (1371-2) were deployed at sea floor without the vent. Water and core was also sampled with NISKIN water sampler and MBARI corer. After that, we found the large bacterial mat, and core sampling was carried out. After movement, the large L. Satsuma colony was found, and collected *L. satsuma* by manipulator. The much part of *L. satsuma* were in the mud. The organisms in the colony of *L. satsuma* were collected with slurpgun. Finally, tubeworms were collected with manipulator, and the vehicle leaved.

4-2. Preliminary results

Norio Miyamoto, Ayuta Shinozaki, Atsushi Nagahori and Yoshihiro Fujiwara (JAMSTEC)

In the present cruise we have succeeded in collecting a large amount of animal. *Lamellibrachia satsuma* attached to the whale bones and a half of *L. satsuma* collected from natural colonies were kept in aquaria. The remaining half of the natural *L. satsuma* were dissected at the anterior part of the trunk region. We collected eggs from the oviduct situated in the posterior end of the ventimental region. Eggs in the oviduct were already fertilized and were incubated in Petri dishes at 12-13ºC. Every 12 hour, embryos and larvae were fixed with 4%PFA/Mops buffer for overnight at 4ºC, dehydrated through a EtOH series and stored in 80% EtOH at -20ºC. We also fixed and stored the vestimentum and trophosome in the same way. For DNA and RNA extraction samples worms were frozen with liquid nitrogen and stored at -60ºC.

Adult females of *Osedax japonicus* were collected from the whale bone retrieved in the HPD#1369 dive and fixed and stored in the same way as *L. satsuma*. Embryos, larvae and dwarf males were found in the mucus of adult females. We removed the mucus by pipetting and collected them. Some of them were fixed in the same way and the others were cultured in the Petri dishes at 12ºC.

Taishi Tsubouchi (JAMSTEC)

In the HPD#1368, we tried to sample sediments just under the whalebone (vertebra) have submerged since 2008 and 5 m distant from it by MBARI corers and succeeded in it. Approximately 240 ml sediments were harvested from both sampling sites. These sediments were halved and stored in refrigerator and deep freezer, respectively. The former will be used for some microbial isolation, and the latter will be for environmental DNA and total RNA extractions. In the HPD#1369, sediments sampling were held in the same way aside from sapling position, in this dive, just beside whalebone (upper jawbone) have submerged since 2003 and 5 m from it. In the HPD#1371, sediment covered with a kind of bacterial mat and its surrounding were sampled. All these sediment samples are kept as described above. The environmental DNA and total RNA will be extracted as soon as get back to the laboratory in JAMSTEC.

Yoichi Yusa and Natsumi Yasuda (Nara Women's University)

We could collect pieces of whale bones using a cutter equipped on Hyper Dolphin, on 13th April 2012 HPD#1369 dive. The bones were from #6 whale deployed in early 2002. We observed many individuals of *Osedax japonicus*, and using a microscope, we dissected a female and found many embryos and several dwarf males on it. This means that the population is still reproducing in a good condition even after 10 years of deployment.

Hiroshi Miyake, Yumiko Tada (Kitasato University) and Aya Fukami (Urawa Business High School)

We collected some tube worms for our studies. Large specimens were used for getting

fertilized egg by dissection. Tube worms which were immature were kept in aquarium on shipboard.

Mitsuru Jimbo, Kazuma Yoshii (School of Marine Biosciences, Kitasato University)
The tubeworms were cut and collected their blood by natural elution. The blood was collected at about 300 ml, and was stored at -80°C. The eggs were picked from the vestment part, and were released to filtrated sea water. Some eggs were dispense 24 well titreplates. These were incubated at 12°C. 4 individuals were cut to three parts, plume, vestment and soft body. Each was stored for RNA preparation and histoimmunochemistry.

Yuji Onishi (Okayama University)
In this cruise, I collected sediment, sea water and animal samples at Kagoshima Bay and off Noma-Misaki by the ROV "HyperDolphin". I froze sediment and animal samples. I percolated sea water samples and measured pH, concentration of ammonia and silica in sea water on board.

Sang-Jin KIM (KORDI)
First the tubeworms were cutted into two parts, anterior and posterior parts. Both parts were used to collect blood and the anterior parts including ovary organ were used to further investigation by other colleagues. After those treatments trophosome was taken out from the posterior part of tubeworm bodies. During the process tubeworms became dry and they were exposed to ambient temperature for a while (more than 1 hr) after death. It seems like that RNA of tubeworm could be easily degraded. Collected trophosomes were soaked in the RNAlater and the samples were preserved at – 60℃. It was difficult to separate the trophosome from the other animal organs to minimize the contamination of eukaryotic cells. To construct the cDNA library the anterior parts were received from Dr. Miyamoto after his study. It was preserved in RNA later and then frozen.

Akira Sasaki and Kazuki Nishida (Kagoshima City Aquarium)
Lamellibrachia satsuma and *Periclimenes thermohydrophilus* were collected by the manipulators and slurp gun of the ROV *Hyper Dolphin* in the HDP#1367, 1368, 1671 dives from the Kagoshima Bay. Then, we kept animals in aquaria.

5. Proposals for the future studies

Norio Miyamoto, Ayuta Shinozaki, Atsushi Nagahori and Yoshihiro Fujiwara (JAMSTEC)

• Observation of development of *Lamellibrachia satsuma* and *Osedax japonicus*.
• Spatial and temporal expression patterns of genes, which play essential roles for morphogenesis, such as endoderm specification, gut patterning, mouth and anus makers, segmentation and antero-posterior patterning.
• Differentiation of bacteriocyte.
• Evolution of cell-cell communications between symbionts and host cells.

Taishi Tsubouchi (JAMSTEC)

Total RNA will be extracted from "whalebone" site (stimulated site) and roundabout site (reference site), respectively. Using these RNA as templates, microbial (Eubacterial and Archaeal) 16S rRNA gene will be amplified and then analyzed microbial community structure. To compare the two sites (stimulated and roundabout), diversity indice such as ACE, Shannon, and Simpson's will be adopted in the alpha-diversity. The microbial diversity in its own site will be evaluated by multidimensional scaling method. Transition of community structure from roundabout to stimulated and vice versa will be calculated based on log normal distribution, primarily.
Isolation of uncultured microbes will be performed using multivariate data analysis, referring to geochemical parameters measured in this cruise and official database registered yet.

Yoichi Yusa and Natsumi Yasuda (Nara Women's University)

We will rear *Osedax* at Nara Women's University using a closed system aquarium, and also at Seto Marine Biological Laboraty using a running sea water system. At the same time, we will fix some females, unfertilized eggs, embryos, and dwarf males (with sperm cells) for chromosomal study. We will study karyotypes of females and males, and their germ cells, to infer the mode of sex determination. Traditional karyotyping and fluorescent in situ hybridization technique will be applied if necessary.
Since the studies are not easy to conduct, we need to visit the study site off Cape Noma again, to further collect the material.

Hiroshi Miyake, Yumiko Tada (Kitasato University) and Aya Fukami (Urawa Business High School)

Lamellibrachia satsuma will be kept in specialized tank for tube worm at Kitasato University and Urawa Business High School. Mud collected from Ariake Bay was mixed with dog food and laid on the bottom of the tank at a thickness of 5 cm. Then mud from Ariake Bay was overlaid to a thickness of 5 cm on top of the dogfood-mud mixture. This tank does not need adding H_2S and need only adding CO_2. We would like to find vest condition for rearing tube worm using our system.
Some of tube worms will be introduced in to transparent vinyl tube to estimate tube production

rate. Few weeks after introducing tube worm into vinyl tube, new tube will be formed in the transparent vinyl tube. Tube production will estimate from the new tubes.

Mitsuru Jimbo, Kazuma Yoshii (School of Marine Biosciences, Kitasato University)
Future plan

1. The Lectin is purified by previously established method.
2. The lectin is digested with trypsin and internal amino acid sequence of the lectin is determined.
3. Based on the determined amino acid sequences, the cloning of the lectin cDNA is carried out.
4. Anti-lectin antibody is prepared by using the lectin.
5. The lectin distribution is observed by immunohistochemistry of the tissue of *L. Satsuma.*
6. Observation of eggs development, lectin emergence.

Yuji Onishi

I will compare the analytical results among each animal species from whale bone in order to estimate how they are supported by whale bone. I will measure isotope compositions of SOM (sedimentary organic matter), POM (particle organic matter) and animal samples.

Sang-Jin Kim (KORDI)

Collected samples will be further studied as followings;

DNA extracted from trophosome will be sequenced by 454 pyrosequencing and then the data will be analyzed to understand the function of endosymbiont in the tubeworm. cDNA library will be constructed from the RNA of animal bodies and the sequenced data will be comparatively analyzed with the endosymbiont's .

Akira Sasaki and Kazuki Nishida (Kagoshima City Aquarium)

We will display the collected animals in the Kagoshima City Aquarium like the *in situ* situation, to make peoples understood and observed their ecology and behavior. We will reveal their ecological features through the long term keeping in aquaria.

6. Notice on Using

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