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Natsushima Cruise Report
NT08-24

Sagami Bay

Dec. 3, 2008, Dec 12, 2008

Japan Agency for Marine-Earth Science and Technology

Contents

Cruse Information

Cruise number NT08-24
Ship name Natsushima／Hyper Dolphine

Title of the cruise Sample collection off Hatsushima, Sagami Bay
Title of proposal The distribution and function of the lectin isolated from Calyptogena okutanii
Molecular mechanism of symbiosis in *Callyptogena okutanii* - sulfur oxidizing bacterium symbiosis.
Microbial succession, multiparametrical study and isolate the useful microorganism on the whale carcass ecosystems.
Cruise period December, 3,2008 ～ December, 12, 2008

Port call JAMSTEC ～ JAMSTEC

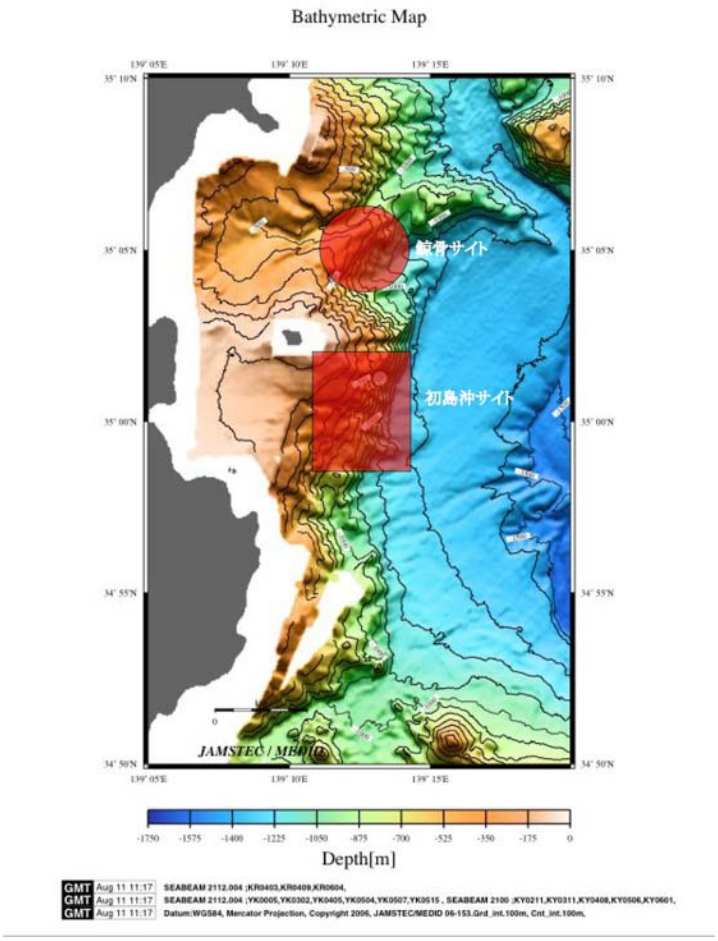
Research Area
①Off Hatsushima, Sagami Bay（Depth：800～1200 m）

The region from 34°58.0'N, 139°11.0'E to 35°02.0'N, 139°15.0'E.

②Sagami Bay, whale fall（Depth：800～1200m）

Circular region which center point is at 35°05.0'N, 139°13.0'E and radius is 1.5 miles.

Research Map



Reseachers

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Mitsuru Jimbo（Kitasato University）

Representative Science Party

S08-41 Mitsuru Jimbo（Kitasato University）

S08-29 Takao Yoshida（JAMSTEC）

S08-63 Yuichi Nogi （JAMSTEC）

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S08-41

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Tadashi Maruyama	(JAMSTEC)
Takao Yoshida	(JAMSTEC)
Hiroya Hasegawa	(Kitasato University)
Hiroki Ikuro	(Kitasato University)

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Tetsuya Miwa	(JAMSTEC)
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Kazue Oishi	(JAMSTEC)
Yoshihiro Fujiwara	(JAMSTEC)
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Masaru Kawato	JAMSTEC
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Rieko Ikeda	JAMSTEC

Observation

Proposal

S08-41 The distribution and function of the lectin isolated from Calyptogena okutanii

Objective

Deep sea organisms often adopts their habits by symbiosing with other organisms. Few substances which involve to symbiosis has been known. Recently, however, it is reported that a carbohydrate binding protein called as lectin, was involved in symbiosis between legume and rhizobia. We hypoathesize that a lectin COL isolated from hemlymph of a symbiotic shell, *Calyptogena okutanii* involved to symbiosis. My objective is to examine the distribution of the lectin and symbiotic sulfer-oxidizing bacteria. also have lectins, we examine the presence of similar lectins isolated from other deep-sea organisms, like *Bathymodiolus* and tubeworm. Scince *Alaysia*, having a kind of lectin, can obtain egg and larvae, we observe the larva development, and examine the distribution of a lectin.

S08-29 Molecular mechanism of symbiosis in *Callyptogena okutanii* - sulfur oxidizing bacterium symbiosis.

Objective

The deep-sea clams belonging to the genus *Calpyogena* have vestigial digestive tracts and are nutritionally dependent on chemoautotrophic sulfur-oxidizing symbiotic bacteria, which they harbor within their gill epithelial cells. Recently, we have reported the complete genome sequence of the symbiont of *C. okutanii* (Kuwahara et al. 2007). The symbiont has genes for sulfur metabolism and inorganic carbon fixation. The symbiont is thought to synthesize amino acids, carbohydrates, and other nutrients and supply them to the host. However, no transporter for such nutrients has been found in the symbiont genome. The major purpose of this proposal is to elucidate the molecular mechanism of symbiosis between deep sea bivalve, *Calyptogerna oktanii* and chemoautotrophic intracellular bacterium. We analyze the gene expressions of gill tissue in *Calyptogena* and incorporation of inorganic carbon by isotope labeling. During the cruise, we collected seep-specific animals including deep-sea bivalves (*Calyptogena species*), deep-sea musells *(Bathymodiolus* species) mainly using a suction sampler and manipulators. For the expression analysises, the samples is dissected and frozen for molecular and biochemical analyses. For the isotope labeling experiment, C14 labelled substance is injected into *Calyptogena* and incubated. After incubation, *Calyptogena* is dissected and frozen for the isotope labeling.

S08-63 Microbial succession, multiparametrical study and isolate the useful microorganism on the whale carcass ecosystems.

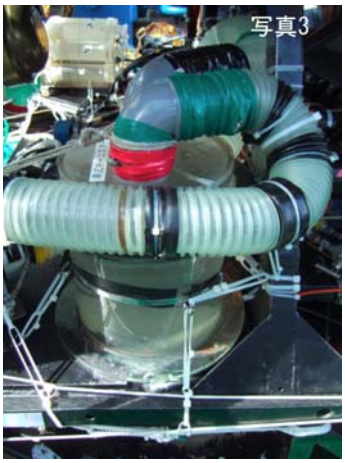
Objective

It is necessary to continuously observed two points where the condition is different to understand the whale carcass ecosystems. The whale carcass ecosystems off Nomamisaki, Kagoshima, Japan, has been investigated for five years or more. There is a necessity for continuously surveying the whale carcass ecosystems in the Sagami bay as the comparison data. The whale carcass ecosystems in the Sagami bay is a place where the whale remains that washed to Atami City in April, 2005 were sunk. Then, it has started the research and the investigation since January, 2006. A large-scale animal of the crab etc. ate whale's soft tissue at the early stage. The Osedax presumed to be a new species from the gene analysis etc. is observed to adhere all over by the costal part made a bleached white bone.

These observation and sampling are done, to confirm the transition of the whale carcass ecosystems afterwards and the change in the microorganism community. The living thing observation, the microorganism diversity analysis, and the transition of microbiofacies of the whale carcass ecosystems in the Sagami bay, is examined as a main goal. Moreover, the samples take from the nearby cold-seep site, and the correlation with the whale bone microbiofacies is examined. The second purpose, isolation of microorganism that symbiosis or adheres to living thing of whale bone, isolation of useful microorganism that resolves chitin, cellulose, and polylactic acid, etc. on whale carcass ecosystems and cold-seep site. A new microorganism such as the difficult culture microorganisms is isolated.

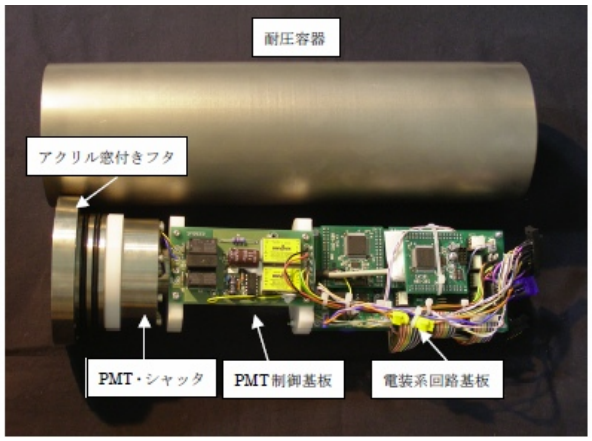
Instrument

Suction sampler with a canister containing six bottles
Like Figure 1, it collect some organisms by suction. By using multiple canister, it collects several samples.

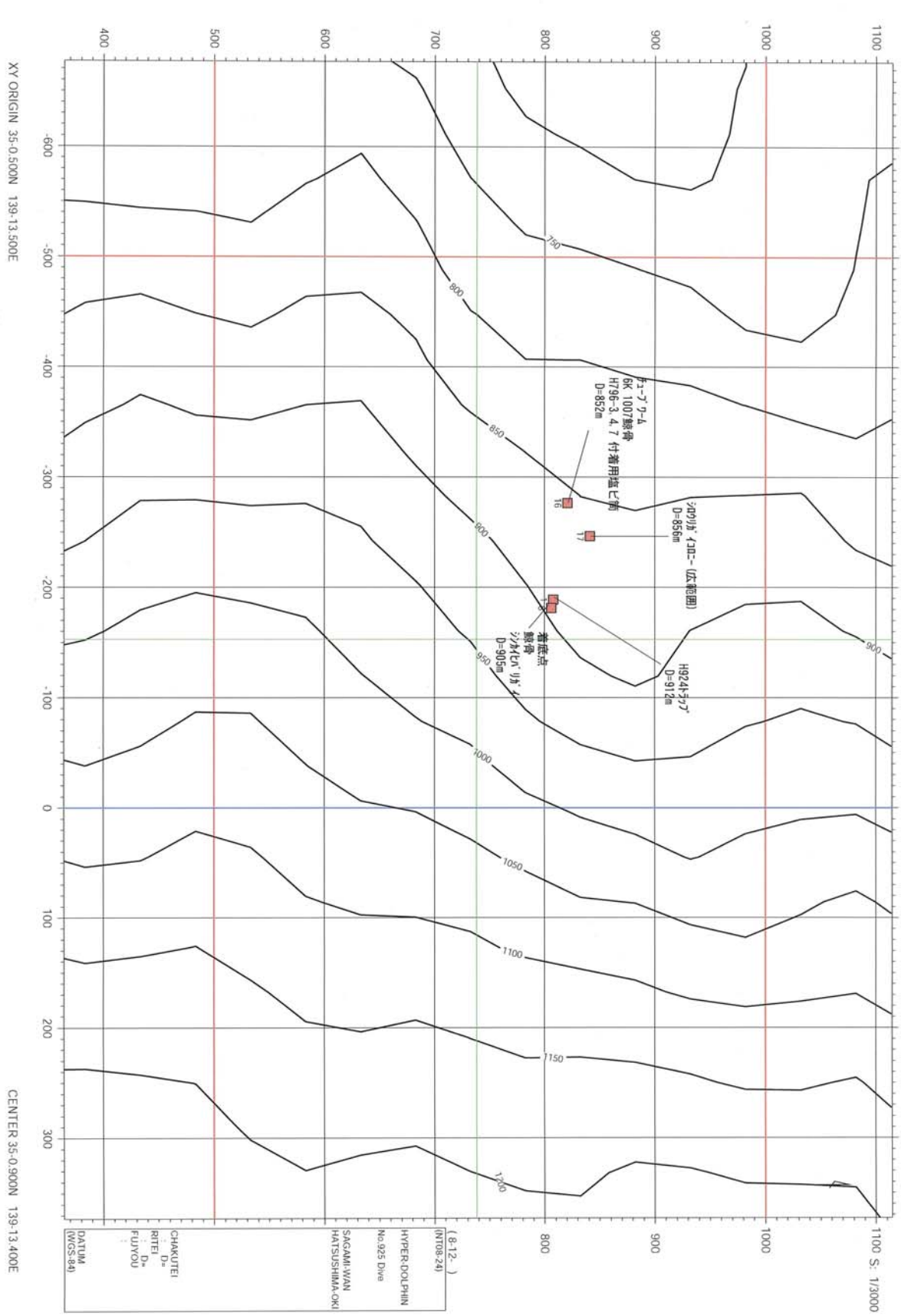


「IISA (Integrated in situ Analyzer) -ATP」

IISA-ATP quantitatively measure ATP concentration, which generally use an indicator of microorganisms at the site.



Dive Log


XY ORIGIN 35-0.500N 139-13.500E
CENTER 35-0.900N 139-13.400E
1100 S: 1/3000
750
800
850
900
950
1000
1050
1100
1150
1200
16
17
着底点
HYPER-DOLPHIN
No.925 Dive
SAGAMI-WAN
HATSUSHIMA-OKI
DATUM
(WGS-84)
CHAKUTEI
RITEI
FUJYOU

Dive 916

Date: December, 4
Objective： The distribution and function of the lectin isolated from *Calyptogena okutanii*

Chief Observer：Mitsuru Jimbo

Research Area：North of long-term deep sea observatory, Off Hatsushima, Sagami Bay,

Payload equipments：syntactic box, suction sampler with a canister with six bottles、Niskin water sampler x 2、MBARI x 2、IISA-ATP, syringe water sampler

Dive summary
08:21 syringe water sampling
9:06 landing
9:14 Niskin water sampling, MBARI core sampling
09:31 recovery of a homer #46
09:50 syringe water sampling
10:14 *Calyptogena* sampling
10:28 Niskin water sampling
10:34 MBARI core sampling
10:50 setting a petri dish
10:51 organism sampling by slurp gun
10:57 tube worm sampling

Dive 918

Date: December, 5.
Objective: Collection of *Calyptogena*, *Bathymodiolus*, other organisms.
Chief observer：Tatsuhiro, Fukuba

Research Area：North part of long-term deep sea observatory,Off Hatsushima, Sagami Bay,

Payload equipments：syntactic box, Suction sampler with a canister containing six bottles、Niskin water sampler x 2、MBARI x 2、IISA-ATP, syringe water sampler

08:50 cable check
10:17 landing
10:39 *Bathymodiolus* sampling
11:14 *Calyptogena* sampling
11:15 leaving

Dive 919

Date: December 6.
Objective： The distribution and function of the lectin isolated from *Calyptogena okutanii*

Chief observer：Mitsugu Kitada

Research Area：North part of long-term deep sea observatory,Off Hatsushima, Sagami Bay,

Payload equipments：syntactic box, Suction Sampler With A Canister With Six Bottles、Niskin water sampler x 2、MBARI x 2、IISA-ATP, syringe water sampler

Dive summary
07:22 Syringe water sampling
09:10 landing
09:17 Niskin water sampling
09:20 MBARI core sampling
10:14 Calyptogena sampling
10:15 syringe water sampling
10:22 recovery of a homer #40
10:32 organisms sampling
10:33 syringe sampling
10:37 MBARI core sampling
10:43 tube worm sampling
10:46 leaving

Dive 920
Date: December, 6
Objective: The distribution and function of the lectin isolated from *Calyptogena okutanii*
Chief observer: Koji Inoue
Research Area: South of long-term deep sea observatory, Off Hatsushima, Sagami Bay.
Payload equipment: Blue box, suction sampler with a canister with six bottles, niskin water sampler x 2, MBARI x 2, IISA-ATP, Syringe water sampler.

13:15 syringe water sampling
14:25 landing
14:42 syringe water sampling
14:55 Calyptogena sampling
15:12 Calyptogena sampling
15:14 MBARI core sampling
15:15 Niskin water sampling
15:17 syringe water sampling

Dive 921

Date: December, 7
Objective: Microbial succession, multiparametrical study and isolate the useful microorganism on the whale carcass ecosystems.
Chief Observer：Yuichi Nogi

Research Area: Whale fall site, Sagami Bay.

Payload: syntactic box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI x 2, sterile core sampler x 2, Kumade sampler, MBARI-type *in situ* culture equipment, petri dish set, anchor clamp x 3

Aim of this dive is to collect a whale bone and to sample water, organisms and core around the whale bones.

09:20 landing
09:21 Niskin water sampling
09:26 MBARI core sampling
09:43 observation of whale bones
10:10 larva sampling
11:09 Niskin water sampling
11:37 core sampling
11:45 fish sampling
12:06 sampling of a whale bone
12:13 Plankton sampling
12:23 MBARI core sampling
12:25 sterile core sampling
12:29 setting of MBARI-type *in situ* culture equipment
12:39 MBARI core sampling
13:05 shrimp sampling
13:18 sampling of plastic bug
13:21 fish sampling
13:37 crab sampling
13:43 leaving

Dive 922

Date: December, 9
Objective: Microbial succession, multiparametrical study and isolate the useful microorganism on the whale carcass ecosystems.
Chief Observer: Mitsuru Jimbo
Research Area: north of long-term deep sea observatory at off Hatsushima, Sagami Bay.
Payload: syntactic box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI x 2, sterile core sampler x 2, Kumade sampler

12:51 landing
12:55 Niskin water sampling
13:05 Bathymodiolus sampling
13:50 MBARI core sampling
13:58 sterile core sampling
14:09 Niskin water sampling
14:37 MBARI core sampling
14:40 sterile core sampling
15:22 Calyptogena sampling
15:41 tube worm sampling
15:41 recovery of petri dishes
15:48 leaving

Dive 923

Date: December, 9
Objective: Microbial succession, multiparametrical study and isolate the useful microorganism on the whale carcass ecosystems.
Chief Observer: Takahiko Nagahama
Research Area: Off Hatsushima, Sagami Bay
Payload: Blue box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI core sampler x 2, sterile core sampler x 2, Kumade sampler, M-type water sampler

08:19 landing
08:41 recovery of shrimp trap
08:59 MBARI core sampling
09:02 sterile core sampling
09:10 M-type water sampling
09:17 Niskin water sampler
09:27 MBARI core sampling
09:30 sterile core sampling
09:37 sampling of organisms by slurp gun
09:45 tube worm sampling
09:56 leaving

Dive 924

Date: December, 10
Objective Molecular mechanism of symbiosis in *Callyptogena okutanii* - sulfur oxidizing bacterium symbiosis.
Observer: Yuichi Nogi
Research Area: Off Hatsushima, Sagami Bay
Payload: syntactic box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI core sampler x 2, sterile core sampler x 2, Kumade sampler, a trap for shrimp

12:17 dive start
18:04 landing
18:05 move
18:10 finding of *Megalodicopia hians*
18:56 organism collection by suction sampler(*Bathymodiolus* sp., Zoarcoidei)
19:35 sterile core sampling (black)
19:42 sampling of Calyptogena sp. using Kumade sampler
19:50 setting a trap for shrimp（Ｈ９２４）
20:00 sampling of Calyptogena sp. using Kumade sampler
20:05 sterile core sampling (blue)
20:14 leaving

Dive 925

Date: December, 11
Objective Molecular mechanism of symbiosis in *Callyptogena okutanii* - sulfur oxidizing bacterium symbiosis.
Observer: Chiaki Kato
Research Area: Off Hatsushima, Sagami Bay
Payload: syntactic box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI core sampler x 2, sterile core sampler x 2, Kumade sampler, a trap for shrimp

09:13 landing
09:18 sampling of *Calyptogena* sp.
09:58 recovery of a trap for shrimp (H924)
10:16 *Calyptogen* sp. sampling
11:08 landing
11:09 resetting of a trap for shrimp (H924)
11:13 leaving

Dive 926

Date:December, 11.
Objective Molecular mechanism of symbiosis in *Callyptogena okutanii* - sulfur oxidizing bacterium symbiosis.
Observer: Hiroshi Miyake
Observer: Chiaki Kato
Research Area: Off Hatsushima, Sagami Bay
Payload: blue box, suction sampler with a canister with six bottles, Niskin water sampler x 2, MBARI core sampler x 2, sterile core sampler x 2, Kumade sampler, a trap for shrimp (recovery)

On the way to sea floor, cable trouble occurred at depth 491.8 m at 14:05. Power of the vehicle turned off and restart. At 16:27, restart diving, the cable trouble occurred 再び. This dive was ended by cable trouble.

Cruise Report

Characterization of *Calyptogena okutanii* and *Bathymodiolus japonicus* lectin

Mitsuru Jimbo, Hirohumi Hasegawa (Kitasato University)

Objective

Lectins are carbohydrate binding proteins, and generally are thought to involve in self-defense. Recently, some lectins from hosts, such as legume and nematode, recognise a specific symbiont. In this selective acquisition, some papers reported that lectins were involved to the process.

A deep sea mollusk Calyptogena okutanii has a degenerated digestive tract, and well-developed gill housed sulfur-oxidising bacteria. Thus, the survival of C. okutanii is mainly dependent on symbiotic sulfur-oxidising bacteria.

We found a lectin activity from hemolymph of C. okutanii, and the lectin was purified and cloned. The lectin named COL mainly detected at not only hemolymph but also gill which housed symbiont. Moreover, the lectin agglutinated the symbiotic bacteria, suggesting that COL may interact with symionts to affect to this symbiosis such as growth control. To examine this hypothesis, I am going to purify the lectin COL to raise antibody of it, and to determine the distribution of COL in the tissue with immunohistochemistry.

Results

The hemolymph and gills were collected from 94 individuals of *C. okutanii*. From a part of hemolymph, COL was purified about 1.5 mg.

Hemolymph was collected from each *C. okutanii*, and hemagglutinating activity was tested against human type A erythrocyte.

The hemagglutinating activity is various according to each individual. The haemagglutinating activity of one is more than hundred times higher than that of the other, although the extracts of gills were same intensity on SDS-PAGE. The haemagglutinating activity tended to be higher according to smells of H_2S. This seems that *C. okutanii* conditioned bad is likely to express more lectins To confirm this hypothesis, it needs to further experiment.

The hemolymph of *Bathymodiolus japonicus* also had a heamagglutinating activity against rabbit erythrocyte. The average activity of each individual was around 32 haemagglutination unit. In this cruise, we collected about 600 ml of hemolymph of 25 *B. japonics*. The hemolymph of *B. platifrons* was also tested on haemagglutinating activity against human and rabbit erythrocytes. Scince the activity is possible to be detected using erythrocytes of other animals, the hemolymph of them were collected to examine it.

Future plan

Purification of COL.

raising COL antibody against rabbit.

tissue distribution detemination by immunohistochemistry.

Search for settlement and morphogenesis inducing substance for tube worm larva.

Hiroki Ikuro, Mitsuru Jimbo (Kitasato University)

Objective

A tube worm, *Alaysia* sp. which belongs to polychaeta, does not have digestive tract, and obtained their nutrients from symbiotic sulfur-oxidising bacteria which occupy in their trunk. Thus, this symbiosis is nessesary for the survival of *Alaysia* sp., but the molecular mechanism of symbiosis was not unclear. To date, some lectins reported to be involved in some symbiosis of organisms like legume, corals and mollusks. We purified a lectin LSL from haemolymph of a tube worm *Lamellibrachia satsuma*. The lectin LSL agglutinated symbiotic bactereia. Thus, it was postulated that the lectin could be involved in symbiosis of the tube worm. To examine this hypothesis, we need to produce a experimental system.

Alaysia sp. which lives in off Hatsushima, Sagami Bay, Japan, can obtain embryos and larvae. They can keep more than one month. Since the embryos does not seem to contain symbiotic bacteria, it is thought that larvae get symbiotic bacteria at a stage after the embryonic stage obtained

The objective of this research is to observe the embryonic development, and to induce settlement and metamorphosis by substances that exists around them.

Methods

Larvae was obtained from sea water in which *Alaysia* sp. was keeped, or from ovary of *Alaysia* sp. They washed with filtrated sea water, and observed them daily. The developmental stage was determined by their form. To examine settlement induction, Some substance from extract of *Alaysia* sp. and *Lamellibrachia* sp. and mud were added to the sea water trochophore larvae kept. The form of the latest stage of trochophore was changed by adding the extract of *Alaysia* sp.

Results

The stage of *Alaysia* sp. larvae was separated to five stage. When the larvae settles, thread-like substance was observed at tail of larvae. Since he thread-like substance was adhesive, it was thought that the substance is useful for settlement of larvae. The adhesion occurred at some stage. When added to carbohydrate-bound resin to sea water, the proportion of settlement tended to increase. The settlement was induced by the haemolymph of *Lamellibrachia* sp. Since *Alaysia* sp. often colonised with *Lamellibrachia* sp., this results might suggest that substance released from *Lamellibrachia* sp. induce settlement of *Alasyia* sp.,

Future plan

Reevaluation of settlement inducing substance.

Protein expression analysis of *Calyptogena* spp.

Takao Yoshida, Yuki Hongo (JAMSTEC)

Objective

We are going to show what proteins from a host and symbionts express highly in gill of *Calyptogena* spp. Thus, Adding inhibitors of protein and RNA synthesis to *Calyptogena* spp., their effects are examined. We also compared the protein expression of gill cells between *Conchocele bisecta* and Solemyidae.

Methods and Results

Calyptogena spp. obtained at HPD#920 were injected with cycloheximide (a protein synthesis inhibitor for eukaryote), chloramphenicol (a protein synthesis inhibitor for prokaryote), actinomycin D (an RNA synthesis inhibitor for eukaryote), and rifampicin (an RNA synthesis inhibitor for prokaryote) by syringe, and kept to 24 h, then the tissues were stored by freezing. Gills were fixed for optical microscopy and electron microscopy. Each tissues of *Conchocele bisecta* and Solemyidae
obtained at HPD#919 was stored by freezing.

Future plan

Protein expression analysis of each tissue, especially gill.

Fates of organic nutritents and protein synthesis in *Calyptogena* symbiotic systems

Research Program for Marine Biology and Ecology
Tadashi Maruyama, D.Sc.
Takao Yoshida, PhD

In *Calyptogena* clam-thioautotroph-symbiotic systems, it is believed that the symbiont supplies fixed organic carbons to the host clam. We have previously published a paper on the genome sequence of *Calyptogena okutanii* symbiont (Vesicomyosocius okutanii) and have reported that the symbiont is likely to be digested by the host clam rather than to secrete the nutrients to the host (Kuwahara et al. 2007).
In this cruise, NT08-24, we aimed to study fates of organic C and of inorganic C in the host cells. For this purpose, we injected ^{14}C-glucose, ^{14}C-glutamic acid, ^{14}C-amino acid mixture and ^{14}C-inorganic carbon to the host in the adductor muscle and chase their fates after incubating them in natural sea water with sediment in a refrigerator. We also planned to trace protein synthesis after injection of ^{14}C-amino acid mixture.

Matrials and methods
Calyptogena clams were collected by hyperdolphin dives, HPD#922 and #924.
Each of the solutions of ^{14}C-glucose in ASW (artificial sea water), ^{14}C-glutamate in ASW, ^{14}C-amino acid mix in ASW, ^{14}C-$NaHCO_3$ (Table 1), was injected to one individual of the collected *Calyptogena* clams via the adductor muscle. The clams were then incubated at 4 degree C for adequate period (a few hours to overnight). After the incubation, clams were dissected and blood were collected in conical centrifuge tubes. Blood cells were collected by centrifugation (2,500 rpm x 3 min) at room temperature. Blood cells and the supernatant were stored in a freezer. Other tissues (gills, adductor muscles, foot, mantle and others) were also stored in a freezer and will be used for tracing the radioisotope in various tissues.

Results
Samples of the injection experiments are listed in table 2. Although at present we have not gotten the radioactivity tracing data yet, it is safe to write that the injection of the these reagents into the adductor muscle is relatively mild for the clams. No clam died in the next day while one bled a little.
For future experiments, we plan to analyze the radioactivity in small molecular fractions, and in protein fractions to understand the fates of small molecular nutrients (glucose, glutamic acids and amino-acid mixture) in the clam-thioautotroph symbiotic system.

1), Kuwahata et al. Curr. Biol. 17, 881-886 (2007)

	Maruyama, T.			T. Yoshida fist exp.			T. Yoshida 2nd exp.		
Reagent solutions for injection	14C-glucose	14C glutamate	14C-Bicarbonate	Amino acid mix	AAmix with cycloheximide	AAmix with chloramphenicol	Amino acid mix	AAmix with cycloheximide	AAmix with chloramphenicol
Abbreviations for the reagent solutions	Gls	GT	BC	AAm	AA+CY	AA+CM	AAm	AA+CY	AA+CM
2x conc. ASW micro-L	250	250	500	100	100	100	200	200	200
20x Tricine Buffer (pH8.0) micro-L	25	25	50	10	10	10	20	20	20
Cycloheximide micro-L	0	0	0	0	20	0	0	40	0
Chloramphenicol micro-L	0	0	0	0	0	20	0	0	40
14C-glucose micro-L	50	0	0	0	0	0	0	0	0
14C-glutamic acid micro-L	0	50	0	0	0	0	0	0	0
14C-amino acid mix micro-L	0	0	0	20	20	20	40	40	40
14C-NaHCO3 micro-L	0	0	100	0	0	0	0	0	0
DW micro-L	175	175	350	70	50	50	140	100	100
Total (micro-L)	500	500	1000	200	200	200	400	400	400
total radioactivity in MBq	0.185	0.185	0.925	0.074	0.074	0.074	0.148	0.148	0.148
Total radioactivity in mCi	0.005	0.005	0.025	0.002	0.002	0.002	0.004	0.004	0.004
Tricine buffer (concentration of Tricine): 400mM									
ASW contains:									

Table 1 Reagents for the experiments

Clam	dive	Size in mm (Length, Hight,Thickness)	Reagent	Injection (μL)	Time(injection)	Time (end)	Period (min?)	Blood vol. in mL	Gill	Foot	Adductor muscle	mantle	Others	Notes
B01-49	HPD922	126x58x43	Gls	250	19:58(081209)	9:56 (081210)		50	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、入出水管は出ていて元気。Injection with 27G needle.培養時の海水量は適当(500mL??)
B01-50	HPD922	133x55x36	Gls	250	20:10(081209)	11:04 (081210)		27.5	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、入出水管は出ていて元気。Injection with 27G needle.培養時の海水量は適当(500mL??)
B01-51	HPD922	120x55x36	GT	250	20:18 (081209)	11:45 (081210)		25.2	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、泥に埋まった状態だが、元気。Injection with 25G needle.培養時の海水量は適当(500mL??)
B01-52	HPD922	123x55x34	GT	250	20:22 (081209)	14:17 (081210)		28	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、上(入出水管の方)は海水から出ていたが、元気の様子。培養時の海水量は適当(500mL??)
B01-53	HPD922	131x63x29	AA mix	200	22:05 (081209)	15:00 (081210)		35.0 (serum=26.0: cells=9ml)	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、培養時の海水量は適当(500mL??)
B01-54	HPD922	139x60x40	AA+CY	200	22:16 (081209)	15:51 (081210)		35	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血なし、培養時の海水量は適当(500mL??)
B01-56	HPD922	126x56x34	AA+CM	200	22:34 (081209)	16:30 (081210)		25	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	出血あり、培養時の海水量は適当(500mL??)
B01-61	HPD922	152x65x45	BC	330	22:46 (081210)	23:50 (081210)		22	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?
B01-62	HPD924	95x46x32	BC	330	22:52 (081210)	09:31 (081211)		12.5	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?.わずかに出血あり
B01-63	HPD924	113x54x36	BC	330	22:56 (081210)	11:51 (081211)		19	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?
B01-64	HPD924	128x63x38	AA mix	400	23:03 (081210)	10:03 (081211)		42	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?
B01-65	HPD924	98x44x27	AA+CY	400	23:09 (081210)	10:42 (081211)		10	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?
B01-66	HPD924	122x53x39	AA+CL	400	23:14 (081210)	11:16 (081211)		25	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	frozen at -30 C.	培養時の海水量は容器のねじのところまで、500mL?

Table 2 samples

Bacterial research

Yuichi Nogi, Takahiko Nagahama, Masayuki Miyazaki, Masaki Sumiyoshi, Hiromi Uchida, Eriko Takahashi (JAMSTEC)

Whale fall community

MBARI core sampling was performed at various depth at the whale fall site, and then variation change of bacteria according to depth was compared by 16S rRNA gene analysis. Compared with previous data, time course of bacterial change at whale fall site are analysed. Moreover, novel bacteria are going to be isolated from a sterile core sample. *In situ* culture instrument were setted at a color-changed region below a whale bone. It will be recovered after 10 days (next cruise).

bacteria isolation from tube worm

Lamellibrachia sp. and *Alaysia* sp. were collected to isolate symbiotic and adhesive bacteria. After plume, trophosome and others of tube worms were cutted and homogenised, the extract was added to ten kinds of medium, and is incubating.

Isolation of bacteria degrading persistent substances

Bacterial traps containing cellurose, chitin, and polylactate membrane was setted at tube worm site and whale fall site. At tube worm site, the trap was recovered after five days. The trap at whale fall site is going to be recovered after 10 days. Recovered traps are incubating until colonies of a bacterium degrading persistent substances appears. we added sterile core sample to various medium and try to bacterial isolation by enrichment culture.

Research for Fungi

To detect uncultured Fungi strain MBF, crustacean were collected by bait traps or suction sampler. We tried to observe microscopically, perform DNA cloning, and isolate fungi from body surface and digestive truct of samples obtained. To culture Malassezia-like fungi, application to medium and embedding treatment were tried *in situ*. Moreover, we tried to isolate fungi from core samples and collected sea water.

Results from these experiment were revealed after arrival to JAMSTEC.

Ecological succession of a whale fall in Sagami Bay

Yoshihiro FUJIWARA, Masaru KAWATO
Florence PRADILLON, Atsushi NAGAHORI

Objective:
To understand
species succession of *Osedax* polychaetes
dominancy of symbiont-harboring mytilids
whale-fall succession
on the *Sagami* whale.

Results:
Species succession of *Osedax* polychaetes
One rib of the *Sagami* whale was collected during the dive # 921. A large number of *Osedax* with white/pink colored palps was attached on the bone, which was morphologically similar to *Osedax* "smooth palps" collected during NT07-22 cruise. Molecular phylogenetic analysis will be conducted soon.

Symbiont-harboring mussels
Several specimens of mussels were found on the rib. The shell length was approximately 5 mm. The density was comparable to that in NT07-22. Molecular phylogeny of host and symbionts will be analyzed in addition to ultrastructural observations.

Whale-fall succession
In comparison with the Nomamisaki whales, degradation of the Sagami whale carcasses was relatively slow (Fig. 1). The most abundant mega fauna was still *Osedax* polychaetes and a little number of mussels appeared. A thyasirid clam was the dominant infauna except polychaetes. Several specimens of a *Munidopsis* crab were firstly observed at the Satomi whale.

Fig. 1. Overview of the Sagami whale at a depth of 925 m.

Operation of IISA (Integrated in situ Analyzer) –ATP and onboard analysis for ATP-based microbial activity assay for deep-sea microbiology

Tatsuhiro Fukuba
(Institute of industrial science, The Univ. of Tokyo)
Yusuke Aoki
(The Univ. of Tokyo)

In this cruise, we operated an integrated in situ analyzer (IISA) –ATP (Fig. 1) to estimate microbial activity in deep-sea environment. IISA-ATP has a "microfluidic device" (Fig. 2) as a core functional element for quantitative determination of microbial ATP with luciferin-luciferase (L-L) bioluminescence based method. The microfluidic device is connected with a pumping device that contains miniature pumps, valves and tubing. The microfluidic device and the pumping device are packaged in an oil-filled housing as an analysis module. Photo intensity of bioluminescence that is emitted from the microfluidic device is measured by photo multiplier tube (PMT) mounted in a cylindrical pressure resistant housing. Electronics to control IISA-ATP is also enclosed in a same housing as a control module. The analysis module and the control module are connected together using underwater cables. The analysis module is also connected to underwater vehicles for power supply and communication.

Fig. 1 IISA-ATP

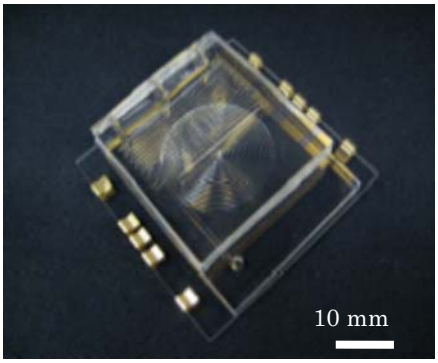


Fig. 2 Microfluidic device for IISA-ATP

IISA-ATP was mounted on an underwater vehicle "HYPER DOLPHIN" for in situ trial operation at off-Hatsushima methane seep site (Fig. 3) during dive #916, 919 and 920. Table 1 shows date, dive#, times of ATP quantification with IISA-ATP during this cruise. As a result, IISA-ATP was successfully operated in surface and deep-sea environment and microbial ATP was determined near methane seep area during dive #916, #919 and #920. In all dives, IISA-ATP had a trouble on its heating system. It was caused by water leakage to one of an underwater cable used for IISA-ATP (#916) and electrical circuit breakdown (#919 and 920). However, detectable signal of bioluminescence caused by luciferin-luciferase reaction was measured in situ under environmental temperature condition. During dive #919 and 920, total and extracellular ATP concentrations were successfully determined by IISA-ATP operations. Raw data that was obtained in dive #919 is shown in fig 4.

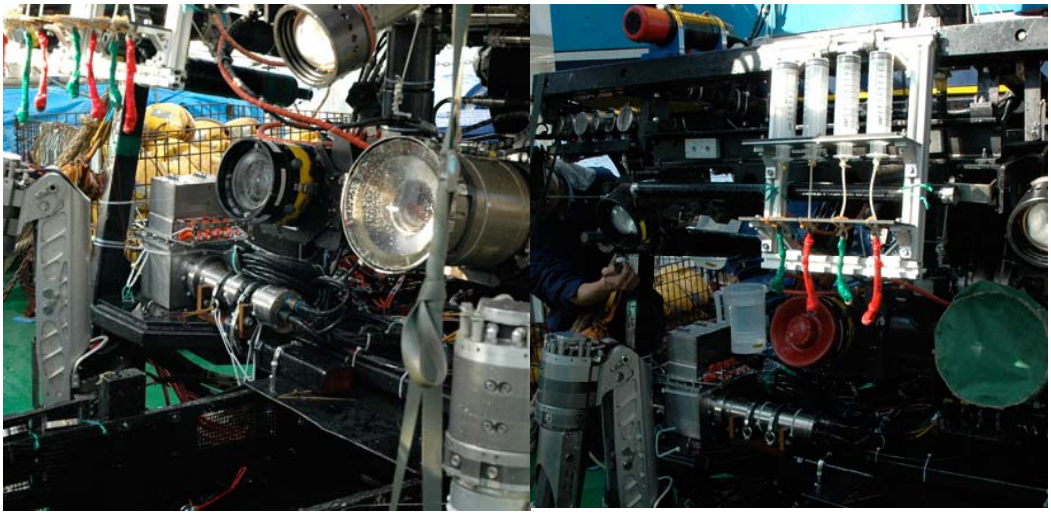


Fig. 3 IISA-ATP and a syringe water sampler mounted on HYPER-DOLPHIN

Table 1 List of IISA-ATP operation during NT08-24 cruise

Date	Dive#	ATP quantification	Note
Dec. 4	#916	13 calib., 3 samples	Heater had a trouble
Dec. 6	#919	15 calib., 6 samples	Heater had a trouble
Dec. 6	#920	7 calib., 6 samples	Heater had a trouble

To obtain control data, water samples were collected utilizing a “syringe water sampler”. 100 ml of water samples were collected at the same position of IISA-ATP operation. The collected samples were processed onboard manually to quantify microbial ATP with L-L method and conventional photo-detector device (Lumi-tester C-110, Kikkoman Co., Japan). Preliminary data is shown in table 2. The data indicates surface water has 15 – 30 times higher ATP contents compare to that of deep-sea water. HPD#920-W04 sample shows slightly higher ATP concentration than that of the other deep-sea samples. The HPD#920-W04 sample was collected at biologically active site.

Table 2 ATP concentration measured by using tabletop apparatus

Date	Dive#	Sample ID	Sample Note	ATP conc.
Dec.4	#916	HPD#916-W03	Surface	3.2×10^{-10} M
Dec.4	#916	HPD#916-W04	Bottom	1.2×10^{-11} M
Dec.6	#919	HPD#919-W03	Surface	2.8×10^{-10} M
Dec.6	#919	HPD#919-W04	Bottom	1.3×10^{-11} M
Dec.6	#919	HPD#919-W05	Bottom	1.3×10^{-11} M
Dec.6	#920	HPD#920-W02	Surface	3.1×10^{-10} M
Dec.6	#920	HPD#920-W03	Bottom	1.3×10^{-11} M
Dec.6	#920	HPD#920-W04	Bottom	1.9×10^{-11} M
Dec.6	#920	HPD#920-W05	Bottom	1.4×10^{-11} M

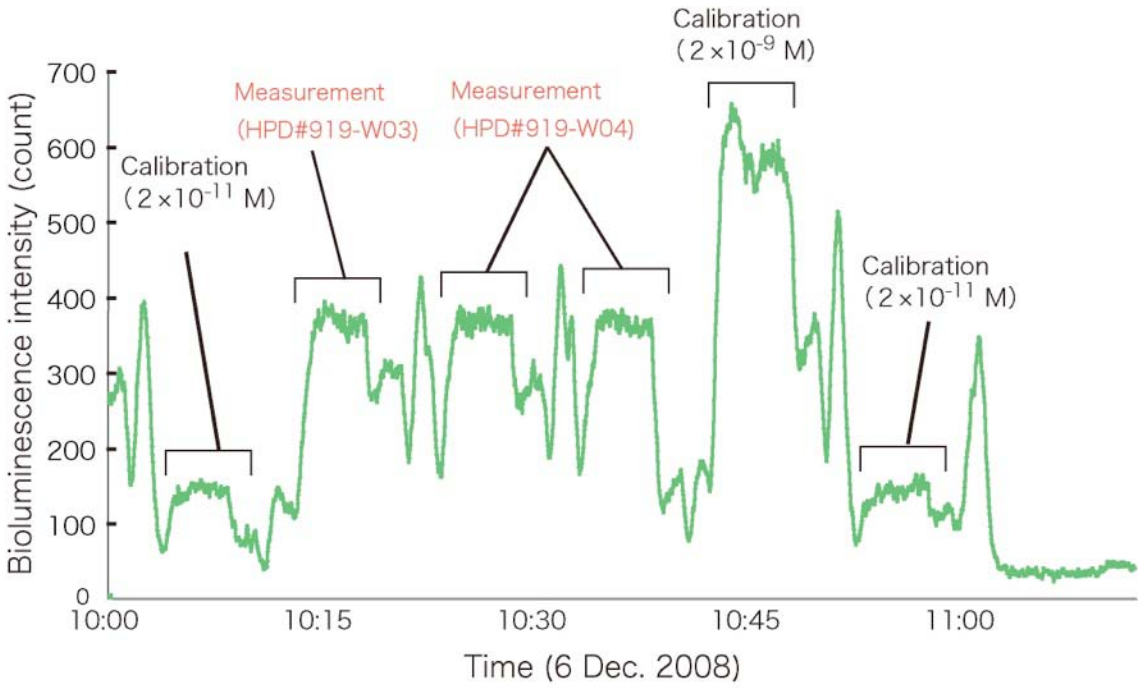


Fig. 4 Raw data (dive #919, 10:00 – 11:00)

Mitsugu Kitada
Enoshima aquarium

In Enoshima Aquarium, we have been trying to cultivate some of the deep-sea animals inhabiting in hydrothermal vent and seep, and establishing a cultivation system to raise these animals.
During this cruise, we have collected animals using the suction sampler system, rake and sorted all the samples. We are going to cultivate these animals long term in Chemosynthetic ecosystem aquarium.

Chemosynthetic ecosystem aquarium

The water temperature is kept around 4°C.
On the left side of the tank, hot water including H_2S and CO_2 venting from chimneys. On the right side of the tank, floor is filled with mud including dog food

Display of the Deep-sea animals collected in NT08-24 cruise.

Deep-sea white clam Calyptogena soyoae

Tube worm Alaysia sp.

Deep water eelpout Ericander sagamia

Deep-sea mussel Bathymodiolus platifrons

- 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 classifications) 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.