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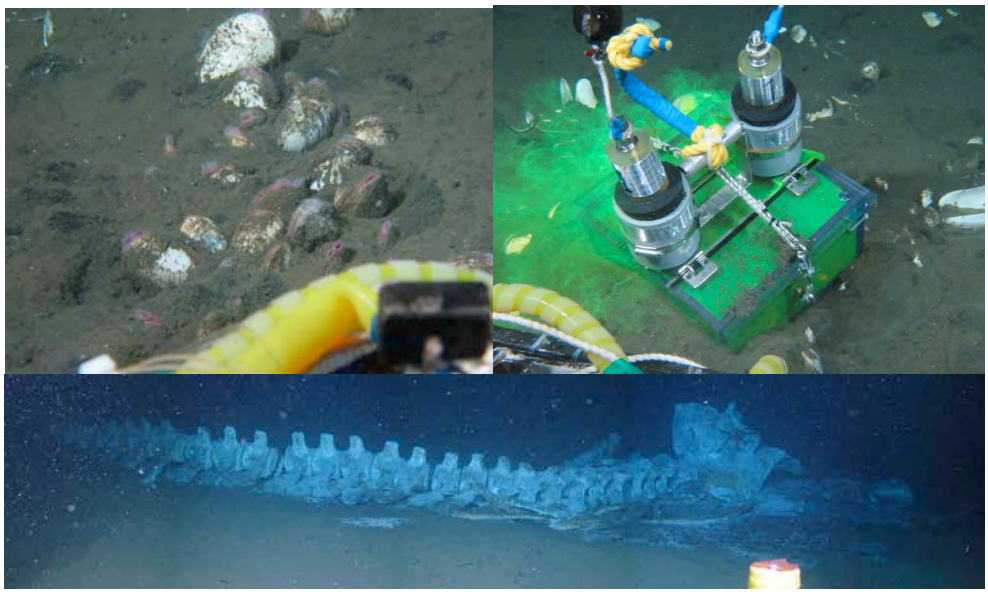
Onboard Report

Of

The Hyper-Dolphin/Natsushima Cruise in Sagami Bay

(NT10-01)



January 12-19, 2010

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

Contents of NT10-01 cruise report

Abstract

The cruise NT10-01 took place from January 12th to 19th, 2010, in Sagami Bay, onboard the R/V Natsushima and with the ROV Hyper-Dolphin. A total of 9 dives were conducted at different study sites including a whale fall site, cold seeps, and a deep-sea observatory near Hatsushima Island. Different projects were carried out.

Biodiversity, dispersal and colonization mechanisms at whale falls were studied at an experimental site where 2 sperm whale carcasses are implanted since 2005 and 2008 respectively. Almost yearly surveys were performed at this site to observe carcass decay, the establishment of chemosynthesis-dependant animal communities and species succession. During this cruise, we continued to document these processes and compared the two carcasses. Bones were collected as well as surrounding sediments, plankton, benthos and water. Preserved samples and live specimens were brought back to our laboratory where species diversity, reproduction, nutrition and symbiosis will be investigated.

Studies at cold seeps sites were focused on *Calyptogena* clams. Distribution patterns of two coexisting species, reproduction, life-history traits, and symbiosis were investigated through sampling and *in situ* experiments.

The third project involved the long-term monitoring of the interactions between organisms and their chemical environment at the sediment-water interface. During this cruise we recovered a lander with a 2 dimension optopode system that monitored the sea bottom near station off Hatsushima Island for 9 months.

要旨

2010年1月12日から19日まで「なつしま」/「ハイパードルフィン」を用いたNT10-01航海を相模湾で実施した．計9潜航により，3つの異なるプロジェクトを鯨骨域，湧水域および初島沖長期観測ステーション近傍で実施した．

2005年と2008年に沈設した2頭のマッコウクジラ遺骸周辺では，生物多様性，分散，蝟集機構を解明するための調査を実施した．このサイトではほぼ毎年調査を実施しており，化学合成生物群集の形成と遷移に関する情報を蓄積している．本航海でもこのようなプロセスの継続的な記録と海底沈設期間の異なる2頭の鯨遺骸間での各種比較を実施した．試料としては，鯨遺骸周辺の堆積物や海水，蝟集するベントス，プランクトン，鯨骨を採集した．試料は冷凍，固定もしくは飼育した状態で持ち帰り，種の多様性，繁殖，栄養摂取，共生について研究室で詳細な研究を実施する予定である．

初島沖湧水域では，生息する2種のシロウリガイ類の分布パターンや繁殖，生活史，共生に着目して試料採集と現場実験を実施した．

また堆積物-水境界の生物活動と化学環境との関係を明らかにするための長期観測実験を実施した．本航海では初島沖長期観測ステーション近傍で9ヶ月間観測を継続していたランダー（2次元オプトードシステムを装備）の回収を実施した．

1. Cruise Information

Cruise number: NT10-01
Ship name: R/V Natsushima
Submersible: ROV Hyper-Dolphin
Title of the cruise: Succession patterns and colonization mechanisms of chemosynthetic organisms associated to whale falls in Sagami Bay
Chief Scientist: PRADILLON Florence (Jamstec)
Proposal numbers and titles:
S09-79: Succession patterns and colonization mechanisms of chemosynthetic organisms associated to whale falls in Sagami Bay (Pradillon F.)
S09-38: What is the biological difference between *Calyptogena soyoae* and *C. okutanii* ? (Fujikura K.)
S09-28: Long term monitoring of bidimentional O2 profiles at the sediment-water interface in Sagami Bay (Oguri K.)
Cruise period: January 12 - 19, 2010
Port call: January 12 Departure from JAMSTEC Yokosuka
January 19 Arrival at JAMSTEC Yokosuka
Research Area: Sagami Bay, within the area located between the following coordinates: 35º00.0'N - 139º12.0'E and 35º05.0'N - 139º14.0'E. Depth: 800 m – 1190 m. See Figure 1.
Cruise schedule:

01/12	Departure from Jamstec
	XBT
01/13	Dives cancelled due to weather conditions
01/14	HD#1074
01/15	HD#1075 & HD#1076
01/16	Scientists exchange at Itoh port
	HD#1077 & HD#1078
01/17	HD#1079 & HD#1080
01/18	HD#1081 & HD#1082
01/19	Disembarking at Jamstec

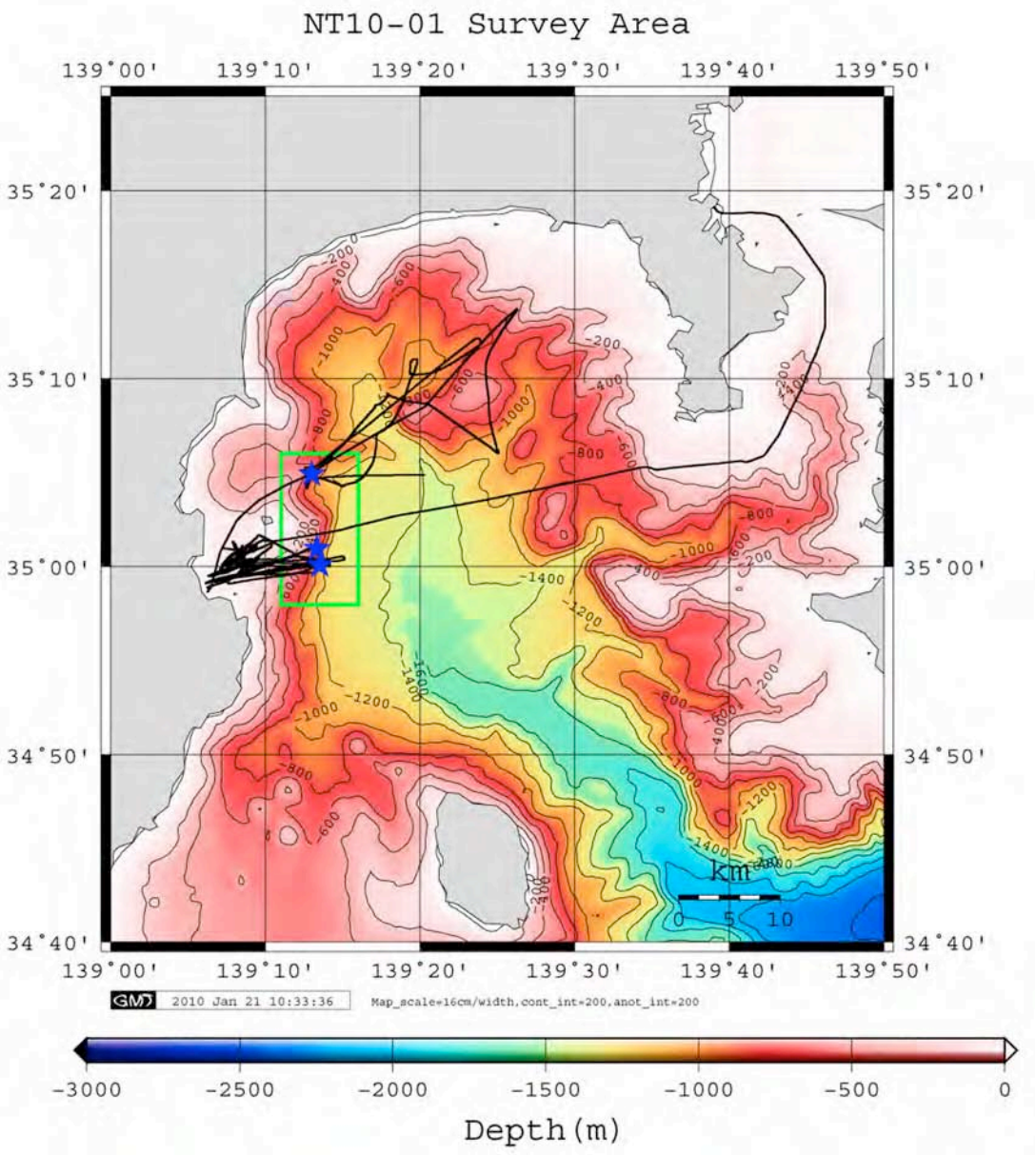


Fig. 1. Survey area and ship track.

2. Research group

Chief Scientist (PI S09-79)	PRADILLON Florence (JAMSTEC) FUJIWARA Yoshihiro (JAMSTEC) KAWATO Masaru (JAMSTEC) MIYAZAKI Masayuki (JAMSTEC) NAGAHORI Atsushi (JAMSTEC) SHINOZAKI Ayuta (JAMSTEC) OKOSHI Kenji (Ishinomaki Senshu University) ITO Nozomi (Ishinomaki Senshu University) SUZUKI Masahiro (Ishinomaki Senshu University) YAMAGUCHI Ryuhei (Ishinomaki Senshu University) SHIMAMURA Sho (Okayama University) NISHIMURA Asuka (Kagoshima University) HIGGS Nicholas (University of Leeds, UK) TADA Satoshi (Tokyo Sea Life Park) AMEMIYA Kentaro (Tokyo Sea Life Park) NODA Chikayo (Aburatsubo Marine Park)
PI S09-38	FUJIKURA Katsunori (JAMSTEC) YOSHIDA Takao (JAMSTEC) KOYAMA Sumihiro (JAMSTEC) WATANABE Hiromi (JAMSTEC) NAKAMURA Yoshimitsu (JAMSTEC) HONGO Yuki (JAMSTEC) SEO Eriko (JAMSTEC)
PI S09-28	OGURI Kazumasa (JAMSTEC) NOMAKI Hidetaka (JAMSTEC)
Marine technician	OKADA Satoshi (Nippon Marine Enterprises, Ltd.)

3. Proposals

3.1. Succession patterns and colonization mechanisms of chemosynthetic organisms associated to whale falls in Sagami Bay

Florence Pradillon, Yoshihiro Fujiwara, Masaru Kawato, Atsushi Nagahori, Ayuta Shinozaki, Masayuki Miyazaki.

Sunken whale carcasses trigger the development of dense animal communities that may persist over decades, due to the massive and sudden amount of organic matter supplied to the seafloor. Such island-like habitats are scattered on the sea floor, and their fast colonization by specialized species remains poorly understood. Several of the most abundant species endemic to whale carcasses live in symbiosis with heterotrophic (polychaete *Osedax*) or sulphur-oxidyzing autotrophic bacteria (mussels *Adipicola*). About 30 species are shared between whale falls and hydrothermal vents or cold seeps. These ecological and zoological affinities were seen as an evidence that organic-based chemosynthetic environments such as whale falls may have been stepping-stones to the colonization of deep-sea chemosynthetic environment. However, our understanding of the dispersal and colonization processes of endemic organisms is still hampered by the difficulties to collect the dispersing larval stages *in situ*, and to culture adult animals in the laboratory.

Lon-term studies on implanted whale carcasses have revealed a faunal succession following the arrival of a fresh whale carcass at the deep-sea floor. Although the succession patterns appear to be relatively conserved, differences in the timing of the succession may arise due to the size of the carcass, the depth at which it sank, or the whale species. Long-term observations of the evolution of whale-fall associated communities are therefore needed to understand the mechanisms governing the succession of the communities, and the rates of organic carbon redistribution from the carcass to the benthic ecosystems.

The polychaete *Osedax* seems to play a major role in bone break down. These animals are able to colonize carcasses as soon as the bones become exposed to seawater. Females harbor symbiotic heterotrophic bacteria in a “root” structure at the posterior end of their body. This root infiltrates bones and extracts lipid and collagen for nutrition. Since the initial description of *Osedax*, 17 species were identified from northern Pacific and Atlantic Oceans, and the genus appears to be spread worldwide.

Recently, *Osedax* populations from 2 sites at different depths (off Nomamisaki 220-250 m, and Sagami Bay 925 m) around Japan were investigated. Both sites were repeatedly surveyed, and animal communities colonizing the bones were sampled. *Osedax* species were consistently present on the bones at each survey (Fig. 3-1).

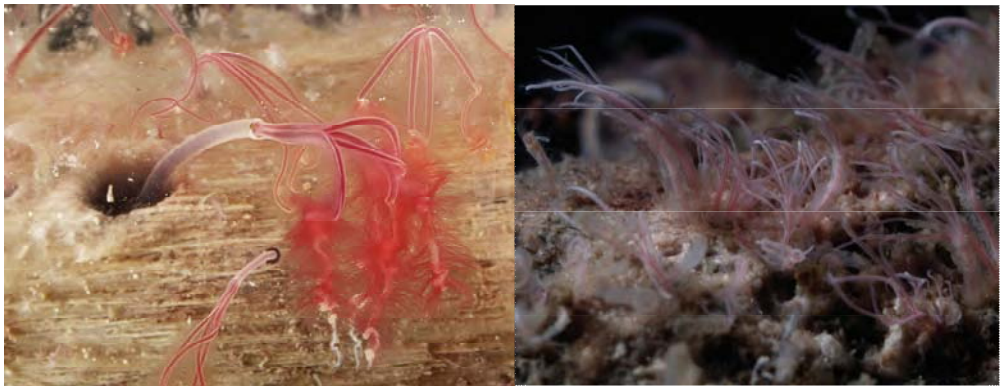


Fig.3-1. *Osedax* species on sperm whale bones at 925 m depth in Sagami Bay. Left: *Osedax rubiplumus* and *O. roseus*. Right: *Osedax* "nude palps".

In Sagami Bay, the succession and coexistence of a large number of *Osedax* species with apparently similar ecological requirements on a very narrow niche is intriguing. On the other hand, only one species, *O. japonicus*, has ever been observed at off Nomamisaki (225-250 m depth). Such differences may be explained by specific reproductive and dispersal strategies, which may influence the geographical and bathymetric range available to each species.

Studies on embryonic development showed remarkably rapid developmental rates even at low temperature. This feature is usually associated with short dispersal range, suggesting that spatial and temporal frequency of suitable bones may be essential for maintaining *Osedax* population.

During this cruise, we will conduct Hyper-Dolphin dives at two sperm whale carcasses implanted at 925 m depth in Sagami Bay. The first carcass was implanted in 2005, after which it was observed and sampled 5 times. Succession of species was observed, and in particular we identified early and late colonizers among *Osedax* polychaetes. In December 2008, a 2nd whale "Satomi" was immersed about 100 m away from the first one. This was a good opportunity to compare species diversity and succession on two carcasses in close proximity and at different degradation stages. With such settings, we have the possibility to answer the following questions: (1) is the succession of Osedax species always identical locally or does species arrival timing changes ? (2) Is the time segregation pattern observed most likely due to differences in dispersal strategies or in the substrates requirement of each species ?

Two dives were already conducted as part of the project S09-79 during the cruise NT09-06 in May 2009.

3.2. Which biological differences exist between Calyptogena soyoae and C. okutanii ?

Katsunori Fujikura, Takao Yoshida, Hiromi Watanabe, Eriko Seo, Yoshimitsu Nakamura, Yuki Hongo, Hidetaka Nomaki, Kazumasa Oguri and others.

Since the discovery of deep-sea methane seep communities at the Off Hatsushima Island sites in Sagami Bay, various biological investigations have been conducted using submersible systems. General information on mega-faunal composition, population density, symbiosis system, reproduction, food web etc … have been accumulated so far. Two species of *Calyptogena* clams are dominant species in the Off Hatsushima Island sites. Both species have symbiotic bacteria in their gills to get nutrient and have similar ecological niche in the community. Generally speaking, species with same ecological niche are hard to maintain in populations due to competition. However, we lack detailed ecological characteristics about both species. The purpose of this investigation is to understand ecological differences between *Calyptogena soyoae* and *C. okutanii*. We have following objectives:

1. Understanding distribution pattern differences,
2. Understanding minimum maturation size,
3. Understanding early life history and dispersal potential,
4. Understanding prevention mechanism of hybridism,
5. Growth rate estimation,
6. Understanding transmission way of symbiotic bacteria from parents to the next generation.

To make clear these scientific objectives, we would like to carry out ecological and physiological investigations at the Off Hatsushima Island sites using ROV Hyper-Dolphin system. We already conducted dive investigations in last April to May (cruise NT09-06). The present cruise is a complement to that previous cruise.

3.3. Long term monitoring of bidimentional O2 profiles at the sediment-water interface in Sagami Bay

Kazumasa Oguri, Hidetaka Nomaki, Sumihiro Koyama

To understand interactions between benthic organisms and chemical (a.k.a. redox, CO_2 production, O_2 consumption etc.) environments at sediment-water interface (SWI), long term monitoring of key elements is significant. In previous studies, measurements of O_2, H_2S, pH and other elements profiles were measured with microelectrodes *in situ*. However, long term monitoring to investigate environmental changes at SWI driven by supply of organic materials from water column, changes of benthic activities and effects caused by other unexpected phenomena such as a shaking by earthquake, were less carried out due to technical limitations of a development of sensors. Our study was focused especially on oxygen dynamics because O_2 is most essential element for oxidation and respiration to sedimentary environments and organisms, respectively. A planar O_2 optode system which measures time lapse two dimensional O_2 profiles can observe such changes, and the purpose of our research is to monitor two dimensional O_2 profiles at SWI for more than half year. To supply power, the optode system was developed to connect extension

power cable from the Hatsushima station. The deployment was started at off Hatsushima deep sea station on 30th April, 2009 during the cruise NT09-06. Since then, the planar O_2 optode system has measured two dimensional O_2 profiles at sediment-water interface at one hour interval. During the monitoring, the optode system was powered from the land station via Hatsushima station, and several thousands of O_2 profile was expected to be recorded by 12th January 2010, although several unexpected shutdown and troubles took place.

The aim of the dive is to recover the optode system and its frame (so called lander system) from the south of Hatsushima deep sea station, and recover the data stored in the optode system.

3.4. Other related projects

- Keeping *Calyptogena* sp. using a piezostat-aquarium system with potentiostat (Sumihiro Koyama).
- Succession of microbial diversity and taxonomic studies in whale-fall community (Masayuki Miyazaki)
- Symbiotic systems and Evolution of deep sea clam *Calyptogena* and intracellular sulfur oxidizing bacteria (Yoshimitsu Nakamura, Yuki Hongo, Akihiro Tame and Takao Yoshida)
- Osedax borings on whale bones (Nicholas Higgs)
- Development, growth and survival in the whale bone attached mussels ((1) larval studies of mussels, (2) shell morphology and microstructure) (Kenji Okoshi, Nozomi Ito, Masahiro Suzuki and Ryohei Yamaguchi)
- Geochemical environment and food web structure at whale fall sites (Sho Shimamura)

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)
1074	Ecology and physiology of seep biota	Seep site Sagami Bay	WGS-84	10:39	35°00.070'N	139°13.527'E	1181
1/14/10				14:17	35°00.072'N	139°13.477'E	1170
1075	Removal of extension cable from Hatsushima permanent station and optode system, respectively. And recovery of the lander system. And collection of two sediment cores.	Seep site Sagami Bay	WGS-84	9:01	35°00.137'N	139°13.523'E	1189
1/15/10				10:21	35°00.154'N	139°13.505'E	1179
1076	Ecology and physiology of seep biota	Seep site Sagami Bay	WGS-84	14:26	35°00.954'N	139°13.329'E	853
1/15/10				16:25	35°00.940'N	139°13.222'E	805
1077	Succession patterns and colonization mechanisms at whales falls	SATOMI whale Sagami Bay	WGS-84	8:52	35°04.933'N	139°13.001'E	923
1/16/10				12:37	35°04.929'N	139°12.990'E	924
1078	Succession patterns and colonization mechanisms at whales falls	SATOMI whale Sagami Bay	WGS-84	13:38	35°04.913'N	139°12.994'E	926
1/16/10				16:27	35°04.929'N	139°12.990'E	925
1079	Succession patterns and colonization mechanisms at whales falls	SATOMI whale Sagami Bay	WGS-84	8:57	35°04.900'N	139°12.988'E	919
1/17/10				10:38	35°04.922'N	139°12.983'E	925

1080	Succession patterns and colonization mechanisms at whales falls	SAGAMI whale in Sagami Bay	WGS-84	13:51	35˚04.989'N	139˚13.046'E	940
1/17/10				15:39	35˚04.992'N	139˚13.006'E	928
1081	Succession patterns and colonization mechanisms at whale falls	SAGAMI whale in Sagami Bay	WGS-84	8:49	35˚04.982'N	139˚12.054'E	940
1/18/10				10:37	35˚04.990'N	139˚12.017'E	934
1082	Succession patterns and colonization mechanisms at whale falls	SATOMI whale in Sagami Bay	WGS-84	13:17	35˚04.897'N	139˚13.987'E	912
1/18/10				14:53	35˚04.935'N	139˚13.987'E	925

Preliminary results of the ROV Hyper-Dolphin dive #1074

Date: January 14, 2010

Site: Off Hatsushima Island site, seep site, Sagami Bay

Landing: Time: 10:39, Lat: 35˚00.07′N, Long: 139˚13.527′E, Depth: 1181 m

Leaving: Time: 14:17, Lat: 35˚00.072′N, Long: 139˚13.477′E, Depth: 1171 m

Chief observer: FUJIKURA Katsunori (JAMSTEC)

Purpose: Growth rate estimation of Calyptogena spp. and foraminifera, symbiotic relationship between Calyptogena and bacteria, early life history of bivalves, cell culture of Calyptogena sp., function of blood cells, pig bone observation.

Dive summary: An *In-situ* staining experiment with chemical marker (calcein and strontium) was conducted to estimate growth rates of *Calyptogena* spp. and foraminifera. *Calyptogena* spp. stained experimentally 8 months earlier (NT09-06) in a similar experiment, were retrieved from the seep site. Sediments were also collected by MBARI cores and scoop sampler, as well as benthic organisms including bivalves, gastropods, polychaetes, crustaceans, sipunculoida and so on, by a scoop sampler and suction sampler. Pig bones deployed in May 2009 (NT09-06) were recovered.

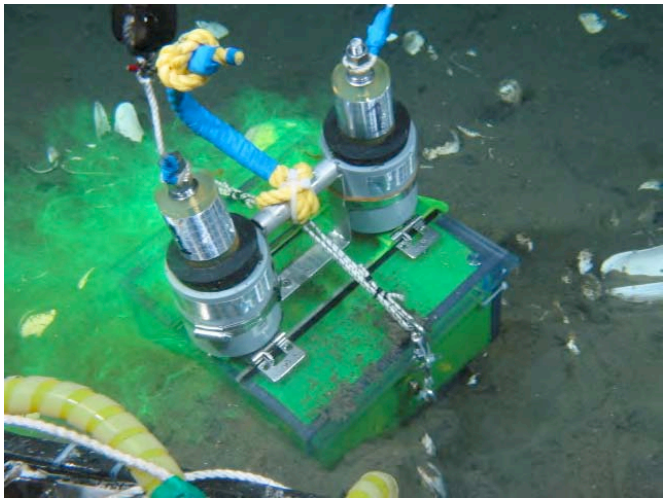


Fig. 4-1. Staining experiment with *Calyptogena* spp.

Preliminary results of the ROV Hyper-Dolphin dive #1075

Date: January 15, 2010

Site: Off Hatsushima Island, Sagami Bay

Landing: Time: 9:01, Lat: 35˚00.137′N, Long: 139˚13.523′E, Depth: 1189 m

Leaving: Time: 10:21, Lat: 35˚00.154′N, Long: 139˚13.505′E, Depth: 1179 m

Chief observer: OGURI Kazumasa (JAMSTEC)

Purpose: The aim of the dive is to recover the optode system and its frame (so called lander system) from the south of Hatsushima deep sea station, and recover the data stored in the optode system.

Dive summary: Prior to the recovery, the ROV Hyper-Dolphin moved to the Hatsushima station and disconnected the extension cable through which the lander was connected. Two push cores were collected at the lander's place, the extension cable was disconnected from the optode system, and the lander was hung with a hook by the ROV before ascent to the surface and recovery on board R/V Natsushima.

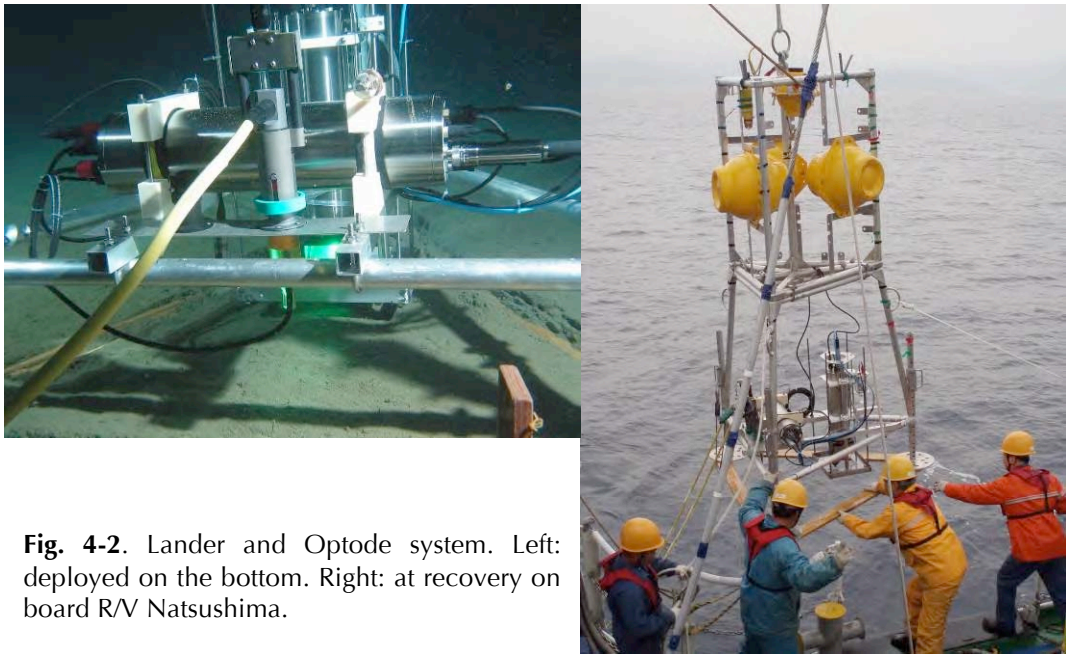


Fig. 4-2. Lander and Optode system. Left: deployed on the bottom. Right: at recovery on board R/V Natsushima.

Preliminary results of the ROV Hyper-Dolphin dive #1076

Date: January 15, 2010

Site: Off Hatsushima Island, 800 m depth seep site, Sagami Bay

Landing: Time: 14:26, Lat: 35˚00.954'N, Long: 139˚13.329'E, Depth: 853 m

Leaving: Time: 16:25, Lat: 35˚00.94'N, Long: 139˚13.222'E, Depth: 805 m

Chief observer: YOSHIDA Takao (JAMSTEC)

Purpose: Life history of *Calyptogena* clams (growth, development of gametes, hybridism), symbiosis of *Calyptogena* clams, and function of *Calyptogena* blood cells.

Dive summary: After landing near the 800 m depth seep-site, we found a *Bathymodiolus* mussels colony on rocks at 858 m depth (35-00.955N, 139-13.317E). Mussels were collected by suction sampler. After that, we tried to collect *Calyptogena* gametes by using an *in situ* gamete sampling box deployed on a small colony at 856m (35-00.960N, 139-13.317E). Sperm was spread after warming. However, eggs were not spawned. After pumping the water and gametes from the sampler, *Calyptogen*a clams in the sampler were collected by scoop. Then, we moved to the *Calyptogena* growth staining box. We found that

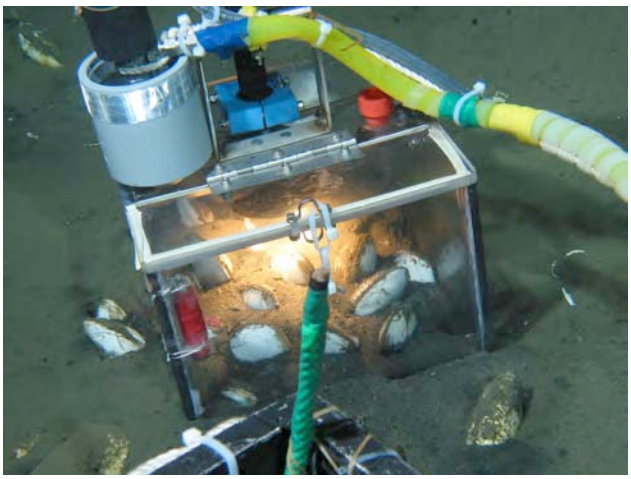


Fig. 4-3. *In situ* gamete sampler deployed on a *Calyptogena* colony, with heater (light) functioning.

the two staining boxes had dropped out. To get the stained samples, mud sample was collected by MBARI-core (red), and *Calyptogena* clams were collected by suction sampler.

Preliminary results of the ROV Hyper-Dolphin dive #1077

Date: January 16, 2010
Site: Off Hatsushima Island, Satomi Whale fall site, Sagami Bay
Landing: Time: 09:41, Lat: 35˚04.933'N, Long: 139˚13.001'E, Depth: 923 m
Leaving: Time: 11:58, Lat: 35˚04.929'N, Long: 139˚12.990'E, Depth: 924 m
Chief observer: PRADILLON Florence (JAMSTEC)
Purpose: Observe the Satomi whale carcass after 13 months on the seafloor.
Dive summary: We observed the whole skeleton, which had been deployed on the seafloor for 13 months, and was surrounded with blackened sediments and white bacterial mats. Bones were still mostly intact, except for the tip of the jaw bones that were heavly colonized by Osedax. We collected a rib partially colonized by Osedax, as well as sediments and plankton.

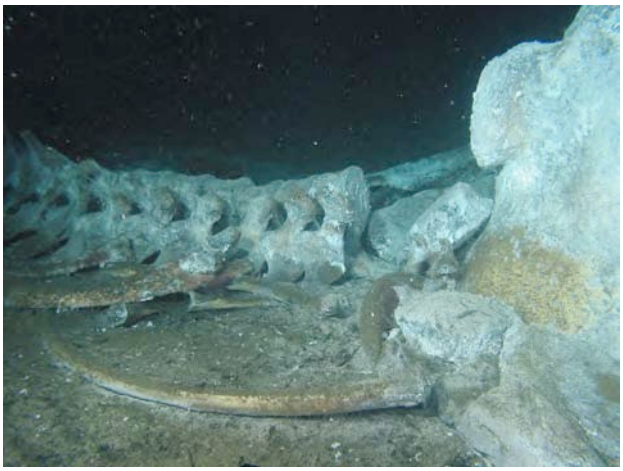


Fig. 4-4. Vertebrae, ribs and back of the skull of Satomi Whale.

Preliminary results of the ROV Hyper-Dolphin dive #1078

Date: January 16, 2010
Site: Off Hatsushima Island, Satomi Whale fall site, Sagami Bay
Landing: Time: 13:38, Lat: 35˚04.913'N, Long: 139˚12.994'E, Depth: 926 m
Leaving: Time: 16:27, Lat: 35˚04.929'N, Long: 139˚12.990'E, Depth: 925 m
Chief observer: FUJIWARA Yoshihiro (JAMSTEC)
Purpose: Observe the Satomi whale carcass after 13 months on the seafloor.
Dive summary: We continued the observation of the Satomi whale skeleton**,** and collected sediments around the carcass, plankton and benthic animals. We also deployed a plankton pump near the carcass (< 1m), which was programmed to start pumping for several hours after the ROV departure.

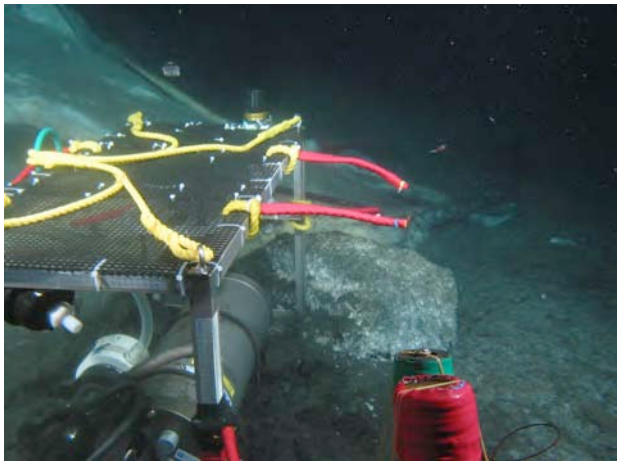


Fig. 4-5. Plankton pump deployed near the head part of the whale carcass.

Preliminary results of the ROV Hyper-Dolphin dive #1079

Date: January 17, 2010
Site: Off Hatsushima Island, Satomi Whale fall site, Sagami Bay
Landing: Time: 8:57, Lat: 35˚04.900′N, Long: 139˚12.988′E, Depth: 919 m
Leaving: Time: 10:38, Lat: 35˚04.922′N, Long: 139˚12.983′E, Depth: 925 m
Chief observer: WATANABE Hiromi（JAMSTEC)
Purpose: Succession patterns and colonization mechanisms at whale falls
Dive summary: The plankton pump deployed on the previous day was partially buried into sediments, but the head part (inlet) looked fine. Before retrieving the plankton pump, sediments and zoarcid fishes were collected, and a colonization device was deployed on the sediment near the head part of the skeleton.

Fig. 4-6. Head part (skull and jaw) of the Satomi skeleton.

Preliminary results of the ROV Hyper-Dolphin dive #1080

Date: January 17, 2010
Site: Off Hatsushima Island, Sagami Whale fall site, Sagami Bay
Landing: Time: 13;51, Lat: 35˚04.989′N, Long: 139˚13.046′E, Depth: 940 m
Leaving: Time: 15:39, Lat: 35˚04.992′N, Long: 139˚13.006′E, Depth: 928 m
Chief observer: AMEMIYA Kentaro（Tokyo Sea Life Park)

Purpose: Succession patterns and colonization mechanisms at whale falls

Dive summary: A plankton pump was set about 20 m away from the carcass. Panoramic video and pictures of the entire whale were taken after landing near it. Sediments, plankton and small fauna attached to the bones were collected, and a baited trap was deployed near the bones.

Fig. 4-7. Thoracic bones of the Satomi Whale skeleton.

Preliminary results of the ROV Hyper-Dolphin dive #1081

Date: January 18, 2010

Site: Off Hatsushima Island, Sagami Whale fall site, Sagami Bay

Landing: Time: 8:49, Lat: 35˚04.982'N, Long: 139˚12.054'E, Depth: 940 m

Leaving: Time: 10:37, Lat: 35˚04.990'N, Long: 139˚12.017'E, Depth: 934 m

Chief observer: OKOSHI Kenji (Ishinomaki Senshu University)

Purpose: Succession patterns and colonization mechanisms at whale falls

Dive summary: Upon landing, about 30 m east of the carcass, the plankton pump deployed during dive #1080 was observed, and a net with pig bones deployed 8 month earlier was recovered. Sediments and water were sampled. The carcass, and especially the head bones were closely surveyed to observe animal communities. Plankton sampling was conducted over the bones, and small epifauna was also collected from the bones. The baited trap deployed the day before was retrieved. Whale bones (2 epiphysis and a piece of vertebral spine) were collected, as well as the sediment underneath. Before ascent to the surface, the plankton pump was hooked by the ROV.

Fig. 4-8. Sampling of the epifauna, by suction sampler, on the skull of the whale carcass.

Preliminary results of the ROV Hyper-Dolphin dive #1082

Date: January 18, 2010

Site: Off Hatsushima Island, Satomi Whale fall site, Sagami Bay

Landing: Time: 13:17, Lat: 35˚04.897'N, Long: 139˚13.987'E, Depth: 912 m

Leaving: Time: 14:53, Lat: 35˚04.935'N, Long: 139˚13.987'E, Depth: 925 m

Chief observer: HIGGS Nick (University of Leeds)

Purpose: Observe the changes in carcass degradation, faunal diversity on the carcass and in surrounding sediments, diversity and life history of *Osedax* polychaetes.

Dive Summary: We began the dive at approximately 20m from the whale skeleton in order to pick up a sack of pig bones that had previously been deployed. We moved to the skeleton and observed the vertebrae in detail and sampled the surfaces using the plankton suction system. We then observed vertebrae in the middle of the spine and in the abdominal part of the skeleton, near the ribs. We sampled sediment using the scoop sampler and MBARI core. We then moved to the front of the whale to take headlong pictures of the skeleton. We observed a spider crab, which we collected alive using the suction sampler.

Fig. 4-9. Caudal vertebrae of the Satomi whale carcass after 13 months on the seafloor.

4.2. On board results

4.2.1. Succession patterns and colonization mechanisms of chemosynthetic organisms associated to whale falls in Sagami Bay

During this cruise, 6 hyper-Dolphin dives were conducted at two whale fall sites at about 920 m depth near Hatsushima Island. Two carcasses were artificially implanted here. They were about 100 meters apart. The first one, called "Sagami" whale, was implanted in April 2005, and surveyed at 9, 16, 32, 44, 49, and 53 (this cruise) months post-implantation. The second whale, called "Satomi" whale was implanted in December 2008, and visited 10 days, 5 months and 13 months (this cruise) after implantation.

Sagami Whale

Fig. 4-10. "Sagami" whale panomaric view after 57 months on the seafloor (mosaic reconstruction. Y. Fuiiwara).

Overall, the skeleton was well degraded: almost all ribs had disappeared (due to both collection during previous cruises and natural breakdown), the spines and lateral processes of vertebrae were missing, and the jaws and top part of the skull were also much damaged. Except for baterial mats, fauna on the bones was relatively scarce (a few small *Osedax* were visible, but only under microscope after recovery).

Satomi Whale

Fig. 4-11. "Satomi" whale panoramic overview after 13 months on the seafloor (mosaic reconstruction, Y. Fujiwara).

Blubber and spermaceti in the head region were the only soft tissues remaining 13 months after implantation. Skeleton was colonized by *Osedax* sp.. The tip of the jaw bones were the most densely colonized parts, but Osedax was also observed at the back of the skull, on the ribs, and on the spines and processes of some vertebrae. One jaw bone (tip) and one rib were collected, both densely covered by *Osedax*. Three morphotypes were identified macroscopically.

Around the skeleton, area of blackened anoxic were visible mostly around the head part, as well as white bacterial mats.

In general, the "Satomi" whale seems to evolve similarly to "Sagami". The carcass hosts many *Osedax*, some belonging to a large morphotype that ressembles *Osedax roseus*. *O. roseus* was sampled on "Sagami" at similar age (i.e. months post-implantation), but is not present anymore on the older carcass.

It is then very likely that other *Osedax* populations than those present on "Sagami" whale are providing colonists for the new "Satomi" whale.

Pig bones

Three nets containing pig bones, deployed 8 months earlier (NT09-06), were retrieved during this cruise. The purpose of this experiment was to examine the potential of the different *Osedax* species detected in Sagami Bay to colonize other substrates than whale bones.

At recovery, a few very small Osedax were observed on the bones deployed at the seep site. These bones were deployed on a rock and remained exposed to seawater during the whole experiment. Pig bones deployed near whale carcasses were found partially buried in sediments, which probably may have prevented Osedax settlement. All bones were kept in tanks in our laboratory in order to further observe Osedax colonization.

Plankton pumps

In order to collect some larval stages of Osedax or other species specialists of whale falls, we deployed plankton pumps for 12 hours near each skeleton. However, due to the sedimentary nature of the bottom, pumps were quickly clogged after starting pumping, and no larval stages could be recovered.

4.2.2. Which biological differences exist between Calyptogena soyoae and C. okutanii ?

(1) Understanding distribution pattern differences

During 2 dives, we took video images and collected both species *Calyptogena okutanii/soyoae* from shallower and deeper sites at the Off Hatsushima Island seep area. After species identification by morphological and genetic analysis, we will discuss relationship between distribution patterns and environmental factors.

(2) Understanding minimum maturation size

Several *Calyptogena* and *Bathymodiolus* bivalve specimens were preserved by Bouin fixation for gonad tissue observation. Also, some specimens were identified sex by gamete collected from gonad. Preliminary result indicated sex ratio of *Calyptogena* spp. was even (Fig. 4-12).

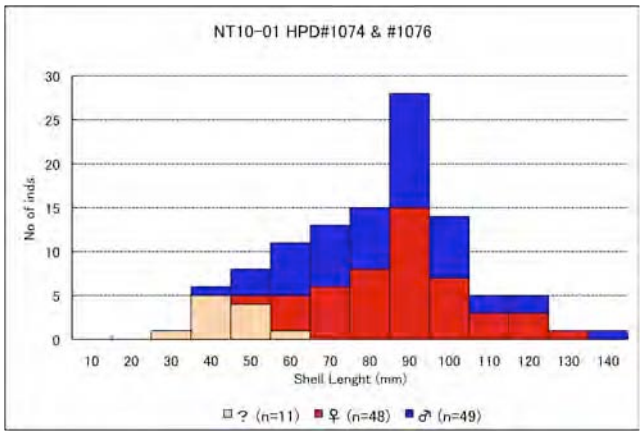


Fig. 4-12. Sex-ratio of Calyptogena spp. By E. Seo.

(3) Understanding early life history and dispersal potential

We tried to collect mature gametes, eggs and sperms of *Calyptogena*, using *in-situ* spawning cage with heating by underwater light (Fig. 4-13). We could get sperm but no eggs. Some gametes were collected from fresh specimens by dissection. Then, we attempted artificial insemination in lab. Embryos were cultured under high pressure environments.

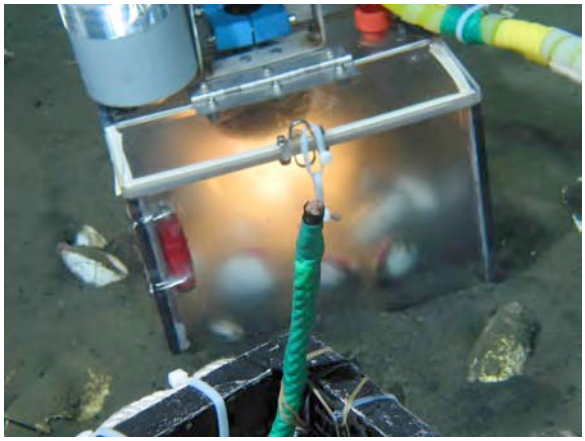


Fig. 4-13. *In situ* experiment to collect mature gametes. Blurred water inside the cage is due to released sperm.

(4) Understanding prevention mechanism of hybridism

Calyptogena soyoae and *C. okutanii* dominantly coexist in a very large community. Sperm release by these clams is induced by a rise in water temperature and eggs are released following male spawning. Both species seem to spawn simultaneously in the community. How do the two species avoid hybridization in spite of similar spawning process and of being phylogenetically close ? The purpose of this study was to understand mechanisms to avoid interspecies hybridization between these two species. As the first step, we will investigate morphological features of their gametes. The gametes collected with the *in situ* spawning cage (Fig. 4-13) will be observed using a SEM and a TEM.

(5) Growth rate estimation

To estimate growth rate of *Calyptogena* spp. and foraminiferan, we conducted *in situ* mark-release-recapture experiment. Firstly, we recaptured marked (by fluorescence and Sr) specimens of 4 cages deployed during a previous cruise (NT09-06). Two of the 4 cages seemed to be in good condition (Fig. 4-14a) but other 2 cages fell down. We attempted a new marking experiment in the same way as previously (Fig. 4-14b). We guessed this new marking was successful.

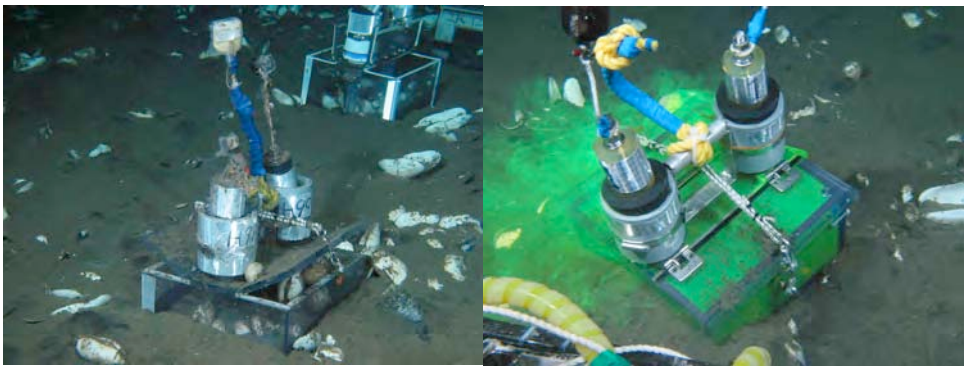


Fig. 4-14. a (left) Marking experiment cages deployed during NT09-06. b (right) New marking experiment conducted during this cruise.

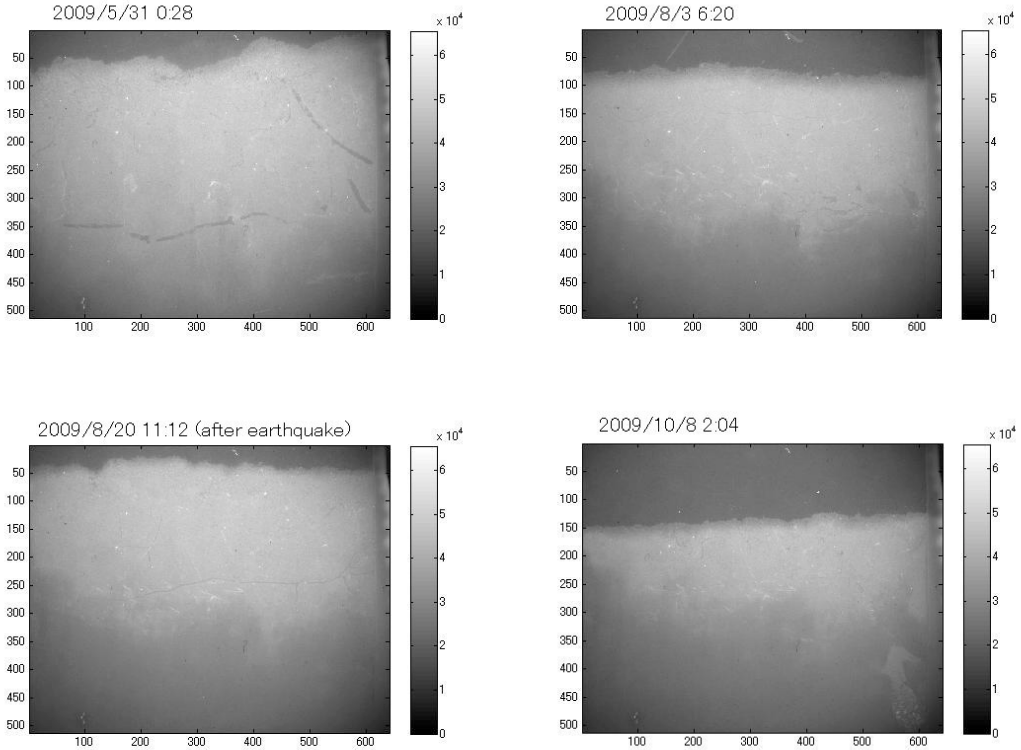
(6) Understanding the transmission way of symbiotic bacteria from parents to next generation

Calyptogena symbiotic bacteria are vertically transmitted via eggs, and though to have co-evolved with *Calyptogena* clams. However, detailed transmission mechanism of symbiotic bacteria is still unknown. To elucidate this, we collected *Calyptogena* clams. Collected clams were immediately dissected, and gill and gonad tissues were fixed with paraformaldehyde or glutaraldehyde for the microscopic analysis. Other tissues were frozen in liquid nitrogen and stored at -80°C.

4.2.3. Long term monitoring of bidimentional O2 profiles at the sediment-water interface in Sagami Bay

The lander system with the planar O_2 optode system was recovered by ROV Hyperdolphin. Prior to the recovery, the extension cable between the lander the Hasushima deep-sea observatory was removed. After removal of the cable, the lander was hung with a hook by the ROV, and it was arrived on deck.

The lander was stored on boat deck of R/V Natsushima. The planar O_2 optode system was removed and washed with fresh water. The computer was removed from the pressure cylinder, and the data in the hard disk was investigated briefly. According to the date stamp, the optode collected O_2 images from 30th/Apr, 2009 to 15th/Dec, 2009. The total number of the images seems over 25,000 at a moment.



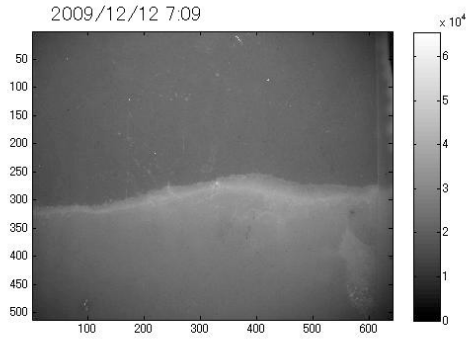


Fig. 4-15. Grayscale (phosphorescent) image taken at sediment-water interface by planar O_2 optode system. These are phosphorescent images from the sensor foil set at the boundary. Principle of the planar O_2 optode using with a transparent sensor foil is shown in Holst and Grunwald (2001).

4.3. Future studies

4.3.1. Succession patterns and colonization mechanisms of chemosynthetic organisms associated to whale falls in Sagami Bay

During this cruise we investigated the overall degradation condition and the animal communities that colonized two whale carcasses implanted in close proximity for different duration. Our main goal was to compare the colonization and succession patterns between the two carcasses, and to investigate whether the presence of another community at an older evolution stage in close proximity might influence the evolution of the more recent community. We collected animal and bone samples, which were either preserved on board, or brought back to our laboratory alive.

Diversity will be investigated using both morphology and genetic approaches (barcoding) both on specimens preserved onboard, or collected later alive from our tanks.

Genetic relationships between individuals of the same species collected on both whales, or at other whale fall sites in the world will also be investigated in order to understand better phylogeography and dispersal of these species.

Development of larvae obtained from live specimens will also be analyzed, in order to gain information about larval life duration, symbiont transmission, development and recruitment processes.

Relationships between host species and their endo/epi-symbiont will be investigated: diversity, localization and abundance of symbiont will be analyzed using molecular biology techniques (cloning, sequencing, ISH). Some bacterial strains from sediment samples or from host metazoan species will also be cultured for further characterization, and for colonization experiments in laboratory controlled conditions.

4.3.2 Which biological differences exist between Calyptogena soyoae and C. okutanii ?

1. Understanding distribution pattern differences.
This subject will be progressed by mainly H. Watanabe, E. Seo, K. Fujikura et al.

2. Understanding minimum maturational size.
This subject will be progressed by mainly E. Seo, Y. Takahashi, K. Fujikura et al.

3. Understanding early life history and dispersal potential.
This subject will be progressed by mainly H. Watanabe, F. Pradillon et al.

4. Understanding prevention mechanism of hybridism
This subject will be progressed by mainly K. Fujikura, T. Yoshida, E. Seo, Y. Nakamura, H. Imai (Univ. of Ryukyus) et al.

5. Growth rate estimation
This subject will be progressed by mainly E. Seo, H. Nomaki, H. Watanabe, K. Fujikura et al.

6. Understanding transmission way of symbiotic bacteria from parents to next generation.
This subject will be progressed by mainly T. Yoshida, Y. Nakamura, Y. Hongo, et al.

4.3.3. Long term monitoring of bidimentional O2 profiles at the sediment-water interface in Sagami Bay

Using the grayscale (phosphorescent) images, visualization of O_2 image will be carried out. The image processing will be made in a laboratory. O_2 irrigations by benthic activities and hydrodynamics will be investigated to compare with the O_2 and the corresponding grayscale images.

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.

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.

Users of data or results on this cruise report are requested to submit their results to the Data Integration and Analysis Group (DIAG) of JAMSTEC.