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RV/Yokosuka and Shinkai 6500 Cruise Report
YK11-04

Kuril Trench (Off South-East Hokkaido)
Japan Trench (Off Sanriku)

August, 16th-20th, 2011

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

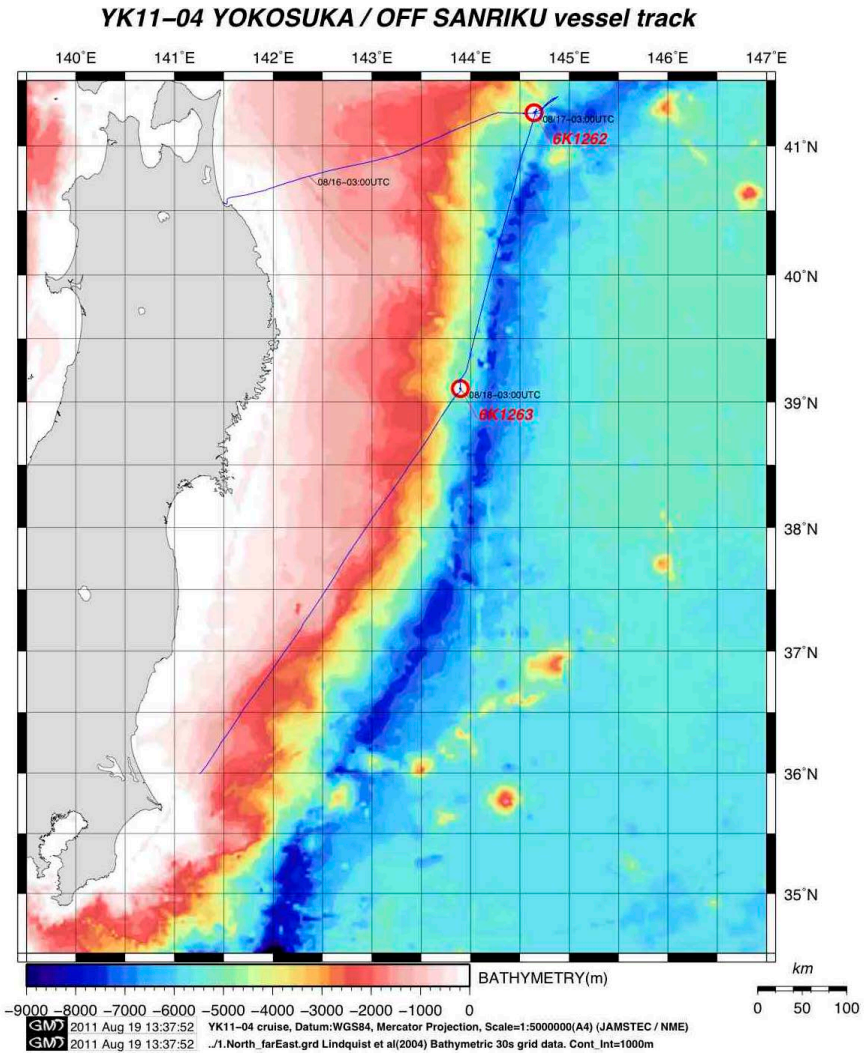
Contents

Acknowledgements

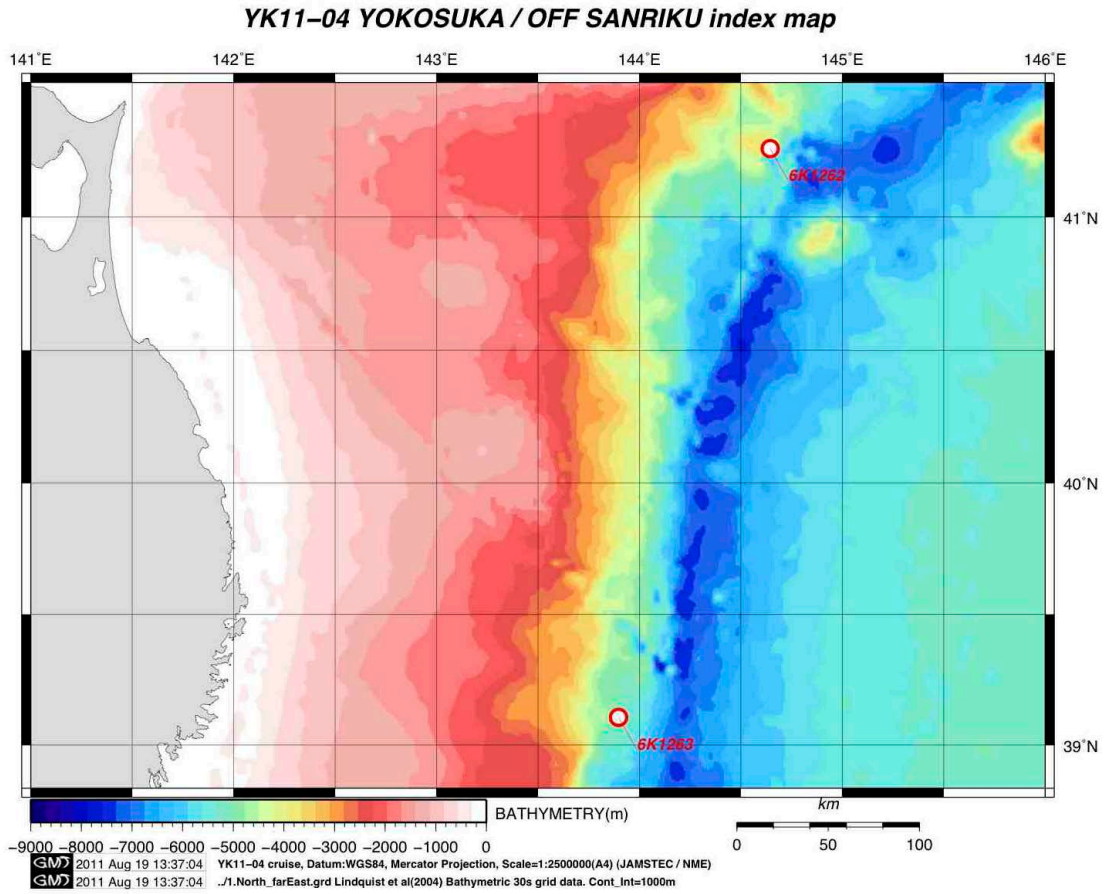
We are grateful to Captain Mr. Koji Samejima, Chief Officer Mr. Takafumi Aoki and Chief Engineer Mr. Hiroyoshi Kikkawa for their safe navigation and their skillful handling of “R/V Yokosuka”. Great thanks are due to Commander Mr. Toshiaki Sakurai and “Shinkai 6500” operation team for their operations in sampling. We also thank Mr. Morifumi Takaesu, Nippon Marine Enterprises, Ltd., for his attentive supports. Finally, we would like to appreciate all the person who supported directly or indirectly this cruise.

1. Cruise Information

1) Cruise ID, Name of Vessel: YK11-04, R/V Yokosuka
2) Title of the Cruise: “Shinkai 6500” Research Dive, Deep-sea Research, FY2011
3) Title of Proposal: Genome diversity in the chemoautotrophic symbiont of *Calyptogena* clams.
4) Cruise Period: August 16, 2011 ~ August 20, 2011
5) Port Call: from Hachinohe (August 16, 2011) to JAMSTEC (August 20, 2011)
6) Research: Area: Off South-East Hokkaido, Kuril Trench and Off Sanriku, Japan Trench



Cruise track of R/V Yokosuka (YK11-04)



The dive position of Off South-East Hokkaido, Kuril Trench and Off Sanriku, Japan Trench

7) Cruise Log (JST)

YK11-04 Shipboard Log
(16.Aug, 2011 - 20.Aug, 2011)

CHISHIMA Trench
JAPAN Trench

Date	Local Time	Note	Description	Position/Weather/ Wind/Sea condition
16 Aug, 2011		**Sail out and Transit**		08/16 12:00 (UTC+9h)
	08:55	let go all shore line, left HACHINOHE	for CHISHIMA Trench	40-45.6'N, 142-21.5'E
	10:00	scientist meeting		Overcast
	10:30-11:00	onboard education and safety training	chief officer, 1st ratio	SW-4 (Moderate breeze)
	14:00	meeting for SHINKAI 6500 operation		2 (Sea smooth)
	17:55	scientist meeting		1 (Low swell short)
	18:00	arrived at research area		Visibly: 6'
	18:10	released XBT	41-15.0628N, 144-25.9641E	
	19:09-19:57	carried out MBES site survey	CHISHIMA Trench	
	20:15	commenced drifting		
17 Aug, 2011		**SHINKAI 6500 dive 1262**		08/17 12:00 (UTC+9h)
	09:38	hoisted up SHINKAI 6500		41-15.3'N, 144-38.6'E
	09:45	launched SHINKAI 6500		Overcast
	09:52	started 6K#1262 dive operation	CHISHIMA Trench	SW-4 (Moderate breeze)
	11:57	landed at sea bottom	Depth: 4816m	3 (Sea slight)
	15:25	left bottom	Depth: 4831m	2 (Low swell long)
	17:08	refloated SHINKAI 6500		Visibly: 5'
	17:30	hoisted up SHINKAI 6500		
	17:39	recovered		
	18:10	commenced proceeding to JAPAN Trench		
	17:15	scientific meeting		
18 Aug, 2011		**SHINKAI 6500 dive 1263**		08/18 12:00 (UTC+9h)
	03:00	arrived at research area		39-06.3'N, 143-53.8'E
	08:42	hoisted up SHINKAI 6500		Overcast
	08:52	launched SHINKAI 6500		NW-3 (Gentle breeze)
	09:00	started 6K#1263 dive operation	JAPAN Trench	3 (Sea slight)
	11:08	landed at sea bottom	Depth: 5348m	2 (Low swell long)
	15:08	left bottom	Depth: 5354m	Visibly: 3'
	17:02	refloated SHINKAI 6500		
	17:21	hoisted up SHINKAI 6500		
	17:29	recovered		
	17:56-18:15	carried out figure eight turn measurement		
	18:20	commenced proceeding to JAMSTEC		
	18:20	scientific meeting		
19 Aug, 2011		**Transit to JAMSTEC**		08/19 12:00 (UTC+9h)
	18:00	scientific meeting		35-20.0'N, 140-40.0'E
	18:30	arrived at off JAMSTEC		Overcast
				NE-5 (Fresh breeze)
				4 (Sea moderate)
				2 (Low swell long)
				Visibly: 6'
20 Aug, 2011		**Cruise completed**		
	09:00	arrived at JAMSTEC		

2. Participants List

Scientists

Principal Investigator

Takao Yoshida
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Institute of Biogeosciences
Japan Agency for marine-Earth Science and Technology (JAMSTEC)

Onboard Scientists

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Haruka Shibata
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Marine Technician

M. TAKAESU	Nippon Marine Enterprises, LTD.

SHINKAI 6500 operation team

T. SAKURAI	Operation Manager
K. CHIBA	Sub Operation Manager
T. YOSHIUME	1st Submersible Staff
K. MATSUMOTO	1st Submersible Staff
M. YANAGITANI	1st Submersible Staff
K. SUZUKI	2nd Submersible Staff
A. ISHIKAWA	2nd Submersible Staff
T. ONISHI	2rd Submersible Staff
Y. TAYAMA	3rd Submersible Staff
H. IKEDA	3rd Submersible Staff
M. KATAGIRI	3rd Submersible Staff

R/V YOKOSUKA Officers and Crew

K. SAMESHIMA	Captain
T. AOKI	Chief Officer
S. FUJII	2nd Officer
Y. KOBAYASHI	3rd Officer
H. KIKKAWA	Chief Engineer
T. OTA	1st Engineer
K. KANEDA	Junior 1st Engineer
T. MORI	2nd Engineer
K. IKEGUCHI	3rd Engineer
T. NASU	Chief Radio Operator
Y. KURAMOTO	2nd Radio Operator
R. KOMATSU	3rd Radio Operator
S. ABE	Boat Swain
N. ICHIKAWA	Able Seaman
M. OHATA	Able Seaman
N. ISHIZUKA	Able Seaman
S. TAKUNO	Able Seaman
S. UZUKI	Sailor
S. SUZUKI	Sailor
K. NAKAI	No.1 Oiler
R. SATO	Oiler
K. MURASE	Oiler
K. HUNAWATARI	Oiler
M. FUJIWARA	Oiler
T. YOSHIKAWA	Chief Steward
T. ABE	Steward
S. TANAKA	Steward
M. NAKANO	Steward
T. WADA	Steward
K. KAWASE	Steward

Member of onboard science party and SHINKAI 6500 operation team

3. Observation

To investigate the genome diversity of *Calyptogena* symbionts, we planned to two dives at Kuril trench and Japan trench. We departed Hachinohe Bay on morning of Aug 15th, and went toward Off South-East Hokkaido, Kuril Trench, and arrived there in the night of Aug 15th. On Aug 16th, we made the 6K#1262 dive in Kuril Trench. Diver was Dr. Takaki. After dive, we sailed toward Off Sanriku, Japan Trench. We arrived research area in the early morning of Aug 17th. We could perform 6K#1263 dive in Japan Trench. Diver was Dr. Yoshida. After dive, we sailed to Yokosuka. We arrived at JAMSTEC on morning of Aug 20th.

During the cruise, several biological samples, such as *Calyptogena* clams, Sea cucumber, etc., and segment by MBARI cores were collected. . After dive, the clams were immediately dissected, and blood, serum, and other tissues were frozen in liquid nitrogen and stored at -80ºC until used. Other samples were also stored at -80ºC or fixed by paraformaldehyde. Detailed analyses of these samples will be performed after the cruise.

4. Dive report

1) Summary of the Shinkai 6500 Dive #1262

Date: Aug. 17, 2011
Site: Off South-East Hokkaido, Kuril Trench
Landing: 41°15.2740'N, 144°38.5687'E, 4816 m (11:57)
Leaving: 41°15.2578'N, 144°38.5960'E, 4831 m (15:25)

Purpose:
Collection of *Calyptogena* clams

Payload Equipment:
Suction sampler（multiple canister） 1
Scoop sampler 1
Sample box 2 (1 large, 1 small)
MBARI-type core 3
Marker 2

Dive Summary
‡ Collected sea cucumber with suction sampler.
The sampling site locate 41° 15.2740N, 144° 38.5687E, 4816 m

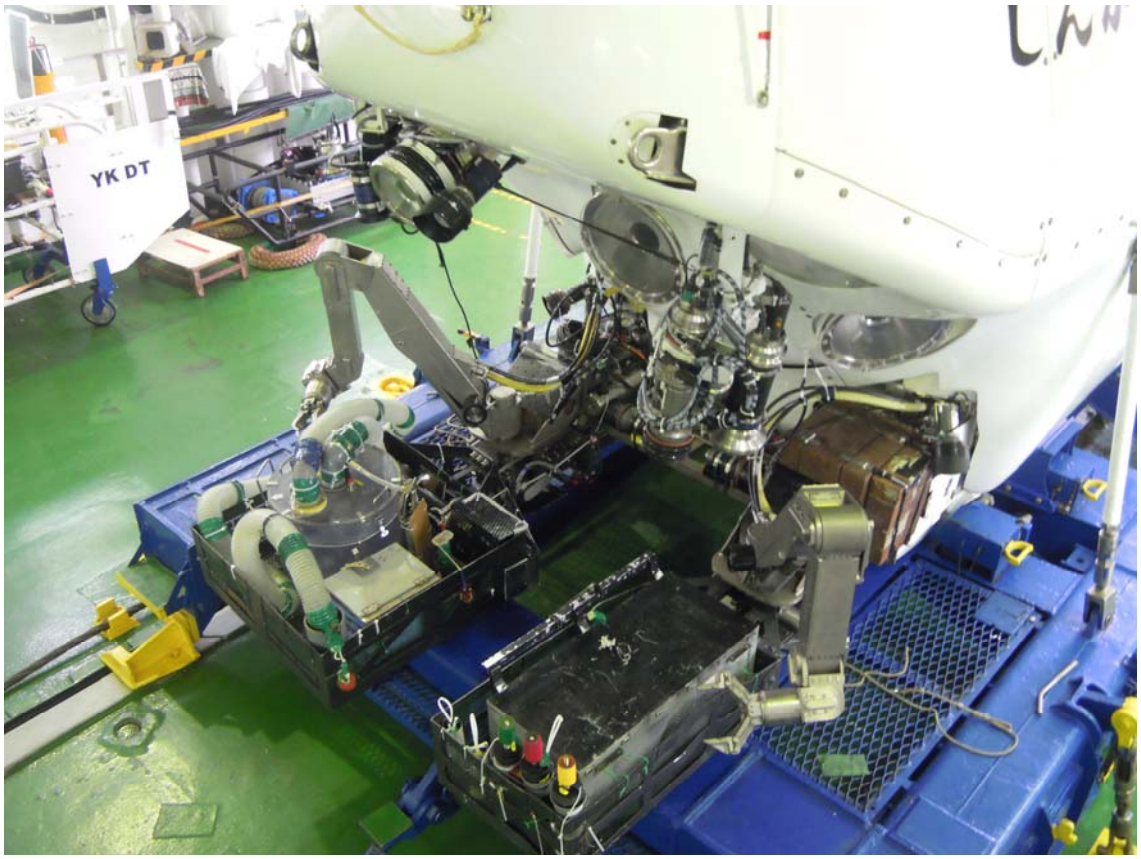
‡ Collected *Calyptogena* clams and seq anemone with scoop sampler into large box (BoxD). The sampling site locate 41° 15.2185N, 144° 38.5931E, 4822 m

‡ Collected *Calyptogena* clams with scoop sampler into large box (BoxB).
The sampling site locate 41° 15.2675N, 144° 38.5940E, 4821 m

‡ Collected *Calyptogena* clams with scoop sampler into large box (BoxC).
The sampling site locate 41° 15.2635N, 144° 38.5845E, 4820 m

‡ Collected *Calyptogena* clams with scoop sampler into small box (BoxA), and sediment cores with MBARI-type corer. The sampling site locate 41° 15.2678N, 144° 38.5824E, 4819 m

‡ Deployment of the marker #128
The site locate 41° 15.2720N, 144° 38.5843E, 4815 m



Payload of 6K#1262

Dive log of 6K#1262

SHINKAI 6500 Dive Log

DIVE NUMBER : 6K#1262 YK11-04

DATE : 2011/08/17 DIVE SITE : CHISHIMA Trench

Time (LCT)	Dep. (m)	Alt. (m)	Head (Deg)	Description	Remarks (position)
10:00	0.0			starting to go into the sea	
11:57	4816.0	3.0	240	reaching the bottom of sea	
11:59	4816.0	1.0	228	brown muddy sediment, Temp. 1.5, no current.	
12:04	4816.0	1.0	193	sea cucumbar sampling using slurp gun started.	
12:09	4813.0	2.0	155	A sea cucumber into No.1 canister	
12:10	4811.0	3.0	154	A small colony of vesicomyids was found. (observation only)	
12:14	4818.0	5.0	242	Rocks? in the vision.	
12:24	4807.0	3.0	182	Rocky cliff-like structure	
12:26	4808.0	3.0	217	Looking around	
12:28	4803.0	2.0	319	Sea anemone on the rock	
12;32				-1200, -120 (Position)	
12;39	4801.0			-1170, 70	
12:44	4818.0	1.0	170	-1200, 90	
12:46	4821.0	3.0	118	Fish	
12:47	4822.0	1.0	118	Sokodara	
12;47				-1290, 110	
12:49	4821.0	3.0	136	Rocky slope	
12:50	4822.0	1.0	094	Vesicomyid-like shell.	
12:52	4822.0	3.0	075	Live vesicomyids were found. Observation.	
12:53	4822.0	3.0	075	Sampling of live vesicomyids using shovel.<Box D>	
13:06	4822.0	2.0	074	star fish is found not collected	
13:15	4822.0	2.0	074	Sampling Sea anemone and Vesicomyid.<Box D> using shovel	
13:18				-1260,133	
13:25	4823.0	2.0	074	Rocks? in the vision.	
13:29	4821.0	1.0	016	Rocky slope	
13:31				-1180,140	
13:34	4822.0	3.0	276	Vesicomyid-like shell clony is found.	
13:42	4921.0	2.0	222	Sampling Vesicomyids <Box B or C> using shovel.	
14:03	4821.0	2.0	248	-1170,130	
14:07	4820.0	1.0	219	Sampling Vesicomyids <Box B or C> using shovel.	
14:27	4820.0	3.0	249	-1180,120	
14:27	4820.0	2.0	240	Move to another vesicomid colony.	
14:33	4819.0	2.0	199	Sampling Vesicomyids <Box A> using shovel.(-1168,120)	
14:54				finish sampling	
14:55				MBARI core(yellow) sampling at the site near vesicimid colony.	
14:59				finish sampling and move to another point.	
15:03		3.0	198	MBARI core (red) sampling at the site far from vesicomid coclony.	
15:06	4819.0	2.0	203	finish sampling and move to another point.	
15:14	4815.0	2.0	159	Setting of Marker 128	
15:16				try to sampling of sea cucumber	
15:19				fail the sampling and move to another point	
15:24	4831.0	3.0	323	Leave the sea bottom	

Event list of 6K#1262

#1262DIVE 高木　善弘 三陸沖海域　SkyFix(WGS-84)SSBL

*** EVENT MARK LIST ***　　2011-08-17 15:34:02

ORIGIN (XY<->LATLON CONVERT)　LAT 41° 15.9000'N　LON 144° 38.5000'E
XY ORIGIN ((X,Y)=(0,0))　LAT 41° 15.9000'N　LON 144° 38.5000'E

NO.	DAY	TIME	LAT	LON	X	Y
1	2011-08-17	10:00:00	41° 15.2462' N	144° 38.6289' E	-1210.0	180.0
	Landing target					
2	2011-08-17	11:57:00	41° 15.2740' N	144° 38.5687' E	-1158.5	95.9
	Landing,Sampling Sea cucumber D=4816m					
3	2011-08-17	13:17:00	41° 15.2185' N	144° 38.5931' E	-1261.3	130.0
	Sampling Calyptogena,Sea annemone D=4822m					
4	2011-08-17	14:03:00	41° 15.2675' N	144° 38.5940' E	-1170.6	131.2
	Finding Calyptogena Colony, Sampling Calyptogena D=4821m					
5	2011-08-17	14:26:00	41° 15.2635' N	144° 38.5845' E	-1178.0	117.9
	Sampling Calyptogena(4) D=4820m					
6	2011-08-17	14:54:00	41° 15.2678' N	144° 38.5824' E	-1170.0	115.0
	Sampling Calyptogena(4), MBARI(1) D=4819m					
7	2011-08-17	15:16:00	41° 15.2720' N	144° 38.5843' E	-1162.2	117.7
	Set #128Marker D=4815m					
8	2011-08-17	15:25:00	41° 15.2578' N	144° 38.5960' E	-1188.5	134.0
	Left Bottom D=4831m					
9						
10						
11						
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16						
17						
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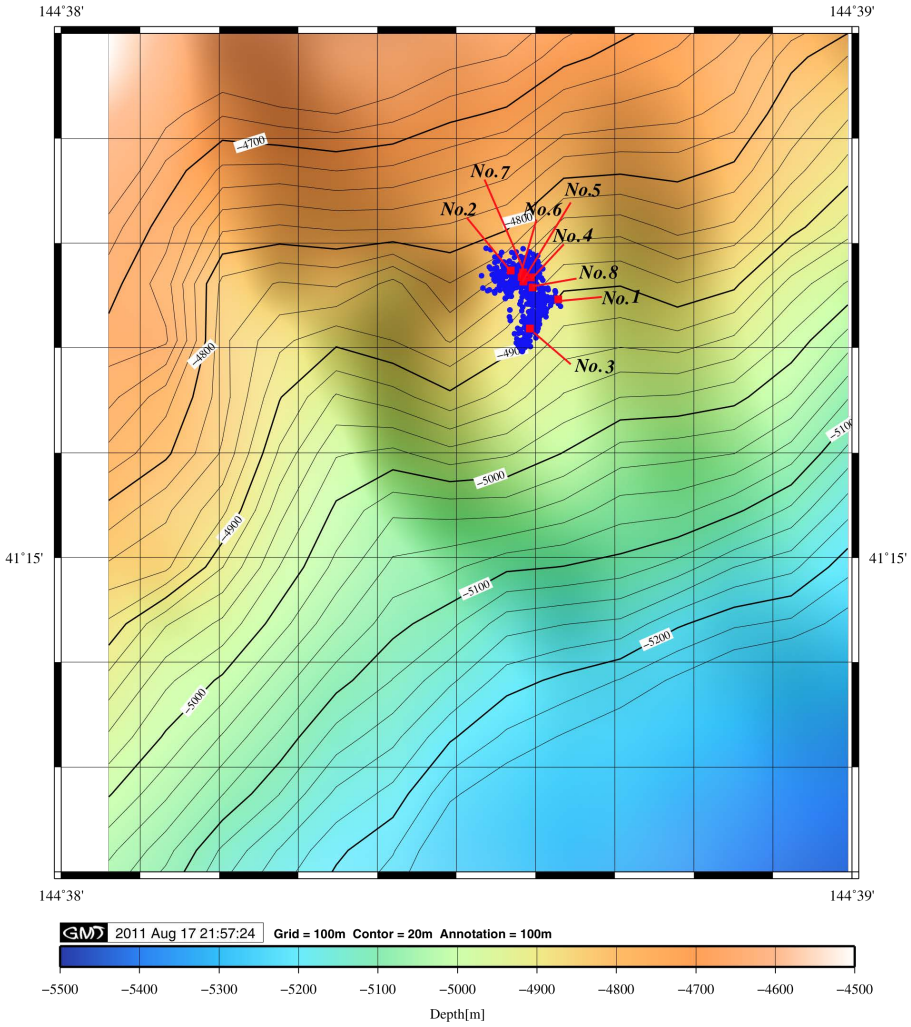
20						

Dive track of 6K#1262

1262DIVE



No.1	41-15.2462N,144-38.6289E	Landing target
No.2	11:57 41-15.2740N,144-38.5687E	Landing,Sampling Sea cucumber D=4816m
NO.3	13:17 41-15.2185N,144-38.5931E	Sampling Calyptogena,Sea annemone D=4822m
NO.4	14: 3 41-15.2675N,144-38.5940E	Finding Calyptogena Colony, Sampling Calyptogena D=4821m
NO.5	14:26 41-15.2635N,144-38.5845E	Sampling Calyptogena(4) D=4820m
NO.6	14:54 41-15.2678N,144-38.5824E	Sampling Calyptogena(4), MBARI(1) D=4819m
NO.7	15:16 41-15.2720N,144-38.5843E	Set #128Marker D=4815m
No.8	15:25 41-15.2578N,144-38.5960E	Left Bottom D=4831m

2) Summary of the Shinkai 6500 Dive #1263

Date: Aug. 18, 2011

Site: Off Sanriku, Kuril Trench

Landing: 39°6.2953'N, 143°53.8491'E, 5248 m (11:08)

Leaving: 39°6.3768'N, 143°53.4051'E, 5354 m (15:08)

Purpose:

Collection of *Calyptogena* clams

Payload Equipment:

Suction sampler（多連キャニスター） 1

Scoop sampler 1

Sample box 2 (1 large, 1 small)

MBARI-type core 3

Marker 2

Dive Summary

‡ Collected some organisms (sea cucumber, shrimp) with suction sampler. The sampling site locate 39° 6.2953N, 143° 53.8491E, 5348 m

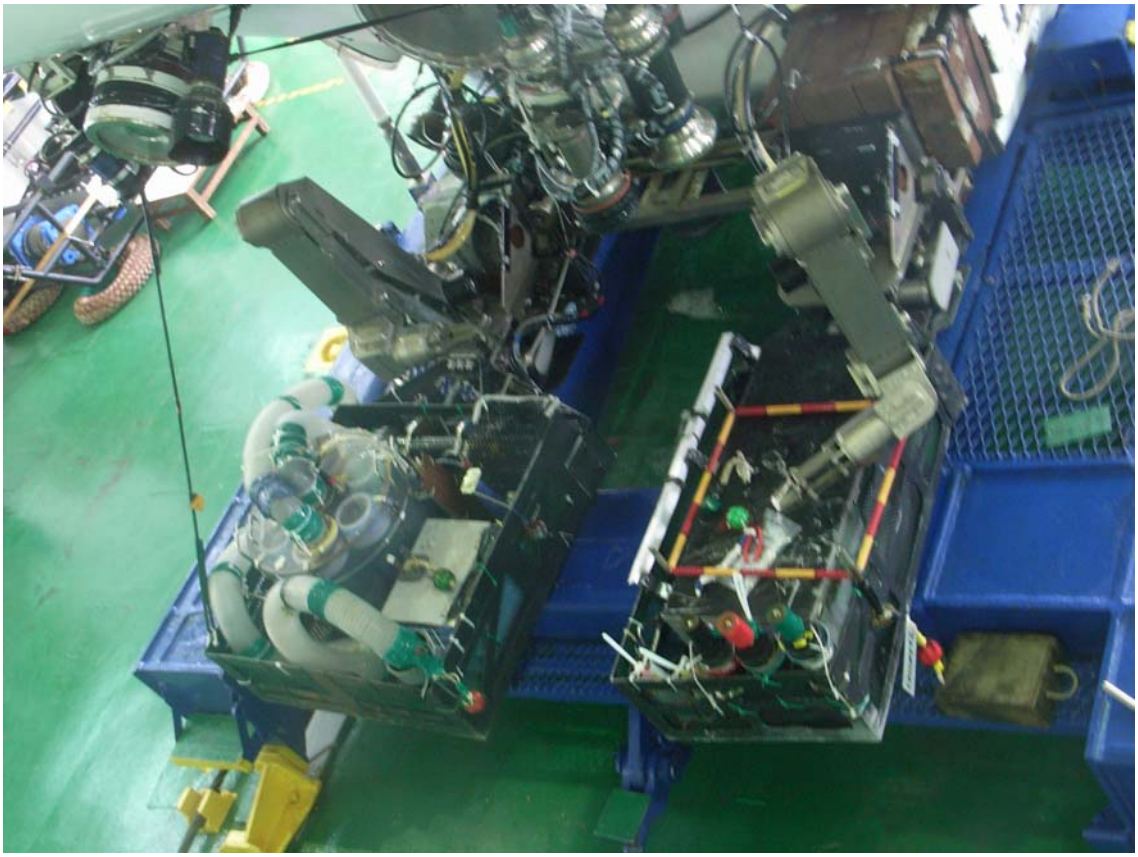
‡ Collected sediment cores with MBARI-type corer from bacterial mat (core black) and non-bacterial mat (core red). Deployment of quadrat in bacterial mat. The sampling site locate 39° 6.3371N, 143° 53.5512E, 5346 m

‡ Collected *Calyptogena* clams with scoop sampler into small box (BoxA). Deployment of Marker #129. The sampling site locate 39° 6.3220N, 143° 53.6165E, 5347 m

‡ Collected some organisms (polychaeta, fish, jellyfish) with suction sampler. The sampling site locate 39° 6.2978N, 143° 53.6928E, 5348 m

‡ Collected *Calyptogena* clams with scoop sampler into large box (Box D). The sampling site locate 39° 6.2553N, 143° 53.5290E, 5352 m

‡ Collected *Calyptogena* clams with scoope sampler into large box (Box B and C). The sampling site locate 39° 6.2759N, 143°53.4722E, 5352 m



Payload of 6K#1263

Dive log of 6K#1263

SHINKAI 6500 Dive Log

DIVE NUMBER : 6K#1263 YK11-04

DATE : 2011/08/18 DIVE SITE : JAPAN Trench

Time (LCT)	Dep. (m)	Alt. (m)	Head (Deg)	Description	Remarks (position)
9:00	0.0			starting to go into the sea	
11:07	5348.0	3.0	10	sea cucumbar observed.	
11:08	5348.0	3.0	13	reaching the bottom of sea(-380,650).	
				muddy bottom, 1.6 deg C.	
11:11				try to sampling sea cucumbar by slurp gun.	
11:15				finish sampling sea cuxumbar (No.1 canister).	
				moving to another point.	
11:22	5347.0	4.0	275	unknown animal observed.(-350, 600)	
11:26	5347.0	3.0	275	sokodara observed.	
11:28	5346.0	3.0	275	-300,500	
11:34	5347.0	3.0	267	-330,420	
11:37	5347.0	1.0	272	unknown fish?? observed.	
11:38	5347.0	3.0	272	-350,360	
11:40	5348.0	1.0	293	sokodara observed.	
11:42	5347.0	3.0	293	-320,320	
11:47	5346.0	2.0	284	-310, 260	
11:56	5346.0	3.0	3	-220,210	
12:01	5345.0	3.0	2	-140,210	
12:06	5345.0	3.0	91	-140,320	
12:08	5344.0	3.0	152	-120,360	
12:14	5345.0	2.0	240	-220,350	
12:19	5346.0	3.0	259	-300,270	
12:22	5346.0	3.0	289	-290,220	
12:24	5346.0	3.0	80	sokodara observed.	
12:25	5346.0	3.0	5	Bacteria mat observed.	
12:29	5346.0	3.0	329	-300,220	
12:32	5346.0	3.0	326	MBARI core(black) sampling at the bacteria mat.	
12:34	5346.0	3.0	326	MBARI core(Red) sampling at the site far from bacteria mat. (control)	
12:39	5346.0	1.0	327	Set the Quadrat and Marker No.122?	
12:43	5345.0	2.0	37	unknown animal? observed.	
12:44	5346.0	3.0	97	sokodara observed.	
12:47	5346.0	1.0	126	-280,290	
12:50	5347.0	3.0	318	Sampling Vesicomyids <Box B> using shovel after observation.(-330,310)	
13:10				set the Marker(number unknown) at the sampling site and move to another site.	
13:19	5346.0	1.0	120	-340, 360	
13:21	5347.0	3.0	120	Sampling a warm?? using slurp gun (No.2 canister).	
				Sampling a Liparid using slurp gun (No.3 canister).	
13:25	5348.0	2.0	114	Sampling a jelly fish using slurp gun (No.4 canister).	
13:32	5349.0	3.0	120	-390, 480	
13:40	5352.0	3.0	192	-520, 500	
13:43	5353.0	2.0	280	-550, 500	
13:50	5350.0	3.0	317	-560, 410	
13:52	5350.0	3.0	320	Jelly fish ??and fish observed.	
13:53	5350.0	3.0	318	-490, 350	
13:56	5349.0	3.0	288	-450. 310	
14:01	5350.0	3.0	276	-450, 250, white matter observed	
14:05	5352.0	1.0	283	trace of something moved on the sandy bottom; it may be clam?	
				-480, 160; landed to look at something vesicomyid-like	
14:09	5352.0	1.0	295	samplingof the clam? into the box C using the shovel	
14:13				Tried again.	
14:20				Sampling finished; -480, 190 (Why position changed during sampling?)	
14:24	5352.0	3.0	338	-460, 140; bacteria mat; crack of the sea floor?	
14:27	5351.0	3.0	3	A marker found (#1223?); -440, 160	
14:30	5351.0	3.0	307	Started to move again.	
14:33	5351.0	3.0	311	Vesicomyid colonev found	
14:34	5352.0	1.0	321	-400, 110; Landed for sampling	

SHINKAI 6500 Dive Log

DIVE NUMBER : 6K#1263 YK11-04

DATE : 2011/08/18 DIVE SITE : JAPAN Trench

Time (LCT)	Dep. (m)	Alt. (m)	Head (Deg)	Description	Remarks (position)
				Some clams have a trace of movement. They may have come here from somewhere?	
14:41				Sampling using the shovel started.	
14:48				The surface layer of the sediment is light brown but the deeper layer is black.	
14:50				-410, 100:	
14:52		3.0	324	Some clams look shorter than typical Calyptogena phaseoriformis.?	
14:52	5352.0	3.0	323	Started to move again.	
14:57	5352.0	3.0	323	Rocks and something like sea anemone?	
14:59	5353.0	3.0	323	-300, 70	
15:02	5355.0	3.0	0	Rocky bottom	
15:06	5354.0	3.0	18	-230, 10: Left the bottom.	

Event list of 6K#1263

#1263DIVE 吉田　尊雄 日本海溝　SkyFix(WGS-84)SSBL

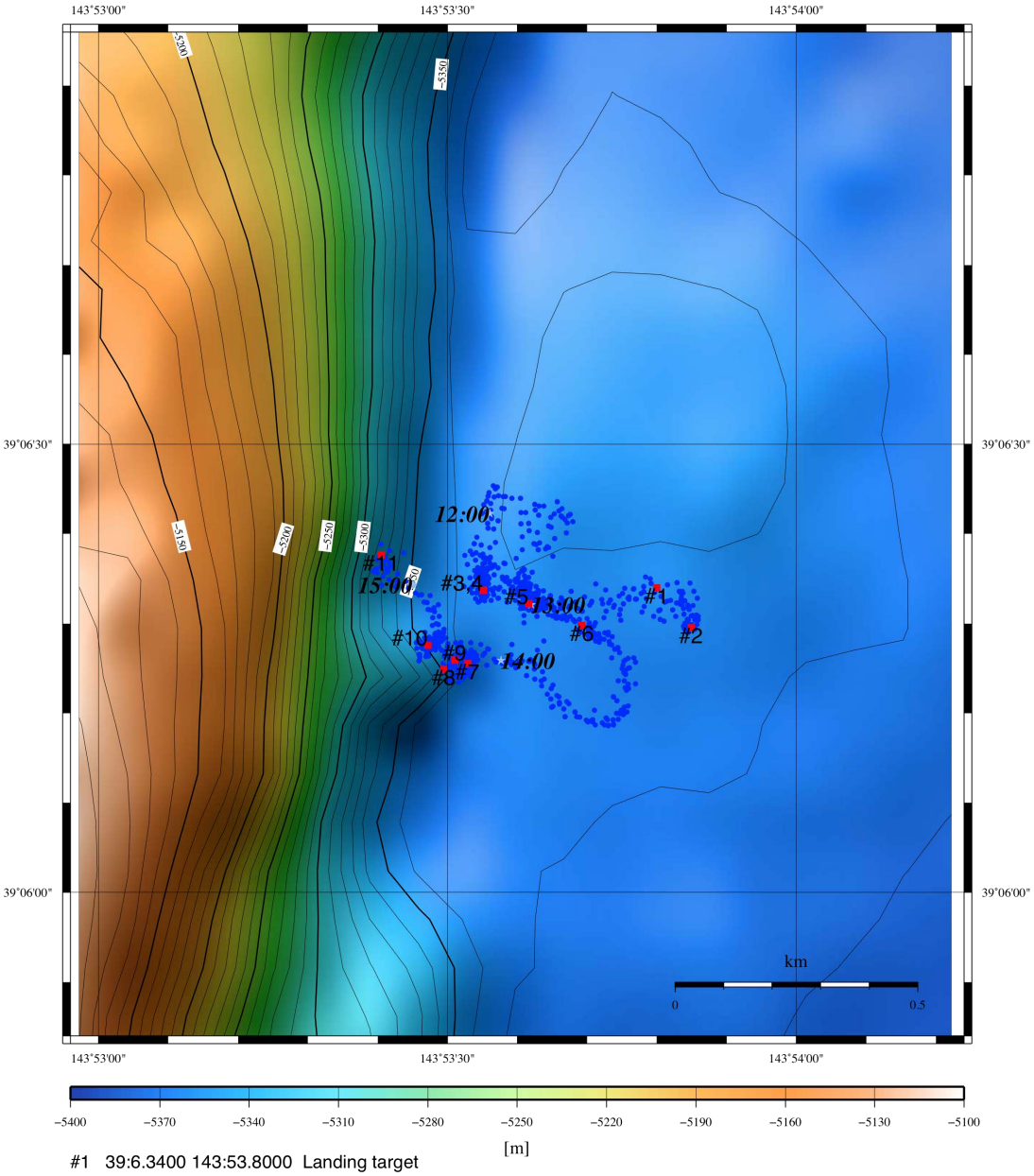
*** EVENT MARK LIST ***　　2011-08-18 15:17:25

ORIGIN (XY<->LATLON CONVERT)　LAT 39°06.5000'N　LON 143°53.4000'E
XY ORIGIN ((X,Y)=(0,0))　LAT 39°06.5000'N　LON 143°53.4000'E

NO.	DAY	TIME	LAT	LON	X	Y
1	2011-08-18	09:00:00	39° 6.3400' N	143° 53.8000' E	-296.0	576.5
	Landing target					
2	2011-08-18	11:08:00	39° 6.2953' N	143° 53.8491' E	-378.7	647.3
	Landing ,Sampling Animals D=5348m　ヤマコ4, エビ2 (スラープガンキャニスター-No.1)					
3	2011-08-18	12:38:00	39° 6.3371' N	143° 53.5512' E	-301.3	217.9
	Finding Bacteria mat, Sampling MBARI(Red,Black) D=5346m　Black: 急速凍, Red: Control					
4	2011-08-18	12:38:00	39° 6.3371' N	143° 53.5512' E	-301.3	217.9
	Deployment Quadrat, #129 Marker D=5346m					
5	2011-08-18	13:10:00	39° 6.3220' N	143° 53.6165' E	-329.3	312.0
	Sampling Calyptogena, Deployment #130 Marker D=5347m　モリが box (A)					
6	2011-08-18	13:24:00	39° 6.2978' N	143° 53.6928' E	-374.0	422.0
	Sampling Animals D=5348m　ゴカイ(キャニスター-No.2), 魚(キャニスター-No.3), クラゲ(キャニスター-No.4)					
7	2011-08-18	14:19:00	39° 6.2553' N	143° 53.5290' E	-452.7	185.9
	Sampling calyptogena D=5352m　Box (D)					
8	2011-08-18	14:25:00	39° 6.2488' N	143° 53.4948' E	-464.7	136.6
	Finding Bacteria mat D=5352m					
9	2011-08-18	14:28:00	39° 6.2593' N	143° 53.5105' E	-445.3	159.2
	Finding 122 Maker D=5350m					
10	2011-08-18	14:49:00	39° 6.2759' N	143° 53.4722' E	-414.6	104.0
	Sampling calyptogena D=5352m　box (B·C)					
11	2011-08-18	15:08:00	39° 6.3768' N	143° 53.4051' E	-227.9	7.3
	Left Bottom D=5354m					
12						
13						
14						
15						
16						
17						
18						
19						
20						

Dive track of 6K#1262

1263DIVE



#1 39:6.3400 143:53.8000 Landing target
#2 39:6.2953 143:53.8491 Landing ,Sampling Animals D=5348m
#3 39:6.3371 143:53.5512 Finding Bacteria mat, Sampling MBARI(Red,Black) D=5346m
#4 39:6.3371 143:53.5512 Deployment Quadrat, #129 Marker D=5346m
#5 39:6.3220 143:53.6165 Sampling Calyptogena, Deployment #130 Marker D=5347m
#6 39:6.2978 143:53.6928 Sampling Animals D=5348m
#7 39:6.2553 143:53.5290 Sampling calyptogena D=5352m
#8 39:6.2488 143:53.4948 Finding Bacteria mat D=5352m
#9 39:6.2593 143:53.5105 Finding #122 Maker D=5350m
#10 39:6.2759 143:53.4722 Sampling calyptogena D=5352m
#11 39:6.3768 143:53.4051 Left Bottom D=5354m

5. Preliminary research reports

1) Genome diversity of *Calyptogena* clams.

Takao Yoshida[1], Yoshihiro Takaki[1], Yukiko Nagai[1], Genki Ozawa[1], Shigeru Shimamura[1], Yoshimitsu Nakamura[1], Shino Suzuki[1], Yousuke Nomura[1], Takashi Kaneko[1], Masaaki Konishi[1], Tadashi Maruyama[1], Akihiro Tame[2], Masatoshi Tsukahara[3], Maiko Nezuo[3] (Biojet co. Ltd.), Morimi Teruya[4], Sei-ichi Okumura[5], Kazuma Kimura[5], Kenta Adachi[5] (School of Marine Biosciences, Kitasato University)

[1] *Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*

[2] *Marine Works Japan, Ltd.*

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[5] School of Marine Biosciences, Kitasato University

Objective and achievement in this cruise

Vesicomyid clams, including *Calyptogena* spp., form dense communities on the deep sea floor near hydrothermal vents and seeps. These clams have vestigial digestive tracts and are nutritionally dependent on chemoautotrophic sulfur-oxidizing symbiotic bacteria, which are harbored within their gill epithelial cells. *Calyptogena* symbionts are vertically transmitted via eggs, and are thought to have co-evolved with the *Calyptogena* clams. Recently, the genomes of two symbionts in *C. okutanii* and *C. magnifica* respectively, belonging to these different *Calyptogena* symbiont clades, were reported. However, the genome diversity of host clam and symbiont in *Calyptogena* was still unknown. To investigate the diversity, we planned to collect the *Calyptogena* clams at Kuril trench and Japan trench. During the cruise, *Calyptogena* clams were collected at several colonies. . After dive, the clams were immediately dissected, and blood, serum, and other tissues were frozen in liquid nitrogen and stored at -80ºC until used. Other samples were also stored at -80ºC. Detailed analyses of these samples will be performed after the cruise.

Future studies

*Analysis of genome diversity of *Calyptogena* host and symbiont

*Analysis of genome size and chromosome of *Calyptogena* clam

*Analysis of expression of several genes in *Calyptogena* clam

*Analysis of blood cells of *Calyptogena* clam

2) Diversity of microorganisms on the deep sea floor near colony of *Calyptogena* spp. and isolation of the microorganisms

Masaaki Konishi[1], Takao Yoshida[1], Yoshihiro Takaki[1], Yukiko Nagai[1], Genki Ozawa[1], Tadashi Maruyama[1], Akihiro Tame[2]

[1] *Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*

[2] *Marine Works Japan, Ltd.*

Objective and achievement in this cruise

Chemoautotrophic ecosystem is observed on the surface of the deep sea. There is little information of the diversity of microorganisms related to the ecosystem. Vesicomyid clams, including *Calyptogena* spp., form dense communities on the deep sea floor near hydrothermal vents and seeps. These clams have vestigial digestive tracts and are nutritionally dependent on chemoautotrophic sulfur-oxidizing symbiotic bacteria, which are harbored within their gill epithelial cells. Therefore, the clams are one of indexes for location of the chemoautotrophic ecosystem. We then attempted to investigate diversity of microorganisms in sediments near colonies of *Calyptogena* spp. at Kuril and Japan Trench. Furthermore, we tried isolate living microorganisms from the sediments.

At Kuril Trench, sediments near a *Calyptogena* spp. colony were also collected for investigating the diversity of microorganisms including bacteria, fungi and yeast. After dive, parts of sediments were immediately inoculated in growth media for isolating microorganisms. Other sediments were stored at 4 and -80°C for chemical and molecular biological analyses. The stored sediments will be analyzed in detail after the cruise.

At Japan Trench, sediment core which observed white bacteria mat on the deep sea floor was collected by MBALI core sampler for investigating diversity of microorganisms. The other sediment core was also collected as the control sample. After the dive, parts of sediments were inoculated to growth media. Other sediments were stored at 4 and -80°C for chemical and molecular biological analyses. The stored sediments will be analyzed in detail after the cruise.

Future studies

*Analysis of diversity of microorganisms in sediments near *Calyptogena* colonies.

*Analysis of diversity of microorganisms in sediments at white bacteria mat.

*Isolation of living microorganisms from sediments by culture dependent method.

3) Isolation of a lectin from a clam *Calyptogena phaseoliformis*

Mitsuru Jimbo
School of Marine Biosciences, Kitasato University

Objective and achievement in this cruise

Many deep sea animals obtain nutrients from symbionts which they harbor. Since the host harbored the specific symbiont species, they must select symbionts. Invertebrates usually use lectins, which binds to some carbohydrate chains, as a first step of self defense mechanism instead of antibodies. If the lectin bound substance was attacked or removed from hosts. Some reports suggested that a lectin of host animals like corals or clams involved in the establishment or maintenance of symbiosis. In the case of *Calyptogena* clams, symbionts were harbored in the cells of host gill. Although they were non-self organisms, they were not rejected by host, and were symbiosed. We previously purified a lectin from *Calyptogena okutanii*, and the lectin agglutinated symbiotic bacteria, and it distributed their gills, suggested that the lectin involved in the symbionts. The objective is the purification and characterization of a lectin from hemolymph of *Calyptogena phaseoliformis* determination of lectin distribution.

In this cruise, I collected hemolymph from 19 individuals of *C. phaseoliformis* and foot, mantle, hemolymph, blood cells and gonads from three individuals.

Future studies

1. Optimization of hemagglutinating activity
2. Purification and cDNA cloning of the lectin.
3. Measurement of haemaggutinating activity of each organs.
4. Comparition of property of the lectins to *C. okutanii* lectin

4) Analysis of genome size and chromosome of *Calyptogena* clam

Takao Yoshida[1], Yoshihiro Takaki[1], Yukiko Nagai[1], Genki Ozawa[1], Shigeru Shimamura[1], Yoshimitsu Nakamura[1], Shino Suzuki[1], Yousuke Nomura[1], Takashi Kaneko[1], Tadashi Maruyama[1], Akihiro Tame[2], Masatoshi Tsukahara[3], Maiko Nezuo[3] (Biojet co. Ltd.), Morimi Teruya[4], Sei-ichi Okumura[5], Kazuma Kimura[5], Kenta Adachi[5] (School of Marine Biosciences, Kitasato University)

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Objective and achievement in this cruise

Vesicomyid clams are very important deep sea shellfish for biology. For development of the deep sea biology, a wide range of knowledge is required. However, few fundamental biological studies have been conducted on Vesicomyid clams. Genome size and chromosomal studies, which are important with regard to the genetics of the clams, are also scarce.

In this cruise, we collected some *Calyptogena* clams, and treated these samples for genome size and chromosome analysis. For the genome size analysis, the mantle and gill tissues were fixed with Carnoy's solution. For the chromosome analysis, the gill tissues were treated with colchicin, KCl, and Carnoy's solutions. Moreover, in this cruise, we obtained some other deep sea invertebrates, such as sea cucumbers, worms, sea anemones, and shrimps. We also treated these samples for genome size or chromosome analysis.

Future studies

*Determination of genome size and chromosome number and karyotype of *Calyptogena* clams.

5) High-pressure adaptation of muscle actins of deep-sea fishes

Koji Inoue and Masaru Kato

Atmosphere and Ocean Research Institute, The University of Tokyo)

Objective and achievement in this cruise

High hydrostatic pressure is one of the major obstacles for organisms to inhabit deep-sea environment because it changes the three-dimensional structure of proteins, resulting in altering the functions of proteins. Thus, it is supposed that the organisms inhabiting the deep sea have developed special mechanisms to adapt to high pressure. Morita (2003) isolated a special actin gene from some deep-sea macrourid fishes, and identified some amino acid substitutions that are likely to be involved in high-pressure adaptation in the sequence. In this study, we try to find similar actin genes in another group of deep-sea fish, Liparidae, to know whether the adaptation mechanism to high pressure is common among distantly related groups of fishes. We also try to reveal the evolutionary history of the deep-sea specific actin genes by phylogenetic and comparative genomic analyses. Fortunately, a liparid fish was obtained in the dive 1263. We sampled the muscle pieces for analyses of actin genes and mitochondrial DNA sequence, and fixed other parts in formaldehyde for morphological analysis.

Future studies

*Analysis of mitochondrial DNA sequence for species identification

*Morphological analysis for species identification

*Analysis of actin gene or cDNA sequences

*Identification of special amino acid substitutions responsible for high-pressure adaptation.

6) Deep-sea litter and floating litter Off Sanriku

Haruka Shibata[1], Hiroshi Miyake[1], Yasuo Furushima[2]

[1] School of Marine Biosciences, Kitasato University

[2] *Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*

Objective and achievement in this cruise

Marine litter is found in the oceans of the world. Marine litter is classified into beach litter, floating litter and benthic litter. Floating litter drifts to the beach or sinks to the bottom. Marine litter causes environmental, human health and aesthetic problems.

Enormous Tsunami caused by the Tohoku-Pacific Ocean Earthquake on 11th March, 2011. The Tsunami flowed out a large number of houses, cars, ships and all property of people lived at Sanriku Coast to the ocean. Satellite imagery shows us the existence of floating litter on the surface of the ocean. However, it was unknown what kind of object in these floating litter. Information of floating litter by the Tsunami off Tohoku is poor after the Earthquake. Therefore we thought that in-site investigation was necessary to identify kind of floating litter and benthic litter. The aim of this cruise is to observe floating litter and deep-sea litter in situ and understand transportation of marine litter to deep-sea floor.

Video recording was conducted for getting quantitative data of floating litter on day time. A video camera (Sony, HDR-SR7) was set at navigation bridge deck. At the same time, kind of litter was checked by visual observation and photo images of floating litter were taken. Some deep-sea litter was collected by the manipulator of *Shinkai 6500*. These collected debris were taken pictures, measured the size, and observed the attached organisms. After observation, Sample was preserved in deep freezer (-80 °C). After this cruise, deep-sea litter will be weigh and attached organisms will be identified.

Future studies

* analysis of the biological and physico-chemical environments

*analysis of the video footages that recorded by *Shinkai6500* camera