

Cruise Report

Natsushima/Hyper-Dolphin NT08-07 LEG2

Studies on adaptation mechanisms to hydrogen sulfide
through analyses of thiotaurine and the taurine transporter
and

Verification of endemicity of animal species in
hydrothermal vent and seamount on the Ogasawara Arc

April 11-17, 2008

Principal Investigator

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1. OUTLINE OF THE CRUISE AND ACKNOWLEDGMENTS

In this cruise (NT08-07, Leg 2), we visited Myojin Knoll, which is about 400 km south of Tokyo, containing hydrothermal vent field in its caldera. We had 7 dives of ROV/hyper-Dolphin (Dive #818-824), during four days distributed to us. Scientists selected from two research proposals, S08-17 (Inoue group) and S08-26 (Watanabe group) participated in this cruise.

The major purpose of Inoue group is to analyze the structure and function of thiotaurine, a sulfur-containing amino acid, and the genes of its transporter (taurine transporter, TAUT), which have been suggested to be involved in detoxification and symbiosis. During the cruise, we collected hydrothermal vent-specific animals including deep-sea mussels (*Bathymodiolus septemdierum*), crabs and shrimps mainly using a suction sampler and manipulators. The samples were dissected and frozen for molecular and biochemical analyses, and live samples were also maintained for laboratory experiments and exhibition in Enoshima Aquarium.

The research objective of Watanabe group is to elucidate endemism, i.e., independency of so-called “hydrothermal vent-specific” species from “seamount-specific” and deep-sea-specific species. For this purpose, we collected plankton samples using suction sampler, benthic animals using core samplers and quadrat frame, at three different types of environments, i.e., hydrothermal vent area, non-hydrothermal area inside the caldera, and non-hydrothermal area on the caldera edge. We will estimate continuity and discontinuity of distribution of each species after sorting and identification of the samples. We expect that we will be able to distinguish “hydrothermal vent-specific”, “sea mountain-specific” and “deep-sea-specific” species.

In addition, we recovered experimental materials set at the vent area of Myojin Knoll in NT06-23 cruise, e.g., cages containing pieces of vent chimney for barnacle transplantation experiments, and plates to attract barnacle larvae for settlement. We also loaded three-dimension camera on ROV during the last dive, and recorded thermal vents and organisms as three dimension movies. We hope that data obtained by these studies will also bring us important information to understand the unique ecosystem around hydrothermal vents.

We would like to thank the crew of RV Natsushima and the operation team of ROV Hyper-Dolphin for their kind assistance with excellent techniques. We also appreciate JAMSTEC for providing us with this opportunity of deep-sea research and its staff for helpful assistance.

2. CRUISE INFORMATION

1) Cruise number/Ship name

NT08-07 (Leg. 2)/RV Natsushima and ROV Hyper-Dolphin

2) Title of cruise

“Hyper-Dolphin” Research Dive, Deep-sea Research, FY2008.

3) Proposal number/ Title of the proposals/ Representative of the proposals

S08-17/ Studies on adaptation mechanisms to hydrogen sulfide through analyses of thiotaurine and the taurine transporter/ Koji INOUE

S08-26/ Verification of endemicity of animal species in hydrothermal vent and seamount on the Ogasawara Arc/ Hiromi WATANABE

4) Period of cruise

From April 11 to April 17, 2008

5) Port calls

From JAMSTEC (Yokosuka, Kanagawa, Japan) to Shimizu Port (Shizuoka, Japan)

6) Investigation area

Izu-Ogasawara Area (Myojin Knoll)

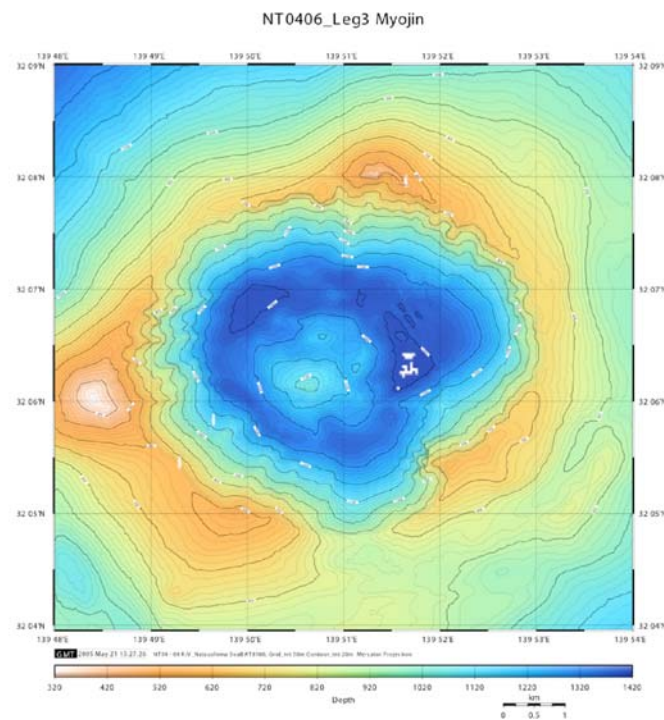


Fig. 1 Map of the caldera of Myojin Knoll.

7) Dive list

Dive #	Observers*	Dive points	Keywords
818	K. Inoue	Hydrothermal vent field in caldera	Mussel collection and recovery of plates for larval settlement
819	H. Toyohara	Hydrothermal vent field in caldera	Collection and observation of vent-specific organisms
820	Y. Ise	Non-hydrothermal area in caldera	Collection and observation of sea mount-specific organisms
821	H. Watanabe	Non-hydrothermal area on caldera edge	Collection and observation of deep-sea-specific organisms
822	N. Endo	Hydrothermal vent field in caldera	Collection and observation of vent-specific organisms
823	T. P. Satoh	Hydrothermal vent field in caldera	Collection and observation of vent-specific organisms
824	H. Miyake	Hydrothermal vent field in caldera	Collection and observation of vent-specific organisms and 3D-movie recording

*Author of dive report. Actually, observation was performed by multiple researchers in the control room on Natsushima, through high-vision and CCD cameras equipped on ROV.

8) Track line chart of the vessel

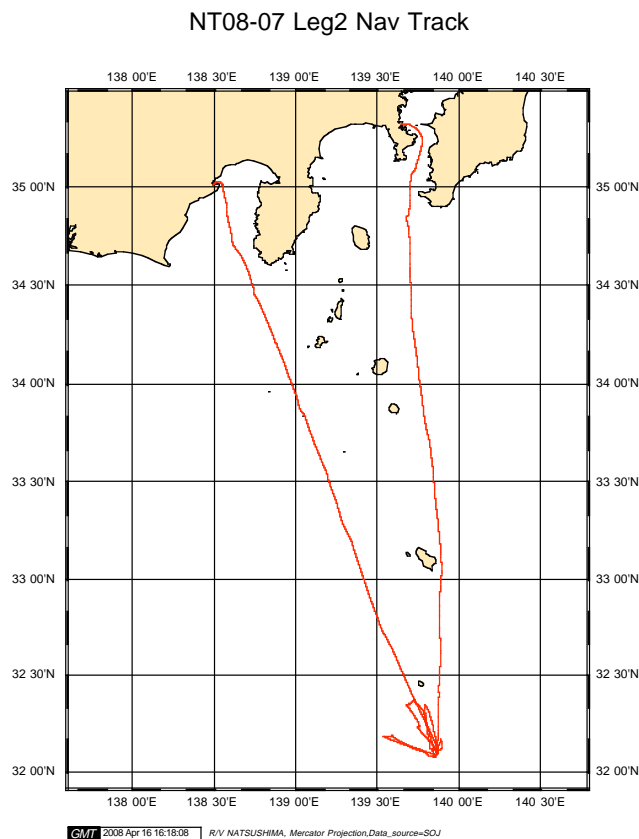


Fig. 2. Track line chart of RV Natsushima during NT08-07 Leg 2.

3. RESEARCHERS

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 Takashi OKUTANI
 Extremobiosphere Research Center, JAMSTEC.
 Motohiro SHIMANAGA
 Center for Marine Environment Studies, Kumamoto University.
 Shinji TSUCHIDA
 Extremobiosphere Research Center, JAMSTEC.

4. SCHEDULE

Mar 6 (Thu)	Meeting for the cruise at JAMSTEC, Yokosuka.
Mar 31 (Mon)	Loading of materials into Natsushima, at JAMSTEC, Yokosuka.
Apr 10 (Thu)	As Natsushima could not access to Hachijo Island because of weather condition, it returned to JAMSTEC, Yokosuka. Thus, the departure port was changed to JAMSTEC, Yokosuka.
Apr 11 (Fri)	Departure from JAMSTEC, Yokosuka
Apr 12 (Sat)	Arrived at Myojin Knoll. Dive #818, 819
Apr 13 (Sun)	Dive #820, 821
Apr 14 (Mon)	Dive #822, 823
Apr 15 (Tue)	Dive #824 and left Myojin Knoll.
Apr 16 (Wed)	Arrived at Shimizu Port
Apr 17 (Thu)	Packing and unloading of samples and materials, and cleaning of laboratories

5. EQUIPMENTS LOADED TO HYPER-DOLPHIN

1) Suction sampler

It was used to collect benthos, planktons and small fish. The nozzle was attached to the left hand of the manipulator, and connected to a rotary canister containing 6 bottles. Mesh bags were put into the bottles for plankton sampling.



Fig. 3. Suction sampler loaded to Hyper-Dolphin

2) Sample box

In addition to the regular sample box, plastic containers were also used to put larger organisms, chimney pieces, attachment plates and so on.



Fig. 4. Sample boxes

3) Shovel

Used for collection of mussels.

4) Bag-type water sampler and RMT thermometer

The nozzle of the water sampler was attached to the right hand of the manipulator with RMT thermometer. The nozzle was connected to two plastic bags through a three direction

connector with a selector lever. Water was evacuated using a perista pump. However, at the last dive, #824, the perista pump was replaced by a manual plastic pump because electric source was occupied by the 3D camera. The technique of the operator of the manipulator hands was excellent so that the small manual pump was nicely controlled.

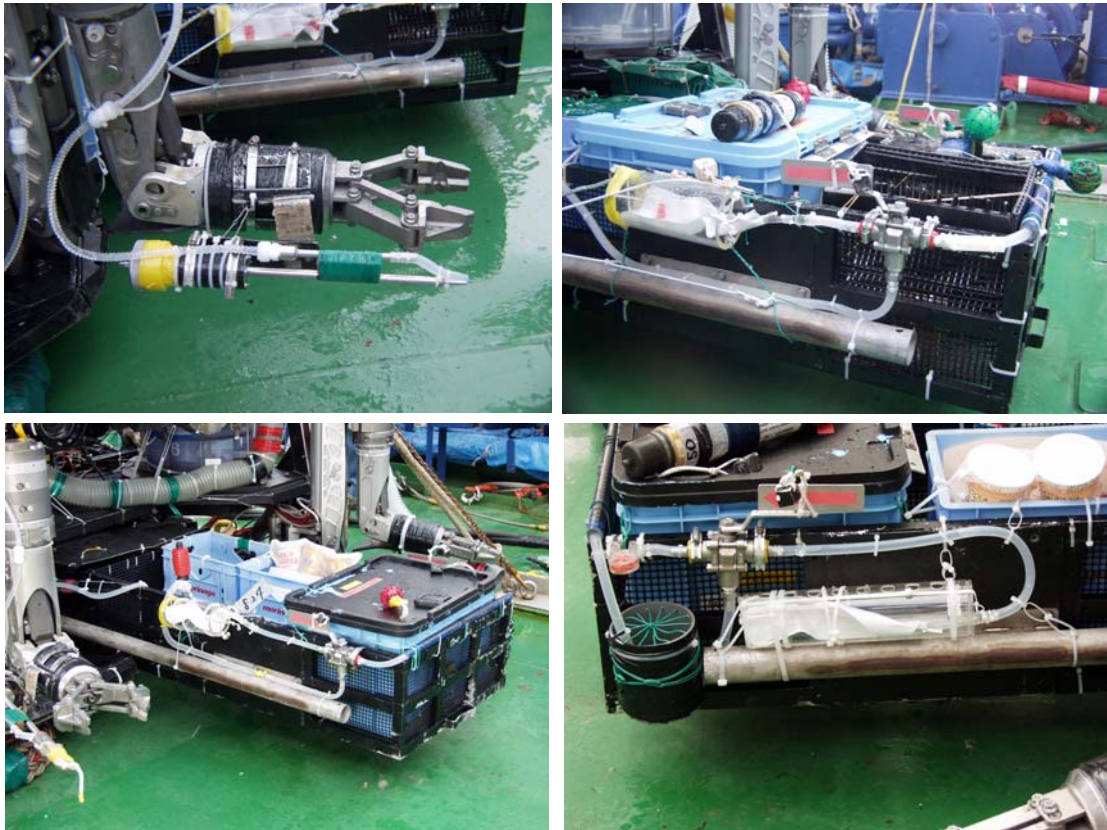


Fig. 5. Bag-type water sampler

5) Bait trap

A small trap, named “Tairyo-kun ver.6”, made from PET bottle and nets to catch crustacean.



Fig. 6. The bait trap, “Tairyo-kun ver.6”

- 6) MBARI core sampler
Six samplers were loaded for sampling of meiobenthos.
- 7) Quadrat frame
30 cm square, with color marking of 10 cm



Fig. 7. MBARI core samplers

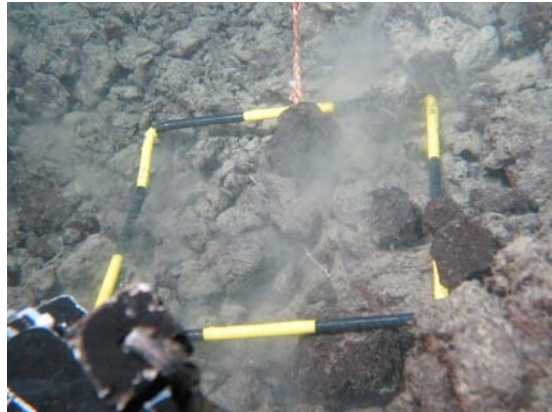


Fig. 8. Quadrat frame

- 8) 3D Hi-Vision video camera
The 3D Hi-Vision camera was developed by Okuno and his colleagues (http://www.jamstec.go.jp/jamstec-j/maritec/rvod/blue_earth/2008/program/pdf/PS34.pdf; in Japanese) and has been tested in a previous cruise, NT08-03. In our cruise, it was loaded to Hyper-Dolphin in the dive 824.



Fig. 9. Three dimension Hi-Vision camera loaded to Hyper-Dolphin

6. DIVE SUMMARY

1) Dive #818, April 12: Mussel collection and recovery of barnacle transplantation cages and attachment plates.

The dive #818, the first dive of leg 2, started under rainy and wavy condition. The vehicle, Hyper-Dolphin, dived into the hydrothermal vent area at the southern part of the caldera toward the point 15 (see Appendix) near a colony of mussels (*B. septemdierum*). Yunohana crabs, squat lobsters and *B. septemdierum* was collected using a suction sampler. Then the vehicle moved to the point 19. A trap for crabs and shrimps was set at this point. We also recovered a cage containing pieces of vent chimney for barnacle transplantation experiments and plates to attract barnacle larvae for settlement, which were set in Dive #630 and #631 of the cruise NT06-23. After taking pictures of a mussel colony to compare with the pictures of the same colony taken two and four years ago, the vehicle left the bottom.

Equipments loaded: Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Boxes, Bags for recovery of plates for barnacle settlement, Bait trap, Shovel.



Fig. 10. Arrangement of research equipments for Dive #818.



Fig. 11. Sampling of the mussel, *Bathymodiolus septemdierum*



Fig. 12. A transplantation cage and the unit of plates for barnacle settlement.

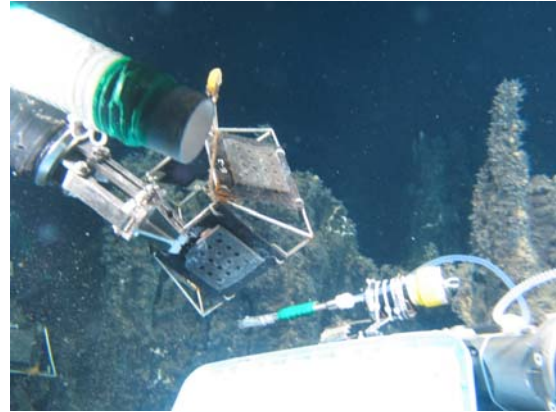


Fig. 13. Recovery of plates for barnacle settlement.

2) Dive #819, April 12: Cage setting for mussel transplantation experiments and recovery of cages for barnacle transplantation experiments.

The dive started at 15:00 under wavy condition. During the diving to the bottom, jellyfishes salps and fishes like conger eels were observed. After passing through a layer of seawater with low transparency, the vehicle arrived at the bottom near the point 15. Plankton was sucked from mussel colonies using the suction sampler, and then some mussels are collected. Two cages containing some mussels collected at the dive #818 were set; one at a position that close to a vent and another at a position far from the vent. We also recovered another cage containing pieces of vent chimney for barnacle transplantation experiments. After the recovery of the cage, the vehicle moved to “Daimyojin” chimney, a giant vent chimney approximately 40 m in height. We took pictures of a mussel colony near the top of the chimney for comparison with previous pictures and also carried out water sampling using the bag-type water sampler.

Equipments loaded: Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Boxes, Bags for recovery of plates for barnacle settlement, Cages for transplantation experiment, Shovel. (Almost the same as #818)

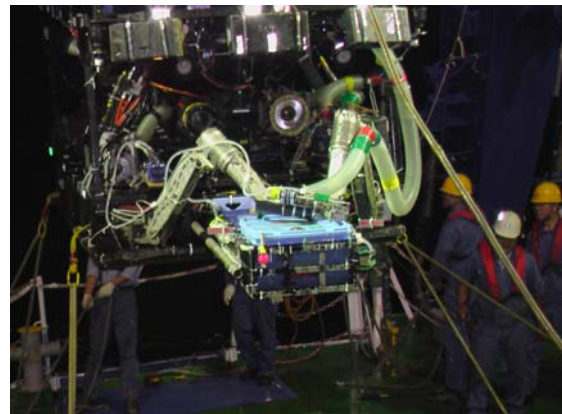


Fig. 14. Arrangement of research equipments for Dive #819. (The picture was taken when the vehicle returned to Natsushima)

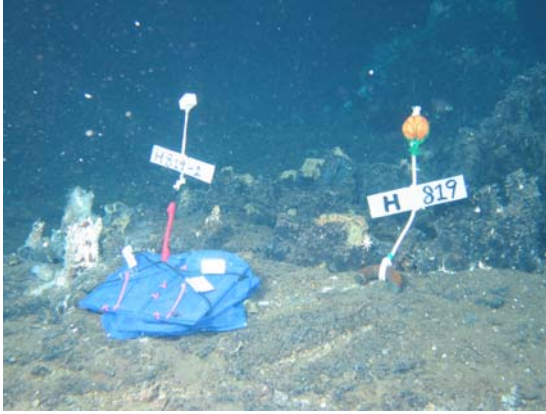


Fig. 15. Transplantation experiment of *B. septemdiarum*.

3) Dive #820, April 13: Observation and sampling of organisms at non-vent area near the edge of the caldera.

After settlement on the bottom (872 m in depth), the vehicle slowly cruised surveying benthic animals. A Gorgonacea species was sampled, to which stalked barnacles and a small crinoid were attached. Although the sampling of plankton to a mesh bottle using the suction sampler was successful, collection of benthos within a quadrat frame was not successful because rocks sucked with benthos stuck in canisters (However, Cladorhizidae sponge was obtained with a rock). Thus, we took pictures and video of area within the quadrat. Sampling of meiobenthos was also carried out using MBARI core sampler. Then we restarted the survey of benthic fauna and collected *Abyssocladia* sp. and Bolosominae gen. sp. After MBARI core sampling at another point and water sampling, the vehicle departed the bottom.

Equipments loaded: Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Box, MBARI core sampler, Quadrat, Shovel.

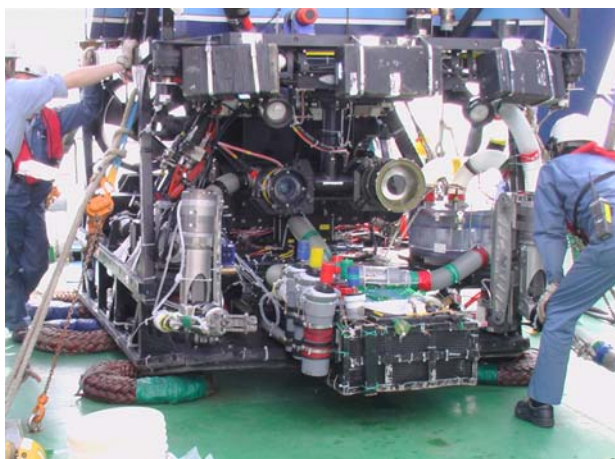


Fig. 16. Arrangement of research equipments for Dive #820.



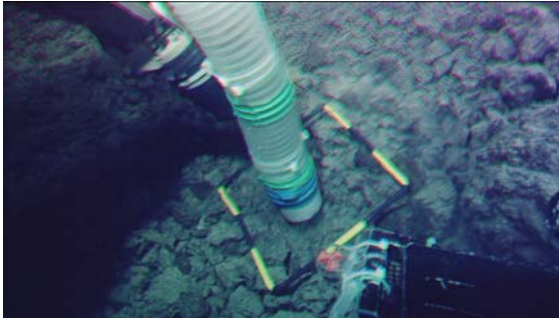


Fig. 17. Sampling of benthic animals using a quadrat frame.

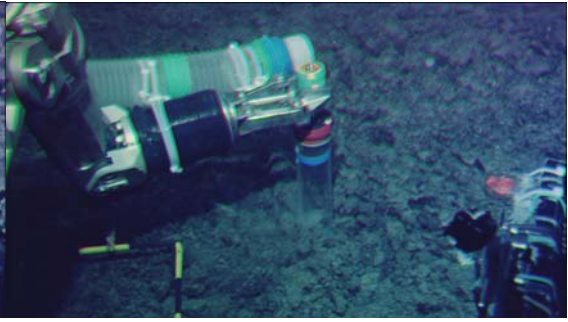


Fig. 18. Sampling of meiobenthos using MBARI core sampler.

4) Dive #821, April 13; Observation and sampling of organisms at non-vent area within the caldera and observation of organisms at the vent area at the north part of the caldera.

We planned sampling of plankton by suction sampler, quadrat sampling of benthos and core sampling of meiobenthos at three points in a line from the point 1 to the point 2 at an interval of 100 m. It was successful until the second points but plankton sampling and quadrat sampling were unsuccessful because the suction sampler became unavailable again due to rock sticking. After the series of sampling at the non-vent area, we visited a hydrothermal-vent field at a northern area of the caldera, which is different from the southern area including “Daimyojin” chimney. The fauna here is essentially similar to the southern area, i.e., *Bathymodiolus* mussels and *Neoverruca* barnacles were dominant and a number of *Yunohana* crabs and squat lobsters were also observed. Zoarcid-like fish was also observed, which was not seen in the southern area. After the sampling of water and rocks around colonies of mussels and barnacles, the vehicle left the bottom.

Equipments loaded: Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Box, MBARI core sampler, Quadrat, Shovel.

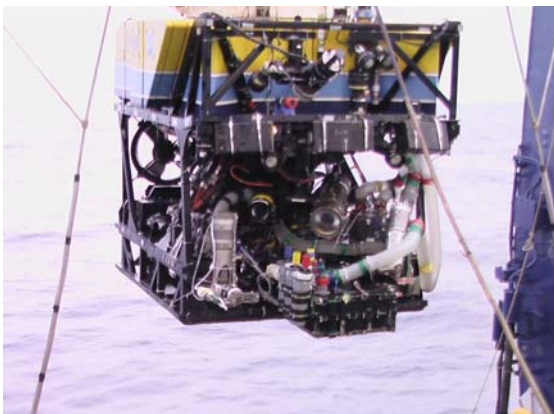


Fig. 19. Arrangement of research equipments for Dive #821.

5) Dive #822, April 14; Observation and sampling of organisms at vent area.

The weather condition became rainy again. The vehicle dived toward the point 8 and reached to the area near “Daimyojin” chimney. Plankton sampling by suction sampler, quadrat sampling of benthos and core sampling of meiobenthos was carried out at muddy area. After observation of the fauna around the giant chimney, the vehicle moved to the point 19, and the bait trap was recovered. The trap successfully captured many crabs. After water sampling and collection of some mussels, the vehicle departed from the bottom.

Equipments loaded: Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Box, MBARI core sampler, Quadrat, Shovel.

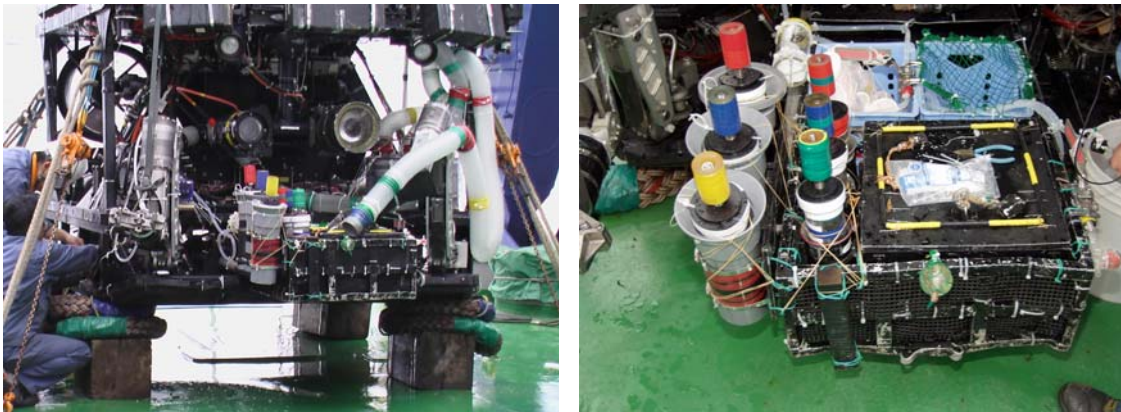


Fig. 20. Arrangement of research equipments for Dive #822.



Fig. 22. The bait trap, by which Yunohana crabs are trapped.

6) Dive #823, April 14; Observation and sampling of organisms at vent area. The vehicle dived toward the point 17 in the southern vent area and proceeded to the point 9. A white-colored chimney was found and water and polychaets were collected from its surface. Yunohana crabs and mussels were also collected. Then the vehicle moved to the point 19. A

fish, *Monomitopus kumae* was also captured during the transportation. At the position 19, the bait trap was set again to catch crabs. A Geryonid-like crab, eating broken mussels, was observed. Sampling of water as well as crabs and shrimps was also carried out.

Suction sampler with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer, Sample Boxes, Shovel.

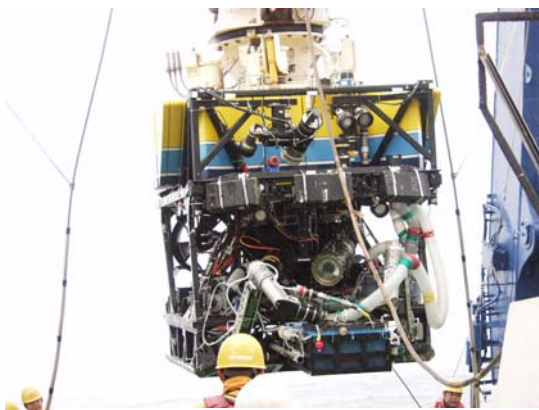


Fig. 21. Arrangement of research equipments for Dive #823.

7) Dive #824, April 15; Hi-Vision 3D movie and final sampling of animals

This is the last dive of this leg. We set a Hi-Vision 3D camera on the vehicle, and kept it recording throughout the dive. The dive was started from the point 15 and moved toward the point 19 to recover the bait trap set yesterday. Only one Yunohana crab was caught, it may be due to the short period after the set of the trap or the large Geryonid-like crab walking around the trap. We also collected shrimps, Yunohana crabs and some chimney samples using suction sampler. After the canister became full, the vehicle moved to “Daimyojin” giant chimney. We got a fine 3D movie of the big active chimney, which has already been used for some exhibitions.

Suction sampler (attached to the left hand of manipulator) with a rotary canister containing 6-bottles, Bag-type water sampler and RMT thermometer (attached to the right hand of manipulator), Sample Box, 3D-Video camera.

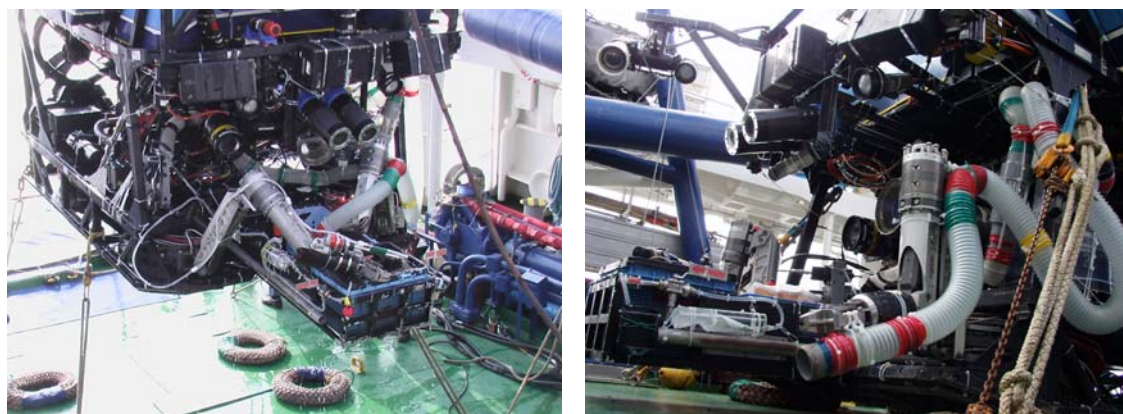


Fig. 23. Arrangement of research equipments for Dive #824. (See also Fig. 9.)

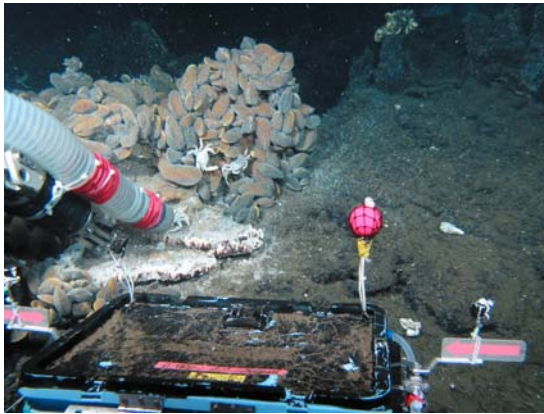


Fig. 24. Sampling of crabs around a mussel colony.



Fig. 25. Thermal vents of Daimyojin giant chimney. Crabs and fish are observed around the vent.



Fig. 26. Benthic animals observed at Daimyojin giant chimney.

7. RESEARCH PEPORPTS (Methods and Preliminary Results)

1) Studies on mechanisms to adapt to the environment of Hydrothermal Vents

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Objective

Various invertebrates inhabiting hydrothermal vents possess sulfur-oxidizing bacteria in their tissues; however, the mechanisms by which toxic sulfides are delivered to these endosymbionts remain unknown. Recently, detoxification of sulfides using thiotaurine, a sulfur-containing amino acid, has been suggested. The objective of our study is to clarify the mechanism of the detoxification by thiotaurine. We have already cloned a cDNA encoding taurine transporter (TAUT) that transport taurine and related amino acids across the cell membrane from the deep-sea mussel *Bathymodiolus septemdierum*, and demonstrated that it transport thiotaurine and its precursors using recombinant expression system in frog oocytes (FEBS letters 582, 1542-1546, 2008). We also established a real-time PCR system to quantify the level of mRNA.

Achievement in this cruise

In this cruise, we collected live *B. septemdierum* and dissected and froze some of them to measure the mRNA level, and also fixed some of them for histological analyses. We are also maintaining live specimens for experiments in laboratory aquarium. We also obtained samples of Yunohana crabs and squat lobsters to clone TAUT cDNA.

At several sampling sites, we sampled seawater just above the mussel colonies using a bag water sampler, and attempted rough estimation of the sulfide level using HACH system.

Future studies

- a) Rearing of the mussels in aquarium under the different condition (e.g., with/without sulfide).
- b) Quantification of TAUT mRNA level in the specimen of rearing experiments and that of specimens frozen immediately after the dive.
- c) Comparison of TAUT mRNA level on samples from the sites containing high level of sulfides with those containing low level of sulfides.
- d) Analysis of free amino acids of the specimen of rearing experiments and that of specimens frozen immediately after the dive.
- e) Histological analyses the specimen of rearing experiments and that of specimens fixed immediately after the dive.
- f) Cloning and characterization of TAUT genes of Yunohana crab and squat lobsters.
- g) Phylogenetic analyses on TAUT sequences of various marine organisms.

2) Studies on endemism and productivity of hydrothermal vent and seamount fauna

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Objective

The endemic species to the hydrothermal vent environment are estimated up to 90% of the fauna of the hydrothermal vent. On the other hand, more than 40% of seamount fauna is considered as endemic to the seamount environment. In general, hydrothermal vents are distributed on the seamount in western Pacific. However, there is no clear guide to distinguish vent-endemic species from seamount-endemic species, except for having symbionts. The objective of our study is to identify hydrothermal vent and/or seamount endemic species in the Myojin Knoll. In addition, with quantitative sampling, we would like to estimate carbon biomass of vent and/or seamount animal communities.

Achievement in this cruise

In this cruise, we tried to collect three sample sets; outside of caldera of the Myojin Knoll, non-vent area inside of caldera of the Myojin Knoll, and vent area inside of caldera of Myojin Knoll, and each sample set consists of quantitative sampling of mega- and meio-benthos and plankton with MBARI-type corers, quadrat and slurp-gun. However, most of the quantitative collections of megabenthos and plankton were not achieved.

Future studies

- a) Comparison of the densities and community structure of animals in above three environments (vent, non-vent and outside of caldera) with the photos of animal assemblage with quadrat
- b) Analysis of species composition and diversity of meiobenthos communities in above three environments
- c) Estimate the carbon biomass of vent animal community in the Myojin Knoll

3) Studies on the settlement-inducing factors of barnacle in hydrothermal vents

Noriyuki ENDO¹, Yasuyuki NOGATA¹, and Kiyotaka MATSUMURA²

¹*Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry;* ²*CERES, Inc.*

Objective

Larval settlement process of intertidal barnacles is affected by several environmental factors (e.g. light, temperature, salinity, surface type, and so on) as well as chemical factors such as settlement-inducing pheromones. Recently, a glycoprotein consisting of 76, 88, and 98 kDa subunits was purified as one of the settlement-inducing pheromone from adult extracts, and the primary structure was determined by cDNA cloning. On the other hand, the knowledge about the settlement-inducing factors of hydrothermal vents species is still poor. The objective of our study is to clarify the settlement-inducing factors of hydrothermal vent barnacle species. In previous cruise (NT06-23), settlement of *Neoverruca* sp. was observed only slate and PVC plates put on hydrothermal vents. Fibrous organisms which look like a hydroid were observed at the same places. These results suggested fibrous organisms and the temperatures around the hydrothermal vent have a close relation to settlement of *Neoverruca* sp.

Achievement in this cruise

In this cruise, we retrieved six sets of slate plates, and collected adult barnacle (*Neoverruca* sp. and *Ashinkailepas seepiophilia*). The slate plates, which barnacles (adult and/or larvae) were settlement, were rearing in laboratory aquarium, and some of them were fixed after observation. Collected barnacles were frozen or fixed for purification of settlement-inducing chemical compounds and subjected to several experiments.

Future studies

- a) Purification of settlement-inducing chemical compounds from the adult barnacles, *Neoverruca* sp. and *Ashinkailepas seepiophilia*.
- b) Rearing of *Neoverruca* sp. and *Ashinkailepas seepiophilia* larvae to cypris.
- c) Laboratory observation of behavior in cypris larvae of *Neoverruca* sp. and *Ashinkailepas seepiophilia* under the various conditions (e.g., with/without sulfide, at various temperature).

4) Mitochondrial genome evolution in the deep sea fishes

Takashi P. SATOH¹, Masaki MIYA² and Mutsumi NISHIDA¹

¹*Ocean Research Institute, The University of Tokyo;* ²*Department of Zoology, Natural History Museum & Institute, Chiba*

Objective

Mitochondrial genome of vertebrates are closed circular molecules spanning a length of approximately 17 kbp and typically containing 37 genes. All 37 genes are arranged in the same

relative order in almost all vertebrate species. However, deviations from typical gene order (gene rearrangement) were subsequently identified in various vertebrate lineages. In the fishes, we have ever found the novel gene orders in 34 species including many deep sea fish (23/34). For that reason, there is a possibility of some sort of correlations between rearrangement and deep sea fish. But more gene order data might be necessary to confirm it.

The objective of our study is to clarify the mechanism and the evolution pattern of mitochondrial gene rearrangement. Therefore, it is necessary to overlap these gene order data with robust phylogenetic tree.

Achievement in this cruise

In this cruise, we collected two deep sea fishes (*Monomitopus kumae* and *Nezumia condylura*) and dissected and froze some of them to measure the mRNA level and to determine the whole mitochondrial genome sequences, and also fixed some of them for histological analyses.

Future studies

- Whole mitochondrial genome sequencing of the specimens frozen immediately after the dive.
- Phylogenetic analysis of covering almost all fish taxon including above mentioned specimens.
- Comparison of quantify the level of mRNA for the specimen of no-rearrangement species and that of specimen (*N. condylura*) frozen immediately after the dive. Because *N. condylura* has mitochondrial gene rearrangement.

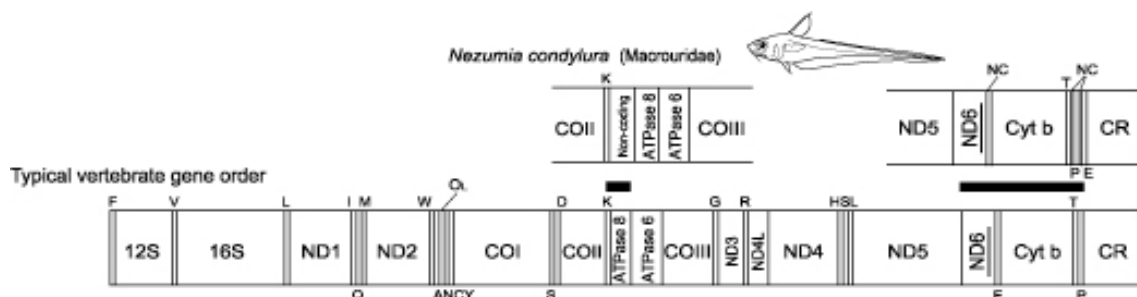


Fig. 27. Mitochondrial gene rearrangement in *N. condylura*

5) Deep sea carnivorous sponges collected by ROV Hyper-Dolphin from Myojin Knoll.

Yuji ISE

Misaki Marine Biological Station, The University of Tokyo

Two sponge specimens of the class Demospongiae were collected by ROV Hyper-Dolphin from Caldera of Myojin Knoll. Spicule character reveals these specimens attribute to two new species of the genus *Abyssocladia* (Poecilosclerida, Cladorhizidae) based on their abyssochelae

and *sigmancistra*. *Abyssocladia* is unique among sponges for their carnivorous habit. The genus is new record from Japanese water.

Abyssocladia sp.1 is 8 cm in total height, composed of a thin peduncle bearing an elongated flattened body with long filaments in its rim. The filament had bulbous object in its tip when the sponge was alive but it shrunk during collection. *A. sp.1* is distinct among 7 known species of the genus because it has cortex packed with microstrongyle which surface is granular.

Abyssocladia sp.2 is small erect sponge in 2 cm in total height, forming a slender, flattened spicular axis with numerous lateral filaments or processes. The species is similar to *A. nadur* Vacelet, 2006 in its gross morphology, however *A. sp.2* lacks sigma and has two types of abyssochelae.

Morphological description of these two new species is in preparation for submission.

6) Development, growth and survival in the deep-sea mussels, *Bathymodiolus* spp.

Kenji OKOSHI, Nozomi ITO and Yu HASEGAWA

Faculty of Science and Engineering, Ishinomaki Senshu University

Objective

Mussels of the Mytilidae are distributed from fresh water to seawater, and from shallow to deep sea. Concerning the deep-sea mussels, various species have been found from sunken wood, whale bones, hydrothermal vents, and cold seeps. Unlike shallow sea species, information is insufficient on development, growth, life span and population dynamics in such deep-sea species. We have studied the fine structure of shells, growth and lifecycle of deep-sea mussels. As for growth, we released some individuals of *Bathymodiolus septemdierum* after marking using strontium in the NT05-06 cruise and attempted to recover in NT06-23. However, only two specimens were recovered mainly due to the shift of the position of the vent. As for lifecycle, we have attempted to find larvae and young individuals of mussels but only small number of specimens have been obtained and it is not sure whether they are *B. septemdierum*. In this cruise, we tried to collect larvae and small individuals of *B. septemdierum* using the suction sampler. We also collect mussel specimens to analyze the growth of the shell.

Achievement in this cruise

We obtained plankton samples using the suction sampler. After sorting on board, bivalve larvae were maintained for observation of growth and behavior. Some individuals with larval shells are likely to be *B. septemdierum* judging from morphological characteristics. We also obtained various sizes of *B. septemdierum* to analyze the changes in proportion. We also treated 20 individuals using tetracycline, which are being maintained in Enoshima Aquarium. We also took pictures of mussel colonies to compare with the pictures of the same colonies in last two cruises.

Future studies

- a) Morphological observation using electron microscopy. Studies on behavior of larvae using video. Species identification by DNA analyses with Prof. Junichi Miyazaki of Yamanashi University.
- b) Addition of new morphological data of *B. septemdirum* to previous data sets. Observation of the maturation of the gonad.
- c) Analyses of shell growth using the marked individuals.
- d) By comparing the present pictures of the mussel colonies with previous ones, growth, disappearance, and recruitment of individuals in the colonies will be analyzed.

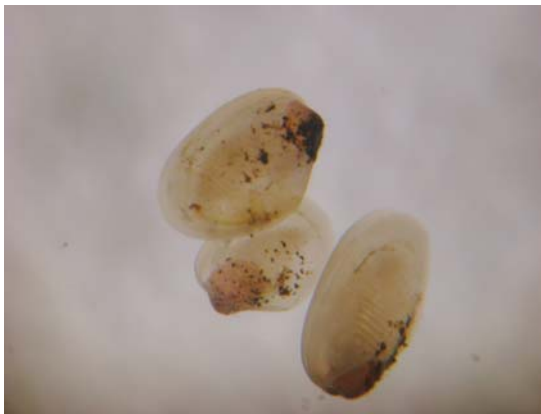


Fig. 28. Mussel larvae shortly after settlement



Fig. 29. *B. septemdirum* specimens marked with

7) Ecological study of *Paralvinella* spp. around Japan.

Takefumi YORISUE¹, Sigeaki KOJIMA¹ and Hiromi WATANABE²

¹*Ocean Research Institute, The University of Tokyo;* ²*Japan Agency for Marine-Earth Science and Technology*

Objective

Hydrothermal vents are discretely distributed on the deep sea floor. At vents, dense animal communities, in which lots of component species are endemic, develop. Evolutional and dispersal processes as well as ecology of the endemic animals are still unknown well. *Paralvinella* is a vent-endemic polychaete and one of the most common animals inhabiting hydrothermal vents in the Pacific Ocean. Therefore, *Paralvinella* worms are important animals to understand evolution and ecology of vent-endemic animals. Around Japan, they are distributed in the Okinawa Trough and the Izu-ogasawara Arc and specimens of the Okinawa Trough were identified as *P. hessleri*. We analyzed genetic population structure around Japan. Recently, we found two morphologically and genetically different types of *Paralvinella* worms within fixed samples collected in the Okinawa Trough. Furthermore, body size of individuals in

the Okinawa Trough is apparently bigger than those in the Izu-ogasawara Arc. In this study, we purpose to elucidate the factors causing such differences.

Achievement in this cruise

We collected many specimens of *paralvinella* sp. and froze some of them for stable isotopic analysis, fixed with PFA for morphological observation and also rearing some to obtain larvae. We took some precious photos and videos of the worm inhabiting on active chimneys.

Future studies

1. Morphological identification of *paralvinella* polychaetes inhabiting the Izu-ogasawara Arc.
2. Analysis of C/N ratio to compare food resources of *Paralvinella* sp. between the Okinawa Trough and the Izu-ogasawara Arc.
3. SEM observation and FISH analysis to find out bacteria attaching to *Paralvinella* sp.
4. Keep rearing to obtain larvae for elucidating larval ecology.

8) Why do hydrothermal vent crustaceans need heart source?

Hiroshi MIYAKE and Shuhei IKEDA
School of Marine Biosciences, Kitasato University

Objective

There are many crustaceans around hydrothermal vent. Some species need hydrogen sulfide for exo-symbiotic bacteria like *Shinkaia crosnieri* and *Rimicaris* species. As for hydrothermal vent crab species, *Gandalfus yunohana* and *Austinograea* species, they do not have symbiotic bacteria and eat other hydrothermal vent animals as a predator. However they were not found at cold seep area, but only at hydrothermal vent area. The heat source from hydrothermal vent may be essence of life of these vent crab.

We would like to clear the necessity of heat for vent crab in the course of long term-keeping in captivity.

Achievement in this cruise

In this cruise, we collected live *Gandalfus yunohana* and kept in aquaria. After the cruise, we will transport G. yunohana to Kitasato university and then keep them in the artificial hydrothermal vent tank to observe the behavior of them to heart source.

Future studies

1. Rearing of the vent crabs in aquarium under the different condition (e.g., with/without heart source).
2. Analysis of necessity of heart source by the difference of crab size.
3. Observing of the life cycle of vent crab.

9) Deep-sea at hand!

Hiroshi MIYAKE¹, Hikaru OKUNO² and Suguru NEMOTO³

¹*School of Marine Biosciences, Kitasato University,*

²*Japan Science Foundation*

³*Enoshima Aquarium*

Objective

Deep-sea realm is very exciting to public people. Today, we can get 2-D HDTV images of deep-sea. Public people is also able to watch these images on screen, TV, DVD and so on. The image of them is very different from the window view of manned submersible like *Shinkai 6500*. We would like to convey the exciting feeling of deep-sea diving using *Shinkai 6500* to public people and would children to be interesting to marine science. So we developed 3-D HDTV camera.

Achievement in this cruise

In this cruise, we collected raw HDTV image data for 3-D with stereo camera set on Hyper-Dolphin.

Future studies

4. Editing the raw HDTV data for 3-D projection
5. Showing the 3-D image to public people at public space like aquarium and museum and also exhibition area of JAMSTEC

10) Biodiversity of animals found in Myojin Knoll

Atsuko SUGITA and Suguru MEMOTO

Enoshima Aquarium

Background and objective

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 vent-specific animals using the suction sampler system and sorted all the samples. We are going to identify these samples and analyze the vent biodiversity in Myojin Knoll.

Summary of animals collected

We have collected deep-sea mussels (*Bathymodiolus septemdierum*), Bythograeid crabs (*Gandalfus yunohana*), Anomura (*Munidopsis myojinensis*) and Alvinocaridid shrimps (*Alvinocaris brevitelsonis*) in vent area to cultivate and display at our aquarium. *B. septemdierum* was a dominant and high population density species in this vent field, but population density of *G. yunohana* and *A. brevitelsonis* was not high. Especially, population density of *A. brevitelsonis* was lower than other above species, and the shrimps inhabit in

limited area. We have found and collected the shrimps at only east vent area in caldera structure (32°6.231 N / 139°52.159) and these shrimps almost inhabited gap of mussel bed. Probably, the shrimp inhabit under a specific condition. A number of collected *G. yunohana* had ripened ovary, but we could not collect any brooding of eggs individual. In the surveys conducted one year ago and three years ago, we have collected brooding of eggs individuals in May and August at Myojin Knoll (NT05-06, NT07-17). It may be because breeding season of the crabs had started.

These animals are being cultivated in Enoshima Aquarium now (Fig. 1, Fig. 2). The tank is displayed in the image of Myojin Knoll hydrothermal vent area with real chimney and estimated particular system. In this system, water temperature is kept about 8°C, in addition, hot water including sulfide is ejected from inside one of the displayed chimney and CO₂ bubble is added in the tank. We've been trying to make the observation of these animal behaviors for a long time. In the future, we would like to have these animals breed in our aquarium.



Fig. 30. *A. brevitelsonis* cultivated in the

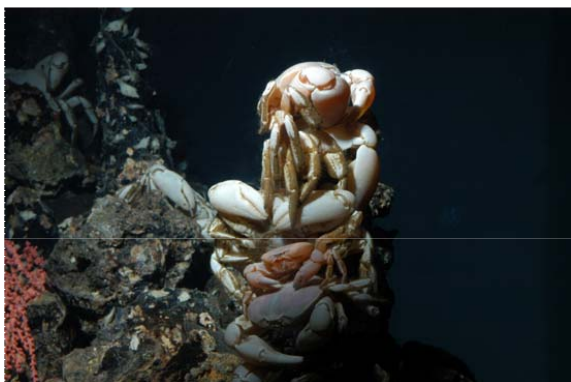
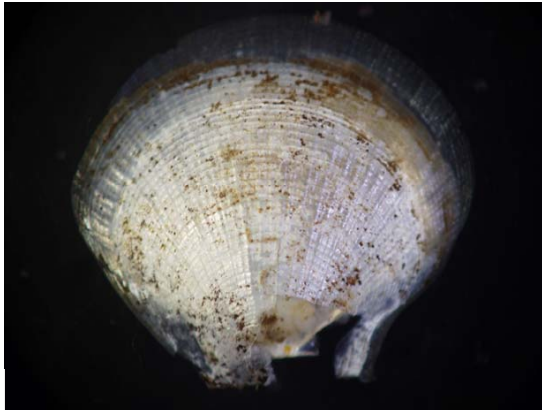


Fig. 31. *G. yunohana* focused in top of chimney

Research of biodiversity

We collected lot of samples in addition to the species mentioned above. The samples were mostly Polychaeta and few bivalve, gastropod and Arthropod. We took pictures of them when they are alive and then fixed using 10% formalin or 99% ethanol.



Propeamussiidae gen. et sp.



Provsnnidae gen. et sp.



Sipuncula gen. et sp



Paralvinella hessleri

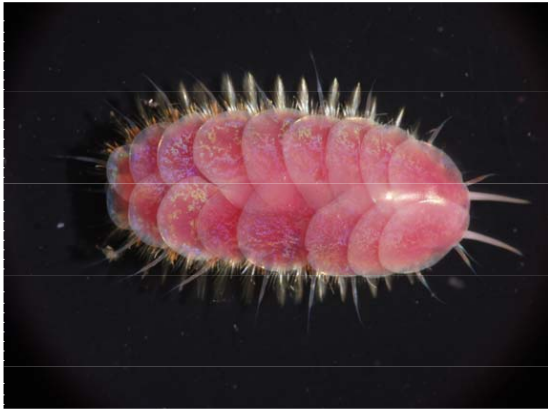


Unidentified Polychaetae



Unidentified Polychaetae

Fig. 32. Examples of collected samples



Unidentified Polynoidae



Unidentified Polychaetae



Unidentified Polychaetae



Unidentified Polychaetae



Unidentified Arthropod

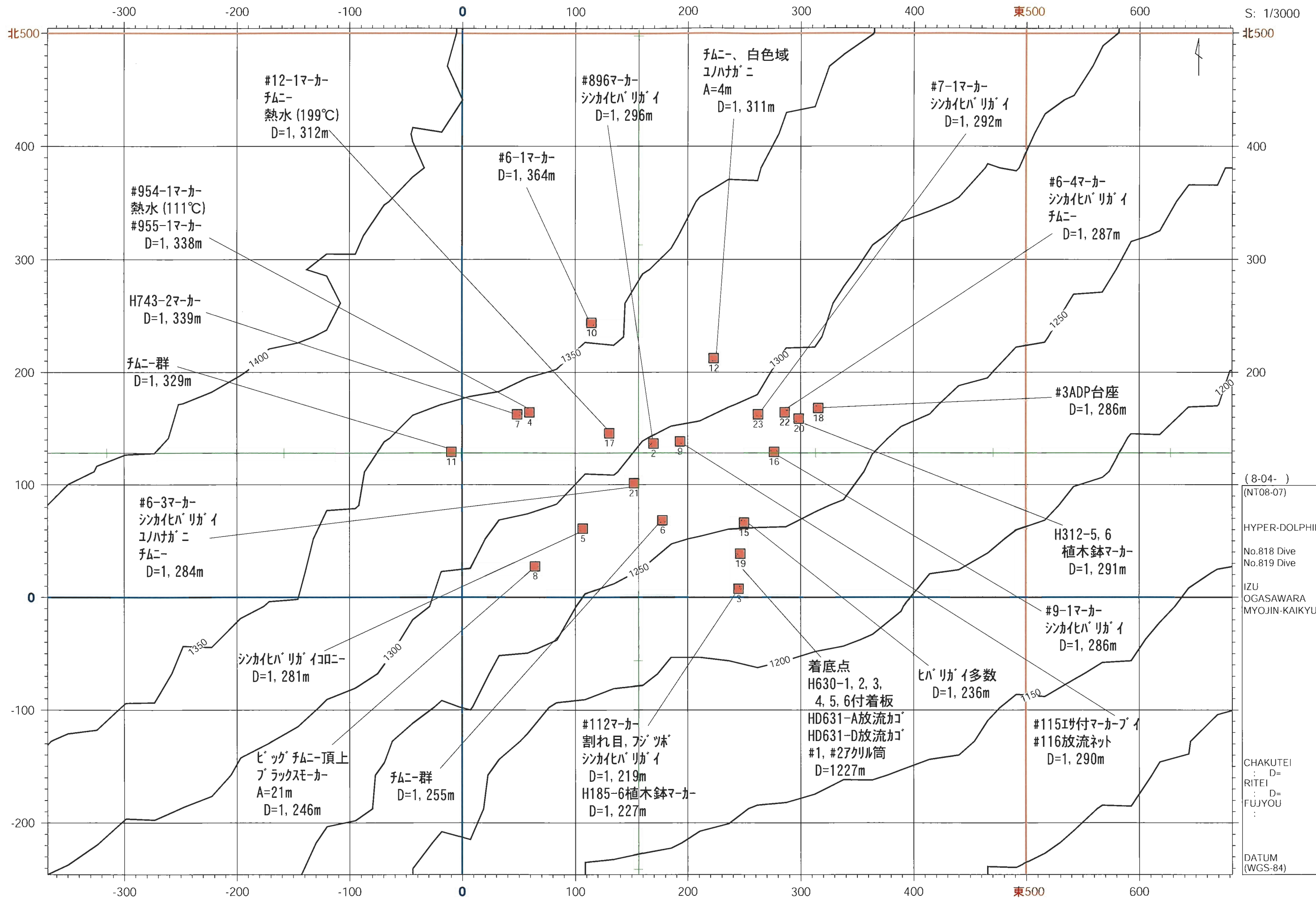


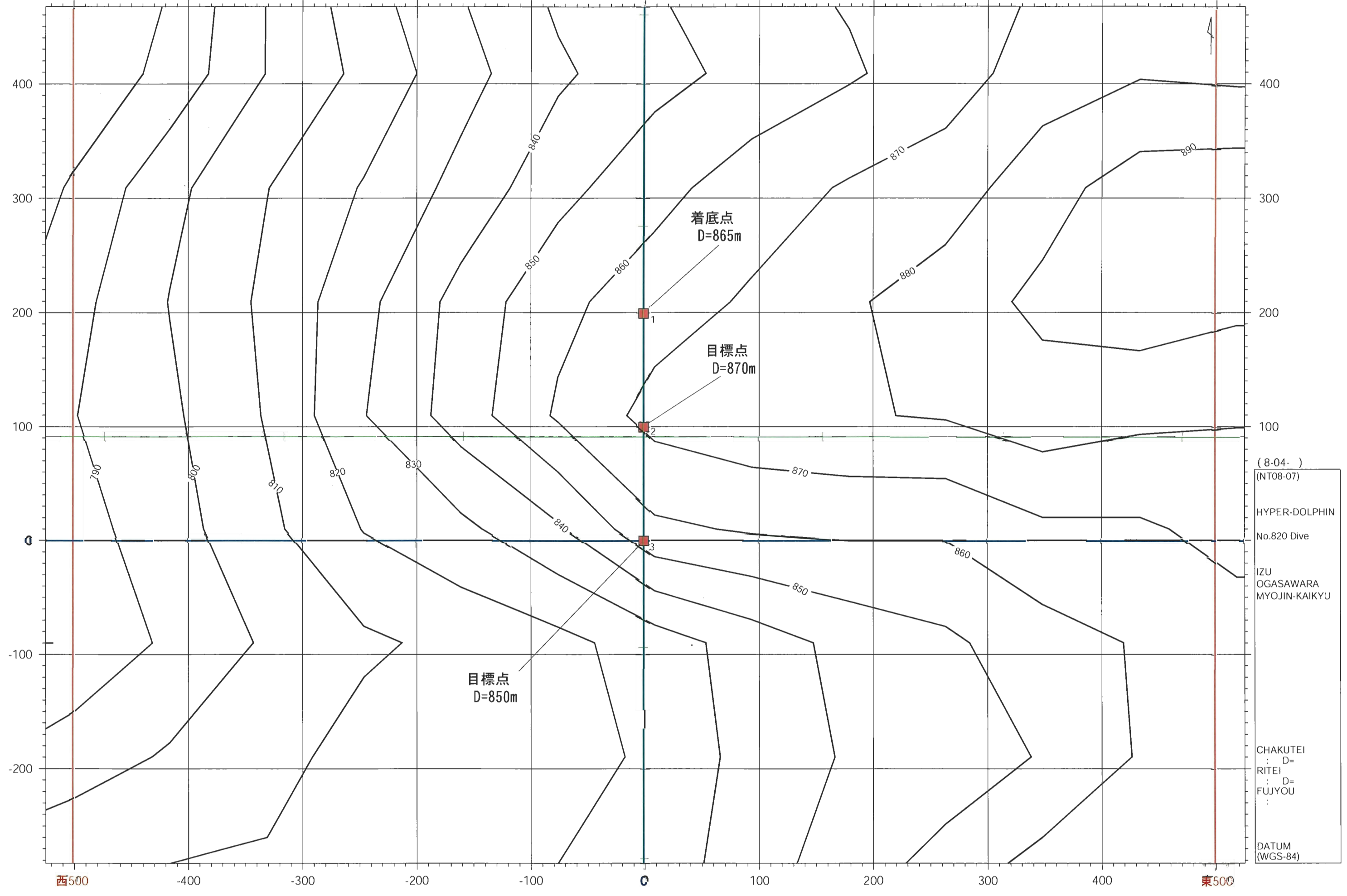
Unidentified Arthropod

Fig. 32. Examples of collected samples (continued)

Appendix

(point maps and route maps)

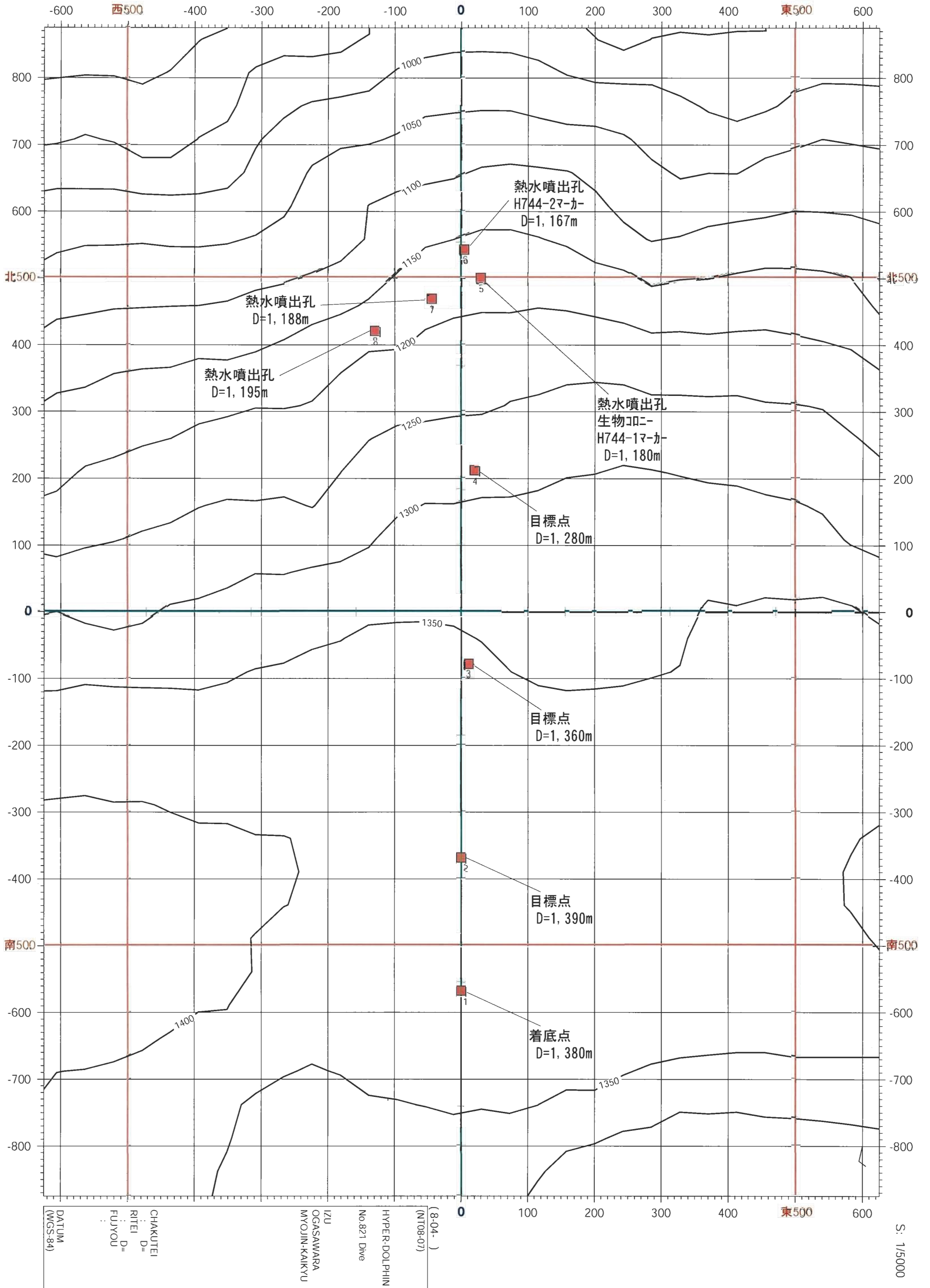




(8-04-)
(NT08-07)
HYPER-DOLPHIN
No.820 Dive
IZU
OGASAWARA
MYOJIN-KAIKYU
CHAKUTEI
: D=
RITEI
: D=
FUJYOU
:
DATUM
(WGS-84)

XY ORIGIN 32-7.200N 139-50.500E

CENTER 32-7.200N 139-50.500E



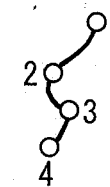
(8-04-)
 (NT08-07)
 HYPER-DOLPHIN
 No.821 Dive
 IZU
 OGASAWARA
 MYOJIN-KAIKYU
 CHAKUTEI
 RITEI
 D=
 FUJYOU
 DATUM
 (WGS-84)

S: 1/5000



1. 09:31 着底 D=1244m
(32-06.247N 139-52.161E)
2. 09:59 D=1235m 生物付岩石採取(1個)
(32-06.236N 139-52.150E)
- 10:18 生物採集(多数)
- 10:27 D=1234m RMT温度計測開始
- 10:28 RMT温度計測終了
- 10:42 D=1233m 生物付フィルム採取(1個)
3. 10:54 D=1223m H818[△]イトラップ設置
(32-06.228N 139-52.154E)
- 10:57 D=1224m RMT温度計測開始
- 11:00 RMT温度計測終了
- 11:07 H630-1附着板回収
- 11:11 「海洋の夢」マーカー設置
- 11:15 HD631-A放流カゴ回収
- 11:19 H630-3附着板回収
- 11:20 H630-2附着板回収
- 11:25 H630-4附着板回収
- 11:27 H630-5附着板回収
- 11:30 H630-6附着板回収
- 11:50 #1Bag採水開始
- 11:52 #1Bag採水終了
- 11:55 #2Bag採水開始
- 12:12 #2Bag採水終了
- 12:21 ヌノハナガニ採集(多数)
4. 13:13 離底 D=1219m
(32-06.220N 139-52.149E)

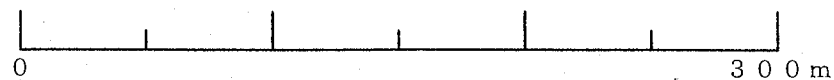
139° 52. 0 E



32° 06. 2 N

ハイパードルフィン
8 1 8 D I V E
2 0 0 8 年 0 4 月 1 2 日
伊豆・小笠原 明神海丘
縮尺 1 / 3 0 0 0

測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1 5 0 2. 7 m / s (D=1 3 0 0 m)

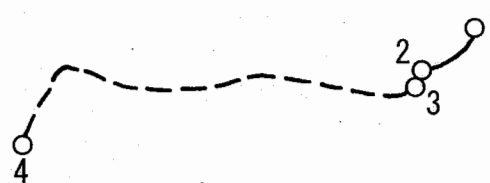


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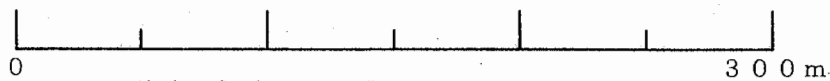


1. 16:26 着底 D=1237m
(32-06.238N 139-52.169E)
- 16:34 D=1235m プランクトン採集開始(#1キャニスター)
- 16:38 プランクトン採集終了
2. 16:48 D=1225m プランクトン採集開始(#2キャニスター)
(32-06.229N 139-52.155E)
- 16:53 プランクトン採集終了
- 16:58 D=1224m シンカイヒバノイ採集(多数)
3. 17:09 D=1223m H819-1放流ネット設置
(32-06.226N 139-52.154E)
- 17:10 HD631-D放流カゴ回収
- 17:13 H819-2放流ネット設置
- 17:14 H819マーカー設置
4. 17:28 D=1244m ビックチムニー視認 A=25m
(32-06.213N 139-52.055E)
- 17:50 D=1245m #1Bag採水開始 A=17m
- 17:52 #1Bag採水終了
- 17:55 #2Bag採水開始
- 17:57 #2Bag採水終了
- 18:02 生物採集(#3キャニスター)
- 18:04 D=1244m 離底

139° 52.0E



32° 06.2N



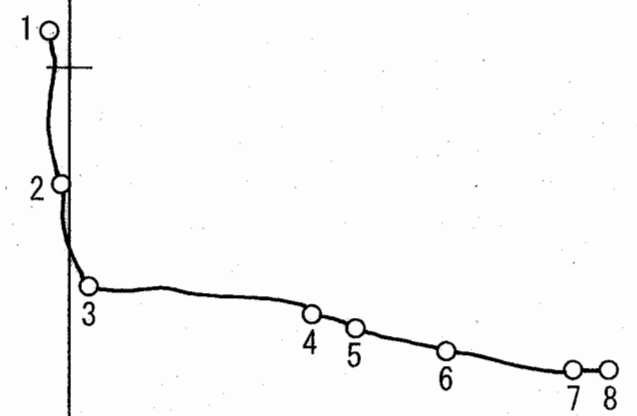
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ハイパードルフィン
#819 DIVE
2008年04月12日
伊豆・小笠原 明神海丘
縮尺 1 / 3000

測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1502.7m/s (D=1300m)



1. 09:06 着底 D=872m
(32-04.608N 139-50.995E)
2. 09:25 D=881m 生物採集
(32-04.575N 139-50.998E)
3. 09:36 D=879m プラクトン採集開始(#4キャスター)
(32-04.553N 139-51.005E)
4. 09:45 D=872m プラクトン採集終了
(32-04.547N 139-51.061E)
5. 09:55 D=870m 定量採集開始(#5キャスター)
(32-04.544N 139-51.072E)
- 09:59 定量採集終了
- 10:03 MBARI採泥(青緑・1本)
6. 10:20 D=862m 生物付岩石採取(1個)
(32-04.539N 139-51.095E)
- 10:31 D=861m 生物付岩石採取(1個)
(32-04.540N 139-51.098E)
7. 10:49 D=859m MBARI採泥(緑・1本)
(32-04.535N 139-51.127E)
- 10:52 MBARI採泥(赤・1本)
8. 11:21 D=859m 生物採集
(32-04.535N 139-51.136E)
- 11:29 D=860m MBARI採泥(赤白・1本)
- 11:38 #1Bag採水開始
- 11:40 #1Bag採水終了
- 11:49 離底 D=859m



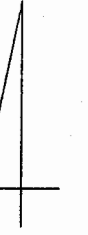
32° 04.5N

139° 51.0E

ハイパードルフィン
#820 DIVE
2008年04月13日
伊豆・小笠原 明神海丘
縮尺 1/3000

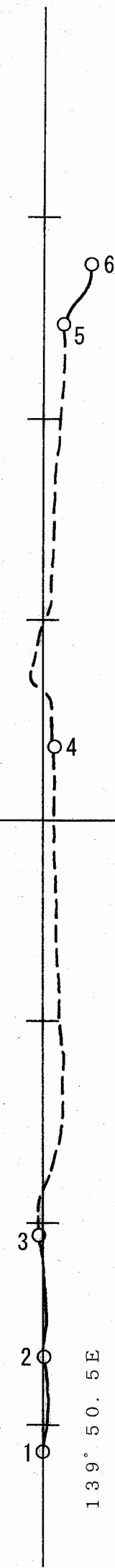
測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1510.1m/s (D=900m)

0 300m
※ 緯度、経度の1目盛りは、0.1分を示します。

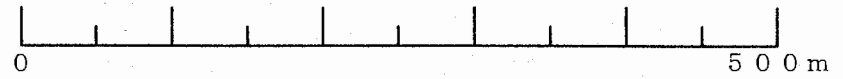


1. 14:38 着底 D=1391m
(32-06.887N 139-50.500E)
14:44 D=1392m 定量採集開始(#1キャニスター)
14:47 定量採集終了
14:50 MBARI採泥(青緑・1本)
14:52 MBARI採泥(赤・1本)
14:55 プラクトン採集開始(#2キャニスター)
2. 15:02 D=1395m プラクトン採集終了
(32-06.934N 139-50.501E)
15:07 定量採集開始(#3キャニスター)
15:09 定量採集終了
15:17 MBARI採泥(赤緑・1本)
15:19 MBARI採泥(黄・1本)
3. 15:35 D=1393m MBARI採泥(黄青・1本)
(32-06.994N 139-50.498E)
15:39 MBARI採泥(青・1本)
4. 15:55 D=1143m
(32-07.237N 139-50.507E)

5. 16:11 D=1190m 海底視認 A=6m
(32-07.447N 139-50.512E)
6. 16:21 D=1181m H744-1マーカー視認
(32-07.477N 139-50.528E)
16:34 D=1179m #1Bag採水開始
16:36 #1Bag採水終了
16:38 #2Bag採水開始
16:48 #2Bag採水終了
16:58 RMT温度計測開始
17:01 RMT温度計測終了
17:11 生物付フィルム採取
17:17 生物付岩石採取
17:19 離底 D=1179m



32° 07. 2N



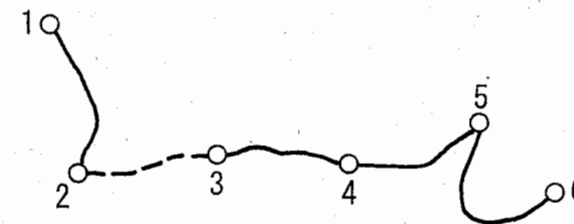
※ 緯度、経度の1目盛りは、0.1分を示します。

ハイパードルフィン
8 2 1 D I V E
2008年04月13日
伊豆・小笠原 明神海丘
縮尺 1 / 5 0 0 0

測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1501.8m/s (D=1400m)



1. 10:29 着底 D=1304m
(32-06.249N 139-52.040E)
- 10:32 D=1305m MBARI採泥(緑・1本)
- 10:33 MBARI採泥(黄・1本)
- 10:42 定量採集終了(#1キャスター)
- 10:44 プラントン採集開始(#2キャスター)
2. 10:55 D=1245m ビックチムニー視認 A=18m
(32-06.217N 139-52.047E)
3. 11:03 D=1266m プラントン採集終了(#2キャスター) A=5m
(32-06.221N 139-52.082E)
4. 11:22 D=1253m MBARI採泥(青緑・1本)
(32-06.219N 139-52.115E)
- 11:24 MBARI採泥(青・1本)
- 11:35 定量採集開始(#3キャスター)
- 11:37 定量採集終了
- 11:42 生物付チムニー採取(1個)
- 11:45 プラントン採集開始(#4キャスター)
5. 11:58 D=1224m プラントン採集終了
(32-06.228N 139-52.148E)
- 12:17 H818⁺イトラップ回収
- 12:25 #1Bag採水開始
- 12:27 #1Bag採水終了
- 12:41 シンカイバカリイ採集(多数)
6. 13:21 D=1224m MBARI採泥(赤緑・1本)
(32-06.213N 139-52.167E)
- 13:25 MBARI採泥(赤・1本)
- 13:26 離底 D=1223m

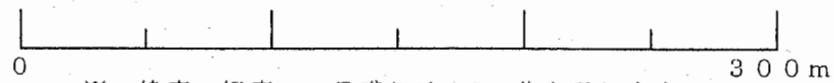


32° 06. 2N

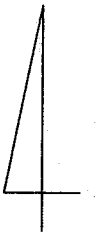
139° 52. 0E

ハイパードルフィン
8 2 2 D I V E
2008年04月14日
伊豆・小笠原 明神海丘
縮尺 1 / 3 0 0 0

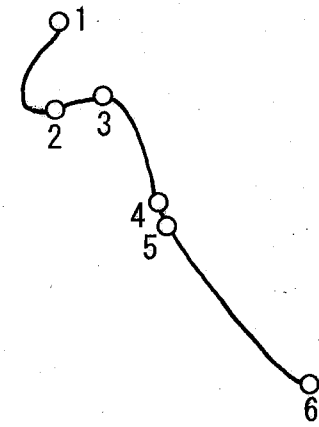
測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1502.7 m/s (D=1300m)



※ 緯度、経度の1目盛りは、0.1分を示します。



1. 16:38 着底 D=1318m
(32-06.299N 139-52.094E)
2. 16:54 D=1310m #1Bag採水開始
(32-06.280N 139-52.093E)
- 16:55 #1Bag採水終了
- 17:01 生物採集(#3キャニスター)
- 17:06 チムニー片採取(1個)
3. 17:16 D=1304m 生物採集(#4キャニスター)
(32-06.283N 139-52.105E)
- 17:17 プランクトン採集開始(#1キャニスター)
- 17:23 プランクトン採集終了
- 17:26 RMT温度計測開始
- 17:29 RMT温度計測終了
4. 17:48 D=1287m 魚採集(#5キャニスター)
(32-06.260N 139-52.119E)
5. 17:53 D=1280m 生物採集(#6キャニスター)
(32-06.255N 139-52.121E)
- 17:59 D=1279m 生物採集(#6キャニスター)
6. 18:19 D=1223m H823エサ付トラップ設置
(32-06.221N 139-52.157E)
- 18:20 ベイトトラップ設置
- 18:23 #2Bag採水開始
- 18:24 #2Bag採水終了
- 18:36 チムニー片採取(1個)
- 18:49 チムニー片採取(1個)
- 18:55 生物採集(多数)
- 18:56 離底 D=1224m

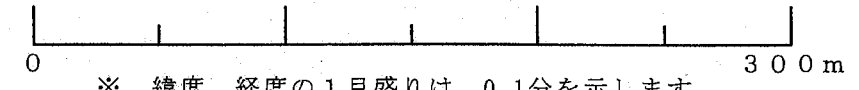


32° 06. 2N

139° 52. 0E

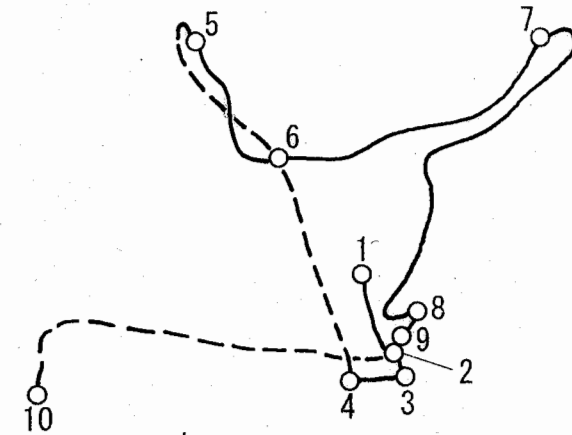
ハイパードルフィン
8 2 3 D I V E
2008年04月14日
伊豆・小笠原 明神海丘
縮尺 1 / 3 0 0 0

測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 15.02. 7 m/s (D=1300m)



※ 緯度、経度の1目盛りは、0.1分を示します。

1. 09:06 着底 D=1242m
(32-06.239N 139-52.145E)
2. 09:16 D=1223m H823^ハイトラップ回収
(32-06.222N 139-52.153E)
- 09:18 H823餌付きトラップ回収
- 09:23 RMT温度計測開始
- 09:27 RMT温度計測終了
- 09:32 #1Bag採水終了
- 09:36 RMT温度計測開始
- 09:44 #2Bag採水終了
- 09:52 チムニー片採取(1個)
- 10:05 ユノハカニ採集(#1キャニスター・多数)
3. 10:21 D=1221m 生物採集(#2キャニスター・多数)
(32-06.217N 139-52.156E)
4. 10:26 D=1217m A=5m
(32-06.216N 139-52.142E)
5. 10:35 D=1306m 海底視認 A=3m
(32-06.289N 139-52.103E)
6. 10:46 D=1286m 魚採集(#3キャニスター・1個体)
(32-06.264N 139-52.124E)
7. 11:15 D=1289m
(32-06.290N 139-52.190E)
8. 12:05 D=1234m ユノハカニ採集(#4キャニスター・多数)
(32-06.231N 139-52.159E)
- 12:12 ユノハカニ採集(#5キャニスター・多数)
- 12:21 生物採集(#6キャニスター)
2. 13:13 D=1227m 生物採集(多数)
(32-06.225N 139-52.155E)
- 13:32 D=1225m チムニー片採取(1個)
- 13:37 生物付チムニー採取(1個)
9. 13:50 D=1254m ビックチムニー視認 A=18m
(32-06.213N 139-52.063E)
- 14:23 D=1246m ヒトデ採集(1個体) A=17m
- 14:51 D=1245m チムニー採取(1個) A=20m
- 14:57 D=1246m チムニー採取(1個) A=23m
- 14:59 H824マーカー設置
- 15:01 離底 D=1246m

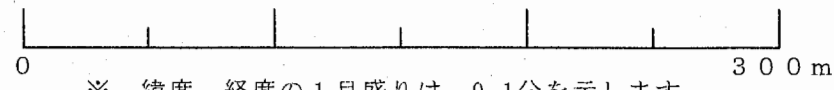


32° 06. 2N

139° 52. 0E

ハイパードルフィン
8 2 4 D I V E
2008年04月15日
伊豆・小笠原 明神海丘
縮尺 1 / 3 0 0 0

測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1502.7 m/s (D=1300m)



※ 緯度、経度の1目盛りは、0.1分を示します。