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NT09-16 Cruise Report

R/V Natsushima – Hyperdolphin
NT09-16 Cruise in the Sea of Japan

"Interaction between chemical environments, biological activities, and material cycling at a well oxygenated sediment-water interface in the Sea of Japan"

September 14th – September 25th, 2009

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

C O N T E N T S

1. Cruise information

Cruise number: NT09-16 Leg2

Research vessel: Natsushima and the ROV Hyperdolphin

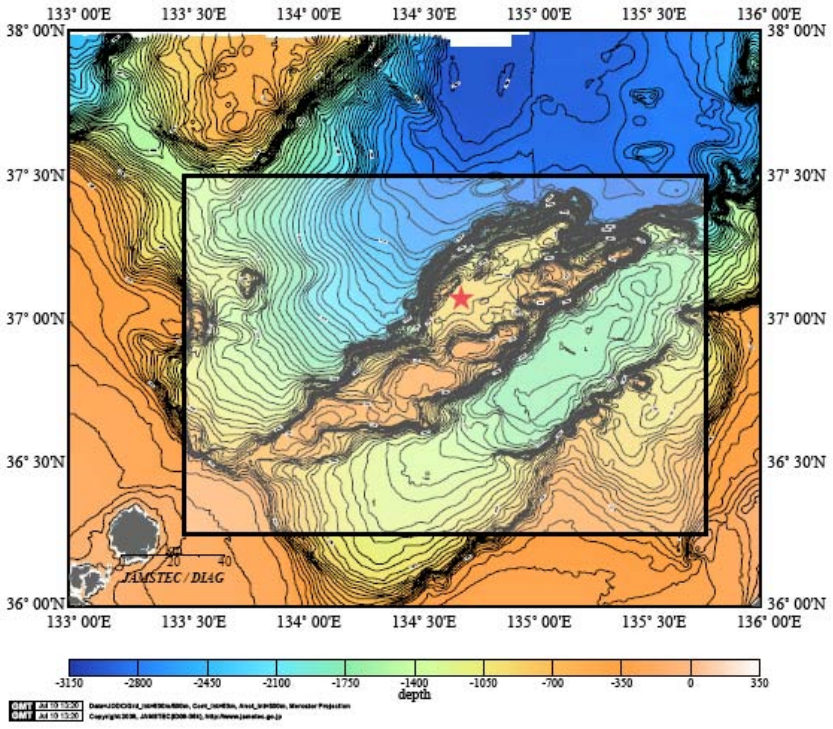
Title of the Cruise: Interaction between chemical environments, biological activities, and material cycling at a well oxygenated sediment-water interface in the Sea of Japan

Cruise period and Ports of call: From September 14th (Naoetsu) to 25th (Hakata), 2009

Research area: Oki-Ridge, Sea of Japan

Water depths: 910-930m

Research Map:



Red star indicates the dive points.

2. Science party

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3. Scientific Report

3.1. Benthic foraminiferal trophic ecology at the Oki-Ridge, the Sea of Japan

Hidetaka Nomaki, Kentaro Innoue, Takashi Toyofuku, Saburo Sakai

3.1.1. Purpose

Benthic foraminifera are major component among deep-sea benthic ecosystems and are also known as major consumers of phytodetritus. We have investigated benthic foraminiferal trophic ecology under low oxygen cocentration area, Sagami Bay, Japan. According to our results, benthic foraminifera plays important roles on the material cycling at the hypoxic area. However, it has not well investigated about their roles on material cycling under well oxygenated area. Here, we carried out in situ ^{13}C-tracer experiments using ROV Hyperdolphin at the well oxygenated deep-seafloor of the Oki-Ridge, Sea of Japan.

3.1.2. Materials and Methods

In situ incubation experiments were carried out at the Oki-Ridge (water depth of 915m) using in situ incubation cores. The surface sediment area of the core is 52.8 cm^2 (ϕ = 8.2cm). Every core has couple of 5ml syringes that can contain ^{13}C-labeled Chlorella or sodium bicarbonate-filtered seawater solution. In total 12 cores were deployed at the site. Culture cores were settled 50 to 100 cm away from each other (Figure 4.4.1). Seven out of 14 cores contained ^{13}C-labeled Chlorella, and the other 5 cores contained ^{13}C-labeled sodium bicarbonate. For Chlorella cores, 2 cores were incubated for 2 days in situ, 3 cores were incubated for 5 days in situ, and the other 2 cores were recovered immediately after deployments as time-0 controls (however, they stayed at the bottom for 2 hours). For sodium bicarbonate cores, 3 cores were incubater for 6 days in situ, and the other 2 cores were taken as time-0 controls. Two reference sediment cores were sampled at few meters away from the incubation station. The inner diameter of the reference cores were same to incubation cores (ϕ = 8.2cm).

After recovery on board, the cores were kept cool (4°C) prior to core processing and processed as quick as possible. Overlying water of the cores were sampled in 3 20ml glass bottles to determine mineralization rates of

Oxygen concentrations in the overlying seawater and in the sediments were measured with microelectrode. The cores were sectioned every half cm depth from the sediment surface to 3cm, every 1 cm from 3 to 5cm, and followed by 5-7, 7-10, and 10-15cm. They were separetd into samples for 1) foraminifera, 2) bulk sediment analysis, and 3) microbial analysis (see Chapter 3.3 by K Inoue).

3.1.3. Future works

The sediment samples will be thawed and then sieved on the 125 μm screen using artificial seawater. Benthic foraminifera will be picked out from the sieved sediments.

3.2. Capturing, gradual slow decompression, and re-compression of deep-sea multicellular organisms using a deep-aquarium system.

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3.2.1. Introduction

The deep sea represents a large portion of the total volume of the oceans and this extreme environment is characterized by the absence of light, low temperature, and high hydrostatic pressure. After the advent of deep-sea vessels opened the door to research on deep-sea organisms, many have been isolated and characterized (Horikoshi and Tsujii 1999). Although increasing attention has been paid to deep-sea animals, no group succeeded in culturing them before 2000 (Koyama and Aizawa, 2000). There are three major obstacles to molecular and cellular biological studies of deep-sea multicellular organisms under atmospheric pressure. The first is the development of devices for capturing and maintaining them, because most succumb as a result of decompression and exposure to the high-temperature surface seawater (Koyama and Aizawa 2000; Koyama et al. 2002, 2005a). The second is acclimation of the captured organisms to atmospheric conditions. The third is culture and freeze-storage of cells extracted from deep-sea organism tissues under atmospheric conditions.

We solved those three problems by developing a novel piezostat aquarium system to capture and maintain deep-sea organisms (Koyama et al. 2002, 2003a, 2003b, 2005a) and succeeded in the cultivation and freeze-storage of pectoral fin cells from the living deep-sea eel *Simenchelys parasiticus* (habitat depth, 366–2630 m; Nakabo 2000) at atmospheric pressure (Koyama et al. 2003a, 2003b, 2005b).

This report describes novel capture and maintenance devices for deep-sea organisms and gradual slow decompression of the organisms using the system.

3.2.2. Experiment

Capture and maintenance of deep-sea multicellular organisms under pressure

Three main approaches have been used to collect deep-sea multicellular organisms for study. The first is remote collection with trawls, corers, and grabs. However, the animals are often injured during decompression. The second is collection by

submersibles with a suction sampler (Hashimoto et al. 1992) or a manipulator (Koyama and Aizawa 2000), but most of the organisms succumb as the result of decompression and exposure to the high-temperature surface seawater. The third method is capture of the organisms in pressure-retaining traps and maintaining them in the laboratory for study within the traps (Yayanos 1978; Macdonald and Gilchrist 1978; Wilson and Smith 1985; reviewed in Smith and Baldwin 1997). Because no piezostat systems were developed for the traps, most organisms could not be kept alive in the pressure-retaining devices for long periods in early studies (Yayanos 1978; Macdonald and Gilchrist 1978; Wilson and Smith 1985).

We developed a novel piezostat aquarium system (Koyama et al. 2002; Fig. 1) to study the behavior of abyssal organisms. The aquarium system was designed for suction capture of deep-sea organisms with a submersible servomotor (Fig. 2). After capture of the organisms, the openings in the inner lids are closed by a spring mechanism that removes the pins (Fig. 2). The calculated minimum-resistance pressure limit of the aquarium is 30 MPa, and the inner seawater can escape through a pressure control valve (Fig. 2) when the inner pressure increases to greater than 20 MPa. Therefore, the aquarium system maintains an inner pressure of 20 MPa (corresponding to a depth of 2,000 m) even when the submersible surfaces from the deepest sea bottom. The organisms are fed on bait worms, and the compressed seawater is circulated and exchanged. Large waste particles such as uneaten bait worms are removed by a filter in the feed box (Fig. 2). Small waste products from the deep-sea fish are eliminated with the exchange of seawater. Pressure is maintained at ± 0.1–0.2 MPa during a 4-month period when the compressed sea water supply is set at the rate of 36 ml min^{-1}. The temperature inside the pressure aquarium is monitored and controlled by a TE electrode sensor and a cooler that cools to 5˚C when the ambient room temperature is higher than 30˚C.

Using the piezostat aquarium system, we succeeded in capturing and maintaining a variety of deep-sea organisms such as deep-sea fish, deep-sea shrimp, deep-sea bivalves, and deep-sea snails under pressure for approximately 2 months (Koyama 2003c).

Gradual slow decompression of deep-sea organisms

Development of the piezostat aquarium system paved the way for the study of living deep-sea organisms under pressure. To accommodate the captured deep-sea organisms at atmospheric pressure, we decompress gradually to the atmospheric condition using the system. At dive 1052, we captured and a lot of copepods using deep-aquarium system with bait trap. The baits in the traps were slice of salmon. All of the

copepods died by increment of temperature, because small copepods block up connecting valve at the bottom of spherical formed aquarium (Fig. 1) and stopped cooled seawater circulation of the system.

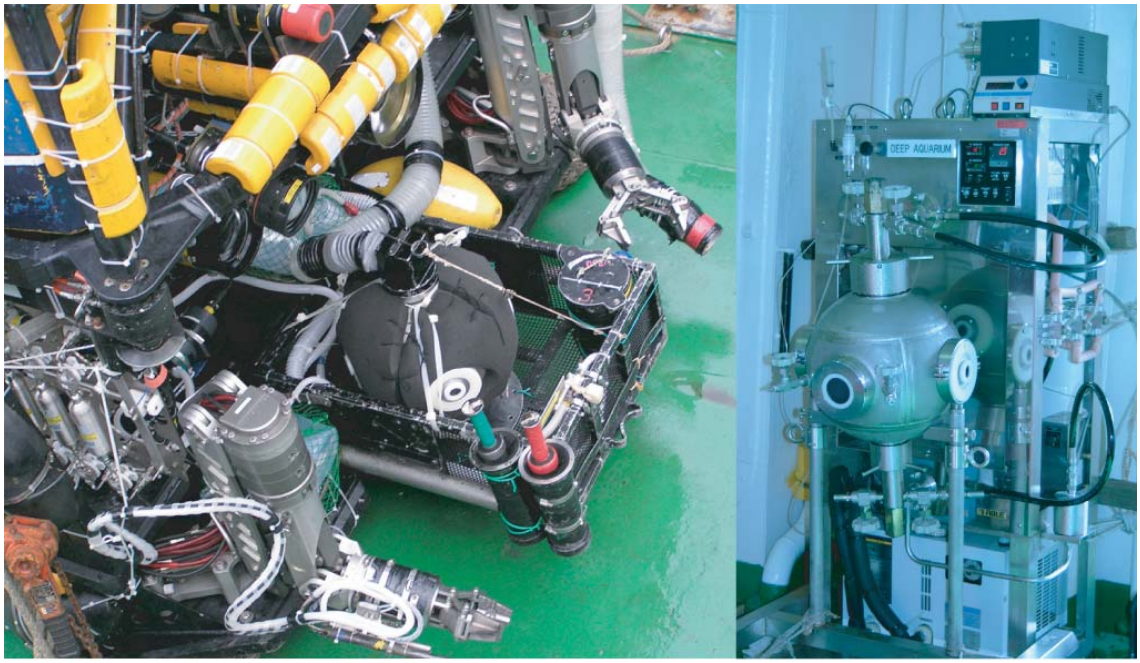

Fig. 1
Koyama, Sumihiro

Fig. 1. Photographs of the piezostat aquarium system.
(*Right*) Piezostat maintenance system. (*Left*) Suction capture system connected to the *RV Hyperdolphin*.

Re-compression of deep-sea organisms

To resurrect captured deep-sea organisms, we re-compressed the organisms up to 500m of hydrostatic pressure using deep-aquarium system. At dive 1055, we captured 6 deep-sea fishes at the depth of 909 m using normal slurp gun device. After the surfacing the unmanned submersible “Hyper dolphin”, the captured fishes recompressed up to 500m within 15 min of the surfacing of the submersible. The captured 2 deep-sea fishes were compressed up to 5MPa that corresponding to 500m depth at compression speed of 25m/min. The other 4 deep-sea fishes bred at atmospheric pressure condition. After 3hr of keeping, all of pressurized 2 deep-sea fishes kept alive.

However, 2 of the 4 deep-sea fishes at atmospheric condition died by decompression stresses. These results indicated that pressurized deep-sea fishes tend to live a long life compared with that of atmospheric condition.

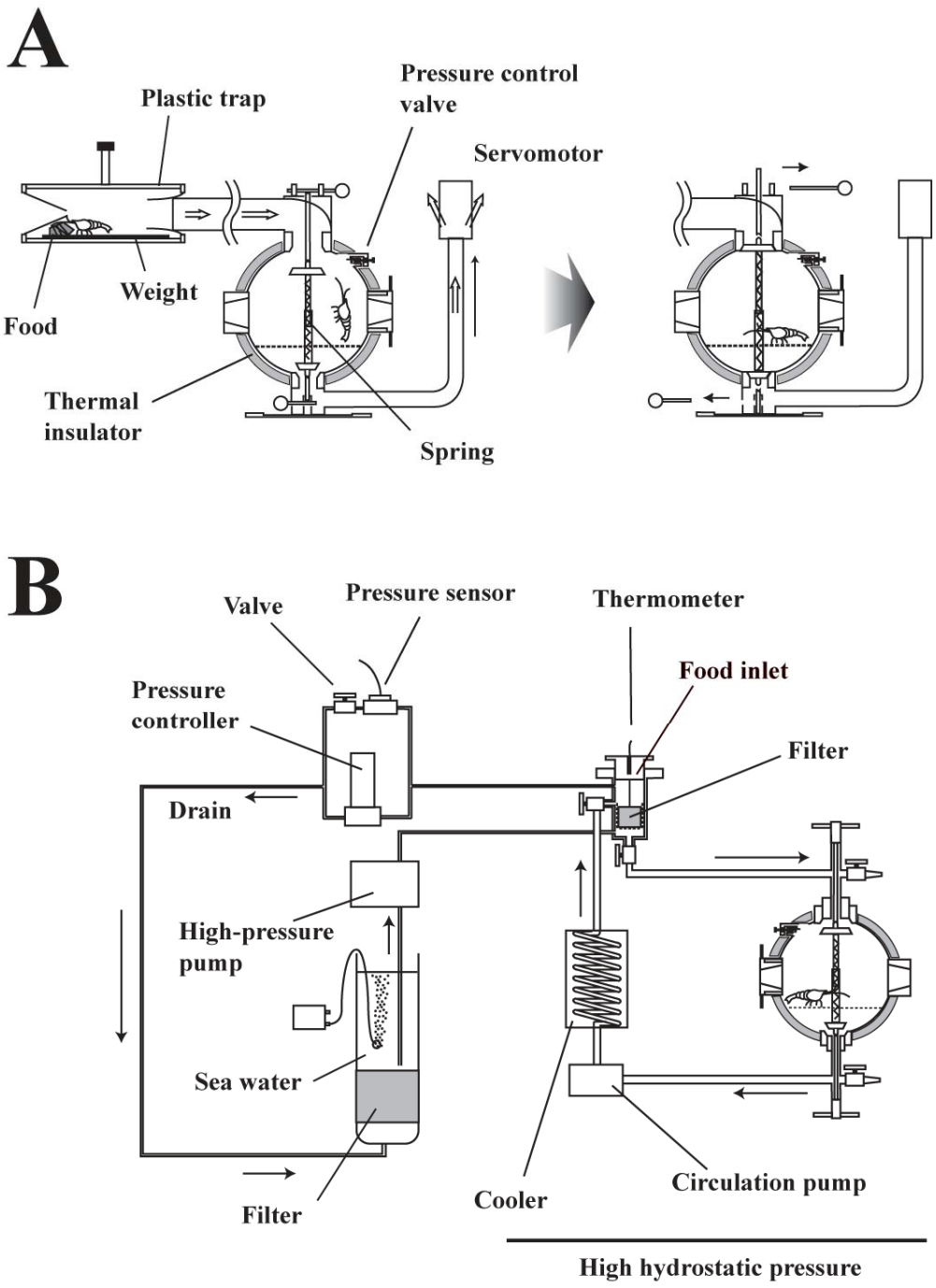

Fig. 2
Koyama, Sumihiro

Fig. 2. Schematic illustration of the piezostat aquarium system.
(A) Suction capture system. (B) Piezostat maintenance system.

3.2.3. Future study

In this cruise, we used Dr. Miwa developed 3rd type of deep-aquarium system. However, some problems were occurred in capturing and maintaining the organisms using the 3rd type of the system. First, there is very weak spring power and difficult to close the lids. Second, mesh in the spherical formed aquarium is very rough and, therefore, small copepods (about 1cm length) easy through the mesh and block up the pipe arrangement of the system. In the future study, we will perform as followings.

1) Development new bait traps.
2) Remodeling of the deep-aquarium system and development of dissemination model of the system.
3) Tissue culture of captured deep-sea fishes.
4) Genomic and proteomic analyses of deep-sea multicellular organisms.

3.2.4. References

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3.3. Bacterial community structure and function at a sediment-water interface in the Sea of Japan

Kentaro Inoue and Kazuhiro Kogure
Ocean Research Institute, the University of Tokyo

3.3.1. Introduction

Sea of Japan has well oxygenated water in deep. The purpose of this study was to clarify ecological characteristics of prokaryotic communities at the well oxygenated sediment-water interface (SWI) in the Sea of Japan. Since the oxygen is the most efficient electron recipient of redox system, it is an important factor for energy metabolisms and biogeochemical cycles in the SWI. In order to achieve the subject, we are investigating microbial community structure and function by molecular biological techniques and *in situ* incubation experiments as described below.

3.3.2 Sample treatment on board

1) The incubation experiments were carried out with using *in situ* incubation cores. Incubation periods were two or five days for the cores added 13C-labeled chlorella, and 6 days for the cores added 13C-labeled bicarbonate. Core samples were carefully sliced into the following layers: 0-0.5, 0.5-1, 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-4, 4-5, 5-7, 7-10, and 10-15. Each layer was frozen at -80 degrees Celsius. Core samples for background data and initial time information were also carried out same methods.

2) Water samples collected by using Niskin bottle were filtrated with Nuclepore filter (pore size 0.2 micrometers). Filers for community structure and other filters were put on agar plates, and incubated at room temperature.

3) Push core samples were carefully sliced into several fractions at one or two centimeters intervals. These core samples were kept at four degrees Celsius.

3.3.3. Sample treatment in laboratory

1) DNA will be extracted from frozen sediment samples. DNA of microbial communities that incorporate stable isotope labeled substrate will be separated by

density gradient equilibrium ultracentrifugation method. Clone libraries will be generated about the heavy nucleic acids.

2) Clone libraries will be generated from frozen filters in order to demonstrate microbial community structures. About other filters, colonies appeared on agar plates will be picked up, and phylogeny, physiology and other properties will be analyzed.

3) Cold storage sediment samples will be used for indigenous strains of bacteria and/or archaea, and their phages.

3.3.4. Potential collaborators

Hidetaka Nomaki (Biogeos3, JAMSTEC)

4. Acknowledgments

We are grateful to the captain S. Ishida and the crews of the R/V *Natsushima* for their invaluable supports during the cruise. We are also grateful to the ROV *Hyperdolphin* operation manager Y. Ohno and the operation team for their skillful operation of the ROV. We thank all the onshore staffs for thier supports on this NT09-16 cruise.

5. Notice on using

This cruise report is a preliminary documentation as of the end of the cruise.

This report may not be corrected even if changes on contents 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 scientists for 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.

Appendix 1. Payload Pictures

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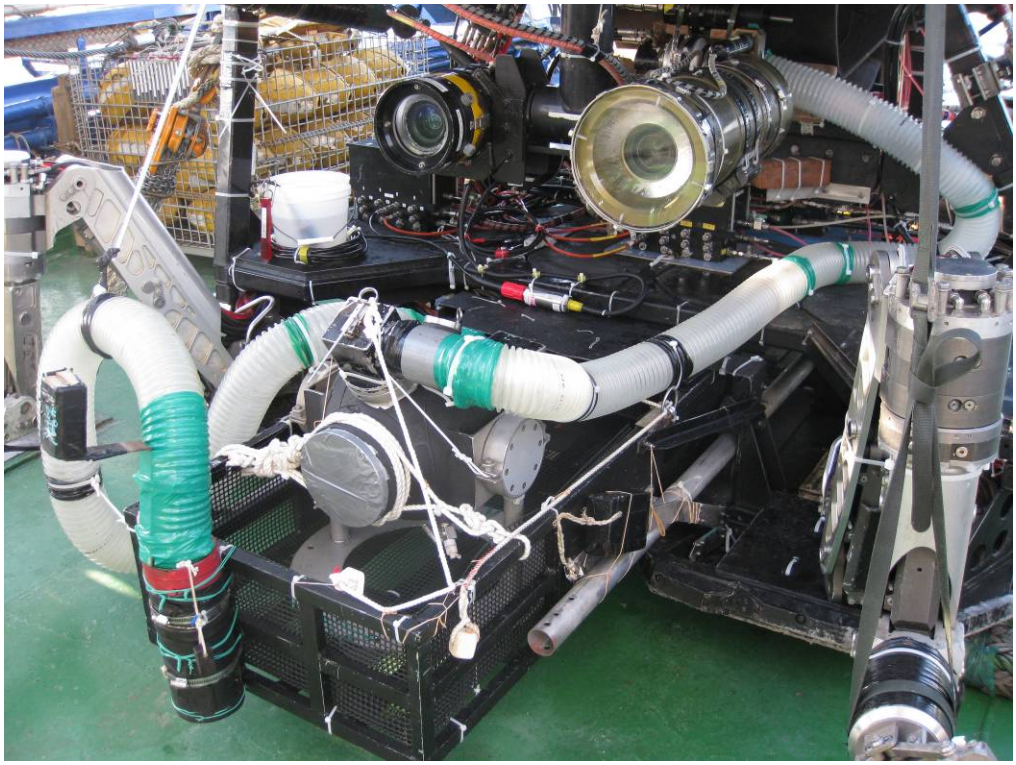
#1049 (20090917)



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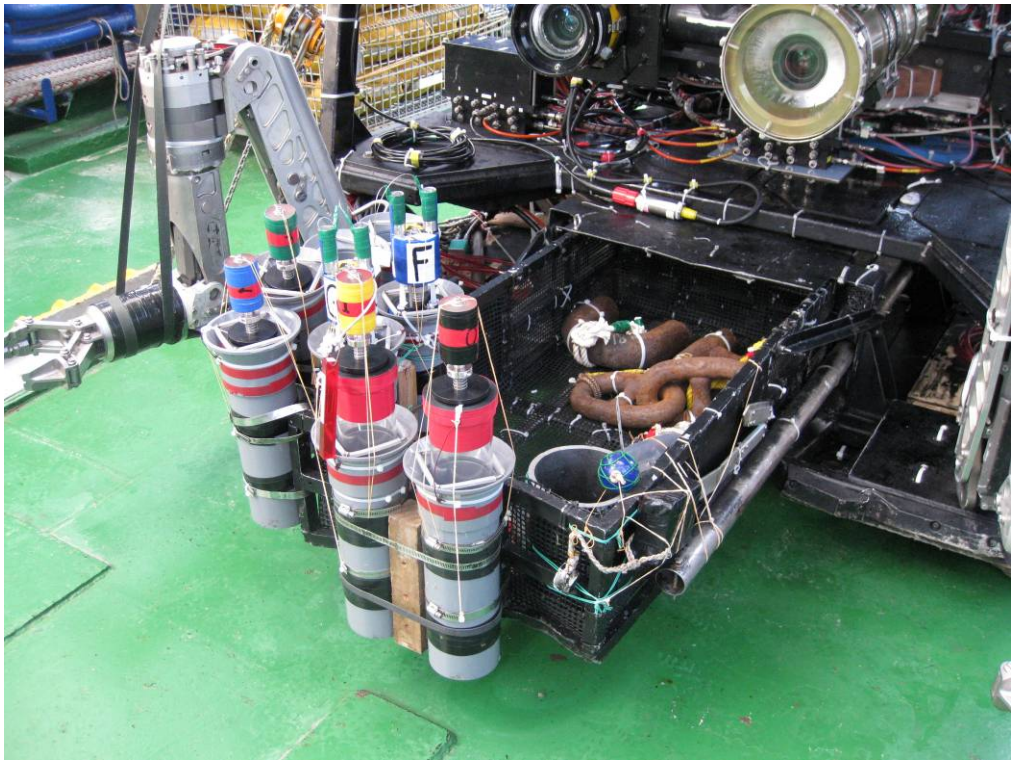

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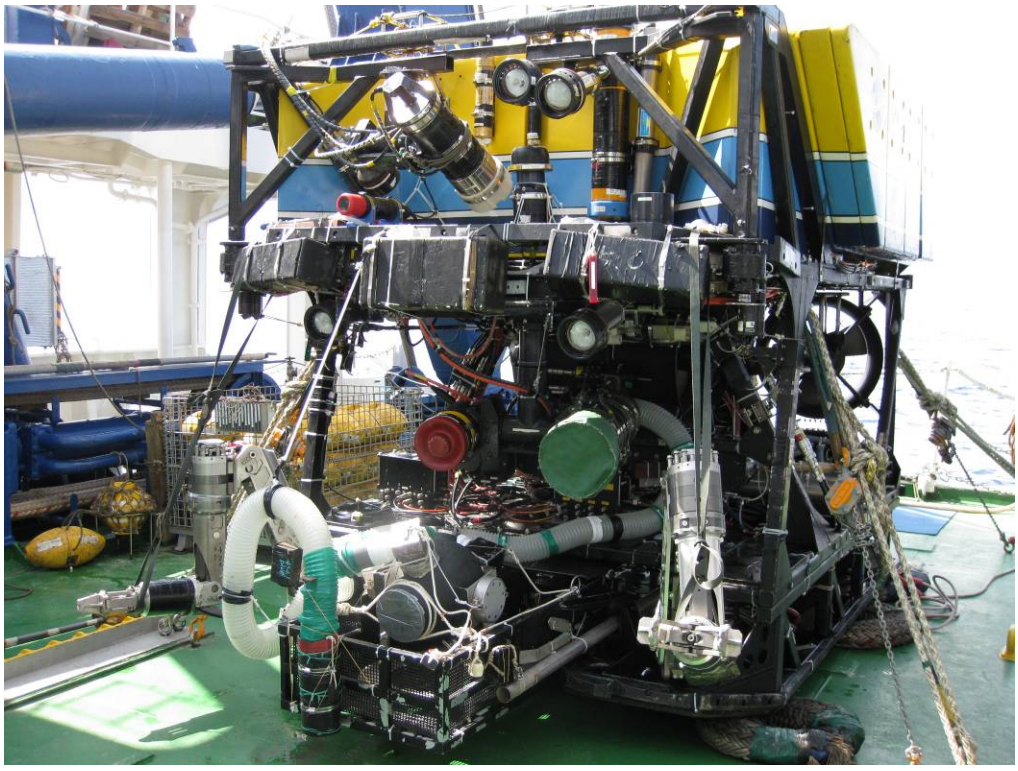

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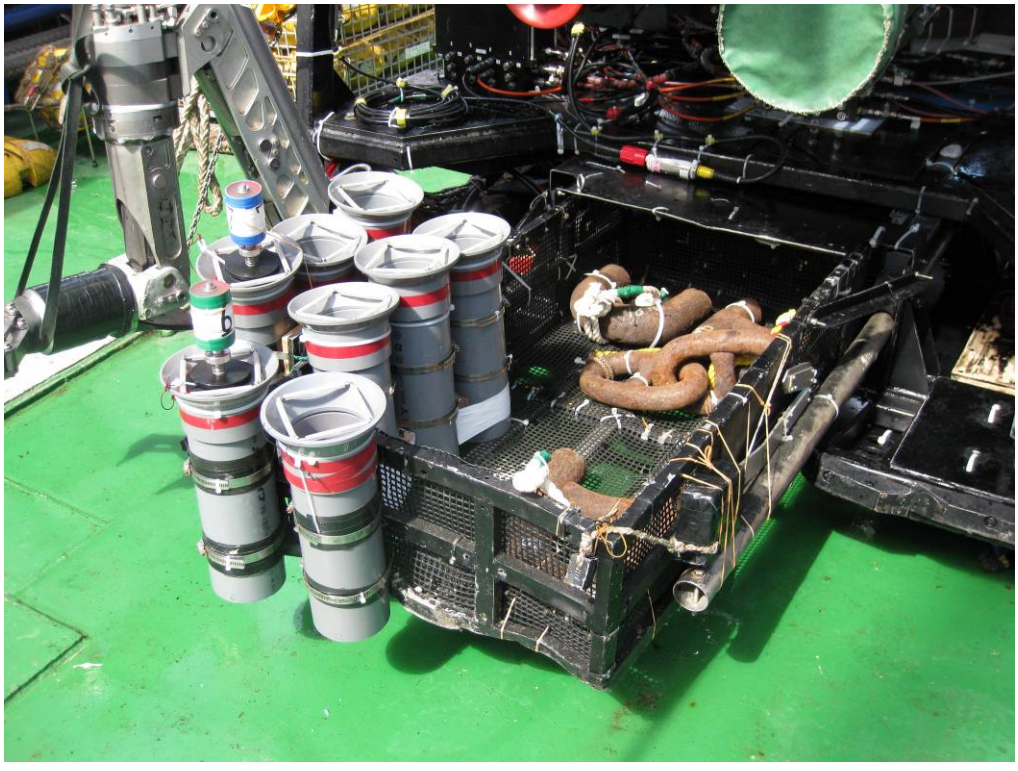

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