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Earthquake Biosphere

KAWAGUCCI, Shinsuke^{1*}

¹JAMSTEC

A novel concept, Earthquake Biosphere, will be introduced in the meeting.

Keywords: Earthquake Biosphere

Impact by the mega-earthquake: the 2011 Mw 9.0 Tohoku-Oki Earthquake, on deep-sea ecosystems in Japan Trench

FUJIKURA, Katsunori^{1*}, Hiromi WATANABE¹, Norio MIYAMOTO¹, Yasuo FURUSHIMA¹, Hidetaka NOMAKI¹, Ken TAKAI¹, Hiroshi KITAZATO¹, Takeshi TSUJI², Kazuno ARAI³, Onboard Party¹

¹JAMSTEC, ²Kyoto University, ³Chiba University

The tremendous March 2011 Tohoku earthquake (Mw 9.0) ruptured a wide area along the plate interface off the Pacific coast of Tohoku, Japan. The tsunami induced by earthquake was extremely huge. Earthquake, after shocks and tsunami have been variously affected to not only coastal marine ecosystems but also deep-sea ecosystems. Expected impacts to deep-sea ecosystems were following:

- 1) The extinction of deep-sea organisms by turbidity currents,
- 2) The extinction and/or new occurrences of chemosynthesis-based ecosystems by chemicals such as CH₄ and H₂, discharge from seafloor,
- 3) Change of faunal composition and distribution patterns of deep-sea organisms by numerous suspended matters.

To estimate and make clear for above expected impacts by the earthquake, we conducted deep-sea investigations using the HOV Shinkai 6500 and Deep-towing TV Camera systems in the Japan Trench from June to August, 2011. Several fissures on the seafloor, new occurrences of bacterial mats associated with CH₄ and H₂S seepages, other bacterial mats associated with decay of dead benthic organisms aggregations, decreasing of *Calyptogena phaseoliformis* colonies and new occurrences of single species holothurian dense aggregations were observed. These phenomena have never found before the March 2011 Tohoku earthquake. So far, we have investigated using some deep-sea research gears. So, we believe, to describe these impacts by the huge earthquake is our important task.

Keywords: the 2011 Mw 9.0 Tohoku-Oki Earthquake, deep-sea ecosystems, bacterial mat, Japan Trench

Growth pattern of the chemosynthetic bivalve *Lucinoma annulatum* based on growthline and isotopic analysis

NAKASHIMA, Rei¹, Mizuho Sato², SAKAI, Saburo³, MAJIMA, Ryuichi^{2*}

¹Geological Survey of Japan, AIST, ²Yokohama National University, ³JAMSTEC

Lucinid bivalve has sulfur-oxidizing bacteria in their gills and are known as a member of cold-seep community. Though they are known as edible in Southeast Asia and a large number of fossil records, their growth process are not revealed at all. Therefore, we examined shell structural and stable isotopic analysis using *Lucinoma annulatum* shells to clarify the growth pattern, physiology, and its relationship with environmental changes.

Shell materials are collected by dredge at 50m in depth of Beppu Bay, West Japan, in November 2005. We observed shell structure of polished section along growth direction using acetate peel method. The shell consists of the outer, middle, and inner shell layers. Width and positions of the growthlines in the middle shell layer and concentric rib and growth disturbance rings in the outer shell layer are measured. Powder samples for stable isotope analysis are milled with 80-500 um intervals in the middle shell layer along shell growth direction by high precision micromill system (GeoMill 326).

As a result of measurement of the width of growthlines, disturbance rings, and concentric ribs, shell growth patterns of the species are divided into early, middle and late growth stages. The early, middle and late growth stages are characterized by narrow intervals of concentric ribs and disturbance rings, wide intervals of concentric ribs and disturbance rings, and drastic decreasing width of growthlines, respectively. The positions (about 25mm from umbo) of boundary between the middle and late growth stages are common to examined specimens. The shell structural change indicates that physiological change from juvenile to adult probably happened at this position.

Oxygen isotope value ranging from 0.5 per mil to 1.5 per mil are calculated to seawater temperature between 15 and 20 degree Celcius. It means that shell growth interruptions are occurred during high and low water temperature extremes, considering temperature range at 50m in Beppu Bay. And the profile of the oxygen isotope shows seven cycles of fluctuations, indicating seven years temperature records during shell growing period.

Keywords: growthline, growth pattern, chemosynthetic community, bivalve, stable isotope

Genetic Population Structure of Alvinocaridid Shrimps in the Okinawa Trough and the Izu-Ogasawara Arc

YAHAGI, Takuya^{1*}, WATANABE, Hiromi², KOJIMA, Shigeaki¹

¹The University of Tokyo, ²JAMSTEC

In hydrothermal vent fields and cold seep areas, most faunal communities are comprised of endemic species (Tunnicliffe and Fowler, 1996). In addition, the hydrothermal vent fields are unstable environments and patchy distributed. Therefore, vent endemic species are thought to have high dispersal ability to maintain gene flow among local populations.

In the northwestern Pacific, the Okinawa Trough and the Izu-Ogasawara Arc are sea areas in which many hydrothermal vent fields are distributed. Species composition of vent faunas and geological background in these two sea areas are strikingly different from each other. Therefore, it seems to be difficult for most species to disperse and maintain gene flow between the two areas. Actually, neoverrucid barnacles in the both fields have been shown to be genetically differentiated by population genetic analysis based on nucleotide sequences of a mitochondrial cytochrome oxidase c subunit I (COI) gene (Watanabe et al., 2005). Although an interspecific comparison of genetic population structures will provide important information to understand the relationship of the communities in the two sea areas, such researches are yet limited.

The purpose of this study is to clarify the population connectivity of alvinocaridid shrimps between the Okinawa Trough and the Izu-Ogasawara Arc by genetic population structure analyses.

An alvinocaridid shrimp, *Alvinocaris* sp. was collected at the Irabu Seamount in the southern Okinawa Trough and the Suiyo Seamount in the Izu-Ogasawara Arc during the NT11-09 and NT11-20 cruises of the R/V 'Natsushima' and the ROV 'Hyper Dolphin', respectively. We determined nucleotide sequences of the COI gene of *Alvinocaris* sp. for 20 individuals of each site.

Alvinocaris sp. populations in the both sites were shown to consist of two genetically deviated lineages. They, however, showed no significant genetic differentiation between two sea areas. In this presentation, we discuss the population connectivity and history of *Alvinocaris* sp. based on the population genetic analyses, ecological information, and geological background of the two sea areas.

Keywords: Okinawa Trough, Izu-Ogasawara Arc, Alvinocaridid shrimps, Genetic population structure

Cultivating approach for understanding symbiont-host linkage of invertebrates in deep-sea chemosynthetic ecosystem

KONISHI, Masaaki^{1*}, WATSUJI Tomo-o¹, NAKAGAWA Satoshi², HATADA Yuji¹, TAKAI Ken¹, TOYOFUKU Takashi¹

¹Institute of Biogeoscience (Biogeos), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), ²Faculty of Fisheries Sciences, Hokkaido University

Chemoautotrophic ecosystem are supported by chemoautotrophs, which are organisms that are capable of fixing carbon by using chemical energy obtained from the oxidation of reduced compounds blowing up from hydrothermal vents, such as sulfuric compounds, methane, and hydrogen. Several benthic invertebrates are well known to be hosts of chemoautotrophic bacteria in cells of specialized tissues and on the surface and obtain nutrition from the bacteria. The behaviors of the symbiont and organisms have been estimated by evidences from the results by field works, phylogenetic analyses, and characterization of isolates related to the symbionts, named 'field work based approach'. However, the approaches are limited by unclear causal associations between environmental and physiological factors and their behaviors. We, therefore, developed a rearing tank system with feed-back control system to provide hydrogen sulfide, which is strong reducing and toxic gas, in order to characterize the deep-sea organisms and their symbionts by 'cultivating approach'.

Using rearing tank system newly developed, we first examined the effect of low concentration H₂S on the episymbiotic bacterial communities of a deep-sea crab, *Shinkaia crosnieri*, by comparison of with/without H₂S feeding. The H₂S concentrations in tank were successfully maintained between 5 to 60 micro mol. per liter for 80 days with the exception of brief periods of mechanical troubles. The survivals of the crab were 44 individuals against initial 60 individuals (73.3%) for 84 days cultivation. On the other hand, the values were three individuals against initial 30 individuals (90.0 %) without the feeding. Even if additional effects of a trouble be considered, the survivals with H₂S feeding seemed to be less than that without the feeding. Busy setae were observed at few days after the continuous feeding of H₂S. Furthermore, white biofilm caused and increased on the surfaces of tank wall and of sea sand in the feeding unit. According to real-time PCR analysis, the copies of partial 16S rDNA of the episymbiont with feeding were three-orders of magnitude larger than those without the feeding. Based on a phylogenetic analysis of episymbiont, several phylotypes were detected in *alphaproteobacteria*, *gammaproteobacteria*, *epsironproteobacteria* and *flavobacteria*, from the crab with H₂S feeding. The symbiont-related phylotypes would be grouped into four different groups; *gammaproteobacteria* in marine epibiont group I, *Sulfurovum*-affiliated *epsilonproteobacteria*, *Osedax mucofloris* endosymbiont-affiliated *epsilonproteobacteria*, and *flavobacteria* closely related to CFB group bacteria epibiont of *Rimicaris exoculata*. Based on the analysis of the biofilm, several phylotypes belong into the above results of epibionts. Growth of these bacteria would be dependent on only chemical and physical conditions except of pressure, and physiological host-symbiont interaction might not be necessary to be significant factor. On the other hand, marine epibiont group I in *gammaproteobacteria*, which has been detected in epibiont phylotypes, was not detected. This fact implies a possibility that the marine epibiont group I tightly bind to their host by somewhat physiological interaction. Interestingly, one of major clades of phylotypes were *Sulfimonas*-affiliated bacteria, and closely related to endosymbiont of a snail, *Alviniconcha* sp. type II that was collected from Vienna Woods site, Manus Basin, where Iheya North field in Okinawa Trough, where *S. crosnieri* was collected, located approximately 3,700 km of geographical distance of Vienna Woods site. The significant growth factors of these bacteria would be also chemical and physical conditions, but not geographical factors. The results demonstrate possibility and clue that the behaviors are able to be discussed against chemical and physical factors, which distinguished from geographical and physiological factors.

Keywords: rearing tank, symbiont-host linkage, *Shinkaia crosnieri*, feed-back control, semiconductor gas sensor

Chronostratigraphy of the *Calyptogena*-bearing, Plio-Pleistocene Miura and Kazusa Groups, central Japan

UTSUNOMIYA, Masayuki^{1*}, TANAKA, Yuichiro², KUSU, Chie¹, Aoba Kobayashi¹, NAKAMURA, Ayako⁵, OKADA, Makoto³, WADA, Hideki⁴, MAJIMA, Ryuichi¹

¹Environment and Information Sciences, Yokohama National University, ²National Institute of Advanced Industrial Science and Technology, ³Department of Earth Sciences, Faculty of Science, Ibaraki University, ⁴Department of Science, Faculty of Geosciences, Shizuoka University, ⁵Ricoh Company, Ltd.

We studied the stratigraphy and geologic ages of the *Calyptogena*-bearing, Plio-Pleistocene Miura and Kazusa Groups, the fore-arc basin-fills, based on calcareous nanno fossil biostratigraphy and magnetostratigraphy.

Samples examined were obtained from twelve horizons of the Ikego Formation (Pliocene) of the Miura Group, five of the Urago Formation (upper Pliocene to lower Pleistocene) and six of the Nojima Formation (lower Pleistocene) of the Kazusa Group exposed in the northern Miura Peninsula.

The following five nanno-fossil datums have been recognized in ascending order (ages from Raffi et al., 2006): FO (First Occurrence) of *Pseudoemiliana lacunosa* (applied as CN11a - 11b boundary: 4.13 to 4.12 Ma) and LO (Last Occurrence) of *Reticulofenestra pseudoumbilicus* (CN11b - CN12a boundary: 3.79 Ma) in the Ikego Formation; LO of *Discoaster tamalis* (CN12a - CN12b boundary: 2.87 Ma) and LO of *Discoaster surculus* (CN12b - CN12c boundary: 2.52 Ma) in the Urago Formation; LO of *Discoaster pentaradiatus* (CN12c - CN12d boundary: 2.39 Ma) in the lowermost Nojima Formations. Gauss - Matuyama boundary (2.58Ma) has been recognized in about 30 to 60 m beneath LO of *Discoaster pentaradiatus*.

Based on the results above, the age of the *Calyptogena*-dominated assemblages from the Ikego Formation can be dated in 4.50 to 3.79 Ma, and those from the Urago Formation in 2.52 - 2.50 Ma.

Keywords: chemosynthetic fossil assemblage, Miura Peninsula, Miura Group, Kazusa Group, calcareous nanno fossil stratigraphy

Distributional gradient of sister species of vesicomid bivalves in chemosynthetic fauna in Japan

WATANABE, Hiromi^{1*}, SEO, Eriko², TAKAHASHI, Yoshimi¹, YOSHIDA, Takao¹, KOJIMA, Shigeaki², FUJIKURA, Katsunori¹, MIYAKE, Hiroshi³

¹Japan Agency for Marine-Earth Science and Technology, ²Atmosphere and Ocean Research Institute, the University of Tokyo, ³Kitasato University

Vesicomid bivalves is one of the important components of the deep-sea chemosynthetic biological communities due to their high biomass based on the chemosynthetic primary production of their symbionts. The restrictive distribution of vesicomid clams by chemical gradient such as sulfide concentration in sediment has been discussed, but the relationships between general environmental properties such as temperature, salinity and oxygen concentration were unclear. With a development of a specific primer set for mPCR to identify concurrent vesicomids, *C. okutanii* and *C. soyoae*, in chemosynthetic biological communities in Sagami Bay, distributional bias of two vesicomids along the environmental factors was examined. Ratio of *C. okutanii* and *C. soyoae* in *Calyptogena* assemblages in Sagami Bay were correlated with depth, temperature and salinity but were not correlated with DO. However, the environmental information of the habitats of *C. okutanii* in Okinawa Trough hydrothermal vent fields revealed that the depth was not the factor to discriminate the habitat of the two *Calyptogena* clams. The present results showed that the distribution of the *Calyptogena* clams which highly depending on chemosynthetic symbionts was also affected by ordinal oceanographic environmental factors.

Keywords: vesicomid, chemosynthetic fauna, Sagami Bay, Okinawa Trough, mPCR

Ethological observation in a *Calyptogena* colony off Hatsushima Island in Sagami Bay, central Japan

HAYASE, Mana^{1*}, NOBUHARA, Takami¹, SUZUKI, Takahiro¹, IWASE, Ryoichi²

¹Shizuoka University (Faculty of Education), ²JAMSTEC

Ethology of *Calyptogena* clams is analyzed on the basis of a serial video-capture images with three-minutes interval from 14th March to 19th June, 1988 on the Long-term Deep Sea Floor Observatory off Hatsushima Island in Sagami Bay. The video camera was fixed to observe the *Calyptogena* colony (yellow bacteria mat and its surroundings of *Calyptogena* gregarious zone).

During the video-capture working time, more than three mud-flows stroke the colony, but the thickness of mud-flow sediments were maybe not over 10 cm. The *Calyptogena* clams pushed up their shells and escaped from the burial by pushing their foot against underground firm bottom. After the mud-flow blanketed the colony, some *Calyptogena* clams randomly moved and disturbed the reducing bottom breaking the bacteria mat, but its significance is unknown.

It is noteworthy that this serial-images record a complete account of prey/predation processes on a *Calyptogena* clam from beginning to end. *Calyptogena* clams were attacked by large buccinid and small turrid gastropods, which wandered in and around the living *Calyptogena* colony and rode on some clams. *Calyptogena* clams escape or protect from their predation by the following three methods: 1) closing the valves; 2) burrowing into sulphide-rich bottom where is not preferable condition for aerobic animals; and 3) hiding behind large dead shells.

Small gastropod-attacks did not cause dynamic escape activity of *Calyptogena* bivalves. Gregarious small gastropods had swarmed on some living *Calyptogena* clams during over 20 days, but the *Calyptogena* clam only once crawled into the reducing bottom and mainly resists the small-gastropod attacks by closing their valves.

On the other hand, attacks by large buccinid gastropods promptly cause escape actions of *Calyptogena* bivalves. We confirm that a *Calyptogena* clam instantly burrowed into the bottom just after large buccinid-attacks. The *Calyptogena* clam stayed in the black reducing sediments over nine hours. During the burrowing time, buccinid gastropods also burrowed into the sediments with their long siphon above the sediment surface, and struggled and tried to prey the clam. After all some buccinid gastropods gave up and leaved the clam burrowing point although others retained around the point. The *Calyptogena* clam once succeeded in escaping from the predation, but unfortunately the clam received next attack by another buccinid gastropod after the clam rose to the surface. The clam maybe had little reserve of energy to burrow again, and alternatively hid behind a large dead *Calyptogena* shell. This way was not efficient for the protect from their predation. After the hiding, the living *Calyptogena* received intensive attacks by many large and small gastropods. It took about 4 days and a half from the start of intensive attacks to completion of the predation. This predation time suggests that old-aged *Calyptogena* colony has enough potential to attract the high-density gastropod population.

Keywords: *Calyptogena*, methane seepage, ethology, Sagami Bay

Nucinella found in a chemosynthetic community off the Sanriku coast, northeastern Japan at 123 m depth

JENKINS, Robert^{1*}, KITAMURA, Akihisa², AMANO, Kazutaka³

¹Graduate School of Environment and Information Sciences, Yokohama National University, ²Institute of Geosciences, Faculty of Science, Shizuoka University, ³Department of Geosciences, Joetsu University of Education

Nucinellids are very small bivalves, generally less than 5 mm in length, related to the Solemyidae, which are typical chemosymbiotic bivalves. Reid (1990) and Amano et al. (2007) hypothesized, on the basis of the Nucinellidae's gutless state and their occurrence in Cretaceous cold-seep deposits, that they have chemosynthetic bacteria in their body. This hypothesis has been partially confirmed by Oliver and Taylor (2012). They found bacteria-like microstructures in their gills. But it has still not yet been fully confirmed that the Nucinellidae have chemosynthetic bacteria or not.

We recovered many dead shells of *Nucinella* sp. with living chemosynthetic lucinid and thyasirid bivalves from the sea off the Sanriku coast at a depth of 123 m during the Taisei-maru (JAMSTEC) cruise KT-11-17 in the summer of 2011. The finding indicates that the Nucinellidae might be a member of chemosynthetic communities even in the Recent, not only in the Cretaceous. Our finding supports the hypothesis that the Nucinellidae have chemosynbiotic bacteria.

Keywords: Nucinellidae, methane seep, cold seep, symbiosis

Evaluation of nutrient sources for the sponges inhabited around seafloor hydrothermal areas in the Okinawa Trough

NAGASHIO, hiromi^{1*}, YAMANAKA, Toshiro¹, WATANABE, Hiromi², YAMAGAMI, Shosei¹, Yuji Ise³, MAKITA, Hiroko²

¹Graduate school of Natural Science and Technology, ²JAMSTEC, ³The University of Tokyo

Since discovery of seafloor hydrothermal vents the dense and endemic animal communities inhabited around the hot vents have been the most impressive feature for many scientists. Such animals have been known as chemosynthesis-based species and studied many investigators. On the other hand, some benthic animals found on abyssal plain have been observed slightly high density at the adjacent area to active vent sites. It implies that those opportunistic benthos may also rely on the chemosynthetic primary production and the hydrothermal chemosynthetic ecosystem may extend widely rather than previous expectation.

In that case, it is an interesting issue how the dense sponge community is sustained around the hydrothermal fields. For clarifying the issue isotope geochemical study has been performed to evaluate food ecology of the sponges and some other animals obtained from the deep seafloor in the Okinawa Trough.

Stable isotope analysis of carbon, nitrogen, and sulfur of the sample organisms obtained from the Tarama knoll show significant low $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values for the sponge samples. Those results suggest plausible contribution of sulfur oxidizing bacteria as food source for the sponges because such low $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values are often observed for thioautotrophic chemosynthesis-based animals. The sulfur isotope ratios of the sponges also imply that the source of sulfur for sulfur oxidizing bacteria is possibly magmatism in origin. It also implies that the observed turbid water at the Tarama knoll is hydrothermal plume. Therefore, active high-temperature hydrothermal emission supplying hydrogen sulfide is expected at the Tarama knoll.

On the other hand, the sponge sample obtained from the Daiichi Kohama knoll shows similar isotopic characteristics observed for the sponges from the Tarama knoll. It may also imply the importance of sulfur oxidizing bacteria as food source for the sponge at the Daiichi Kohama knoll, therefore, hidden hydrothermal emission may be expected at the knoll.

Keywords: stable isotope, sponge, deep sea, seafloor hydrothermal system, Okinawa Trough

Estimation of trophic position in marine ecosystems based on nitrogen isotope of amino acids: prey-predator interaction

CHIKARAISHI, Yoshito^{1*}, FUJIKURA, Katsunori¹, TSUCHIYA, Masashi¹, YOSHIDA, Takao¹, FUJIWARA, Yoshihiro¹, KITAMURA, Minoru¹, LINDSAY, Dhugal J.¹, UMEZU, Yuichi², NAGABORI, Atsushi¹, SHINOZAKI, Ayuta², OGAWA, Nanako O.¹, OHKOUCHI, Naohiko¹

¹JAMSTEC, ²Hiroshima University, JAMSTEC

Estimate of the accurate trophic position of organisms in food webs allows better understanding of not only biomass flow and trophic linkages in complex networks of natural ecosystems but also function and niche of individual organisms, species, and communities in the ecosystems. Combination of ultra-sensitive carbon and nitrogen isotope analyses of bulk whole samples (or tissues) and nitrogen isotope analysis of amino acids of the samples is a potential powerful tool to access the above subjects.

In this presentation, we will show a comprehensive data set on the isotope signatures for various organisms collected from marine surface photosynthetic and deep-sea chemosynthetic communities in Sagami bay, and will discuss 1) applicability of this method to chemosynthetic communities, 2) nitrogen flow into the deep-sea food web from photosynthetic (i.e., sinking particle) and chemosynthetic (i.e., seep water) communities, and 3) characteristics of specific nitrogen (or amino acids) transfer system during symbiosis between chemosynthetic bacteria and host organisms.

Estimation of trophic position in marine ecosystems based on nitrogen isotope of amino acids: host-symbiont relationship

TSUCHIYA, Masashi^{1*}, Takao Yoshida¹, CHIKARAIISHI, Yoshito¹, Yoshihiro Fujiwara¹, Yuichi Umezu², NAGAHORI, Atsushi², FUJIKURA, Katsunori¹, OHKOUCHI, Naohiko¹

¹Japan Agency for Marine-Earth Science and Technology, ²Graduate School of Biosphere Science, Hiroshima University

This study aims to clarify the trophic position (TP) of marine organisms to understand food web structures in marine ecosystems based on the stable isotope analysis of amino acid that we mainly focused on 1) the food web structures of marine ecosystems, and 2) the host-symbiont interactions. Combined analyses using stable isotopes along with ecologic, biochemical, and genetic analyses will clarify the relationships among prey-predator interactions in marine organisms and illustrate the dynamics and evolution of marine ecosystems.

We clarified the host-symbiont interactions between *Calyptogena* clam, and endosymbiotic bacteria. TP of *Calyptogena* muscle and symbiotic bacteria shows 2.0 and 3.1, respectively. TP increases 1.1 from symbiotic bacteria to muscle. We assumed that host *Calyptogena* muscle obtained almost 100 % of nutritional source from symbiotic bacteria as amino acid. On the other hand, *Bathymodiolus* species have same TP value (=1.2) among the host *Bathymodiolus* (muscle, mantle and foot), symbiont-bearing gill, and symbiotic bacteria. It means that *Bathymodiolus* may have different mechanisms of nutrient requirement than *Calyptogena*.

Keywords: nitrogen isotope of amino acids, trophic position, host-symbiont relationships, chemosynthetic ecosystem, *Calyptogena* species, *Bathymodiolus* species