

## TOWARDS THE NEXT GENERATION OF CARBONATE-BASED PROXIES

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Reconstructions of past climate and environments are largely based on stable isotopes and trace element concentrations measured on fossil foraminiferal calcite. Element and isotope composition of foraminiferal calcite roughly reflects seawater composition and physical conditions, which in turn, is related to paleoceanographic parameters. Additional biological controls on test composition biases such correlations and needs to be corrected for when aiming at precise and accurate reconstructions. The various physiological processes involved in foraminiferal biomineralization have, however, different impacts on different elements and isotopes. For instance transmembrane transport of Ca-ions has a large impact on Mg fractionation (and hence the Mg-temperature proxy), whereas it has very little effect on Na/Ca ratios (a novel proxy for salinity). Many foraminifera-based proxies are thus impacted by more than one physiological process, which can only be corrected for by 1) quantification of the impacts of these processes (ion pumping, photosynthesis, pH regulation, etc) on calcitic element and isotope composition and 2) combine high-resolution multi-element and isotope analysis to simultaneously correct for these impacts. Since trace metals and isotopes are affected by multiple parameters, combining analyses not only makes reconstructions more robust, but also fundamentally more accurate.

## The evolution of shell microstructure of protobranch bivalves

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Molluscs are the second largest taxa and most of them have the shell of calcium carbonate. Molluscan shells are composed of the complex structural units that are called shell microstructures. Molluscs demonstrate a great variety of microstructures which are similar in phylogenetically close taxa. Thus, investigations of shell microstructures can provide clues for systematic and phylogenetic analyses of molluscs, including fossil taxa. Additionally, these trends suggest the possibility that the shell microstructure had a crucial role in the evolution of Mollusca.

The Protobranchia is an ancestral group of the Bivalvia and comprise four superfamilies (Nuculoidea, Nuculanoidea, Manzanelloidea, and Solemyoidea). However, the systematics of protobranch bivalves has been also problematic, because their simple external shell morphology can provide an insufficient number of informative characters. Therefore, Comprehensive investigation of the shell microstructure and molecular phylogenetic study of protobranch bivalves are required for understanding molluscan evolutionary history. The purpose of this study is to reveal the relationship between the shell micro-structure of protobranch bivalves and molecular phylogeny, and to discuss the evolution of the shell microstructures and their significance as novel morphological characters.

As the result of molecular phylogenetic analysis, it is revealed that the species of protobranch bivalves formed a distinct clade with long branches expect for one exception. One species of Sareptidae were included in Nuculanoidean clade while Sareptidae is placed within Nuculoidea in earlier systematics. SEM observation revealed that each of four superfamilies has a distinct trend in the composition of shell microstructures. And the results of the molecular phylogenetic analysis and the observation of the shell microstructure were consistent with each other. This condition indicates the shell microstructures of the Resent protobranch bivalves show a phylogenetic constraint. Nevertheless, previous study shows this trend is imperfect in fossil taxa. Some fossil nuculoids have nacreous structures and some fossil nuculids possess homogeneous structures. The foliated aragonite that resembles nacreous structure is known as the most primitive shell microstructure. Ancestral nacreous structure was first originated in the Paleozoic protobranch bivalves prior to any other structures that are found in protobranchs of younger ages. Thus, the absence of the nacreous structure may represent the secondary condition in protobranchs. However, the loss of nacreous might be unreasonable, because nacreous structure is considered to be the strongest shell microstructure. In further studies, the evolution of the shell microstructure of protobranchs should be discussed in terms of the habitats and the production costs of the shells as well as protective functions of shells.

Keywords: shell microstructure, mollusca, bivalve, protobranch

## Tube mechanical properties and structural design of *Hydroides elegans* under multiple stressors

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Most marine calcifiers construct robust calcareous skeletons or shells through biomineralization to protect themselves from predatory attacks. Due to increased anthropogenic emission of CO<sub>2</sub> in recent years, reduced global ocean pH and decreased carbonate concentration in seawater are expected to impede the CaCO<sub>3</sub> accretion in shell formation and produce a mechanically brittle shell structure. In addition, the effect of elevated pCO<sub>2</sub> level can act synergistically with temperature and salinity changes in seawater, further affecting the calcification process adversely. To investigate the combined effects of multiple environmental stressors on calcifying marine organisms, we studied the effects of pH (8.1 and 7.8), salinity (34 and 27 ‰), and temperature (23 °C and 29 °C) on the mechanical properties of the tubes built by the tubeworm, *Hydroides elegans*. By employing Micro-CT scanning and micro-force testing, information on tube topography and mechanical properties were analyzed using finite element analysis (FEA). Markedly, despite the structural deterioration observed in reducing pH and salinity, the level of elevated temperature counteracts these effects and even strengthen the overall mechanical properties. This may suggest that warming conditions in the early subtropical summer seawater may rescue the tapeworms from decreasing pH and salinity in the near future.

Keywords: calcifiers, biomineralization, stressors, *Hydroides*, tubeworm

## The mechanical consequence of ocean acidification - the application of finite element analysis

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We studied the effects of low pH (near-future average pH 7.8) seawater on the structure and mechanical properties of the calcifying serpulid tubeworm, *Hydroides elegans*, compared to normal pH (current average pH 8.1).

We found that tubes produced at pH 7.8 altered tube ultrastructure, volume and density, and decreased the mean tube hardness and elasticity to a large extent by ~80% and ~70%, respectively. Specifically, mechanical properties of the outer and inner surfaces of the tube were curbed by pH 7.8, and the tube breaking force required to damage the tube was reduced by 64%.

Nano-indentation to spatially map the micromechanical properties of tubes built by the biofouling serpulid tubeworm, *Hydroides elegans*. The mechanical information was analyzed by computational model, finite element analysis (FEA). In order to study the details of strength properties of the shell, finite element analysis (FEA) was used to simulate the consequence of predatory attack in nature for both shells produced in the control and treatment seawater. The finite element analysis provided a reasonable answer to this phenomenon: altered mechanical properties shifted the stress development and distribution within the tubes and therefore resulting in mechanical weaker part of that were suffering from higher stress concentration.

Keywords: Hydroides, ultrastructure, tubeworm, calcifyer, mechanical properties

## Visualization approach on foraminiferal calcification under various pH

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Foraminifera, marine unicellular organism, have been thought as one of the major carbonate producer in ocean. Their calcareous tests are commonly utilized as paleo-environmental indicators in various studies of earth science because their tests have been archived as numerous fossil in sediment for long time and various environmental information are brought by population, morphology and geochemical fingerprints. The calcareous test itself is interested by many foraminifer scientists. The knowledge about the cytological process on carbonate precipitation has been described for couples of decade using by multi approaches. Foraminiferal regulations of calcium and carbonate ion uptake into calcareous tests from ambient seawater under different pH conditions are of great interest. Our previous studies showed the potential to understanding the biomineralization of foraminifera by the application of fluorescent indicators. Recently, we apply the method to show the spatial distributions of cytological calcium and pH in living cell at several pH conditions (7.5-8.1). Observed results show that foraminifera controls pH variation and concentration of calcium at even different environmental pH. These observations results will help to consider how the geochemical compositions arranging on the foraminiferal test, sensitivity of pH proxy of boron and others.

## Live confocal imaging of cytoplasmic structure and calcification processes in *Amphisorus kudakajimensis*

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Although complex processes of calcification processes have been reported in Foraminifera, details of the cellular events generating organic templates and causing calcification are still unknown. To better understand cellular mechanisms in foraminiferal calcification, it is important to observe the molecular dynamics in vivo (e.g., calcium ion, matrix proteins). Here we report confocal microscopic observations of cytoplasmic structures in a live cell of a *porcelaneous symbiotic foraminifer Amphisorus kudakajimensis* and discuss the application of calcium imaging combined with pharmacological manipulations to study intracellular calcium dynamics. In addition, we succeeded in observing the elevated pH (pH 9.0) in organic templates, and lowered pH (pH 6.0) around thread-like cells using a cell-impermeable fluorescent pH indicator (HPTS).

Keywords: calcification, calcium imaging, Live-cell imaging, confocal microscopy

## Internal pH distribution and post-metamorphic biomineralization in the tubeworm, *Hydroides elegans*

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The serpulid tubeworms produce a diverse tube structure through controlled calcification. Cellular environment associated with actively calcifying serpulid tubeworms at metamorphosis were studied using pH and calcium sensitive indicators. With a notable degree of compartmentation, the thoracic region between the collars showed a high pH value above 8.5 and elevated calcium ion levels. As suggested by SEM-EDX results, such region also demonstrated a higher Ca signal. To analyze the presence of crystalline CaCO<sub>3</sub>, the unpolished sample was characterized using SEM-EBSD at 20kV, this low voltage and non-destructive approach showed the direct formation of aragonite. Applying in situ lift-out technique at the calcified region, TEM specimen was prepared for structural analysis using selected area diffraction pattern. This study documents the cellular environment during the first calcification event in the serpulid tubeworm at the transition of metamorphosis and the subsequent aragonite formation.

Keywords: imaging, serpulid tubeworms, visualization, calcifier, biomineralization

## Genomic Exploration of the Nautilus' Shell Matrix Hydrophilic Proteins: An Insight To Their Evolution in Mollusks

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The presence of a calcium-carbonate-based shell is a defining feature of most members of Mollusca. Thus, research on the genomic aspects of biomineralization of this group is interesting, since the resulting knowledge can be useful for understanding their evolutionary success. Interestingly, most members of cephalopods have secondarily lost their external mineralized shells. The nautiloids, however, is one of the two extant cephalopod groups still maintaining their true shells. Phylogenetically, the nautiloids had diverged from the ancestors of non-shelled, extant cephalopods (Neocoleoidea) in the mid-Paleozoic (Silurian/Devonian boundary,  $\pm 416$ MYA), older than the split between ammonoids and neocoleoids. This makes studies on nautiloid shell biomineral-proteins important and interesting, since insights from the nautiloids might shed light on how shell internalization and de-mineralization events evolved in cephalopods, while at the same time, might help to elucidate the evolution and identification of core components of mollusk shell biomineralization proteins, through comparisons with other molluscan biomineral-related protein data. In this talk, we are reporting our result of the genomic explorations to identify biomineralization-related proteins in the nautiloid *Nautilus pompilius*. To do so in our research, we first determined the total transcriptome sequences from the mantle tissue using pyrosequencing, while simultaneously did a total proteome analysis of the shell's hydrophilic proteins by orbital-trap mass-spectrometry. We then conducted a transcriptome-proteome comparative analysis in order to identify the hydrophilic components of shell biomineral-related proteins in the Nautilus, where we identified 51 distinct shell specific EST/proteins sequences. In the talk, we are also going to discuss how the findings provide an insight to the study of the evolution of mollusk shell biomineralization.

Keywords: Shell matrix protein, Nautilus, Transcriptome, Proteome, Biomineralization



## Using *Acropora digitifera* to bridge the gap between genome biology and geochemistry

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Coral's calcification mechanism has been receiving great attention in the fields of both geochemistry and biology. In geochemistry, high-resolution proxies using coral skeletal elements have been developed to reconstruct climate history (Gagan et al, 2012). In parallel, coral genomes have been sequenced progressively. However, trials that connect these two different fields of studies focusing on coral calcification have not been conducted yet. In this study, we focused on *Acropora digitifera* as the target species because enough genomic information is available (Shinzato, 2011) and its potential as geochemical proxies (Inoue, 2011). First, using ZoophyteBase, which has been recently developed as coral's proteome database (Dunlap et al, 2013), we investigated the genes that are potentially related to metabolism using inorganic minerals in seawater and analyzed their gene components and the correlations with seawater chemistry. Second, using next-generation sequencing, we are currently comparing *Acropora digitifera*'s gene expression between fast and slow calcification lineages of this species. In addition, coral skeletal elements of these materials have been analyzed by ICP-AES. In this presentation, we report the progress of these analyses focusing on calcification related genes and skeletal elements.

References: [1] Dunlap et al, 2013.BMC Genomics. DOI: 10.1029/2011PA002215 [2] Gagan, et al, 2012. Paleoceanography. DOI: 10.1029/2011PA002215 [3] Inoue et al, 2011. Geophysical Research Letters. DOI: 10.1029/2011GL047786 [4] Shinzato et al, 2011. Nature. DOI:10.1038/nature10249

Keywords: *Acropora digitifera*, Calcification, Gene, Skeletal elements

## Comprehensive identification of shell matrix proteins in brachiopods

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Brachiopods are marine invertebrates that appeared in the Cambrian, and they have two shells like bivalves composed of calcium carbonate or calcium phosphate. Shells contain organic matrix, which have important roles in the biomineralization processes. Recently, many shell matrix proteins in molluscs have been identified, and their roles in shell formation have been discussed. On the other hand, shell matrix proteins in brachiopods have not been identified, except for partial amino acid sequences of a chromoprotein, named ICP-1. In this study, we performed comprehensive identification of shell matrix proteins of the brachiopod *Laqueus rubellus* using proteomics combined with transcriptomics. As a result, we identified a total of 18 shell matrix proteins. BlastP search showed that these proteins have no homologues in skeletal proteins identified from other phylum, suggesting that brachiopod and mollusc shells are different in origin.

## Utility of nitrogen isotopic composition of amino acids in shell protein

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Stable isotopic composition of sedimentary organic nitrogen has been employed as a proxy to understand biogeochemical nitrogen cycles in marine and lacustrine environments. However, modification of the isotopic signals during early diagenesis (including heterotrophic assimilation/disassimilation, recycling, and reproduction) in water column and sediments always leads to much uncertainty on the interpretation of bulk isotope data. Recently, we found that a proteinogenic amino acid, phenylalanine, shows little change in the nitrogen isotopic composition during heterotrophic degradation even in long-length grazing food webs, whereas the other proteinogenic amino acid, glutamic acid, shows significant <sup>15</sup>N-enrichment at each step of food webs. Moreover, the isotopic signals of these amino acids in shell protein are always identical to those of biomass protein (e.g., muscle tissue) when the shell was produced. These results imply that the nitrogen isotopic composition of phenylalanine and glutamic acids from shell protein (e.g., in microfossils of foraminifera) captures (1) primary isotopic signals of organic nitrogen in the environment where the shell was produced and (2) trophic position of the shell-owner in ecosystems when the shell was produced.

In the presentation, we will show comparative data sets on the isotopic composition of amino acids between muscle and shell protein from various organisms, and discuss its applicability as a proxy to estimate the primary isotopic signals in environments and the trophic position of organisms of interest.

Keywords: amino acid, nitrogen isotope, food web

## Variation of North Atlantic nitrogen fixation in Caribbean coral skeletons

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Oceanic nitrogen fixation is important as new nitrogen in oligotrophic oceans and balances with denitrification in global nitrogen cycle controlling primary production. North Atlantic ocean is known to have higher nitrogen fixation rates, although the controlling factors have been debated by modern observations and sediment cores in geological time scales. Reef corals have been widely used as paleo-environmental proxy in oligotrophic oceans. Recent studies suggested that nitrogen isotopes of organic matter preserved in coral skeletons  $\delta^{15}\text{N}_{\text{coral}}$  have the potential to record coral nitrogen sources on decadal to millennia scale. In this study, we report recent 90-year records of nitrogen isotopes in *Diploria* sp. coral cores from Cayman Islands.  $\delta^{15}\text{N}_{\text{coral}}$  values were  $+1.9 \pm 2.6$  ( $\sigma$ ) ‰ (n=139), which suggested that the variation of  $\delta^{15}\text{N}_{\text{coral}}$  was controlled by nitrogen fixation ( $\sim 0$  ‰) in ambient seawater. The trend line of  $\delta^{15}\text{N}_{\text{coral}}$  increased  $\sim 4$  ‰ from 1920s to 2010s. This result suggests that nitrogen fixation rate in Caribbean Sea decreased during the past 90 years. Detrended  $\delta^{15}\text{N}_{\text{coral}}$  showed a negative correlation between Atlantic Multi-decadal Oscillation (AMO) index ( $R=-0.71$ ,  $P \ll 0.001$ ), which suggested that nitrogen fixation rate increased in higher SST condition leading an index for hurricane activity on multi-decadal scales. In this presentation, we discuss the relationship between nitrogen fixation and hurricane activity in global warming state.

Keywords: Coral skeletons, nitrogen isotopes, nitrogen fixation, Caribbean Sea, North Atlantic Ocean

## Fluorometric analysis of photosymbiosis: Toward quantitative validation of ecological proxy of planktic foraminifers

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Endosymbiosis of planktic foraminifers with photosynthetic algae (photosymbiosis) is established especially among species which dominate in warm, low-nutrient surface water. Here, photosymbiosis probably plays an important role for host foraminifers, and can be considered as an adaptive ecology to live in such oligotrophic oceans. Therefore, back in geologic time, photosymbiosis could have been involved with species adaptive radiation as well. In such viewpoint, stable isotopic change of foraminiferal test through ontogeny, attributed to change of symbiont photosynthetic effect, has been used as an indicator to detect fossil photosymbiosis. However, how host-symbiont association change through ontogeny, if any, is practically unknown and has never been quantified. Here, we offer new insights for photosymbiosis based on photosynthetic characteristics of symbionts, obtained by in vivo fluorometric analysis (Fast Repetition Rate Fluorometry, FRRF).

We cultured two symbiont-bearing species, *Globigerinoides sacculifer* and *Globigerinella siphonifera*, and conducted FRRF measurement on individual host-algal consortium during the culture period. FRRF can identify photosymbiosis of individual foraminifer instantly in a non-destructive manner, and gives us various photosynthetic characteristics of symbionts, i.e., maximum fluorescence yield ( $F_m$ , index of chlorophyll content), photochemical efficiency ( $F_v/F_m$ , index of potential photosynthetic activity), and effective absorption cross-section of photosystem II ( $\sigma_{PSII}$ , capability of the absorbed energy to promote a photochemical reaction).

Sequential FRRF analyses on single individuals revealed that  $F_m$  increases with growth, and then decrease drastically at the end of their life, which means that the algal biomass per individual foraminifer increases through ontogeny, but the symbionts are rapidly digested at the end.  $F_v/F_m$  and  $\sigma_{PSII}$  values were constant through ontogeny, though  $F_v/F_m$  drops in correspondence with the decrease of  $F_m$ . Compared between the two species, average values of both  $F_v/F_m$  and  $\sigma_{PSII}$  showed statistically significant differences.  $F_v/F_m$  was significantly higher in *Gs. sacculifer*, which means that symbionts are more actively photosynthesizing in *Gs. sacculifer*. Because  $F_v/F_m$  is mainly depends on nutrient availability, it is a direct evidence of nutrient (metabolite) flow from host to symbionts. On the other hand,  $\sigma_{PSII}$  was higher in *Gn. siphonifera*, indicating that this species can utilize low light energy more efficiently, i.e., more " low-light-adapted " than *Gs. sacculifer*. Actually, it is consistent with inferred habitat preference of *Gn. siphonifera*, which is relatively deeper than *Gs. sacculifer*.

These FRRF results provide us information of foraminiferal photosymbiosis both quantitatively and qualitatively. When the information is combined with test geochemistry mentioned above, it will presumably enable us to quantify the photosynthetic activity from foraminiferal tests. Then, it can be applied to fossil specimens as a validated ecological proxy of photosymbiosis.

Keywords: planktic foraminifers, photosymbiosis, Fast Repetition Rate Fluorometry

## Skeletal isotope compositions of *Acropora* coral primary polyps experimentally cultured at different temperatures

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We investigated temperature and growth-rate dependency of skeletal oxygen and carbon isotopes in primary polyps of *Acropora digitifera* (Scleractinia: Acroporidae) by culturing them at 20, 23, 27, or 31 °C. We cultured primary polyps of *A. digitifera* at Sesoko Station, University of the Ryukyus, Motobu, Okinawa Prefecture, Japan for 10 days. From the results of the polyp weight and polyp area, calcification was most rapid at 27 and 31 °C. The  $\delta^{18}\text{O}$  — temperature relationship ( $-0.18\text{‰}/\text{°C}$ ) is consistent with reported ranges for *Porites*, indicating that juvenile *Acropora* polyps can be used for paleotemperature reconstruction. We found a gap between curves for the experimental polyps and the equilibrium curves for inorganic aragonite of about 3.0 ‰ for  $\delta^{18}\text{O}$  and 8.0 ‰ for  $\delta^{13}\text{C}$ , with the primary polyp values being lower than the equilibrium values of inorganic aragonite. The kinetic isotope effect was evident in the polyps cultured at low temperature but disappeared at high temperatures, despite relatively low light levels. The estimated upper calcification flux limit for a kinetic isotope effect ( $\sim 0.4 - 0.7\text{ g CaCO}_3/\text{cm}^2\cdot\text{y}$ ) was similar to that of *Porites* colonies with a linear extension rate of  $<5\text{ mm/y}$ , suggesting that the calcification flux may be used as a measure of kinetic isotope effect dominance in different genera at different growth stages.

Keywords: coral, temperature, stable isotopes, polyp, kinetic effect

## Corals at marine volcano of Satsuma iwo-jima: Implication for a new proxy of hydrothermal events and biological adaptati

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Coral cores from massive corals could record marine environmental and ecological changes in their annual bands with monthly temporal resolution in the present and/or the past. We discovered large massive Porites corals living at active volcanic island of Satsuma Io-Jima, located 50 km south from Kyushu area, southern part of Japan. Satsuma Io-Jima provides a unique opportunity to observe marine organism living under extreme environments of volcanic gases emission and different types of hydrothermal activities from sea flower. We collected eleven coral cores from four different conditions around the island to test if corals could record volcanic and hydrothermal activities and how corals could survive in extreme environments such as very low pH condition with CO<sub>2</sub> emission. Coral annual bands recorded in x-ray images revealed that these corals have been survived at least during last a few hundreds years. Coral extension rate for the site near hydrothermal vent was significantly small (1-2mm/year) relative to that for general condition of Porites corals (ca. 10-20 mm/year), suggesting that coral growth was influenced by hydrothermal activity. We will demonstrate our preliminary results of geochemical approaches of  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ , Sr/Ca, Mg/Ca, Ba/Ca, and F/Ca in coral skeletons and in surrounding seawater and discuss the possibility for reconstructing the past hydrothermal events and relationship between marine ecosystem and extreme environments at volcanic activity as the analogues for coral adaptation to future ocean acidification.

Keywords: Coral geochemistry, hydrothermal activity, coral adaptation, ocean acidification

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## Sediment ecosystems dynamics on proxies development of foraminifera

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I would like to discuss how sediment ecosystems dynamics give affection to foraminiferal environmental proxies developments.

Keywords: Sediment ecosystems, dynamics, deep-sea foraminifera, environmental proxies



## Benthic Foraminifera from the deep-water Niger delta (Gulf of Guinea): Assessing activity of hydrate pockmark

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We present an ecological study of foraminifera from 4 deep-sea stations sampled in a pockmark field from the deep-water Niger delta (Gulf of Guinea, Equatorial Atlantic Ocean). All stations are located very close to each other (less than 1.2 km distance). Both sites GMMC-01 and GMMC-02 settle in an active pockmark where methane seepages were recorded by ROV observations. A third station (GMMC-03) is located in a topographic depression which is interpreted as a collapsed pockmark where no gas seepage takes place. The site GMMC-04 is a reference station, without past or present seepages. The main objective of this study is to define whether fossilizing benthic foraminifera are reliable and relevant proxies to detect gas emission in relation to hydrocarbon resources. We focus on living (stained) and dead individuals from present environments, and combine our observations with an outstanding analysis of stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) in tests of living and dead foraminifera. Our observations show that degraded organic matter with low bio-availability is present at all stations with a preferential burial of organic compounds in topographic depression (GMMC-03 station). Mudclast breccias cemented by authigenic carbonates (mainly aragonite) are recorded at both station of active pockmark (GMMC-01 and -02). There, prokaryotic consortia involved in both sulphur and methane cycles underline that both sulphide production and methane oxidation take place in the sediment close to sediment-water interface. Compared to the reference site GMMC-04, living foraminifera recorded at active and inactive pockmark show only minor changes in terms of diversity, standing stocks and faunal composition. However, the  $\delta^{13}\text{C}$  signal of some living and dead (but well-preserved) foraminiferal species (*Ceratobulimina contraria*, *Melonis barleeanus*, *Uvigerina peregrina*) is moderately depleted in active pockmark compared to both other stations. This depletion may be related to (1) a discrete geochemical imprint of anaerobic methane oxidation in upper sediments and (2) a potential effect of prokaryotic  $^{13}\text{C}$ -depleted biomass as a potential food source for benthic foraminifera. Overgrowth of authigenic carbonate on badly preserved foraminifera generates an important shift to lower  $\delta^{13}\text{C}$  values. Whereas living faunas reflect "snapshot" environmental conditions at the sampling period (November 2011) when seepages were likely discrete, dead faunas (modern thanatoconosis) carry a reliable message integrating temporal variability of gas emission. They reveal major faunal differences that are quite reliable to detect gas hydrate seepages in different pockmark stages with some key-species (i.e., *Bulimina marginata*, *Bolivina albatrossi*) underlining periods of enhanced methane emission and pockmark collapsing.

## Potentials and challenges on the use of environmental DNA to reconstruct deep-sea ecosystem and environmental changes.

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Deep sea is one of the most difficult to access environment, and consequently one of the most poorly known. However, deep-sea sediments and the organisms inhabiting this environment play a crucial role in the oceans geochemical cycles. Benthic communities are often well adapted to their local environment and therefore can reflect accurately the present conditions but also can provide insights into the past history of environmental changes. Unfortunately, except for a few specific taxa, most knowledge on deep-sea biodiversity is still missing. Deep-sea fauna is very patchy and rarity of most taxa adds to the sampling difficulty using traditional methods. Environmental DNA (eDNA) presents the advantage not to rely only on living organisms present in the sample. The presence of a species in an environment can also be detected using trace DNA left by the organism in the sediments (fragment of dead organisms, fecal pellets, etc). Recent development of DNA sequencing technologies led to promising results in the large-scale exploration of biodiversity from deep-sea environments based on eDNA using environmental DNA.

Here we will examine the use of environmental DNA as a proxy to reconstruct deep-sea communities and estimate environmental conditions in the deep-sea ecosystem. We will present data obtained from deep-sea (500-9000 m) around Japan as well as from worldwide deep-sea oceans to explore the potential use of eDNA as a proxy at various geographical and historical scales and levels of resolution. The data obtained from Iheya North vent field in the Okinawa Trough allowed us to compare the signal of eDNA along extreme environmental gradients at a very restricted geographical scale, while worldwide deep-sea eDNA survey provided us with information of on the global deep-sea environment history and colonisation. Potential of eDNA obtained from sediments to obtain information on water column processes such as plankton blooms will also be discussed.

Keywords: Deep Sea, Environmental DNA, Biodiversity, Sediment, Hydrothermal vent

## Long term monitoring of oxygen distributions at sea floor, Sagami bay, Japan.

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Oxygen (O<sub>2</sub>) distributions at the sediment water interface (SWI) are fluctuated by physical, chemical and biological interactions. Especially, bioturbation and bioirrigation at SWI enhance O<sub>2</sub> supply into the sediment, and such benthic activities play significant role on maintaining oxic environment at sediment surface. However, studies of these interactions in deep sea SWI have been limited due to technical limitations for the instrument developments and the operations. In order to investigate the SWI, we constructed a planar O<sub>2</sub> optode system to visualize O<sub>2</sub> distributions across SWI. This system was optimized for low O<sub>2</sub> concentrations, which value was equivalent to the typical O<sub>2</sub> minimum zone, ~50 μM. Using with a platform (so-called lander) to mount the planar O<sub>2</sub> optode, the system was set on the sea floor. On 21/Jan/2008, the deployment for the measurement was stated at Sagami bay, 1170m in water depth by extension of the power cable from Hatsushima deep-sea observatory. Until 31/Jan/2008, the two dimensional O<sub>2</sub> profiles were obtained at 1 hour interval. Throughout the deployment, 245 O<sub>2</sub> profile images and the corresponding grayscale images were obtained. Throughout the analysis of the images, we found the following aspects and phenomena: (1) O<sub>2</sub> penetration depth ranged 5~8mm. (2) O<sub>2</sub> irrigations sporadically enhanced the O<sub>2</sub> penetration depth to ~10mm. (3) O<sub>2</sub> concentrations in the sediment were fluctuated by time. (4) Microtopography and hydrodynamics affected to the O<sub>2</sub> concentrations on the sediment surface. (5) Meiobenthic activities suggesting anoxic metabolism were found below O<sub>2</sub> penetration depth. In the presentation, we present these characteristics with the O<sub>2</sub> images obtained from the *in situ* measurement.

Keywords: sediment-water interface, oxygen, optode, meiobenthos

## New evidence for halite co-precipitation during coral calcification

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In the last JpGU Meeting, we reported halite grains in coral skeleton through the observation of massive coral skeleton of *Porites lobata* by Analysis Transmission Electron Microscope (ATEM). Each halite grain typically shows a square shape and its grain size is around 80 nm. The spatial distribution of halite grains is inhomogeneous and seems to be independent on the arrangement of growth lines.

We observed new evidence that the halite grains in coral skeleton could precipitate during coral calcification. The electron diffraction patterns from some selected areas including both an aragonite and a halite grain show that there are special crystallographic orientation relationships between them. In consideration of misfit ratios between some selective bond lengths of halite and those of aragonite, crystallographic orientations of halite and aragonite seem to be a kind of hetero-epitaxial relationship.

This is the first observation for a primary precipitated mineral phase other than aragonite in coral skeletons. The halite phase in coral skeleton will provide a new perception for understanding the process of coral calcification.

Keywords: reef-building coral, calcification, biomineral, aragonite

## Ocean acidification in the tropical Northwest Pacific since the mid-20th century reconstructed from coral boron isotope

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Ocean acidification has been accelerating as a result of absorption of increasing anthropogenic CO<sub>2</sub> in the atmosphere emitted by fossil-fuel combustion and land-use practices since the Industrial Revolution, which can be resulting in decreased surface ocean pH and posing a critical threat to marine ecosystems (e.g., Sabine et al., 2004; Orr et al., 2005). By the end of the 21st century, predictions based on different scenarios indicate that ocean pH will decrease by 0.3-0.4 pH units. Only a few long-term continuous observations of sea surface pH have been derived from Station ALOHA off the Hawaiian Islands in the North Pacific, near the Bermuda Islands, and near the Canary Islands in the North Atlantic. A decreasing pH trend in the western North Pacific surface waters for 1983-2007 was estimated from the observational data of oceanic CO<sub>2</sub> partial pressure and related properties (Midorikawa et al., 2010). To elucidate the natural variability of ocean pH and assess the actual trend in ocean acidification more accurately, we must go further back in time. For these reasons, we rely on paleo-pH archives or other related parameters.

Massive corals, an informative archive of past ocean environments, precipitate annually banded calcium carbonate skeletons at a relatively rapid rate (about 1 cm per year), allowing for accurate chronological control and high-resolution sampling. Because of pH-dependent isotopic fractionation between the two dominant boron species in seawater, boron-isotopic systematics in marine carbonates provide a potential proxy for ocean pH in the past (e.g., Hemming and Hanson, 1992). Nevertheless, only two previous investigations provided boron-isotope time series from long-lived corals from the Great Barrier Reef in the South Pacific for the last 300 years. Unlike seawater temperature and salinity records (Asami et al., 2005; Felis et al., 2009), no coral-based reconstruction of long-term pH variation in the North Pacific has been reported.

Here, we generated an annually resolved 60-year-long (1940-1999 A.D.) record of seawater pH from boron isotope composition in a *Porites* coral collected in Guam Island, located in the Western Pacific Warm Pool which contains the highest annual sea surface waters and serves as a heat engine for the earth climate. The first long-term continuous boron isotope-pH proxy record in the North Pacific from the coral provides evidence of a slight ocean acidification trend (equivalent to 0.05-0.08 pH units for surface water) since the mid-20th century, although the critical factors that affect interannual variability remain unknown (Shinjo et al., 2013). From this perspective, the results of this study will provide improved constraints on global atmosphere-ocean interaction models and understanding of the future coral reef ecosystems.

Keywords: coral skeleton, boron isotope composition, pH, ocean acidification, North Pacific

## 15N/14N mapping of the isotope labeling cultured foraminifera using ultra thin section

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Shallow water benthic foraminifera, *Ammonia beccarii*, survive under anoxic conditions in conjunction with possible endobionts. Based on the amino acid analysis, those endobionts are expected to utilize nitrate pool in the foraminifera. However, nitrogen cycles in the foraminiferal cell and endobionts are still unclear. Here, we obtained two dimensional-nitrogen isotopic compositions of *A. beccarii* which had been incubated under oxic and anoxic conditions with <sup>15</sup>N-labeled nitrate. After observing with transmission electron microscope to confirm cellular ultrastructure and endobiont distribution, same ultra thin section was examined for nitrogen isotopic composition analysis using secondary ion mass spectrometer. Nitrogen isotopic compositions were measured with spatial resolution better than 400 nm. <sup>15</sup>N-enriched parts were found in certain structures in the cell, but not in the endobionts in this experiment.

Keywords: Benthic foraminifera, nitrate respiration, symbiotic microbe, NanoSIMS