Foraminifera: Story teller of both Earth's environments and biotic evolution

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Foraminifers are marine protists that are evolved since the late Proteozoic. They take part in marine biogeochemical cycles in particular to either carbon or nitrogen cycles. Foraminifers have appeared in the begining of eukaryotic evolution, and flourish up to the present. Both marine environmental changes and biotic evolution are able to know from foraminifera as they have quite good fossil records. However it has still not known well about foraminiferal biology. I try to focus in biological features of foraminifera and show its unique ecological and biological features.

Keywords: Foraminifera, Earth's history, biotic evolution

The variable ambient ph during chamber formation process of benthic foraminifera

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Some of major studies recently showed that calcifying marine organisms respond differently to ocean acidification (OA). Prediction of OA impact is getting tangled by this variability. Moreover, this complicates modelling future ecological cycles. Carbon usage is key to understanding calcification and hence understanding impact of OA. Using fluorescent techniques to visualize pH gradients, this shows foraminifera actively pump protons to promote passive CO2 uptake. This appearance the basis of calcification and effectively support carbon uptake independent from seawater pH. The resulting fundamentally new calcification model has major implications for understanding past changes in atmospheric CO2 as well for predicting future CO2.

Keywords: Foraminifera, Calcification

Living (stained) benthic foraminifera from the Mozambique Channel (eastern Africa): Exploring deep-sea biodiversity and biogeochemistry of unicellular fossilizing meiofauna

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This study was done in the framework of the PAMELA project (“Passive Margin Exploration Laboratories”) funded by TOTAL and Ifremer. Live (Rose-Bengal stained) deep-sea foraminiferal faunas have been studied at four stations between 530–3200-m depth in the Mozambique Channel (eastern Africa) to understand how complex environmental conditions (e.g., organic matter, oxygenation) control (1) their ecological structure (i.e., diversity, standing stocks, and microhabitats) and (2) their geochemical signatures (stable isotopes, and trace elements). Two upper-slope stations, located at 530- and 780-m depth off Madagascar, are bathed by well-oxygenated bottom waters. They are characterized by fine sediments enriched in highly degraded organic matter (low amino-acid bio-availability and reduced chlorophyllic freshness). Mineralization of organic compounds results in relatively moderate oxygen penetration depth (i.e., 15 and 30 mm) in sediment. Interestingly, foraminiferal simple diversity (S) is exceptionally high at both sites. The higher standing stocks are observed in the 780-m deep station, where peculiar sedimentary facies of organic matter focusing are recorded (OC >2.0% DW). Redox conditions and sedimentary organic matter control the composition and the vertical distribution (i.e. microhabitat) of benthic faunas at both upper-slope sites. Bolivina alata, Bulimina marginata, Haplophragmoides bradyi and Nouria compressa are relevant bio-indicators of enhanced burial of organic matter prevailing at the 780-m deep station (i.e., eutrophic settings), whereas Uvigerina hispida and Uvigerina semiornata are dominant at the 530-m deep station (i.e., relatively mesotrophic settings). Two other stations are located on well-ventilated terraces from the deep-sea canyons of Tsiribihina and Zambezi (>3000-m depth). They are characterized by carbonate ooze, which is depleted in degraded organic matter and, where oxygen penetration depth is relatively deep (i.e., > 80 mm). Because of food scarcity, foraminiferal simple diversity (S) and standing stocks are relatively low, and agglutinated and organic-walled taxa dominate foraminiferal faunas. Hospitella fulva, a foraminifera belonging to Allogromiida, occupy very deep infaunal microhabitat, what disrupts the classical scheme of microhabitat patterns in oligotrophic settings.

Keywords: Mozambique Channel, Foraminifera, Diversity, Microhabitat, Sedimentary Organic Matter, Stable Isotopes and Trace Elements

Reconstruction of foraminiferal calcification process by effective use of restricted data

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In this presentation, We propose the method of reconstruction of calcium dynamics around foraminiferal test
from a small data like obtained by calcium probe, and report the result of application of our method to real calcification data.

Relation between the organic network and the crystal defects in the calcite of the prismatic layer of *Pinctada fucata*

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Biominerals are biogenic mineralized tissues containing not only inorganic components but also a small amount of organic matrices that play an important role in formation of biominerals. Molluscan shells, which are typical biominerals consisting of calcium carbonate and organic matrices, have various kind of microstructures. The shell of a pearl oyster, *Pinctada fucata*, has two different layers. The outer layer of the shell is a prismatic layer consisting of calcite crystals. Each prism, which is surrounded by the organic framework, is composed of some single crystals of calcite. The single crystal has the small distortion of crystal orientation because this crystal has some small-angel grain boundaries inside of the crystal which cause the minim lattice distortion or defect and divide the crystal into subgrain units of a few hundred nanometers. Such minim lattice distortion and defects may increase the toughness of the shell by inhibiting the cleavage and fracture of the crystals in the prismatic layer. The small-angle grain boundaries were observed by TEM along with the localized organic matrices like networks, indicating that the organic networks may cause the small-angle grain boundaries. TEM observations showed that the organic networks are a few dozen nanometers in thickness and divide the crystal into subgrain units of a few hundred nanometers. However, what the organic networks consist of or how they cause the small angle grain boundaries has not been reported yet.

To reveal the components of the organic networks and formation mechanism of the small angle grain boundaries in the calcite crystal of the prismatic layer, we extracted the organic network from the prismatic layer and tried to identify the components. The IR spectrum of acetic acid-insoluble materials from the prisms revealed that the major component of the organic network was chitin. LC-MS/MS analysis of acetic acid-insoluble and SDS/DTT-soluble fractions showed that the chitinolytic enzymes such as chitinase and chitobiase were involved in the intracrystalline organic matrices of the prismatic layer. These results suggested that the chitinase and chitobiase regulate the formation of chin fibers that interact with the calcium carbonate to make the small-angle grain boundaries. To understand the function of the chitinolytic enzymes in the prismatic layer, calcium carbonate was precipitated in the chitin hydrogel after being treated with the commercially available enzyme. The calcite crystals precipitated in the chitin hydrogel appeared to contained larger crystal defects as the chitinolytic enzyme concentration increase and crystal defects similar to those in the prismatic layer were observed as to some extent concentration. In addition to observation, a variance of lattice spacing was calculated from peak broadening in powder X-ray diffraction and compared among the these calcite crystals. As a result, a variance lattice spacing tended to increase depending on chtinolytic enzyme concentration, implying that chitinolytic enzyme decrease the thickness of the chitin fiber to increase the interaction between chitin fibers and calcium carbonate. Such interaction is probably important to produce the small-angle grain boundaries in the calcite crystal and strengthens the toughness of shell.

Keywords: biomineral, shell, chitinase

Lateral and Vertical Textures in Shells of Calcitic and Aragonitic Foraminifers

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Although shells of various foraminifers were reported to be composed of unique layered structures, details of the crystallographic structure have not been clarified sufficiently. Here, we investigated the shells of foraminifers, calcitic *Ammonia beccarii* and aragonitic *Hoeglundina elegans*, using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and selected area electron diffraction (SAED). Despite the difference in their crystal polymorph and shapes, they exhibited similar textures in micro- and nano-scales. The shells were revealed to consist of single-crystalline micrometric domains with their c axes perpendicular to the shell surface from SAED patterns of platy samples 100 nm thick prepared using a focused ion beam (FIB) technique. Cross-sectional SEM and TEM observations showed the presence of lateral organic layers ~10–20 nm thick with ~200–400 nm intervals in the single-crystalline domains. Whereas a micrometric layered structure has previously been reported as a periodic growth pattern, we discovered finer lateral textures. On the other hand, a columnar structure ~100–400 nm wide derived from vertical textures was observed on the cross-sections after mild etching of the shells in a diluted acetic acid solution. We also found the vertical textures which originated from strains of the crystal lattice as a difference of contrast in the TEM images of the FIB-cut samples. The columns were produced through selective dissolution of the strained area. Therefore, the foraminifer shells of the two species were deduced to consist of micrometric single-crystalline domains having lateral and vertical textures with organic thin layers and strains, respectively, regardless of the difference of polymorph.

Morphospecies, cryptic species or biological species…? Searching for the evolutionary significance of diversity in foraminifera

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Many groups of foraminifera are characterized by the formation of elaborate shells. These shells provide detailed morphological features that can be used for species classification. Since the majority of works on foraminifera focuses on their fossil record and their application as proxies in micropaleontological studies, a comprehensive morphotaxonomy has been established, describing tens of thousands of extant and extinct morphospecies. On the other hand, genetic analyses of the group revealed an even higher diversity on the molecular level, hidden within the traditional morphospecies. These cryptic species are usually marked by large genetic distances, differentiated biogeographic distribution patterns and ecological adaptations, implying that cryptic species rather than morphospecies represent the level of biological species.
Biological species are defined as reproductively isolated groups of organisms and their identification forms the basis for all studies on the biodiversity, biogeography and ecology of any group of organisms. Yet so far in foraminifera the evolutionary significance of both the morphological as well as genetic diversity and their relation to reproductive isolation remain uncertain, even though this knowledge is indispensable to improve the application of foraminifera as paleoceanographic proxies. E.g. the amount of genetic variation that represents species level divergence instead of intra-population variability is not yet known. In addition, it cannot be objectively stated which morphological features represent species-level differences and which are the result of environmental adaptations. Thus, in order to achieve an objective identification of the level of biological species in foraminifera, culturing experiments are needed to observe reproductive isolation.
We apply a single cell approach to survey the extent of cryptic diversity within foraminifera morphospecies and to examine their distribution and ecological adaptations. In addition, we conduct morphometric analysis in order to establish a connection between morphological and genetic diversity. Furthermore, we carry out mating experiments in order to determine the degree of genetic divergence that corresponds to reproductive isolation and thus represents the level of biological species in foraminifera. In addition, we will try to investigate the existence of different mating types, in order to elucidate the influence of the mating system on the evolution and diversification of the group.

Keywords: foraminifera, genetics, morphology

Proteomic analysis of shell matrix proteins in the pond snail *Lymnaea stagnalis*: identification of potentially functional proteins

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Matrix proteins have important roles in molluscan shell formation, and their amino acid sequences have been characterized for some species. However, the mechanisms of shell formation have barely been clarified. In order to setup a platform for a systematic functional analysis of shell matrix proteins, we performed a combined transcriptome and proteomic analysis of the shell matrix proteins for the pond snail *Lymnaea stagnalis*. We found a total of 203 shell matrix proteins from the shell matrix of *L. stagnalis*. A total of 161 amino acid sequences of them showed sequence similarities to known proteins, including four paralogs of dermatopontin, which was previously reported from the shell matrix of *L. stagnalis*, when searched against public databases, while the remaining 42 showed no similarity to the proteins in the current databases. Next, in order to discriminate 'functional' shell matrix proteins from those that were accidentally entombed in the shells, we compared the levels of expression of these shell matrix proteins between the right side and the left side of the mantle, which makes the shell, underlying assumption being that genuine functional shell matrix protein genes would be more strongly expressed in the right hand side of the mantle in the dextral shells, while there would be no such differential expression pattern for the proteins which were accidentally trapped within the shells. Actin is the most abundant shell matrix protein found in the shell of *L. stagnalis*, but the expression patterns of the actin gene showed no difference between right and left of the mantle. On the other hand, the second most abundant shell matrix protein called Pif-like protein, which is an acidic shell matrix protein identified also in *Pinctada fucata* and *Crassostrea gigas*, showed that its gene is more strongly expressed in the right hand side than the left hand side of the mantle. Our results suggest that Pif-like protein is a functional shell matrix protein, while actin is a protein occluded in the shell accidentally. Finally, we searched for conserved domains of these amino acid sequences. We found various domains and classified them into six categories. Those are extracellular protein, enzyme, cation-interaction protein, polysaccharide interaction protein, proteinase inhibitor, and others. Conserved domains allow us to estimate possible functions of novel shell matrix proteins. Characterization of those novel proteins, and functional analyses of all those proteins identified in this study for this 'model organism' will help understand the mechanisms of biomineralization as well as the evolutionary processes of shell formation in molluscs.

Keywords: Transcriptome, Proteomic analysis, Biominelarization, Shell formation, Matrix protein

Reefal microbial crusts found in a Holocene reef sediment core, off Okinawa Island, the Ryukyu Archipelago

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Reefal microbialites are fine-grained, non-skeletal crusts, locally common in late Quaternary coral reef deposits. They are interpreted as microbial carbonates produced by heterotrophic bacterial communities. The carbonate crusts are typically up to 10 cm thick and form the late stage of reef developments, covering on framework skeletons. Major questions still remains unanswered concerning their uneven distributions in space and time, formation process and controlling factors. Here we first report fine-grained, non-skeletal carbonate crusts in a Holocene reef core drilled at Naha New Port area, off west of Okinawa Island, the Ryukyu Archipelago, which are similar in texture and fabric to reefal microbialites reported from deglacial reef deposits from other coral reef regions. The carbonate crusts are up to several centimeters in thickness, and mainly developed in a particular stratigraphic horizon (depth: 5~6 mbsl, age: ca. 7 ka), from which the crust thickness decreased downward and upward. Based on their external surfaces and internal sections, these crusts are classified mostly as a digitate type (thrombolite) and partly as a weakly layered type (stromatolite). Surface elemental analysis showed that crusts are composed mainly of Ca and Mg (Mg calcite). Microscopic observations clearly showed a biological succession from in situ bioeroded corals, overlain by coralline algae and encrusting foraminifers, finally to fine-grained crusts. Silt-sized grains were mainly made of peloids, with subordinate bioclastic and siliciclastic grains. SEM-EDX analysis recognized four different elemental spectrum patterns in the carbonate crusts: sulfur (S)-rich pattern (interpreted as trapped skeletal Mg calcite grains), poor S pattern (precipitated Mg calcite crystals with Mg/Ca ratio of ~0.14), Si and Al common pattern (precipitated Mg calcite crystals with trapped siliciclastic grains or clay minerals), and no Mg pattern (trapped coral aragonite grains). These petrographic and geochemical features are very similar to reefal microbialites found in deglacial reef deposits from other coral reef regions. Some differences in thickness and elemental compositions are possibly related to environmental settings (a volcanic hinterland vs. a mixed carbonate-siliciclastic hinterland).

Keywords: microbialite, Ryukyu Archipelago, Holocene

Syndepositional formation of calcareous nodules on muddy sea floor: elucidating depositional history by C, O, S isotope characterization

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Calcareous nodules are commonly observed in mudstone sequences, however, paleodepositional condition including paleothermometry have never been discussed based on its chemistry without exceptional case such as methane seepage. It partly owes to their complicated deposional history. We characterized dark grey muddy carbonate nodules collected from Cretaceous strata in Hokkaido, Japan with carbon, oxygen, sulfide sulfur and sulfate sulfur isotopes, and demonstrated the syndepositional formation of some nodules. It is notable that initial formation of carbonate “precursor” for all muddy nodules studied here were syndepositional near oxic/anoxic boundary at shallower depth of muddy sea floor.

Some outcrop observations demonstrate calcareous nodules can be formed just below the sea bottom (JBSB). Structure suggesting consolidation JBSB includes burrows that eject calcareous precursor material from nodule. JBSB origin of the nodules consolidated associated with anaerobic oxidation of methane (identified by carbon isotope ($d^{13}C$) values) with sulfate reduction are also demonstrated with oxygen isotope ($d^{18}O$) and sulfur isotope ($d^{34}S$) values. Such nodules show the exactly same $d^{18}O$ values with that of benthic foraminifers. A bivalve fossil found on one of the methane seep nodules preserved aragonite of the shell and yielded close $d^{18}O$ paleotemperature with that of host nodule.

The cross-plot of the $d^{13}C$ and $d^{18}O$ data can emerge “upper limit line” of $d^{18}O$ values showing syndepositional formation. On the other hand, nodules from the Oyubari area characterized with $d^{34}S$(sulfate) lower than -18 permil indicating JBSB formation had conflicting $d^{18}O$ values. It can be ascribed to the difference of burial depth between sediments of the Haboro and Oyubari areas. Even if precursor of the nodules initially crystalized JBSB, strong compaction during burial would have caused permeation of pore water into the incomplete nodules. Carbon dioxide or bicarbonate ions derived from decomposed organic matter would have caused recrystallization of calcite with $d^{18}O$ as low as -10 permil in the nodule.

Keywords: calcareous nodule, oxygen isotope, sulfur isotope, carbon isotope

A novel approach in utilising marine methane seep derived bivalve shells for developing local ΔR corrections in deep sea environments

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Shells and corals are commonly used as proxies for sea surface temperature reconstructions in paleoclimatology. These species utilise the dissolved inorganic carbon (DIC) in seawater making it suitable for analysing ocean circulation through the measurement of radiocarbon ^{14}C. It is also possible to estimate the age of the shell through analysing the growth increments within the shells. Yet calibration models in deeper marine environment remains complicated due to the multiple variables that must be considered which include problems such as carbon sinks, ocean current flux and dead carbon effect from seafloor vents.
This study proposes a novel approach in ^{14}C dating shells found in a deeper marine environment (600m) located near methane vents off Tokai using a Single-Stage Accelerated Mass Spectrometer (AMS), to develop a suitable calibration model with a greater goal of observing local marine environmental changes in the deep sea. This method may allow bivalves living in deeper marine environments to be used as future tools for measuring samples located in complicated environments such as deep hydrothermal vents and cold seep vents, possibly leading to assessing local shallow fault movements occurrences in the past.
Sclerochronological analysis of the shells proved difficult since growth increments of deep sea shells are not determined according to simple changes in temperature such is the case with most surface marine shells which often reveal daily, fortnightly or annual bands during seasonal temperature extremes. The radiocarbon ^{14}C age of these bivalve shell measurements ranged between three age groups of 1396±36-1448±34, 1912±31-1938±35 and 5975±34. The ^{14}C age of shells that were alive upon collection and the dissolved inorganic carbon (DIC) in seawater show little difference (around 100 14C age) indicating that shells are not heavily affected by the dead carbon effect from cold seeps that is of biogenic or thermogenic origin, which can make the age of the shells to become considerably older than their actual age. The novel calibration model used was therefore based on the seawater DIC collected above the *Calyptogena* spp. colony site (1133±31), resulting in the dead shells to be clustered around 1900 Cal AD. This age group proves to be interesting as the Ansei-Tokai earthquake (M 8.4) in 1854 is extremely close to the bivalve colony site. Based on the theory that the bivalve shells formed sometime after the venting of methane fluids, it may support the validity for applying such novel calibrations methods in complicated deep sea environments. Using geological data obtained using visual analysis and sub-seafloor structural analysis that show multiple shallow faults and chaotic sediment structure below the colony site, the *Calyptogena* spp. shells have a strong connection to the coseismic faulting activity and could show potential for radiocarbon dating to be applied on marine samples providing the necessary calibration tools are available.

Keywords: Radiocarbon dating, Active fault, Cold seep, Bivalves, Methane

Oxygen isotope and Mg/Ca ratio of high magnesium calcite of benthic foraminifera as a proxy for water temperature

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Although metal/Ca or oxygen isotopes ($\delta^{18}O$) of organic precipitated calcium carbonate (calcite and aragonite) of marine species have been used to estimate paleotemperature, the relationships between components of large benthic foraminiferal shells (high-magnesium calcite) and seawater temperature has yet to be established. We investigated the possibility as a proxy for seawater temperature through culture experiments of three species of reef-dwelling large benthic foraminifera in a laboratory. Three species found commonly in the western Pacific were selected including perforate species *Calcarina gaudichaudii*, and imperforate species *Amphisorus kudakajimensis*. All of them are host to algal symbionts. They grew sufficiently during culture experiments and showed a maximum in terms of shell length and weight at 27℃ and 29℃ while they were significantly small at 30℃. Mg/Ca ratios of three species in similar range showed high correlation to water temperature, suggesting Mg/Ca ratios as a precise proxy for paleo-temperature in shallow-reef environment. In terms of $\delta^{18}O$, *C. gaudichaudii* showed strong correlation versus temperature while $\delta^{18}O$ of *A. kudakajimensis* showed less significant correlation possively caused by poor growth of unhealthy individuals. The trends in the temperature and oxygen isotope ratios were similar for both species, suggesting the potential of oxygen isotope ratios in the tests of reef-dwelling foraminifera as a paleo-thermometer. Species-specific calibration may be necessary for the use of $\delta^{18}O$ of reef-dwelling large benthic foraminifera as a proxy of paleo-temperature.

Keywords: high magnesium calcite, proxy for water temperature, large benthic foraminifera

Culture experiments to better understand biomineralization under varying geochemical conditions
--Zombie factory in Japan: the first trial on Scandinavian foraminifera--

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Ocean acidification is a consequence of ongoing global climate change, and it may have severe impacts on calcifying organisms. This process may be amplified in coastal regions where erosion, land run-off and eutrophication contribute in lowering the pH.
The aim of our study is to define how bottom-dwelling foraminifera will respond to pH changes in the Skagerrak/Baltic Sea region in addition to other environmental stressors such as temperature and salinity changes.
First results demonstrate variations in shell preservation of the living foraminiferal fauna. The more marine foraminifera have pristine shells, while the low salinity Baltic ones are more or less dissolved and only inner organic linings are still visible. However, these “zombie” foraminifera are still alive, as determined from the CellTracker Green labelling. The dissolution can be linked to the lower pH in the Baltic.
In order to investigate the zombie foraminifera further, we set up culture experiments on healthy foraminifera under controlled geochemical parameters. We discovered that lowering the pH is not sufficient to create zombie foraminifera and that abrasion between foraminifera and sediment is probably involved in the shell loss. Those results highlight the multiple factors affecting the balance of benthic ecosystems subjected to environmental stressors.

Keywords: benthic foraminifera, ocean acidification, laboratory experiment

Approach to comprehensive analyses of molecular phylogeny, morphometrics, and geochemistry of planktonic foraminifera

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Planktonic foraminifers have been widely used for the studies of paleontology and paleoceanography. Stable oxygen and carbon isotope ratios of planktonic foraminiferal shells are the major proxies for paleo-environment, as these isotopes are affected by inhabiting water temperature and the chemical components. Most of previous studies have been conducted with the traditional species concept based on the morphological differences of their calcareous shells. However, molecular phylogenetic studies have unveiled the presences of multiple cryptic species in a single morphospecies of planktonic foraminifers. Such a high diversity of planktonic foraminifers suggests that the current paleoceanographic proxies are underestimated due to the mixed information of multiple biological species. Therefore, it is the urgent task to re-assess the ecological and geochemical characters at each biological species. For this purpose, we need to work out the implementation method, which combines the multiple analyses: DNA, morphological, and geochemical analyses, for a single individual. The DNA extraction method by using the buffer based on the guanidium isothiocyanate now enables us to preserve the calcareous shells after the extraction. By using this method, we can detect the morphological and geochemical characters on a same individual, which is identified by the molecular technique. However, the thermal and chemical reactions of this DNA extraction method to the calcareous shells are still unknown. In this study, we test whether or not the method of the molecular experiment physically and chemically damage the calcareous shells. We collected the living specimens of planktonic foraminifers and divided them into three experiment sets. In the first set, the specimens were applied to the DNA extraction with incubation process at 70 °C for 40 minutes as usual. In the second set, the time for incubation was three times longer than the first one. Through the comparison between these two patterns, the effect of the incubation time to the calcareous shells can be detected. We also prepared the specimens just collected from the sea-water but without the process for the DNA extraction, as a control. The densities of the calcareous shells were measured by the micro-focus CT scanning, and then their stable oxygen and carbon isotopes were analyzed one by one. The comparisons of physical and geochemical characters of the planktonic foraminiferal shells showed that the heat and chemical treatments concerning the DNA extraction never changed the shell component. Thus, we succeed to establish the experimental methods, which investigate the morphological and geochemical features for each biological species from single individual.

Keywords: planktonic foraminifers, Molecular phylogeny, micro-focus CT scan, Stable isotopes

A study on chemical composition of living acantharian (Radiolaria) shell

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Radiolaria is one of marine zooplanktons having skeletons/shell of opal (SiO_2+nH_2O) and/or celestite ($SrSO_4$), ranging from Cambrian to Recent. Living Radiolaria is divided into 5 Orders, Collodaria, Nassellaria, Spumellaria, Acantharia, and Taxopodia. The first three orders, have siliceous skeletons and then become to be microfossils in pelagic sediments. While Acantharia has a skeleton consisting of celestite, and thereby this order group cannot become fossils. In this study, we have conducted radiolarian culturing in laboratory at Ehime University for direct observation of their skeletal growth system. The living radiolarian specimens were obtained from surface waters of warm Kuroshio Current near off Kashiwajima Island, Kochi Prefecture, Shikoku Island, Japan. The detection of additional growth on skeletons was used a fluorescent compound following the method by Ogane et al. (2009, 2010). Through this direct-observation study, a possibility that acantharian shells contain siliceous compound was suggested. We therefore focus on to present the data of living Acantharia obtained in the poster.
Living acantharian cells were collected from surface waters (ca. <5-m depth) on 12the July, 30thh November,2015 and 11th January, 2016 by using a plankton net (85 micron meters mesh). We picked up individual cells from sample materials at Kashiwajima, and then performed on culturing experiments for radiolarian cells at a laboratory of Ehime University. The samples were incubated under an artificial day-night cycle (12hours day/12hours night) with white and blue LED lights at 27dgree Celsius.
After 24hours when we left radiolarian cells within incubators, we added a solution including fluorescent compound called HCK-123 into culturing dishes (final concentration ca. 0.5-1 micron mole). After 24-30 hours culturing, we mounted slides for observation with a confocal laser scanning microscope (Carl Zeiss LSM510) at Department of Biology, Faculty of Science of Ehime University. Some fluorescent compounds such as PDMPO and HCK-123 have been used one of the tracers for biological silicification (ex. Shimizu et al., 2001; Desclés et al., 2007; Ogane et al. 2010), which can stained newly formed siliceous skeletons of microplanktons because these agents are incorporated in siliceous depositions during biogenic mineralization. Therefore we can detect Silicon distributions in additional parts in siliceous shells. We also conducted FE-SEM and WDS analyses to check Si-distribution in the acantharian shells.
[Results]
Four specimens of Acantharia, identified, *Acanthometra muelleri*, *Amphilonche complanata, Acanthostaurus conacanthus* and *Acanthometron pellucidum*, among culturing radiolarian specimens clearly emitted fluorescence using by a fluorescent compound HCK-123 labeling. Each specimen shows fluorescence emission at the following parts of living cells: surface of spines and central soft parts for *A. muelleri*, proximal parts of spines and centrals for *A. complanata*, proximal surface parts of spines for *A. conacanthus*, and broken spines including its tip parts for *A. pellucidum.* WDS analysis was also performed on *A. complanata* shell for distribution mapping of Sr, S, O and Si contents. Sr, S and O, which are considered as major compositions of acantharian shells ($SrSO_4$), occurred from whole skeletal part. Si signals were also detected from a tip and surface of spines. Considering these results from our culturing experimental works and WDS analyses on living Acantharia above-mentioned, it is revealed that living acantharian cells contain Silica, in

particular on an active growth part of skeletons. This fact suggests that SiO_2 is one of the important components on skeletal forming of Acantharia.

Keywords: Radiolaria, Acantharia, skeletal composition

Effects of ocean acidification on shell and somatic growth, and stable isotopes of shell carbonate of two species of abalones

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Ocean acidification is now one of the important issue to appreciate the impact on marine calcifiers (IPCC, 2013), and potentially affects their survival, calcification, growth, physiology and development. To evaluate the effects of elevated CO_2 levels on shell and somatic growth, and stable isotope compositions of two species of abalones (*Haliotis discus discus*, *Haliotis gigantea*), we conducted culture experiments at three treatment levels of seawater pCO_2 (400, 750, and 1200 µatm), at approximately 23 °C.

The effects of seawater pH on calcification (shell width, shell weight) was non-significant in both species. On the other hand, the positive relationships between pH and wet weight of soft tissue of two species were observed. Their adjusted wet weight of soft tissue at 1200 µatm was significantly greater than that at 400 µatm. These results suggest that elevated pCO_2 affected their metabolism (e.g. higher metabolic rates to maintain homeostasis).

Stable oxygen isotope compositions of outer (calcite) and inner (aragonite) shell layers of two species showed non-significant relationships with pH. The negative correlations between carbon isotope compositions and pH of both layers appeared in both species, and the slopes of these relationships of shells were lower than that of dissolved inorganic carbon (DIC) of seawater. We estimated the equilibrium values of carbon isotope compositions at each pCO_2 treatment, and the difference between the carbon isotope compositions of shell and equilibrium values showed gradual increases in shell carbon isotope compositions with decreasing pH. Thus, the pCO_2-induced change in metabolism of abalones might appear in carbon isotope compositions of shells as the metabolic effect.

Keywords: ocean acidification, culture experiment, stable isotopes, abalone, metabolism, biomineralization

