

JpGU-EGU Biogeosciences Joint Session: aims and challenge

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Since JpGU Biogeoscience Section was established on 2010, we are continuously holding international session in collaboration with EGU. This session aims to discuss ecological and environmental information through geochemical data of proxy organisms, such as calcifier or silicifier. We encourage to carry out both biological and goescientific approaches. I will shed light on foraminiferal biodiversity and its application to environmental proxies.

Keywords: Biogeosciences, foraminifera, proxy, natural history, basic

Seasonal variation in genetic types of a planktonic foraminifer

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Planktonic foraminifers have been employed in paleoceanographic studies due to two major characters: wide geographic distributions of species and chemical composition of their calcareous shells, which reflect temperature and chemistry of ambient seawater. Accumulating phylogeographic knowledge, high genetic diversity of planktonic foraminifers has been known today and these genetically isolated species are distributed in narrower geographic area than we expected. The previous studies re-established temperature dependences of genetic types according to the pattern of their geographic distributions in many area. Moreover, genetic differentiation among geographically distant populations examined the role of ocean currents in dispersal of planktonic foraminifers. In order to improve the use of planktonic foraminifers as ocean environmental indicators, understanding ecological characters of genetic types is a crucial issue. In the previous studies for molecular phylogenetic analyses, living specimens of planktonic foraminifers were collected at each site as occasion arises. The present study therefore collected samples every month for 1 and half years at same location, the Tosa Bay, where the branch of the Kuroshio Current reaches. We focused on *Globigerinoides ruber*, the most commonly used species for paleoceanography, and found this morphospecies during the whole years in the study area. Four genetic types are detected throughout our survey, though the frequency of each type varied at each sampling time. We demonstrate the effect of external environment to productivity of planktonic foraminifers by analyzing the relationship among water-temperature, chlorophyll concentration, and frequency of genetic types. We furthermore test whether the distance from the main path of the Kuroshio Current is efficiently caused to transport a population of specific genetic type. These examinations provide us the exact information to address seasonal variation of planktonic foraminifers at genetic-type level.

Keywords: time-series collection, planktonic foraminifer, genetic type, ecological feature

Cryptic diversity in planktonic foraminifera and the relationship between molecular and morphological classification

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Planktonic foraminifera are marine single celled eukaryotes that are found globally in the ocean. They construct calcite shells that accumulate in the sediment and are used for micropaleontological studies to reconstruct past climate conditions. Modern planktonic foraminifera have been classified into about 40 species on the basis of morphological characteristics of their calcite shell. Molecular investigations however uncovered an unexpectedly high cryptic genetic diversity within these morphospecies, implying that their biodiversity has been largely underestimated. These cryptic species show distinct biogeographic distribution patterns and ecological adaptations. Therefore, understanding the extent of genetic diversity within a morphospecies as well as the ecological adaptations of its cryptic species is highly important in order to enhance the applicability of foraminifera as proxies in micropaleontological studies. We are applying a single cell approach to survey the extent of cryptic diversity within the SSU rDNA of individual morphospecies and to examine the biogeography, habitats and ecological adaptations of cryptic species. In addition, we are trying to establish a connection between genetic diversity and morphological variability of the calcite shells by morphometric analysis in order to taxonomically revise the morphospecies and to create a connection between living specimens and the fossil record.

A fundamental function of calcareous spine of large benthic foraminifers for lighting inside

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Major groups of foraminifers precipitate calcium carbonate and form calcareous shells with unique and complicate structure. One characteristic structure is a spine, covering the exterior surface of the shells. These shells and spines are though to have a function for the protection of the cell from predators or for the prop to extend the pseudopodia. However, in other organisms (e.g., land plants), calcareous crystals play a role for increasing light intensity into the photosynthetic tissue more efficiency by scattering light. Our question is what are ecological functions of the shell and spines of calcareous crystals in foraminifers.

Large benthic foraminifers (e.g., *Calcarina*) are the dominant species in the tropical reef waters, where is under exposure of strong light with nutrient depletion. They harbor a vast amount of photosynthetic symbionts inside the cell and utilize these symbionts for uptake recycle-nutrients to survive in the oligotrophic waters. Morphologically, *Calcarina* have blunt spines, which help to spread the pseudopodia, extended in a single circle round a central axis. Their pseudopodia attach to the basement, and avoid to be flowed over by tidal wave. However, our knowledge to ecological function of calcareous spines of *Calcarina* is still limited. We investigated the crystal orientation of calcareous spines of *Calcarina gaudichaudii* by using Electron Back Scatter Diffraction (EBSD) analysis. Calcareous crystals are arranged horizontally to the axis of the spine in the center and gradually vent to the edge. This orientation of calcareous crystals makes a pathway of light through spines to the interior of the shell. Our finding suggests that calcareous spines of *C. gaudichaudii* have ecological function to focus light into the cell and promote photosynthesis of their harboring symbionts.

Keywords: Calcarina, calcareous, Electron Back Scatter Diffraction analysis

Functional analysis of shell proteins using transgenic pearl oysters

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For the study of biomineralization, the mollusc *Pinctada fucata* is arguably an attractive genetic model system, with its draft genome sequence having been deciphered recently. It produces two crystallographically different shell layers, including the industrially important nacreous layer. We are interested in developing knock-out pearl oysters to analyze the functions of shell matrix proteins, the key players in biomineralization processes, including the unusually acidic protein Aspein. In this study, the widely used Minos transposon system is applied to generate insertional mutagenesis. The first step in developing transposons as tools for mutagenesis is to demonstrate their mobile elements function efficiently and stably in the target organism. Therefore, green fluorescent protein (GFP) is integrated into the transposon to reflect its efficiency.

Morphological observation of cytoplasm with acidic and alkaline vesicles in large foraminifera cell by confocal imaging

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Algal symbiont-bearing large benthic foraminifers are primary and carbonate producers as well as paleoenvironmental indicators in tropical and subtropical reef environments. Despite their importance, their cellular physiology is not well known. In the present study, we have developed methods to observe in vivo images of a living symbiotic porcelaneous large foraminifer, *Amphisorus kudakajimensis*. The Nikon A1 confocal laser scanning microscope with Calcein-AM was used as a fluorescent indicator for visualizing the morphology and streaming of cytoplasm in living *A. kudakajimensis*. The observations indicated that the cytoplasmic density decreased and reticulopodia were formed at the aperture in the marginal part of intrashell cytoplasm. We also observed vesicles with elevated pH (pH 9.0) and lowered pH (pH 6.0) in reticulopodia-like cytoplasm using a pH-sensitive probe molecule, 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS). The present study demonstrates the use of confocal microscopy in studying cytoplasmic dynamics and the initial calcification processes such as seawater endocytosis and alkalization of seawater vacuoles.

Keywords: Large foraminifera, Confocal imaging, Live imaging, pH, Calcification

Calcification mechanisms in foraminifera and proxy incorporation

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Calcifying organisms, such as pteropods, bivalves, corals and foraminifera provide a rich resource for pale-oceanographers and climatologists because their geochemical make-up (proxies) can be used to reconstruct past ocean history and evolution during and after natural carbon perturbations. However, it has been shown for all geochemical proxies that the main assumption of only one environmental variable controlling a target proxy is too simple. Empirical calibrations introduce a lot of uncertainty because the mechanisms of proxy incorporation are not well understood. The major problem is that the calcification mechanisms are still a black box. In this presentation I will review our current understanding of calcification and proxy incorporation in foraminifera, specifically with respect to the impact of carbonate chemistry and the new opportunities arising from this.

Keywords: Calcification, proxy

Minor element partitioning and mineralogy in limpets from a CO₂ vent site

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Specimens of the patellogastropod limpet *Patella caerulea* were collected within and outside a CO₂ vent site at Ischia, Italy. The shells were sectioned transversally and scanned for polymorph distribution by means of confocal Raman microscopy. Minor element to calcium ratios were measured using laser-ablation-inductively-coupled-plasma-mass-spectroscopy (LA-ICPMS). Mg/Ca, Sr/Ca, and Li/Ca ratios were determined in calcitic as well as aragonitic parts of the shells. This approach allows for investigating the effects of the polymorph and the seawater carbonate chemistry on minor element partitioning separately.

Keywords: calcification, proxy, CO₂, mineralogy, patellogastropod limpet

Mathematical analysis of proton, carbon, and calcium transport during calcification process of benthic foraminifera

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Foraminifera are marine unicellular organism responsible for approximately 50% of today's ocean calcium carbonate production, which implies that they play a considerable role in the global carbon cycle in ocean chemistry. However, we have not fully understood how their calcifications proceed and change their chemical environment.

To understand the calcification process, we investigate the dynamics of the pH around the foraminifer. The visualized spatial pH distribution shows that the calcification accompanies a rapid decrease of the surrounding pH, which implies that the calcification accompanies active proton pumping. The resultant spatial pH distribution is used to estimate the flux of proton released out of the protective envelope. Combining the estimated proton flux and the calcium carbonate responsible for a new chamber, we propose that the proton/H⁺ exchange process is the key process driving the foraminiferal calcification.

Keywords: benthic foraminifera, calcification, proton transport, mathematical analysis

An experimental approach to understand trophic interaction of photosymbiosis in planktic foraminifers

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Planktic foraminifers are marine heterotrophic protists. Of about 50 species of modern planktic foraminifers, about 10 species that especially dominate in warm and low-nutrient surface water harbor autotrophic algae as endosymbionts (photosymbiosis). It is generally considered that foraminifers benefit from photosynthates of symbionts, and in return, they provide nutritious environment for symbionts to live. At the same time, however, the host's degree of dependence on symbionts is still enigmatic. This is because growth of the host primarily depends on food (prey) availability. In this context, a common assumption that photosymbiosis is an advantageous ecology for host foraminifers to live in oligotrophic oceans still has room to discuss. To understand trophic interaction between host and symbionts, we conducted culture experiments and analyzed vitality of host-symbiont consortia under controlled nutrient conditions.

We cultured dinoflagellate-bearing species *Globigerinoides sacculifer* for two weeks. Assuming the two sources of nutrients for symbionts, i.e., from the host's metabolites and from the ambient seawater, we controlled feeding regime (fed *Artemia* every other day or unfed) and nutrient concentration of culture media (0.22- μ m filtered seawater [SW] or nutrients-added filtered seawater [NSW]). Four experimental groups are set; (a) fed and SW, (b) unfed and SW, (c) fed and NSW, and (d) unfed and NSW. Nutrient concentrations of SW and NSW were respectively 0.2 and 16 μ mol L⁻¹ of NO₃+NO₂, and 0.07 and 1.0 μ mol L⁻¹ of PO₄. Temperature was set to 26.5-27.5 °C. Photosynthetic active radiation was set to 170-220 μ mol quanta m⁻² s⁻¹, and its light/dark cycle was 14/10 hours. Test growth of the host, chlorophyll content, and photo-physiology of the symbionts were used as criteria of their vitality. We measured maximum test length of host foraminifers and chlorophyll fluorescence of individual host-symbiont consortium during the culture period almost every day. For fluorometric analysis, we used fast repetition rate (FRR) fluorometry. From FRR measurement, F_m (an index of chlorophyll content), and F_v/F_m (an index of potential photosynthetic activity) were obtained and analyzed for each individual consortium.

During the culture period, foraminifers grew and formed new chambers in the fed groups (a, c). On the contrary, specimens in the unfed groups (b, d) gradually decreased their cytoplasm volume, and in accordance with the decrease they often shed chambers one by one. The chlorophyll content, thus the biomass of symbionts per foraminifer, tended to increase in the fed groups (a, c), whereas it decreased or kept nearly constant in the unfed groups (b, d). Despite the apparent diminishment of the unfed groups, F_v/F_m was significantly higher in the unfed groups (b, d) than that in the fed groups (a, c). It indicates that symbionts in starved foraminifers photosynthesized more actively. Nutrient concentration in the culture media (SW or NSW) did not necessarily affect on F_v/F_m .

Considering the fact that foraminifers maintained their life and symbionts were capable of photosynthesis in starved condition, it can be said that foraminifers have survived only by photosynthates derived from the symbionts or digesting the symbionts themselves for about two weeks of the culture period. If this relationship is true in natural environment, photosymbiotic interaction should help foraminifers to survive for certain duration even if they cannot capture any prey. This should be an advantage for them to live in low-nutrient and well-lit environment.

Keywords: planktic foraminifers, photosymbiosis, nutrients, FRRF, culture

Strong typhoon in 2005 recorded in the shell growth lines and geochemical signals of *T. maxima* from Okinotori Island

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The giant clam (*Tridacnidae*) widely distributes over the coral reefs in Indo-Pacific oceans and forms the largest shells in bivalves. The giant clam has symbiotic algae, facilitating fast growth rate and forming daily shell growth patterns. By measuring and counting the daily growth lines, we can know exactly when the daily growth lines were deposited. This growth pattern analysis is useful for reconstruction of the past environments at daily resolution. The aim of this study was to validate whether the shell of the giant clam (*Tridacna maxima*) in Okinotori Island, the southern edge island in Japan, could record daily environmental events such as typhoons.

The shell samples were collected alive in Okinotori Island on 5th Jun 2006. The shells were cut into two slices along the maximum growth axis. The number and interval width of microgrowth lines were measured under a digital microscope (KYENCE VHX-2000). Sub-sampling for oxygen stable isotope measurements were performed along the growth direction on another slice of the shell by using dental drill. The powder samples were introduced to carbonate preparation device (Kiel Device IV), and the produced CO₂ was analyzed by a stable isotope ratio mass spectrometer (Thermo Scientific MAT253). Stable oxygen isotope ratios ($\delta^{18}\text{O}$) were compared with sea surface temperature (SST) and maxima values were assigned the lowest SST in February. The correspondence between reconstructed SST and daily observed SST indicated that the microgrowth lines were formed daily basis. Growth disturbances were observed as v-shaped breaks in the shell outer layers. The high $\delta^{18}\text{O}$ peaks corresponded with these growth disturbances suggested that this specimen recorded the strong typhoon in Okinotori Island on 3th September 2005. Trace element analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Agilent 7700) with 100 μm and 30 μm spots (Mg, Ca, Mn, Sr, Ba) revealed that the specimen has Ba/Ca positive peaks as an after-signature of strong typhoons. Moreover, the significant positive peaks observed in Ba/Ca profile suggested the upwelling events due to typhoons in Okinotori Island.

These results indicated that sclerochronological and geochemical record in giant clam shells could be useful for reconstruction of past typhoon events.

Keywords: Giant clam, Oxygen isotope, Carbon isotope, Trace elements, Growth line

Effects of recent ocean acidification in the western North Pacific on *Porites* coral calcification

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One third of carbon dioxide emitted to the atmosphere as a result of human activities has been incorporated by the surface ocean. Subsequently, the seawater is acidifying at an unprecedentedly faster rate (ocean acidification); seawater pH (pH_{SW}) has declined by ~ 0.1 since the Industrial Revolution (1750 AD). As a pH_{SW} decline reduce saturation state of calcium carbonate, it will probably lead to severe consequences for scleractinian corals, important reef builders in the tropical and subtropical oceans. Up until now, many studies have evaluated effects of ocean acidification on scleractinian corals through culturing experiments, but few studies evaluated it in the natural environment. To better understanding how corals and coral reef ecosystems will adapt to or be damaged by the resulting changes in environments, field observations are crucial. Using thermal ionization mass spectrometry, we measured a 100 year record of boron isotopes ($\delta^{11}\text{B}$) of massive *Porites* coral obtained from Chichijima, Ogasawara Archipelago, western North Pacific. The result revealed a rapid decline of $\delta^{11}\text{B}$ since 1960 (-0.17 ± 0.07 ‰/decade), suggesting a decrease of pH of extracellular calcification fluid (pH_{CF}) due to ocean acidification. The result also showed that pH_{CF} has been decreasing rapidly (changing sensitively to pH_{SW}) than estimation from culturing experiments of *Porites* corals. Thus it suggests the calcification fluid of *Porites* coral become corrosive to aragonite in the future ($\text{pH}_{\text{CF}} = \text{ca. } 8.3$ when $\text{pH}_{\text{SW}} = \text{ca. } 8.0$ in 2050) at an earlier point than previously expected, despite the pH_{CF} up-regulation mechanism of corals. Therefore, it is likely that ocean acidification has already had negative influences on corals in addition to various environmental stressors represented by regional warming.

Keywords: boron, coral, *Porites*, ocean acidification, calcification

Proton Management of Foraminiferal Calcification

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Marine calcification plays an important role in the global carbon cycle. Currently, approximately half of all carbon buried in the seafloor is the result of biogenic calcium carbonate production. Perforate foraminifera are a prime example of marine carbonate producers and responsible for a large portion of today's production. The physiological processes involved in calcification, however, are still unclear. Here we present some results on the intra- and extracellular pH changes in benthic perforate foraminifera during calcification. These observations allow for calculating the budgets of ion fluxes that are taken up and removed from the calcification space, which are placed in the context of previously obtained results and published calcification models to construct a unifying model for perforate foraminiferal calcification. This model also accounts for general patterns in observed fractionation factors of various elements.

Keywords: foraminifera

Inorganic precipitation mechanism of calcium carbonate polymorphs and their precursors

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Calcium carbonate, CaCO₃, occurs in six different forms: three crystalline polymorphs (calcite, aragonite, and vaterite), two hydrate phases, and amorphous calcium carbonate (ACC). These polymorphs are important both in life and material sciences, especially the occurrence of CaCO₃ in living organisms has received considerable attention. As a basis for understanding biomineralization, inorganic precipitation mechanism of these polymorphs has been extensively investigated for over a hundred years. Recently, crystallization pathway through non-classical mechanism such as stable prenucleation cluster aggregation has been proposed, which give a new picture of the early stages of calcium carbonate growth. However our knowledge of formation process of CaCO₃, especially that of the mechanism of polymorph selection, is far from complete.

We have investigated experimentally and theoretically the metastable formation of CaCO₃ polymorphs and their precursors. In particular, the effect of Mg²⁺ on the nucleation and growth of CaCO₃ polymorphs has been focused and the quantum chemical calculations of Mg-containing CaCO₃ surfaces and clusters appearing in the early stages of CaCO₃ formation have been performed. As a result, Mg²⁺ substituted for Ca²⁺ affects the structure of surfaces and clusters, and may have significant effect on the polymorph selection of CaCO₃. In this presentation, we will report our results in detail based on the recent progress in this field.

Keywords: calcium carbonate, metastable phase, precursor

Mesoscopic textures of biogenic and biomimetic calcite crystals

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The mesoscale granular textures having a single crystalline feature are generally observed on the continuous body of various biogenic and biomimetic calcite crystals. The distribution of the organic phase and lattice strain in the textured crystals vary with organism species or the growth conditions. The prismatic layer of a fan mussel, *A. pectinata*, exhibits a relatively homogeneous, low-strain texture consisting of the nanoscale grains with discrete organic inclusions; the prism structure of a pearl oyster, *P. fucata*, and an avian eggshell have a high-strain granular texture with localized organic phases. A variety of the mesoscopic textures similar to the biogenic calcite crystals are artificially produced in a supersaturated solution containing specific organic molecules. The high-strain textures were produced through mesoscopic dendritic growth of calcite by physical impedance of a rigid gel matrix and subsequent thickening of the branches. Continuous growth of the crystal involving nanoscale segregation of soluble polymers would result in the formation of the low-strain body having mesoscopic textures. The chemical durability of the low-strain biogenic and biomimetic textured calcites are enhanced by the combination of the inorganic crystal and the organic molecules.

Keywords: Biomineral, Calcium carbonate, Mesocrystal

Intraspecific variation in isotopic composition and trace element concentrations of Pleistocene brachiopods

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Carbon and oxygen isotope composition ($\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VPDB}$) in fossil rhynchonelliform brachiopod shells has been used as a powerful tool to reconstruct paleoenvironmental conditions. Several petrographic and chemical techniques, such as transmitted light microscopy, cathodoluminescence, scanning electron microscope (SEM), and trace element analysis were applied in those studies to select brachiopod shells that have not been diagenetically altered and retain their original isotopic and chemical composition. However, there are few references that showed how the isotopic and chemical composition is modified by several processes operating during meteoric diagenesis. Therefore, we conducted a comparative study of isotopic composition and trace element (Na, Mg, Sr, Mn and Fe) concentrations in modern and fossil brachiopod (*Kikaithyris hanzawai*) shells. The modern and fossil specimens were collected off Amami-o-shima and the Upper Pleistocene Wan Formation in Kikai-jima, respectively. The isotopic profiles of inner shell surface along the maximum growth axis can be divided into three stages that were likely to be related to changes in life mode and shell morphology of this species. The trace element concentrations are irregularly varied on the sampling transects. There are some spots on the sampling transects, where Mn and Fe concentrations are anomalously high. These spots are likely generated by metabolic factor(s) because they are not associated with decreases in $\delta^{13}C_{VPDB}$ and $\delta^{18}O_{VPDB}$ values which are indicative of meteoric diagenesis. Our results suggest that brachiopods with complicated shell morphology which may be related to the change in life mode during the growth are not suitable for paleoenvironmental reconstructions based on their isotopic composition. It is also suggested anomalously high Mn and Fe concentrations cannot be used to identify diagenetically altered portions within brachiopod shells.

Keywords: carbon isotope, oxygen isotope, trace element concentration, brachiopoda, proxy, Pleistocene

Assessment of skeletal compositions in *A. digitifera* coral as temperature proxies

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Introduction

While biogenic carbonates such as foraminifera and coccolithophorid are attractive tools to reconstruct the past environments, scleractinian corals also provide environmental data around tropical to subtropical region with much higher time resolution. For example, oxygen isotope ratio ($\delta^{18}\text{O}$) and strontium-calcium (Sr/Ca) ratio have been used for reconstructing sea surface temperature and salinity by generally using *Porites* spp. In this study, we investigate the effect of temperature on skeletal $\delta^{18}\text{O}$ and Sr/Ca ratio in *A. digitifera* corals for evaluating temperature proxies for one of paleoceanographic applications.

Materials and Methods

Three colonies of *A. digitifera* were collected at Sesoko Island, Okinawa, Japan. We reared coral samples in seawater with 5 different temperature settings (18, 21, 24, 27, 30 °C) and set 2 tanks in each temperature treatment (3 coral nubbins \times 3 colonies in a tank). Calcification rate of coral nubbins was measured by buoyant weight technique every two weeks during the period of experiments. Oxygen and carbon isotope ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) were analyzed by a stable isotope ratio mass spectrometer, and the ratios of trace elements (Sr/Ca, Mg/Ca, U/Ca and Ba/Ca) were measured by an inductively coupled plasma mass spectrometer (ICP-MS).

Results and discussion

Skeletal $\delta^{18}\text{O}$ of corals is often used as a seawater temperature proxy. In this study, a strong negative correlation was found between $\delta^{18}\text{O}$ and water temperature, and the temperature dependency was comparable with that of *Porites* spp. Thus $\delta^{18}\text{O}$ of *A. digitifera* is suggested to be useful as a temperature proxy without clear influence from growth rate. A negative correlation was also observed between Sr/Ca ratio and temperature, which is compatible with that of *Porites* spp., although the correlation was weaker than $\delta^{18}\text{O}$. But variation of Sr/Ca ratio was not controlled by skeletal growth rate, suggesting that the dominant factor controlling the skeletal Sr/Ca ratio is water temperature. Thus, skeletal $\delta^{18}\text{O}$ and Sr/Ca ratio of *Acropora* spp., at least *A. digitifera*, can be useful as a proxies for seawater temperature as well as *Porites* spp., although more investigation would be required for Sr/Ca-thermometer of *Acropora* spp.

Keywords: coral skeleton, temperature, proxy

Reliable stable isotopic compositions of individual *Uvigerina* spp. as sea environmental proxy

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The stable carbon and oxygen isotopic composition ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of benthic foraminiferal carbonate shells have been used to reconstruct the past sea environment. Especially, *Uvigerina* spp. is often used to estimate the sea level changes and deep-sea circulations. However, it is difficult to analyze the isotopic compositions of individual foraminifera in the traditional analytical method, and thus the reliability of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of individual *Uvigerina* spp. as sea environmental proxy is still unclear. In this study, the isotopic compositions of individual *U. akitaensis* and *U. ochotica* were analyzed by using the micro-scale isotopic analytical system.

Surface sediment samples were collected from the four sites in the Sea of Okhotsk during cruise MR06-04 of R/V Mirai (JAMSTEC) in October 2006. Those samples were cut for each 1 cm (0-8cm below the seafloor) and used in this study. Then the living (Rose Bengal stained) individuals of *U. akitaensis* and *U. ochotica* from samples were analyzed by the micro-scale isotopic analytical system (Ishimura et al. 2008).

As a result, the isotopic dispersions of *Uvigerina* spp. indicated about ± 0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in all sampling sites. In addition, isotopic values of *Uvigerina* spp. showed almost constant values in same species regardless of sediment depth, individual weight, and variation of shell structure. The observed homogeneous isotopic compositions of *Uvigerina* spp. were comparable with isotopic homogeneity and dispersions of NBS-19 (international isotopic standard). Therefore, we concluded that the individual *Uvigerina* spp. have highly reliable isotopic composition as sea environmental proxy. However, $\delta^{13}\text{C}$ values represented about 0.7 ‰ differences between *U. akitaensis* and *U. ochotica*, thus we have to consider this $\delta^{13}\text{C}$ difference in *Uvigerina* spp. when we reconstruct the past sea environment based on $\delta^{13}\text{C}$ of *Uvigerina* spp.

Our main conclusion in this study is that the individual *Uvigerina* spp. have highly reliable stable isotopic compositions as sea environmental proxy.

Keywords: stable isotope, benthic foraminifera, microscale analysis, *Uvigerina*