

## 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.

Keywords: calcification, foraminifera

## Environmental variation during foraminiferal calcification

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Foraminifera have been considered as one of the major carbonate producer in ocean. Their calcareous tests are broadly utilized as paleo-environmental indicators in various studies of earth science because their tests have been archived as numerous fossil in sediment for long time and various environmental information are brought by population, morphology and geochemical fingerprints.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 knowledge about the cytological process on carbonate precipitation has been described for couples of decade using by OM, SEM and TEM. Foraminiferal calcium and carbonate ion managements are of great interest for broad field of biogeosciences. Our studies showed the potential to understanding the biomineralization of foraminifera by the application of fluorescent indicators. Recently, we visualize the spatial distributions of cytological calcium and pH in living cell at same time. Observed results show that foraminifera controls very detailed timing of pH variation and concentration of calcium at any stage of chamber formation dynamically. These observations results will help to consider how the geochemical proxy on the foraminiferal test working as paleo environmental indicators.

## Distribution of intracellular pH and algal endosymbionts during chamber formation in symbiont-bearing reef foraminifers

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Calcification mechanisms of algal symbiont-bearing reef foraminifers have not yet been well understood. In particular, it is cytologically not clear how the photosynthesis by algal endosymbionts enhance the calcification of host foraminifers. We visualized the distribution of intercellular pH and algal endosymbionts using a fluorescence probe HPTS as well as chlorophyll autofluorescence. High pH vesicles were gradually stored around symbionts before chamber formation. Our observations suggest that the photosynthesis by symbionts enhances the production of calcite needles or carbonate pools in vesicles by removing CO<sub>2</sub> around them.

## Autoclave aquaria allow for high-pressure culture experiments on deep-sea benthic foraminifera

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Our understanding of palaeodeep-water circulation modes and deep-water renewal in the world's oceans is essentially based on isotopical and geochemical ratios recorded in tests of calcareous deep-water benthic foraminifera that precipitate their shells in a constant ratio to the surrounding water mass. However, as more field data are assembled it appears that the documentation of deep-water in benthic foraminiferal shells is not always straightforward. Therefore, culture experiments on deep-sea benthic foraminifera are needed to verify the established palaeodeep-water proxies. However, to our knowledge, barophilic species like *F. wuellerstorfi*, the most trusted species for reconstructions of palaeodeep-water circulation and ventilation, neither formed new chambers nor reproduced in mesocosms kept at 1 bar. To accommodate this problem we have developed facilities and procedures that allow long-lasting high-pressure culture experiments on undepressurized deep-sea sediments and associated fauna and flora. In this presentation I will describe the experimental set-up and present results from methane-seepage simulations.

Keywords: pressure, culture, foraminifera

## Laboratory culture experiments: effect of dissolved oxygen concentration on planktonic foraminifera

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Planktonic foraminifera shows significant species turnover ratio related Cretaceous oceanic anoxic event (OAEs), however, the direct effect of dissolved oxygen (DO) concentration on planktonic foraminifera remain obscure. Although culture experiments can investigate quantitatively the relationship between foraminiferal ecology and environmental parameters, DO-controlling experiment has never been conducted because of the difficulty of observation and/or control of dissolved oxygen under the modern ocean condition. In the present study, we cultured two planktonic foraminifera species (*Orbulina universa* and *Globigerina bulloides*) under six different DO conditions. Both species have extremely high tolerance to low DO than we expected before, and it suggests that at least "dysoxic" condition (more than  $0.7 \text{ mg O}_2 \text{ l}^{-1}$ ) could not be a direct cause of the extinction of planktonic foraminifera at OAEs. Their high tolerance to extremely low DO would be caused by the evolutionary descendant of benthic foraminifera. Final shell weight increased with DO despite almost the same culture duration among treatments, thus it suggests foraminiferal fossil shell weight could reflect the DO conditions.

Keywords: planktonic foraminifer, culture experiment, dissolved oxygen concentration

## Subarctic and Arctic *Emiliana huxleyi* coccolith morphological responses to the growth temperatures

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Coccolithophore is a key calcified phytoplankton for biogeoscience because of their specific products such as calcified scales, coccolith, and unsaturated ketones, alkenone. As a biomarker, the chemical compositions of alkenones are often used to estimate paleo ocean temperatures and to identify the changes of the marine environments (e.g., Harada et al., 2012). In particular, the polar region is an important area to recognize the changes of the global environments and the sea-ice melting is known to be accelerated by the global warming. MIRAI subarctic expeditions have been continued to investigate the subarctic to Arctic environment and concurrently the culture experiments of marine organisms including coccolithophores have been performed to predict the future changes of marine ecosystem. In this study, two *Emiliana huxleyi* strains, MR57N and MR70N, isolated by MIRAI subarctic expedition in 2010 were cultured at the different temperatures (5, 10, 15 and 20°C) and salinities (25, 32, and 35 per mill) and investigated from the viewpoint of the coccolith morphology. As the results, these strains showed the similar growth properties that the growth rate increased with raising the temperature. According to SEM observations and the image analyses, the size (length of distal shield: LDS) and the numbers of distal shield elements of the coccolith decreased with raising the temperature. The central area of the coccoliths was also changed from grill structures to completely calcified structures. Moreover, the cell size of *E. huxleyi* decreased with raising the temperature. Thus, the subarctic and Arctic *E. huxleyi* strains showed the correlations between cell sizes and coccolith morphometric parameters with variable central area morphology depending on the growth temperatures. For the salinity experiments, there is almost no variation on the growth properties and a little variation on the morphometrical parameters. These results imply that the subarctic and arctic *E. huxleyi* strains can maintain enough to grow in the temperature range between 5 to 20°C and the salinity range between 25 to 35 per mill, suggesting that these strains can ideally survive under the warmer and/or the less salinity environments. The coccolith morphologies and the parameters vary in response to the growth temperature but not to the salinity. This relationship was applied to the natural plankton samples reported by Bollmann et al. (2009) and the part of their data was correlated with sea water temperatures but the data shallower than 15 m depth was not. This may imply the importance of the light intensity and the further culture experiments with the different light intensity are required.

Harada et al. (2012) *Global Biogeochemical Cycles* 26, GB2036.

Bollmann et al. (2009) *EPSL* 284, 320.

## Morphological and ecological characters of two cryptic genetic types in the radiolarian *Spongotrochus glacialis*.

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The discovery of cryptic species often helps in grasping the true diversity and detailed ecological preferences of pelagic planktons. Radiolarians are a good environmental proxy for paleontology because of their worldwide oceanic distribution and the good preservation of their shells. However, cryptic species have not yet been recognized in this planktonic protist group. In the present study, we focused on the morphospecies *Spongotrochus glacialis* with specimens collected from different layers in the Equatorial-Subtropical Pacific. A molecular phylogeny based on the internal transcribed spacer of rDNA (ITS-rDNA) sequences recovered two clades of cryptic species (types I and II) from this single morphospecies. These two distinct types were separately distributed, either in the oligotrophic surface water (type I) or below the chlorophyll maximum layer (type II). Moreover, the types showed morphological differences in the shells. Our morphometric analyses established lengths of spines as a morphological criterion to distinguish between the two types: type I with longer spines and type II with shorter spines. The length of spines is apparently associated with the habitat of each type. Type I with longer spines could be suitable for extending flagella and floating on the surface, whereas type II with shorter spines are appropriate to protrude only short flagella and dwell in deep water. Such morphological and ecological features at the cryptic species level of Radiolaria could provide new proxies for paleoceanographic studies.

Keywords: Radiolaria, cryptic species, ITS rDNA, morphometric

## The Phylogenetic Affiliations of Radiolarian-like Environmental 18S rDNA Genes from the Northern South China Sea

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To determine the molecular taxonomic affiliations of radiolarian-like environmental 18S rDNA genes from the northern South China Sea, we constructed phylogenetic trees by the 18S rDNA sequences of identified radiolarian species, correlating environmental sequences from other sea areas with those full length 18S rDNA gene of representative clones from the northern South China Sea. In our previous work, a great number of our radiolarian-like sequences from the South China Sea were placed within the RAD-III, named by Not et al. (2007) of environmental sequences from the Sargasso Sea size-fractionated samples (< 2 micrometre). Now we found 18S rDNA sequences of two identified species *Arachnosphaera myriacantha* and *Astrosphaera hexagonalis*, collected from Okinawa Island by Yuasa et al. (2009) also belongs to RAD-III. There is a unique clade composed solely of environmental clone sequences formed basal to the Taxopodida. It does not belong to RAD IV or V, still remains unknown. Our Nassellaria-like environmental clone from the northern South China Sea was joined with the group of described Plagoniid species. And the clones from Cariaco Basin, Caribbean clustered with Pterocorythid and Theoperids groups. Comparison of our sequences with two recent survey of Acantharia 18S rDNA, the position of northern South China Sea clones was still difficult to distinguish. It belonged to the clade composed of subgroups Arthracanthida, Sphaenacanthida and RAD I (Unidentified Clade 1). These ambiguities may be because the genetic diversity of radiolarian in warm waters sea areas is still poorly known.



## Fossil cyanobacteria as a potential pH proxy for Phanerozoic ocean

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Fossil cyanobacteria appeared in the most part of Phanerozoic era, and its abundance changed significantly through time. Their fossilization related to calcification in the living state, which requires optimum chemistry in the ambient water. Therefore, the fossil record of cyanobacteria potentially reflects past ocean chemistry. Recent study revealed that cyanobacterial calcification depends mainly on pH, dissolved inorganic carbon,  $\text{Ca}^{2+}$  concentration (Shiraishi 2012, GCA). Using well-established proxies of  $\text{Ca}^{2+}$  and partial pressure of  $\text{CO}_2$ , oceanic pH was estimated from the fossil record of calcified cyanobacteria. Estimated range and trend of pH are similar to those of previous studies, but exhibited more frequent changes. In a future study, it is necessary to understand the relationship between  $\text{CaCO}_3$  saturation state achieved by photosynthesis and calcification amount, in order to increase the reliability of estimation.

## Benthic foraminifera as Quaternary palaeoclimate proxies in the New Caledonia Basin

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In 2001, gravity core GC4 was extracted from the New Caledonia Basin and the uppermost 141 cm, representing approximately the last 140 000 years, was investigated for chemical isotopic, carbonate, non-carbonate and trace element signatures. In this study, sub samples of the same uppermost 141 cm of GC4 were picked and sorted for all foraminiferal taxa. A total of 161 species of benthic foraminifera were obtained from the core and 46 species occurred in sufficient numbers to investigate changing patterns and trends in biodiversity and relative abundance. All changes can be related to broad scale oceanic and palaeoclimatic fluctuations during the last 140 000 years. Within GC4, two distinct foraminiferal assemblages were detected using Bray-Curtis cluster analysis and Multidimensional Scaling (MDS) analysis. Factors that influence the foraminiferal assemblages include environmental setting, SSP, oxygen levels at the sediment-water interface and transportation by oceanic current systems operating within the region.

Keywords: foraminifera

## Daily light cycle reconstructed by Sr/Ca in a fossil giant clam, *Tridacna gigas*, living in 4.6 ka, southern Japan

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Giant clams are long-lived bivalvia living in a shallow tropical ocean. Their aragonite shells are potential paleo-environmental archives, i.e., oxygen stable isotopic values record paleo-seawater temperatures. Generally, trace elements in calcium carbonates are also considered as a paleo-thermometer. However, the case of Tridacninae may not follow the traditional temperature dependent fractionation, but potentially reflects insolation. This is explained by "trans-calcification", which is one of photosynthetically induced calcification mechanisms. Enzymatic reaction pumping  $\text{Ca}^{2+}$  to host liquid of calcification results in relatively depleted Sr/Ca ratio in day time when photosynthesis activates. Following this hypothesis, we have reconstructed the past daily light cycle.

A fossil *T. gigas*, Stg04-b was collected at Ishigaki-jima Island in Okinawa Prefecture, Japan (N24 20'0.4" E124 09'22"). Two horizons of the specimen were dated by carbon-14 method at the accelerator mass spectrometry (AMS) center of Yamagata University. The micro analyses were performed for the outer layer of 1.4 cm thickness using an electron probe micro-analyzer (EPMA), JXA8900, and a micrometer-scale secondary ion mass spectrometer (NanoSIMS), CAMECA NS50 at the Atmosphere and Ocean Research Institute (AORI), the University of Tokyo. As a result, we found clear Sr lamination parallel to the daily increment of about 20  $\mu\text{m}$  thickness. The hourly insolation (P) was calculated from the difference of Sr/Ca ratio ( $\Delta\text{Sr/Ca}$ ) following the equation:  $\Delta\text{Sr/Ca} = -a \times P$ , where the parameter,  $a$ , is defined by analysis of modern *T. maxima* [1]. Using the technique, we distinguished the sunny and cloudy seasons in 4.6 ka.

### Reference

[1] Sano, Y., Kobayashi, S., Shirai, K., Takahata, N., Matsumoto, K., Watanabe, T., Sowa, K., Iwai, K. 2012, Past daily light cycle recorded in the strontium/calcium ratios of giant clam shells. Nature Commun. 3, DOI: 10.1038/ncomms1763

Keywords: giant clam, Sr/Ca, NanoSIMS

## The cesspool preservation hypothesis as a key to preservation of exceptionally well preserved orsten-type fossils

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Recently, an ostracod (Crustacea, Arthropoda) specimen with exceptionally well preserved soft parts was discovered from fecal pellets rich layer at a borehole core recovered from Oppama Park, Kanagawa Prefecture, central Japan (Tanaka et al., 2012). Similar soft part-preserved arthropods have been found in the Late Cambrian Orsten limestone (Maeda et al., 2011). Such "cesspools" were exceptionally phosphatized during early diagenesis owing to the high local phosphorus levels produced by the accumulated fecal pellets. The "cesspool preservation hypothesis" provides an explanation for this kind of exceptional fossilization, found in the marine sediment record from the Late Cambrian onward.

Keywords: Fossil, Cambrian, Cesspool, Arthropoda, Paleontology, Holocene