Paleobiology of Foraminifera: Towards Better Understanding of Ecosystems and Biotic Evolution Through Foraminifera

Chaired by Hiroshi Kitazato and Joan M. Bernhard

Foraminifera are known as some of the most suitable organisms for reconstructing paleo-environments and evolutionary histories of marine organisms. However, we are not sufficiently informed about presently living foraminifera, their evolution and their response to environmental changes. What kind of information do foraminifera record within their test or cell? How do foraminifera survive and evolve during times of rapid, severe environmental change on Earth? What is the role of foraminifera in marine ecosystems? Biological approaches such as culture experiments, studies of foraminiferal anatomy and/or genetic analysis are direct ways of answering these questions. In this session, we aim to present information on biological features of foraminifera with the purpose of finding new keys to decode historical records within sediments or in the tests/cells. The following topics or approaches are covered this session:

1. Culture experiments under controlled environmental factors such as temperature, salinity, dissolved oxygen content, nutrient content, trace element concentration, or isotope values. In situ experimental studies in the field are also welcome.

2. Genetic analysis in order to understand phylogenetic relationships among foraminiferal genera or species.

3. TEM/SEM anatomy of the foraminiferal cell/test.

4. Biology and ecology of foraminifera in extreme environments, such as hydrothermal vents, cold seeps, or at abyssal to hadal depths.
Foraminiferal growth models: 
Towards a fully integrated record of calcification

Heather Anne Austin & William E.N. Austin

1Sediment Ecology Research Group, Gatty Marine Laboratory, 
University of St. Andrews, Fife, KY16 8LB, Scotland, U.K. 
ha8@st-andrews.ac.uk

2School of Geography & Geosciences, University of St. Andrews, Fife, KY16 9AL, Scotland, U.K.

Multilocular benthic foraminifera express growth by the intermittent addition of chambers and many individuals typically live for several months or longer. Foraminiferal tests therefore hold the potential to record information on changing seawater chemistry and temperature, particularly in the mid- to high-latitude shelf seas, where seasonality is most pronounced. Results from preliminary laboratory culturing experiments are presented, where field-collected individuals of *Elphidium williamsoni* were observed to have asexually reproduced and released juveniles. The growth and growth rates of these juveniles were determined over a period of 179 days in culture; growth rates, expressed as a change in maximum test diameter, were initially high (14 μm.d⁻¹) but tended to decrease asymptotically with time. Observations of growth and chamber addition in this planispirally-coiled species are used to develop a simple, generalised growth model where the amount and rate of calcification are determined over a six-month period. We compare these data with observations of growth in field-sampled populations of *E. williamsoni* and conclude that growth rates and growth models derived from culturing experiments are very similar to field data. Knowledge of the timing and rates of growth in benthic foraminifera may significantly improve our understanding of palaeoceanographic data based on whole-test measurements and, as microanalytical methods become more widely available, will help to constrain so-called ‘seasonal effects’ and other intra-test variability observed in shell chemistry.
Extracellular cracking and content removal of benthic diatoms by intertidal foraminifera

Heather Anne Austin¹; William E. N. Austin² & David M. Paterson¹

¹Sediment Ecology Research Group, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife, KY16 8LB, U.K. ha8@st-andrews.ac.uk

²School of Geography & Geosciences, Irvine Building, University of St. Andrews, St Andrews, Fife, KY16 9AL, U.K.

Field-collected living specimens of the benthic foraminifera Haynesina germanica were maintained in the laboratory and fed a naturally occurring motile benthic diatom assemblage dominated by Pleurosigma angulatum. The extracellular removal of diatom contents was inferred for P. angulatum in controlled experiments. A characteristic pattern of fracturing of the diatom frustule was observed that was directly attributed to foraminiferal feeding/sequestration mechanisms. These feeding/sequestration mechanisms have a potentially important bearing on our understanding of foraminiferal aperture morphology, foraminiferal evolution and the preservation of diatoms in marine sediments. Recognition of this characteristic breakage pattern of diatom frustules may provide insight into the natural importance of foraminifera in grazing diatom biofilms.
Benthic foraminifera as proxies of temperature impact and dystrophic crisis affecting survival of Pacific oysters

*Crassostrea gigas* (Thunberg) in Marennes-Oléron bay

Vincent Bouchet1,2; Jean-Pierre Debenay2; Pierre-Guy Sauriau1; Joël Radford Knoery & Patrick Soletchnik3

1CRELA (UMR 6217 CNRS-IFREMER-ULR), BP 5, 17137, L’Houmeau, France
vincent.bouchet@ifremer.fr
2UPRES EA 2644, 2, Boulevard Lavoisier, 49045, Angers cedex, France
3IFREMER (LERPC), BP 133, 17390, La Tremblade, France

Among hypothesis explaining Pacific oyster *Crassostrea gigas* summer mortality, sediment feedback to *C. gigas* was investigated. It is well known that huge amounts of biodeposits accumulated during autumn and winter under oyster culture areas lead to sedimentary organic enrichment, and in turn alteration of sediment physical structure and geochemical functioning. Thus, due to temperature increase during spring and early summer, organic matter accumulated on intertidal mudflats is remineralized and this may induce toxic fluxes (ammonia and sulphur) from sediments to the water column. Foraminiferal assemblages have been reported to react to physical disturbances or chemical pollution. Thus, the aim of the study was to determine how living (stained) benthic foraminifera react to organic matter remineralization. A field experiment was conducted in the oyster farming bay of Marennes-Oléron (Charente-Maritime, France) in order to monitor both sediment (Chl *a*, total organic carbon and organic nitrogen contents, δ13C and δ15N composition, sulphur and ammonia contents, pH, Eh, temperature) and water column (salinity, turbidity and percentage of saturation of dissolved oxygen) from April to August 2004. Foraminifera core samples were taken from one site in the top 8 cm sediment and sectioned in 5 slices: 0-0.5, 0.5-1, 1-3, 3-5 and 5-8 cm. Values of POC and PON, Chl *a* and ammonia contents increased between May 25 and June 10, whereas bottom-water oxygen saturation became severely depressed (O2 saturation < 20%). Average temperatures increased from 12°C to 21°C, high daily thermal amplitude (maximum=12.3°C) was noted between May 15 and May 25 and during emersion time, sediment temperature reached a maximum value of 28°C on May 20. At the same time, turbidity was high (800 to 1400 NTU). During the short-term dystrophic crisis, at the end of this period, living (stained) foraminiferal abundances severally decreased from 10000 to 4000
individuals in 50 cm³ of sediment. Increasing temperature associated with high daily temperature amplitude and short-term hypoxia affected benthic foraminiferal species survival. *Ammonia tepida* was still the most abundant species whereas *Haynesina germanica*, *Brizalina variabilis* and *Quinqueloculina seminula* declined. Instead of benefiting from high Chl $a$ content and increasing temperature, foraminiferal assemblages were clearly disturbed when the average temperature reached $20^\circ$C, which is a temperature theoretically favourable to foraminifera growth and reproduction. This suggests that temperature, even if theoretically favourable to the benthos, including foraminifera, may have an indirect negative impact, mainly by leading to dystrophic conditions. One month after the dystrophic crisis, benthic foraminiferal species recolonized intertidal sediments, and *Cribroelphidium gunteri* appears as the most opportunistic species during recolonization.
Long-term observations of foraminifera in situ

Sam S. Bowser¹; J. Blair²; J. Mastroianni² & A. Hansen²

¹Wadsworth Center, NY State Dept. Health, Albany, NY 12201 U.S.A. bowser@wadsworth.org
²Magee Scientific Company, Berkeley, CA 94703 U.S.A.

ROMEO — the world’s southernmost cabled underwater observatory, located in Explorers Cove, Antarctica, was used to image Cibicides refulgens living epizoically on the scallop Adamussium colbecki during the 2002/3 and 2004/5 field seasons. This work represented an initial test of the system, and was designed to gather long-term data on Cibicides life habits (mobility, reproductive events, etc.) and physical/biological parameters encountered in this natural setting. Time-lapse movies confirmed the sedentary nature of this species, as well as its resistance to disturbance by megafauna. Future work is aimed at investigating the trophic behavior of the giant agglutinated foraminiferans unique to Explorers Cove.

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Laboratory experiments are performed in the Laboratory of Recent and Fossils Bio-Indicators at the University of Angers in order to qualify the ecological and geochemical responses of benthic foraminifera to the physico-chemical forcing of the ocean. By unravelling vital and environmental effects a better understanding of the response to anthropic and climatic forcing will be established.

Since about 20 years, the isotopic composition, and trace element ratios of foraminiferal shells have served to retrace past climatic changes. But unfortunately, the relations between the composition of the foraminiferal shell and the specific physico-chemical parameters of the environment inhabited by the organism when it calcifies are still poorly understood. Studies in natural environments, comparing the ecology of recent benthic foraminifera with the stable isotopic composition and trace element ratios of their shells, have been performed, but the results are often ambiguous. This is mainly due to the fact that race element partitioning and isotope fractionation during calcification are influenced by numerous interfering factors. Studying foraminifera under controlled laboratory conditions potentially allows us to separate the effects of single environmental parameters on the isotopic composition and trace element ratios of the foraminiferal shell.

Furthermore laboratory studies can also help to understand the ecological responses of benthic foraminifera to specific events such as hypoxic to anoxic events which can result of anthropic impact and/or climatic changes.

At present, in our laboratory, experiments under controlled conditions are focussed to study specifically:

1) The temperature effect on the oxygen isotopic fractionation ($\delta^{18}O$). Growth experiments with *Bulimina marginata/aculeata* are carried out at various temperatures (8, 10, 12, 14° C). Living adult specimens
marked with calcein are isolated and incubated at different temperatures. Food (dried green algae) is added in order to stimulate reproduction. After 4 months, the $\delta^{18}$O and Mg/Ca ratio of specimens which were grown under controlled conditions are analysed.

2) The salinity effect on the trace element partitioning in the foraminiferal shells. Experiments using *Ammonia tepida* intend to obtain formation of new chambers at various salinities (range between 10 to 45 ‰). Chambers added under controlled conditions are analysed using LA-ICP-MS at the Utrecht University (The Netherlands).

3) The influence of the oxygen-depleted conditions. Hypoxic to anoxic conditions will be simulated to see the effect on the composition of the foraminiferal assemblages. This will determine specific species or assemblages which could act as bio-indicators of oxygen-stress related to environmental change. Fluorescent-marker methods are applied for accurate determination of the living versus dead number of specimens. This is needed since the traditionally used Rose Bengal stain is not suitable for repetitive sampling in a low oxygen context, where protoplasm may be preserved for a prolonged period of time.
Hidden diversity of allogromiid foraminiferans 
in low-latitude environments

Andrea Habura1,3; Susan T. Goldstein2; Sarah Broderick1 & Samuel S. Bowser1,3

1Division of Molecular Medicine, Wadsworth Center, New York State Department of Health, Albany, NY 12201, U.S.A. habura@wadsworth.org; bowser@wadsworth.org
2Department of Geology, University of Georgia, Athens, GA 30602, U.S.A.
3Department of Biomedical Sciences, University at Albany, 1400 Washington Avenue, Albany, New York 12222, U.S.A.

Foraminiferal species distribution and richness is usually judged by examining tests (Sen Gupta, 1999). It has long been known that some groups of foraminiferans, particularly the allogromiid taxa, are not easily identified in this manner (Gooday, Bowser & Bernhard 1996; Pawlowski et al. 2002). Previously, an environmental DNA study was used to explore the comprehensiveness of morphological sampling methods. This analysis showed that in a deep-sea-like high-latitude environment (Explorers Cove, Antarctica), at least 75% of the foraminiferal species present were not identified by traditional techniques, and most of these “hidden” species were allogromiids (Habura et al. 2004).

In order to investigate whether this domination of the foraminiferal assemblage by allogromiids is a feature of high-latitude environments alone, we used a series of targeted DNA-based surveys and allogromiid-focused morphological searches at several locations along the US eastern seaboard. Both methods of survey revealed the presence of large numbers of new allogromiid taxa. Morphological screens and specific PCR primers targeted against particular foraminiferal clades also documented substantial differences in the allogromiid assemblage between the locations. The fact that these low-latitude sites exhibit considerable cryptic allogromiid diversity has significant implications for nutrient cycling in the benthos and reconstruction of paleoenvironments.

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Living benthic foraminifera in methane- and sulfide-enriched sediments at cold seeps and hydrothermal vents

Petra Heinz1; Stefan Sommer2; Olaf Pfannkuche3; Christoph Hemleben1 & Hiroshi Kitazato3

1Institute of Geosciences, Tübingen University, Sigwartstr. 10, 72076 Tübingen, Germany
petra.heinz@uni-tuebingen.de

2GEOMAR Research Center for Marine Geosciences, Wischhofstr. 1-3, 24148 Kiel, Germany

3Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15, Natsushima-cho, Yokosuka, 237-0061, Japan

Methane- and sulfide-enriched sediments at cold seeps and hydrothermal vents show a high variability of environmental conditions in a very narrow area. This leads to the formation of ecological niches and a biological zoning of the fauna. While the distribution of seep communities like chemoautotrophic bacteria or macrofaunal groups is well investigated, little information is available concerning the biology and population of benthic foraminifera in these habitats. Living (Rose Bengal stained) benthic foraminifera in different methane- and sulfide-enriched sediments were investigated in this study. Foraminiferal assemblages, densities, species composition, diversity, and distributional patterns of several vent sites in the Northeast and Northwest Pacific (Hydrate Ridge off Oregon; cold seeps off Hatsushima, Sagami Bay and off Kuroshima Knoll; hydrothermal vent at Hatoma Knoll) were compared to search for possible characteristic foraminiferal zones, endemic species, or other common community structures. Living foraminifera, dominated by species which can tolerate or prefer low oxygen conditions, were found at all sites, but they showed high variable densities and taxa composition, indicating strong local geochemical, environmental and faunal influence on each sampling site, despite of similar methane- and sulfide-enriched concentrations in the sediments.
Diversity of rDNA in *Chilostomella*: Molecular differentiation patterns and putative hermit types

Vera Hemleben¹; Guido W. Grimm²; Hiroshi Kitazato³ & Christoph Hemleben⁴

¹Department of General Genetics, Centre of Plant Molecular Biology (ZMBP), University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany vera.hemleben@uni-tuebingen.de
²Institute of Geosciences, University of Tübingen, Sigwartstrasse 10, 72076 Tübingen, Germany
³Institute for Research and Earth Evolution, Japan Agency for Marine-Earth Science and Technology, Natsushima-cho 2-15, Yokosuta 237-0061, Japan
⁴Institute of Geosciences, Kernlager, University of Tübingen, Sand 6/7, 72076 Tübingen, Germany

We screened nuclear DNA sequences coding for the 3’ region of the small subunit ribosomal RNA (SSU rDNA), which were obtained from *Chilostomella* individuals (Foraminifera) for apparent sequence similarities and discrepancies. Sequence characteristics were verified with an enlarged SSU data set and correlated with sequences from the internal transcribed spacer (ITS) regions. Cloning allowed us to evaluate the intraspecific and intraindividual variability. For *Chilostomella spec.* from various biogeographic areas (North Atlantic, Mediterranean Sea, North Pacific) several highly divergent SSU genotypes were obtained, which could have been misinterpreted as genetically distinct cryptic species or SSU paralogs and produced artificially high intrageneric sequences divergence. Actually, one specific SSU rDNA sequence type (called “domestic” type) was found in most *Chilostomella* individuals that exhibits the general genotypic characteristics of calcareous test-building foraminifers; this SSU rDNA type showed a convincing biogeographic differentiation pattern, also supported by the ITS data. The other SSU types could be pro parte assigned to agglutinated or monothalamous taxa suggesting contaminants or “hermit” forms associated with the *Chilostomella* shells. Our findings support the capability of cloning to reveal misleading environmental sequence data and provide, in combination with ITS data, decent insights in population differentiation of benthic foraminifers. In contrast, the benthic foraminifera *Virgulinella* showed no sequence diversity among the populations from Japan, New Zealand, and Namibia.
Long-term benthic foraminiferal culture: Strategies for carbonate-system control and experimentation

Christopher J. Hintz¹; G. Thomas Chandler¹; Timothy J. Shaw²; Daniel C. McCorlke³; Joan M. Bernhard³ & Jessica K. Blanks¹

¹Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC, U.S.A.
²Chemistry and Biochemistry, University of South Carolina, Columbia, SC, U.S.A.
³Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, MA, U.S.A.

The effect of oceanic pCO₂ and carbonate system chemistry on benthic foraminiferal biomineralization and paleoproxy incorporation is not well understood. Carbonate ion concentration is a significant control of stable isotope incorporation in planktonic foraminifera. Moreover, low calcite saturation state, directly related to carbonate ion concentration, appears to alter trace metal proxy incorporation into foraminiferal calcite. Past laboratory benthic foraminiferal culture studies relied on ambient laboratory or compressed medical-grade air to maintain constant culture seawater pCO₂ and δ¹³CDIC with limited success. Improvements made to the system over 15 years (e.g., artificial instead of natural sediment substrate; high volume aeration with atmospheric air; and high volume/low velocity seawater flow) have greatly improved the long-term stability of carbonate system and seawater δ¹³C(DIC). However, even under stable conditions, we have observed several significant differences in cultured and core-top benthic foraminifera calcite proxy signatures from these two calcification environments (e.g., δ¹³C; D_Ba). Current culture efforts are focusing on alkalinity manipulation to experimentally control carbonate ion concentrations and induce biological responses in the recorded stable isotope and trace metal signatures. In as few as 4 months, foraminiferal reproductive events have been observed in supersaturated (Ü ~ 3) and saturated (Ü ~ 1) treatments with greater than 50% of culture populations thriving in the highly-constrained artificial culture system. Thus, the experimental technique to test carbonate concentration influence on benthic foraminifera stable isotope and trace metal proxies was initially successful. Still, the reliance on atmospheric air for this culture system, with seasonally varying pCO₂ and δ¹³C(CO₂) may cause significant temporal variation in cultured foraminifera paleoproxy...
incorporation confounding the results. An unclear record of the calcification environment during precipitation would limit the ability to fully understand the complex interaction of the carbonate system and preserved proxy signatures. Therefore, the next generation of long-term culture techniques for definitively evaluating the role of carbonate system chemistry, paleoprocess incorporation, and preservation may need additional control methods to adequately constrain the calcification environment.

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The “unusual” reproduction of planktic foraminifera:
An asexual reproductive phase of

*Neogloboquadrina pachyderma* (Ehrenberg)

Katsunori Kimoto & Masashi Tsuchiya

*Japan Agency for Marine-Earth Science and Technology, JAMSTEC, Japan. kimopy@jamstec.go.jp*

*Neogloboquadrina pachyderma*, one of the planktonic foraminifera that lives in temperate to subpolar water in the world ocean is widely used in high-latitude paleoenvironmental analyses. However, their life cycles and reproductive behavior are not understood sufficiently well. Here we present the first example of asexual reproduction in a planktonic foraminifer [*Neogloboquadrina pachyderma* (Ehrenberg) right-coiling form] in culture.

Twenty six specimens of living *N. pachyderma* for cultural experiments were collected from surface water in the Tsugaru Strait, between mainland Japan and Hokkaido (lat 41°28.8’N, long 141°14.2’E, water temperature: 4.7°C) using plankton tow on Mar. 2003. One week after from collecting, one *N. pachyderma* released a lot of bispherical bodies in the culture vessel. All released individuals consisted of two chambers and were uniform in shape. Moreover, we could see streaming cytoplasm in them and what appeared to be rhizopodia protruding from their spherical shells. We kept these offsprings in different temperatures and succeeded in growing six specimens at 4.7 °C and 8 °C. Particularly, in spite of the right-coiling morphotype of the parental shell, all these offsprings developed the left-coiling morphotype. Moreover, three matured offsprings produced gametes. The SSU rDNAs of these gamonts were classified as *N. pachyderma* (sin.) genotype. Thus, *N. pachyderma* may have alternating sexual and asexual reproductive phases and exhibit both coiling directions. Our findings suggest we must reconsider the phylogenetic and morphologic uses of both *N. pachyderma* coiling variations as paleoclimate proxies.
Roles of benthic foraminifera in carbon cycling at marginal oceans with active tectonic forcing: in situ experiment and observations

Hiroshi Kitazato1; Kazumasa Oguri1; Ronnie N-. Glud2 & Hidetaka Nomaki1

1Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15, Natsushima-cho, Yokosuka, 237-0061, Japan
kitazatoh@jamstec.go.jp

2Marine Biology Laboratory, Copenhagen University, Strandpromenaden, Helsingør, Denmark

Forearc region along arc-trench system is an active area of sedimentation. Sedimentary basins with thick piles of hemipelagic sediments are typically distributed in the area. Sagami Bay is one of typical sedimentary basins at forearc region. High rates of sedimentation at the basin are sustained by the lateral input of organic and inorganic particles from land or coastal areas. Hydrocarbon ore deposits are characteristically developed in the basins, as carbon burials actively take place at sediment-water interface (SWI). Benthic organisms, in particular to benthic foraminifera, should play roles to mineralize organic carbon at the sediment-water interface.

The aims to evaluate roles of benthic foraminifera in carbon budget at deep-sea floor, we have long been observing material cycles around sediment-water interface at a permanent station in Sagami Bay, Japan. Our basic strategy for research is to carried out both observations and experiments directly at deep-sea floor. For this purpose, we have developed both in situ feeding devices and planer optode systems. Mineralization potentials are measured through in situ feeding experiment with carbon-13 labeled food materials. Two dimensional images of both oxygen and pH are visualized through planer optode system. Respiration signals both by O₂ and CO₂ are excellent proxies for mineralization of organic carbon.

In this presentation, we try to figure out our deep-sea observation systems and to explain results obtained through the systems. Our observation shows that benthic foraminifera play important roles in carbon cycling at SWI. Benthic foraminifera consume and mineralize one third of deposited POM at SWI. Benthic foraminifers are also playing big roles in carbon burials at SWI by active plough of sediment particles.
The influence of labile and refractory C-org on benthic foraminifera: A laboratory mesocosm study

Karoliina A. Koho1; A.M. Langezaal1; Y.A. Van Lith1; T.J. Kouwenhoven1 & G.J. Van Der Zwaan1,2

1University of Utrecht, Utrecht, Netherlands - koho@geo.uu.nl
2Radboud University Nijmegen, Nijmegen, Netherlands

The vertical distribution of benthic foraminiferal communities is believed to be controlled by food supply (C$_{org}$ fluxes to the sea floor) and/or dissolved O$_2$ concentrations. However, the importance of quality or freshness of organic carbon has largely been neglected in previous studies. The C$_{org}$ flux to the deep sea sediment is already degraded, as remineralisation begins immediately at the sea surface and continues through the water column. The extent of the degradation depends on several factors including O$_2$ exposure time, water column length and sedimentation rate (Hedges & Keil, 1995, and references within. Marine Chemistry, 49: 81-115). Therefore, even in locations where C$_{org}$ concentrations are relatively high the nutritional value of organic matter may be low. This experiment is designed to explore whether benthic communities respond differently to varying qualities of organic matter.

A phytoplankton bloom was simulated using the diatom species *Thalassiosira pseudonana*. A ‘labile’ component consisted of fresh freeze dried algae, while a ‘refractory’ part was prepared by salinity shock treatment of diatoms with UHQ water. This process causes rupture of the diatom cells and releases labile carbon as CO$_2$. 39mg of either labile or refractory diatoms were added in the fed cores. In addition, blank (non-fed) cores were observed as a reference. The experiment was conducted under controlled laboratory conditions with stable oxygen concentrations.

In total the experiment ran for eight weeks after the feeding. Sampling was carried out at four time intervals: at experiment set up, before feeding, at four weeks and at eight weeks after feeding. Two cores were also collected from the field site for background information.

At each sampling time a replicate core was processed for foraminiferal studies. In addition, a core was sampled for geochemical analyses, including porewater and overlying water NO$_3^-$, NH$_4^+$ and DOC concentrations and fluxes,
The influence of labile and refractory C-org on benthic foraminifera: A laboratory mesocosm study

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and bulk sediment $C_{\text{total}}$, $C_{\text{org}}$, and $N_{\text{total}}$ concentrations. The porewater oxygen concentrations were monitored periodically.

Bacterial potential activity is investigated in the different treatments and at each sampling time to ensure that the activity was consistent between the cores. Aerobic bacteria are quantified by plate counts whereas most probable number (MPN) technique is used to determine the activity of sulphate reducers and denitrifiers.

The results to date indicate that the microhabitat of benthic foraminifera deepens after feeding, as the oxygen content is not limiting and more $C_{\text{org}}$ is available. This agrees with the predictions of the TROX model (Jorissen et al. 1995. Marine Micropaleontology, 26: 3-15). The initial assemblage is heavily dominated by Melonis barleeanum, however at four weeks after the feeding some species such as Bolivina alata and Glomospira charoides start to show higher relative abundances. In addition, in the high quality setting some living foraminifera show severe signs of dissolution, which may be related to $CO_2$ release from the degrading labile $C_{\text{org}}$. The porewater fluxes of $NO_3^-$ and $NH_4^+$ become negative around 30 days after feeding, indicating that the breakdown of algae on the top of the cores has ceased. Highest fluxes of $NO_3^-$ and $NH_4^+$ are observed in the high quality cores in proportion to the quantity of added carbon.
Low $\delta^{13}C$ in tests of live epibenthic and endobenthic foraminifera at a site of active methane seepage

Andreas Mackensen1; Jutta Wollenburg1 & Laetitia Licari1,2

1Alfred Wegener Institute for Polar and Marine Research, 27568 Bremerhaven, Germany
amackensen@awi-bremerhaven.de

2CEREGE, Europôle de l’Arbois BP 80, 13545 Aix-en-Provence cedex 4, France

To investigate the use of benthic foraminifera as a means to document ancient methane release, we determined the stable isotopic composition of tests of live (Rose Bengal stained) and dead specimens of epibenthic Fontbotia wuellerstorfi, preferentially used in paleoceanographic reconstructions, and of endobenthic high-latitude Cassidulina neoteretis and Cassidulina reniforme from a cold methane-venting seep off northern Norway. We collected foraminiferal tests from three pushcores and nine multiple cores obtained with a remotely operated vehicle and a video-guided multiple corer, respectively. All sampled sites except one control site are situated at the Håkon Mosby Mud Volcano (HMMV) on the Barents Sea continental slope in 1250 m water depth.

At the HMMV in areas densely populated by pogonophoran tubeworms, $\delta^{13}C$ values of cytoplasm-containing epibenthic F. wuellerstorfi are by up to 4.4 ‰ lower than at control site, thus representing the lowest values hitherto reported for this species. Live Cassidulina neoteretis and C. reniforme reach $\delta^{13}C$ values of -7.5 and -5.5 ‰VPDB, respectively, whereas $\delta^{13}C$ values of their empty tests are by 4 ‰ and 3 ‰ higher. However, $\delta^{13}C$ values of empty test are never lower than those of stained specimens, although still lower than empty tests from the control site. This indicates that authigenic calcite precipitates at or below the sediment surface are not significantly influencing the stable isotopic composition of foraminiferal shells. The comparatively high $\delta^{13}C$ rather results from upward convection of porewater and fluid mud during active methane venting phases at these sites. These processes mingle tests just recently calcified with older ones secreted at intermittent times of less or no methane discharge.

Since cytoplasm-containing specimens of suspension feeder F. wuellerstorfi are almost exclusively found attached to pogonophores, which protrude up to three centimeters above the sediment, and $\delta^{13}C$ values of bottom-
water dissolved inorganic carbon (DIC) are not significantly depleted, we conclude that low test $\delta^{13}C$ values of *F. wuellerstorfi* are the result of incorporation of heavily $^{13}C$ depleted methanotrophic biomass that these specimens feed on, rather than due to low bottom-water $\delta^{13}C_{\text{DIC}}$. Alternatively, the pogonophores, which are rooted at depth in the upper sediment column, may serve as a conduit for depleted $\delta^{13}C_{\text{DIC}}$ that ultimately influences the calcification process of *F. wuellerstorfi* attached to the pogonophoran tube well above the sediment/water interface. The lowest $\delta^{13}C$ of live specimens of the endobenthic *C. neoteretis* and *C. reniforme* are within the range of pore water $\delta^{13}C_{\text{DIC}}$ values, which exceed those that could be due to organic matter decomposition, and thus in fact document active methane release in the sediment.
Degradation and alteration of phytodetritus by benthic foraminifera: in situ $^{13}$C-tracer experiments

Hidetaka Nomaki$^{1,2}$, P. Heinz$^3$, N. Ohkouchi$^1$, T. Nakatsuka$^4$, H. Suga$^1$, K. Matsumoto$^1$, Y. Chikaraishi$^1$ & H. Kitazato$^1$

$^1$Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan
nomakih@jamstec.go.jp
$^2$Ocean Research Institute, University of Tokyo, Minamidai 1-15-1, Nakano-ku, Tokyo 164-8639, Japan
$^3$Institute of Geosciences, University of Tuebingen, Sigwartstrasse 10, 72076, Tuebingen, Germany
$^4$Institute of Low Temperature Science, Hokkaido University, N19 W8, Kita-ku, Sapporo 060-0819, Japan

The fate of organic matter on the seafloor is crucial both for understanding carbon cycle in the ocean and for the application of sedimentary organic matter as a recorder of paleoenvironment. Since benthic foraminifera often dominate large biomass on the deep-sea floor, they are expected to play a significant role in organic carbon consumption at the sediment-water interface. Here, we evaluated the role of the benthic foraminifera by operating in situ $^{13}$C tracer experiments in Sagami Bay, Japan.

The in situ $^{13}$C-tracer experiments were operated in Sagami Bay (water depth 1450 m) by supplying $^{13}$C-labeled unicellular algae ($Dunaliella tertiolecta$, as a model of phytodetritus) onto the surface sediments in closed chambers. We examined the incorporation of $^{13}$C-labeled carbon into total biomass and each lipid compound extracted from the bulk sediment and benthic foraminiferal cell. The benthic foraminifera ingest large quantity of phytodetritus (~3.8 mgC m$^{-2}$ d$^{-1}$) as much as by bacteria. Furthermore, degradation rates of C$_{18}$ and C$_{18:1}$ fatty acids in the foraminiferal cells of two phytodetritus feeding species were nearly 10 times faster than those in the bulk sediments, indicating that foraminiferal feeding enhances the degradation of phytodetritus on the deep-sea floor. On the other hand, benthic foraminifera synthesized some fatty acids and sterols (stigmasterol, methylencholesterol, etc.) within 4 to 6 days after being fed by $^{13}$C-labeled algae. A total abundance of sterols in benthic foraminiferal cell accounts for 20 mg m$^{-2}$ in the surface 5 cm of the sediments, corresponding to ~4% of total sterols in the surface sediments. Considering days- to weeks- scale turnover of foraminifera, organic matter on the seafloor should be largely altered by foraminiferal ingestion, digestion, and synthesis in time scale of few days.
Living benthic foraminifera from methane seep environments: A case study from the Adriatic Sea

Giuliana Panieri

Dipartimento di Scienze della Terra e Geologico-Ambientali, Università di Bologna, Via Zamboni 67, 40126 Bologna, Italy
panieri@geomin.unibo.it

Multiple abrupt, global climate shifts that correlate with highly negative $\delta^{13}C$ anomalies recorded in fossil foraminifera lead to the assumption that these negative excursions might reflect past intervals of massive dissociations of gas hydrates. In this context, the present work is focused on the ecology and stable isotopic compositions of living/recently living (protoplasm-containing) and dead (fossil) benthic foraminifera (>63µm) from modern or relatively recent settings. In particular, I investigated several areas in the northern Adriatic Sea where hydrocarbon gases are currently released and seepage-related, carbonate-cemented sediments occur at 30 m water depth. On the basis of present data, foraminiferal assemblages at these seeps are not unique, although some taxa appear to be more adapted to the prevailing conditions. Total foraminiferal density is higher in seeps than in background samples; this can be plausibly attributed to trophic conditions. Carbon isotopic signatures in shells of living/recently living benthic foraminifera do not conform to the very negative, methane-influenced carbon isotope values of the pore waters; similar values obtained in seep and background samples for the same species indicate biological factors. Differences in $\delta^{13}C$ values in foraminiferal cytoplasm from seep and control sites clearly indicate ingestion of different kinds of food. The $\delta^{13}C$ values in foraminiferal cytoplasm at the seep sites (ca -25.52‰) are lower than those in cytoplasm of the same species in control samples (ca -22.82‰), suggesting that *Beggiatoa* (which are known to produce lipids with very light $\delta^{13}C$ signatures; e.g., $\delta^{13}C = \text{ca} -65‰$), may be a food source for the foraminifera, and explaining the higher foraminiferal density at seep sites. Overall, however, on the basis of my present data, it is not clear what is the influence of biological and physical factors on carbon isotopic signatures of foraminifera living at methane seeps. Thus, additional biological and geochemical data are needed to assess the effect of past methane releases on benthic foraminifera.
Mg/Ca micro-distribution in foraminiferal test –
Implication of laboratory culture experiments

Takashi Toyofuku & Hiroshi Kitazato

Institute for Research on Earth Evolution (IFREE4), Japan Agency for Marine-Earth Science
and Technology (JAMSTEC), 2-15, Natsushima-cho, Yokosuka,
237-0061, Japan - toyofuku@jamstec.go.jp

The magnesium/calcium (Mg/Ca) ratio in the tests of foraminifera has
rapidly developed as a paleo-temperature proxy in paleoceanography because
the magnesium content of calcite is a function of its precipitation temperature.
Many studies have established the equation between Mg/Ca and temperature
using sediment trap, plankton net, sediment core top and culture specimens.
These empirical equations are useful to apply paleoenvironmental reconstruction.
However, the foraminiferal uptake of elements has not fully understood yet.

Pelagic foraminiferal test are consist of almost pure calcite. A general
magnesium content of foraminiferal test is smaller than expected magnesium
contents in calcite which is precipitated from seawater by two orders of
magnitude, according to results of inorganic precipitation experiments. Moreover,
magnesium amount in test is also different among species. Meanwhile, the
relationship between Mg/Ca and temperature is different from species to
species. These suggest that uptake of elements from seawater is positively
controlled by foraminiferal biology.

In this study, we studied about chamber formation processes and
magnesium and calcium micro-distributions of foraminiferal test in order to
investigate an incorporation of elements from seawater to calcareous tests
through foraminiferal organisms. The foraminiferal chamber formation process
was observed under inverted microscope from first to last in laboratory. The
elemental measurements were carried out by electron probe microanalyzer
(EPMA) on seven cultured specimens and natural populations. We used juvenile
specimens produced during asexual reproduction in the laboratory. The whole
tests of cultured specimens are calcified under well-controlled physicochemical
conditions. It is advantage of living culture in order to evaluate the influence of
ambient environments on foraminiferal test chemistry because any factors are
variable systematically by an experimental design.
Elemental micro-mapping measurement indicated heterogeneous distribution of magnesium. There are several magnesium concentrated bands in both planktonic and benthic species. High magnesium bands correspond to organic layers in the test. The intrachamber variation was also observed in addition to high magnesium bands. The variation was not random, but showed a weak planar distributional pattern. Microscope observations documented the precipitation of calcite does not occur evenly over the organic membrane of the developing chamber during their calcification process. We speculate variable Mg/Ca values within their test walls, which resulted from the pattern of chamber formation and from minor variations of Mg/Ca within one chamber wall.
Molecular characterization of bacteria and kleptoplast within *Virgulinella fragilis*

Masashi Tsuchiya¹; Takashi Toyofuku²; Kiyotaka Takishita¹; Hiroyuki Yamamoto¹; John Collen³ & Hiroshi Kitazato²

¹Extremobiosphere Research Center (XBR), JAMSTEC, Japan
tsuchiyam@jamstec.go.jp
²Institute for Research on Earth Evolution (IFREE), JAMSTEC, Japan
³School of Earth Sciences, Victoria University of Wellington, New Zealand

To understand the survival strategy of *Virgulinella fragilis* in oxygen-depleted and sulfide-enriched environments, molecular phylogenetic analyses and ultrastructural observation (TEM) were conducted. It is thought that existence both of endosymbiotic bacteria and kleptoplasts allow *V. fragilis* to survive in low oxygen environments. However, our observations suggest *V. fragilis* have similar bacteria and kleptoplast that exist both in oxygen-depleted and in oxygenated environmental specimens.

Samples were collected both from Namako-ike Lagoon, Kagoshima, Japan, and Wellington Harbor, New Zealand. The water depth of Namako-ike is 22 m, isolated from surrounding seas by a gravel bar. Lake water is stratified throughout the year, and shows low oxygen concentration (<0.1 mg/l) below a depth of 15m, where a hydrogen sulfide enriched environment is present. Light partially reaches the bottom of the lake due to existence of a discontinuity layer. On the other hand, well-oxygenated sea water (7 mg/l at 19 m) is present in Wellington Harbor. However, in the 1970’s, environmental conditions were extremely degraded and an oxygen-depleted and sulfide-enriched environment predominated in the harbor. In this period, *V. fragilis* dominant in this harbor, which is the type locality of *V. fragilis* (Grindell and Collen, 1976). Environmental improvement has occurred since this time; the harbor is now a well-oxygenated environment.

Bacteria and kleptoplasts were determined from both Namako-ike and Wellington specimens. In Namako-ike specimens, a single morphological type of large-sized bacteria exists in host foraminifers. In contrast, two morphological types of bacteria exist in Wellington specimens. One morphotype is large-sized, and shows features similar to Namako-ike specimens. The other morphotype consists of small-sized bacteria. The large-sized bacteria from both locations exist in the cell cortex singularly in vacuoles whereas small-sized bacteria in
Wellington specimens were rapidly digested. In the results of clone analyses for bacterial 16S rDNA, most common were *Desulfobacterium corrodens* (α-proteobacteria, similarity=94%) in both Namako-ike Lake and Wellington specimens, which we presume to be equivalent to the large-sized bacterium. Other clones from Wellington specimens were uncultured α-proteobacteria (similarity=90%) that we presume to be small-sized bacterium. Kleptoplasts existing inside the foraminiferal cells have a double membrane chloroplast-like structure located close to the foraminiferal cytoplasm, and are not exist inside vacuoles. Kleptoplasts were determined by a central pyrenoid and thylakoid lamellae. These morphological features were thought to be of diatom origin. In Wellington specimens, kleptoplasts had an accumulation of starch-like features. This suggests that some of the kleptoplasts in light conditions have a photosynthesis function. In the results of clone analyses, kleptoplasts were shown to be different diatom species; *Skeletonema pseudocostatum* (similarity=99%) for Namako-ike specimens and *Coscinodiscus radiatus* (similarity=99%) for Wellington specimens. *V. fragilis* obtain diatoms from each environment, and keep them in their cytoplasm. Specimens from both environments have similar bacterial species and kleptoplast that are kept in the foraminiferal cell. Thus, it is not necessary to keep both endobionts in oxygen-depleted condition; both endobionts may have different behavior in each environment. In light or partially light conditions in shallow waters, we suggest two hypotheses:

1) In oxygen-depleted environment, the occurrence of hydrogen-sulfide due to α-proteobacteria in the foraminiferal cell cortex is oxidized with kleptoplastic photosynthesis oxygen.

2) In oxygenated environment, bacteria show parasite-like behaviors that utilize organic materials likely caused by host foraminiferal activities or photosynthesized products in kleptoplasts.