# Microbialites in chemosynthetic ecosystems

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Microbialites are macroscopically identifiable the earliest evidence of life, which are formed with association of benthic microbial communities and some environmental factors (1). Most microbialites consist of carbonate, but also consist of silica, phosphate, iron- and manganese-oxides, iron sulfides (2), and magnesium hydroxide (3). Previous investigations for microbialites in terrestrial (rivers, lakes, and hot springs) and shallow marine settings revealed that photosynthetic microbes such as cyanobacteria and algae play an important role for microbialite formation (2). However, microbialites are also occurred in some chemosynthetic ecosystems at subsurface and deep-sea settings. Due to the sample availabilities, reports and descriptions of such unusual microbialites were limited so far. Here, I summarize the studies on microbialites at the dark environments, and introduce characteristics on a microbialite that have newly found on 2013 at the Shinkai Seep Field, Southern Mariana.
Some carbonates at deep-sea methane seep sites (4, 5) and in pipeline at the landfill sites (6, 7) contained microbialite textures. Carbon isotope ratio of the carbonates and biomarkers indicated the association of methanogen and/or methane-oxidizer for making microbialite textures. Some iron sulfide deposits near deep-sea hydrothermal vents also exhibited microbialite textures, which have been interpreted as organosedimentary structures associated with various chemosynthetic microbes. A newly discovered chimney at the Shinkai Seep Field consisted of magnesium hydroxide and calcium carbonates, which were interpreted as a product of serpentinite-associated alkaline fluid. Microbialite textures were locally found in the chimney and only consisted of magnesium hydroxide. Scales of microbialites at the chemosynthetic ecosystems were ranged from several mm to cm. In addition, some of them occurred with bivalves and tubeworms. On the other hand, microbialites at the photosynthetic ecosystems occur larger scales at a maximum of several km (2). Further studies for understanding microbialites at the chemosynthetic ecosystems will provide useful information for reconstruction on early life from ancient microbialites.
Acknowledgements: The chimney samples of the Shinkai Seep Field were obtained through the cooperative efforts of the captain and crews of the support ship R/V Yokosuka and the operation team of the DSV Shinkai 6500. In addition, the scientific party on the cruise YK13-08, YK14-11, and YK15-11 lead by Dr. Yasuhiko Ohara provided the chimney samples and tireless supports during the cruises. I would like to thank all these relevant persons.
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Keywords: microbialite, chemosynthetic ecosystems

 

# Decomposition of the plant in the ocean of the Late Cretaceous -Focused on the Upper Cretaceous Yezo Group distributed in Nakagawa Town, Hokkaido, Japan-

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Land plants, mainly composed of cellulose and lignin, are an important carbon reservoir on the earth. However, those organic compounds are relatively difficult to decompose for most organisms. In modern marine environments, wood-boring bivalves, which have symbiotic microbes to decompose cellulose, can mechanically break up the wood by their shells and consume cellulose. Then, decomposed organic compounds are useful for benthic organisms which have no ability to decompose original wooden material. Based on such degraded organic matter and other chemical compounds produced during decay of woods, sunken-wood communities will be established. Thus, it is important to know role of wood-boring bivalves through the ages. The wood-boring bivalves have appeared in the Early Jurassic and diversified in late Cretaceous. However, degradation process of the wood by boring bivalves in the Cretaceous ocean hasn't been fully revealed. Thus, the current study aim to reveal the degradation process of the wood in the ocean using the fossils from the Cretaceous Yezo Group distributed in Nakagawa Town, Hokkaido, Japan.
Totally 67 carbonate concretions were collected from the localities where Cretaceous deposits were exposed. Surface of the observation, cut polished surface and thin section of the observation, X-ray CT imaging, such as the cleaning of containing invertebrate fossils were carried out in the laboratory. Within the sample, ca. 70 % of concretions contained wooden trunk fossils. Within the concretions contained wooden fossils, about 34 % were bored by wood-boring bivalves. Detailed observations of borings suggest that the all bore holes were made by xylophagous wood-boring bivalves which are known as deep-sea wood borers. Within the bore holes, aggregations of framboidal pyrites, indicating activity of sulfate reducing bacteria were frequently found, and pyrites accumulated within the wood and around the wooden fossils. Fecal pellets, sometimes contain wood fragments, were also accumulated around the woods. It indicates that it was revealed that at least about 30% of the wood is degraded by deep-sea wood-boring bivalves and sulfate reducing bacteria in the deep sea zone of Yezo basin of the Cretaceous.

Keywords: Wood-boring bivalves, Sulfate reducing bacteria, Cretaceous

 

# Influence of cold seep methane on the forming of echinoderm skeletons

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Recently, a few modern species of echinoderms have been reported as a member of the chemosynthetic community organisms, and at least two fossil species of echinoderms have been found from cold seep deposits (Pawson and Vance, 2004; Gaillard *et al*., 2011; Landman *et al*., 2012). In order to understand how these echinoderms from cold seep environment take carbon to form their skeletons, two analyses were done; 1) stable carbon isotope analysis of the fossil skeletons, and 2) stable carbon isotope analysis of a living sea urchins in aquaria under environments of controlled stable carbon isotope ratios ($\delta^{13}C$).
The fossil echinoderms have been collected and studied from two cold seep areas; the Upper Cretaceous (Campanian) in South Dakota, USA and the coeval formation in Hokkaido, Japan. The both crinoids from South Dakota and Hokkaido have clearly lower $\delta^{13}C$ values than normal echinoderms, suggesting their skeletons were influenced by cold seep methane which has extremely low $\delta^{13}C$ values (Kato and Oji, 2015). However, it was still unknown how these crinoids formed their skeletons with such low $\delta^{13}C$ values.
A lot of marine organisms such as many mollusks, are considered to precipitate their skeletons in equilibrium with the ambient sea water in regards to their carbon isotope (Epstein *et al*., 1951). For example, bivalves shells from a chemosynthetic community show ±5‰ $\delta^{13}C$ values (e.g. Mae *et al* ., 2007). However, echinoderm skeletons, even from a normal (non-seep) environment, are known to be formed not in isotopic equilibrium, and the $\delta^{13}C$ values of the echinoderms skeletons are different depending on the classes or species (e.g. Weber, 1968). Thus, in echinoderm biomineralization, an isotope fractionation due to vital effect or some carbon sources other than from sea water should be possible.
In order to clarify the cause of inequilibrium of the $\delta^{13}C$ value in the skeletons of echinoderms and to estimate degree of isotope fractionation in forming skeletons, specimens of modern echinoid (*Strongylocentrotus intermedius*) have been raised in two experiments; 1) the echinoids has been raised under three types of sea water with differently controlled $\delta^{13}C$ value of DIC (dissolved inorganic carbon), and 2) with two different types diets (a kelp (*Saccharina longissima*) and a land plant (*Fallopia sachalinensis*)) with different $\delta^{13}C$ value.
Compared with the controlled case of culture with normal sea water with seaweed diet, $\delta^{13}C$ value of the echinoid test changed in both cases (in waters with high $\delta^{13}C$ value, and with different diets. Thus, it suggests that skeletons $\delta^{13}C$ of this echinoid, are influenced by isotope ratio of both sea water and foods. This result clearly shows that the process of isotope fractionation and forming skeletons of echinoderms should be different from those of most of mollusks. It is thought that the echinoderms in cold seep environment depend on bacteria mat for their diet, or detritus that have low $\delta^{13}C$.

Keywords: cold seep, echinoderms, chemosynthetic community, biomineralization

 

# Mass occurrence of the enigmatic gastropod *Elmira* in the Late Cretaceous Sada Limestone seep deposit in southwestern Shikoku, Japa

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*Elmira* is a medium- to large-sized gastropod genus, which has so far been recorded only from a presumably Eocene methane-seep deposit in Cuba, and its systematic affinity and paleoecology are unknown. We report a mass occurrence of *Elmira* sp. from a Late Cretaceous seep deposit in Shikoku, Japan, called Sada Limestone, with its mode of fossil occurrence, carbonate petrology, and stable carbon isotope analyses. Sada Limestone is characterized by the dominant occurrence of a large-sized thyasirid bivalve "*Thyasira*" *hataii* and serpulid worm tubes. The mass occurrence of *Elmira* sp. occurs as a lens-shaped carbonate body, 6.5 m in length and less than 2 m in thickness, intercalated in the thyasirids-rich limestone. The *Elmira*-rich lens body has a flat top and a concave base, and consists of multiple shell accumulation layers, which were formed by winnowing and filling of a depression in slope mud. The rare occurrence of *Elmira* sp. elsewhere in the Sada Limestone suggests that it lived in local aggregations in the vicinity of the depression. The matrix of the *Elmira* mass occurrence is rich in dolomite and ankerite and is less depleted in $^{13}C$ ($\delta^{13}C$ values of calcite: -5.3 to -2.4‰; of dolomite: -8.3‰) than the calcitic matrix of the surrounding limestones. This suggests that the *Elmira* mass occurrence was cemented below the sulfate reduction zone and thus with little influence of anaerobic methane oxidation. It is, therefore, difficult to consider that *Elmira* sp. harbor chemosymbiotic bacteria. As some trochiform gastropods do in seep sites, *Elmira* sp. was maybe a bacteria grazer gregarious on bacteria mats and/or hard bottoms.

Keywords: chemosynthesis, cold seep, Gastropods, Cretaceous, Shikoku

 

# Meiofaunal community compositions around hydrothermal vents in three seamounts in NW Pacific –Vent copepods want to stay with vent polychaetes?

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In contrast to specific large benthic invertebrates in chemosynthetic ecosystems around deep-sea hydrothermal vents, meiofaunal communities in such habitats have only recently been investigated. This is especially true in the Northwest Pacific Ocean, even though there are many seamounts with active hydrothermal vents in their calderas. We studied the variations in meiofaunal composition around hydrothermal vents on chimney structures (vent chimneys) and in the adjacent non-vent fields in the calderas of three neighboring seamounts (Bayonnaise Knoll, Myojin Knoll, and Myojin-sho Caldera), in Izu-Ogasawara Arc, NW Pacific. A typical meiofaunal composition (nematodes as the most abundant taxon; harpacticoid copepods as the second) was observed in the sediments on sea-floor in the non-vent (control) fields, and even in the sediment at the base of chimneys. On the surfaces of vent chimneys, on the other hand, copepods and their nauplii were the most abundant. There were significant differences in copepod compositions on vent chimneys between the three seamounts, and even between different substrates on the same chimneys. *Stygiopontius* (Dirivultidae, Siphonostomatoida), a typical vent copepod group, was abundant and often predominant on the dense mats of tubes of *Paralvinella* polychaetes living in the closest proximity to vent fluids among vent metazoan macrofauna. These copepods, however, showed the lower density on the beds of *Neoverruca* barnacles although their beds were adjacent to *Paralvinella* mats at cm scales. No significant genetic difference was observed among local populations of *Stygiopontius,* suggesting they are the same species. Furthermore, stable carbon and nitrogen stable isotopic ratios and radiocarbon abundances in meiofaunal soft tissues revealed the dirivultids utilized mainly chemolithoautotrophic microbes at vents. These results show that *Stygiopontius* copepods prefer *Paralvinella* mats as their habitats or have similar environmental preferences (such as higher water temperature or existence of chemolithoautotrophic microbes) to *Paralvinella*.

Keywords: hydrothermal vent, meiofauna, community compositions

 

# Nutrition sources of meiofauna revealed by stable carbon and nitrogen isotope ratios and natural radiocarbon abundances explain meiofaunal distribution patterns at hydrothermal vent fields

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Deep-sea hydrothermal vents host unique marine ecosystems which mainly rely on organic matters produced by chemolithoautotrophic microbes. Although there are abundant meiofauna at the hydrothermal vent field, studies on their distributional patterns and nutritional sources are still limited due to their small body sizes. In this study, we investigated dietary sources of meiofauna at hydrothermal vent fields in the western Pacific using stable carbon and nitrogen isotope ratios ($d^{13}C$, $d^{15}N$) and natural radiocarbon abundances ($D^{14}C$). Based on these data, we evaluated how much do the hydrothermal vent meiofauna gain their nutrition from the chemolithoautotrophic microbes and how does it related to their distributional patterns around hydrothermal vent fields. Bacterial mats of the hydrothermal vent chimney typically exhibited heavy $d^{13}C$ values (up to -10‰) and depleted $D^{14}C$ values (~600‰). The $d^{13}C$ and $D^{14}C$ values of Dirivultidae, an endemic copepod family inhabiting hydrothermal vent chimney, exhibited similar values to the bacterial mat but distinct from those of sediments at surrounding area or water column plankton, suggesting that they exclusively rely on bacterial mat at the vent chimney. Other copepods at the vent chimneys may also rely on bacterial mat to some extent. To the contrary, $d^{13}C$ values of nematodes at vent chimneys were -26.6 and 23.2‰, which were similar ranges to those at non-vent sites, suggesting vent nematodes did not gain their nutrition from the chemolithoautotrophic microbes. Those nutritional facts obtained from isotopic compositions explain well about distributional patterns of these meiofauna; while dirivultid copepods exclusively distribute at vent chimneys and other copepods are more abundant at vent chimneys than in non-vent sediments, nematodes showed similar abundances between vent chimney and non-vent sediments.

Keywords: Hydrothermal vent ecosystem, meiofauna, feeding habit, stable isotope ratios, natural radiocarbon abundance

 

# Species diversity of vesicomyid bivalves from the middle Miocene seep carbonates in the Bessho Formation, Nagano Prefecture, Japan

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Vesicomyid bivalves have been dominant animals in submarine cold seeps through the Cenozoic age. Although coexistence of two or more vesicomyid species in a modern single seep site is considered to be due to different preferences in sulfide flux, salinity, and water temperature among species (Barry et al., 1997; Watanabe et al., 2013), co-occurrence of two or more vesicomyid species and its cause have rarely been discussed for ancient seeps. The middle Miocene Bessho Formation in Nagano Prefecture, central Japan is composed of slope mudstone deposited at a back-arc basin and contains many seep carbonates in various sizes. Two fossil vesicomyid species, *Adulomya uchimuraensis* and "*Calyptogena*" *akanudaensis*, were previously recorded from the Bessho Formation. This study makes genus reassignment of "*C*". *akanudaensis* and newly reports two vesicomyid species, *Pliocardia* sp. and *Adulomya* sp. and notes that relative abundance of the four vesicomyid species depends on carbonate size. The large seep-carbonate mounds more than 20 m in diameter is characterized by abundant occurrence of *A. uchimuraensis* with rare occurrences of "*C*". *akanudaensis* and *Pliocardia* sp. From the smaller, about 1 m in diameter carbonate body, "*C*". *akanudaensis* dominantly occur in association with *A. uchimuraensis*. The siltstone containing several cm-sized small carbonate concretions yields abundant shells of *Adulomya* sp. in scattered occurrence. The difference of carbonate size suggests that of fluid flux and/or longevity, and the species diversity of vesicomyid clams in the Bessho Formation might be caused by variation of seep activities among sites.

Keywords: Pliocardia, Adulomya, Vesicomyidae, Middle Miocene, Cold seep

 

# Reconstruction of seepage history in the Eocene Poronai Formation, Hokkaido, Japan

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The Eocene Poronai Formation, central Hokkaido, is known to mark the oldest fossil records of vesicomyid bivalves in Japan. This study made an outcrop mapping of lithology, mode of fossils occurrence, and stable isotopic analyses to make clear the seep-habitats in initial stages of vesicomyid diversification through the Cenozoic age.
A series of irregular-shaped seep-carbonate rocks, about 1-2 m in diameter, vertically piled up in the outcrop section of massive siltstone. The carbonate rocks and the surroundings yield abundant shells of chemosynthetic bivalves, *Hubertschenkia ezoensis* and *Conchocele bisecta*. Their modes of fossil occurrences are divided into two types, 1) shell-concentrated lens and 2) sporadic patches and scattering, which alternate each other in the vertical section. The shell-concentrated lenses are mainly recognized in siltstone, whereas the scattering type is encompassed in the carbonate bodies.
It is noteworthy that all the shell-concentrated lenses were associated with calcitic concretions partly containing fluidized texture just below them. The fluidized part consists of mixture of 1) white-colored detrital micrite depleted in $\delta^{13}C$ (–38.05 to -22.91‰), 2) gray-colored micrite not depleted in $\delta^{13}C$ (4.87 to 9.01‰) and 3) black-colored sparitic cements with widely ranging values of $\delta^{13}C$ (-42.09 to 1.88‰). Detail lithological mapping show that such fluidized texture tends to be formed avoiding shell-rich part. These suggest that the alternating pattern of two modes of fossil occurrences was controlled by intermittent fluidizing events as follows. Sporadic biofacies was formed under the diffusive phase resulting in gradual rise of pore-water pressure. Over the critical point of pore-water pressure, fluidization was triggered in unconsolidated parts not rich in buried shell remains, which acted as a nucleus of precursor concretions. Such local fluidization caused a focused flow fostering shell-concentrated mode.

Keywords: chemosynthetic, Vesicomyidae, methane seepage, Poronai Formation, Eocene

 

# Succession of whale-fall ecosystems at shallow waters: mainly based on one year monitoring of deployed whale carcasses in aquarium tank and natural environments in Tsukumo Bay, Ishikawa, Japan

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A whale carcass which sank into the sea-floor supports unique ecosystem called "whale-fall community" (Smith, 1989, Nature). Some of the communities are supported by microbial activities during degradation of organic matter within the bones. The community is thought to be change in its community structure over time through degradation process of carcass (Smith and Baco, 2003). Those whale-fall communities are well known in deep-sea environments and such schematic faunal succession has been proposed based on research in the deep-sea. Because of technical limitation of observations in the deep-sea, changes of microbial mat hasn't been fully analyzed. In addition, whale-fall community in the shallow waters are also not well known so far.
So, we tried experiments on deployed whale bones in aquarium tank. Fresh sea water continuously flow into the tank. In addition, we put whale-bones at 11 m in depth in Tsukumo Bay, Ishikawa, Japan.
As a result, the whale bones floated within few days after deployment. Many bubbles of gas, probably hydrogen sulfides, gushed out from the bones. A week later, green algae and white giant bacteria appeared on the bones. Two to three weeks after the deployment, the chemosynthetic cilliate (*Zoothamnium niveum*) and Hyalogyrinidae gastropod (*Xenoskenea* sp.) iappearred in the tanks. *X.* sp. grazed on both microbial mats, white bacterial mat and green algal mat. After appearance of those biota, abundance of those changed through a year. The green algae and the gastropods decreased in the winter and flourished in summer. Whale bones deployed in the natural environments, ca. 11 m deep, more than 1.5 years had encrusting animals such as barnacles, and very few area of the bone surface were covered by microbial mats.
We succeed to make a whale-fall community from fresh bone in aquarium tank with continuous supply of fresh sea water. It allow more detailed observations. Based on the observations, the shallow water whale-fall communities including chemosynthetic biota have establish within few weeks.

Keywords: Whale-fall community, ecosystem, reduced environment

 

# Population connectivity of benthic copepods in deep-sea chemosynthetic communities

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Meiobenthos are small (<1mm) benthic animals living on seafloor. Although their considerable diversity in marine environment, only less attention is paid for meiobenthos than macro- and mega-benthos. Copepod is the most abundant crustacean in meiobenthos in deep-sea hydrothermal vent environment, and a copepod family Dirivultidae is one of the most successful vent taxa. However, only little is known about ecology and evolution of dirivultids. In the present study, we examined efficiencies of several DNA extraction methods available for copepods, and estimate population connectivity of dirivultid copepods of the genus *Stygiopontius* in the western Pacific hydrothermal vent fields. DNA extraction efficiency was increased when the morphology of copepod was destructed. However, even the DNA extraction with the lowest concentration (~5ng/μL) was sufficient to obtain sequence data by Sanger sequencer. Genetic diversities of *Stygiopontius* copepods were different among oceanographic regions, higher in back-arc basins in the southern Pacific than in volcanic arc. In both regions, local populations were not significantly separated genetically based on partial mtCOI sequence. The present result contributes to our knowledge of ecology of meiobenthos, and establishment of environmental assessment tool using meiobenthos in deep-sea hydrothermal vent fields.

Keywords: hydrothermal vent, environmental assessment, meiobenthos

 

# Evaluation of nutrient sources of the biological community in the Shinkai Seep Field, Southern Mariana Forearc using C, N and S stable isotopes

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The Shinkai Seep Field (SSF), located in the inner trench slope of the southern Mariana Trench, ~80 km northeast of the Challenger Deep, is a serpentinite-hosted chemosynthesis-based community composed mainly of *Calyptogena* bivalves. It has been considered that the community is supported by the following mechanism; serpentinization of mantle peridotite produces $CH_4$-rich fluids, and anaerobic oxidation of $CH_4$ by sulfate-reducing bacteria (SRB) generates $H_2S$. Then, sulfide-oxidizing bacteria (SOB) oxidize the $H_2S$ in order to obtain energy and produce organic matter (Ohara et al., 2012). However, there have been no geochemical studies on the energy and nutrient sources supporting this community. Therefore, the purpose of this study is to elucidate nutrient sources of the community in SSF using C, N, and S isotope analyses.

Nine animal species and particulate organic matter (POM) obtained around the colony and sediments obtained beneath the colony by *Shinkai* 6500 during YK13-08 cruise were analyzed. The animal samples were used to measure C, N, and S isotopes of their soft tissues, and the POM and sediment samples were measured total organic carbon (TOC) and total nitrogen (TN) and their isotope ratios. Acid volatile sulfide (AVS) was extracted from the sediment samples to measure its concentration and sulfur isotopic ratio.

TOC and AVS concentration increased with decreases in their isotopic ratios, suggesting that SOB produces organic matter using $H_2S$ derived from SRB activity in sediment beneath the colony. Because isotopic ratios of POM are within the range of the common marine phytoplankton, it is thought that the POM was produced within the phobic zone by photosynthesis.

$\delta^{13}C$ value of *Calyptogena* bivalves within the range of a typical chemosynthesis-based animals relying on SOB for a nutrient source, while $\delta^{34}S$ value is approximately equal to that of AVS in the sediment. Thus, the energy source supporting the bivalves is $H_2S$ derived from SRB activity. Also, isotopic composition of the amphipod, polychaete, and anthozoan samples show intermediate values between the POM and sedimentary organic matter, suggesting that the nutrient sources of these organisms are organic matter derived from both photosynthetic and chemosynthetic productions. The sibogrinid has clearly different isotope composition, implying that this organism relies on SOB using the different carbon fixation pathway and/or methanotroph as nutrient sources.

Keywords: isotopic composition

 