# Seasonal distributions of archaeal membrane lipids and $TEX_{86}$ thermometry in the modern shallow coastal ocean

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While a large number of studies have documented rapid increase in the annual sea surface temperature (SST) and global mean sea level rising, global warming effect on enclosed coastal seas, such as Seto Inland Sea or Chesapeake Bay, is less understood. For understanding historical SST records in these shallow basins, we need to utilize a certain SST proxy preserved in sediments. However, paleo-thermometry in the shallow coastal basin is rather difficult because carbonate microfossils typically used for paleotemperature proxies are practically absent in the shallow ocean. On the hand, it is expected that organic compound based paleo-temperature proxies, such as $TEX_{86}$, can be utilized even in the shallow coastal sea. However, since $TEX_{86}$ thermometry has rarely been used for such shallow marine sediments, applicability of $TEX_{86}$ in the coastal ocean is still uncertain.

Here we test potential ability of $TEX_{86}$ paleothermometry in shallow coastal anoxic basin. We collected particulate organic matters from the water column of Beppu Bay (Seto Inland Sea) (~70m deep) at every 10 m. The vertical depth profile of glycerol dialkyl glycerol tetraethers (GTGTs) distribution within the anoxic and oxygen-enriched water columns was determined. We also calculated $TEX_{86}$ values, $TEX_{86}^{H}$ and $TEX_{86}^{L}$ temperatures which were compared with the in situ measurements of water temperatures.

Comparing total GDGTs in the anoxic water mass to those of the oxygen-eiriched water mass, those are significantly abundant in the anoxic bottom water. $TEX_{86}^{H/L}$ derived temperatures correlate with the in situ measurements of water temperatures. Therefore it is expected that temperatures delived from $TEX_{86}^{H/L}$ represent in situ water temperatures in Beppu Bay.

# Technological challenges for the advanced study of subseafloor life

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During the past decades, scientific ocean drilling has explored the subseafloor biosphere at some representative drilling sites: on the ocean margins, organic-rich anaerobic sedimentary habitats (e.g., Shimokita coalbeds) harbor sizable numbers of microbial cells over 1,000 meters below the seafloor whereas microbial populations in ultra-oligotrophic aerobic sedimentary habitats of the oceanic gyre (e.g., South Pacific Gyre) are several orders of magnitude lower. Molecular analysis at the community level demonstrates the occurrence of subseafloor microbial ecosystems that consist of phylogenetically diverse microbes; however, metabolic functions of individual microbial components and strategies for long-term survival under the energetically and geophysically severe condition have remained largely unknown. To address these issues, analytical developments customized for subseafloor life are of our essential challenges for the advanced microbiological and biogeochemical analyses.

One of the most fundamentally significant techniques is the precise detection and enumeration of indigenous subseafloor life. Recently, we have standardized an improved cell separation method, which effectively detached the cells from mineral grains of the sedimentary habitat, by applying multiple density gradient layers. Based on the cell-derived fluorescent wavelength pattern [1], the detached cells can be precisely enumerated using an automated fluorescent microscopic system [2] and/or a flow cytometry. The combined use of this technique with a cell sorter allows us to separate the cells for single cell genomics and mass spectrometry (e.g., NanoSIMS) analyses. The systematic analytical scheme currently applies to some representative deep-biosphere samples such as the South Pacific Gyre and Shimokita coalbeds (i.e., IODP Expedition 329 and 329, respectively), from which results may open a new window to the study of subseafloor life.

[1] Morono *et al.* (2009) *ISME J.* **3**, 503-511. [2] Morono *et al.* (2010) *Sci. Drilling* **9**, 32-36.


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