Expedition Log for IODP Expedition 347
Baltic Sea Paleoenvironment
7th October 2013

Exploring the deep biosphere

Bo Barker Jørgensen

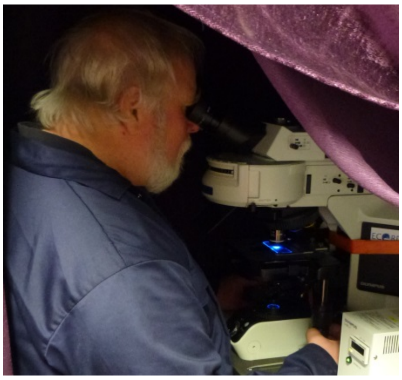
A main objective of this expedition is to understand how the Baltic Sea environment developed over the past hundred thousand years. Yet, we are also exploring the microbiology of the drilled Baltic Sea sediments, the deep biosphere. This is done by six microbiologists on board the drillship and by many shore-based colleagues. Why we are here is explained in the *Info-box* at the end of this logbook.

Seen from the perspective of the geoscientists on board the *Manisha*, microbiologists use drill cores in a destructive manner because we take away whole round core pieces that subsequently lack in the stratigraphic record. We and the geochemists therefore get a whole bore hole for ourselves at four of the sites visited. Cores from these holes are either subsampled on board or are deep frozen to be shipped to scientists around the world.

The microbiology team is very international:

Nan Xiao (Chinese)
Kochi Core Repository, Japan

Ray Zhang (Chinese)
Xiamen University, China



Barry Cragg (British)
Cardiff University, UK

Ian Marshall (Australian)
Aarhus University, Denmark

Andrea Torti (Italian)
Aarhus University, Denmark

Bo Barker Jørgensen (Danish)
Aarhus University, Denmark

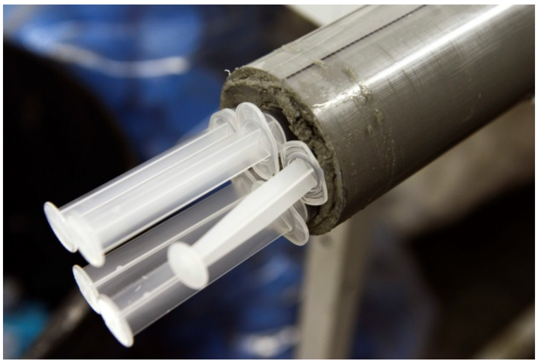
Many more microbiologists than the six who are on board have requested samples from this expedition to study a large number of research questions related to the deep biosphere. Thousands of samples must therefore be collected and preserved in many different ways as specified by all the scientists. This is quite a challenge to those of us on board who handle, log and curate the material. Already during spring 2013, long before the expedition, the microbiologists met in Aarhus to exchange their research plans and discuss how to achieve their objectives. Even a few days before we left the hotel in Copenhagen to board the drillship, planning and discussions continued (photo).

BoBarker@ECORD/IODP: Before the expedition. Left: Serious thoughts by the planning of microbiology sampling. Right: The theory is ready on the whiteboard - core flow for microbiology samples from drill floor to deep freezer.

Our first chance to start the microbiology sampling came already with the second hole drilled on our first station, located in southern Little Belt.

CarolCotterill@ECORD/IODP: As soon as a core is on deck it is cut into 1.5 m long sections and the first syringe samples are taken from the exposed core end. Curator Holger is holding the core while Andrea takes a syringe sample – or is he injecting something?

We had meticulously worked out schemes in advance of how the many samples should be taken in each 3-m long core that came up from the hole – one scheme for the syringe samples and one scheme for the 5-10-cm long cylinders of core to be cut off. These schemes were printed out in color in two copies, one page per core. It all looked very colorful and well organized and we were all well prepared. Yet, to be honest, there was quite some stress in the beginning as the cores landed on deck and the complicated sampling started. But already after a few cores a good work flow developed and we were all proud that no samples were lost.

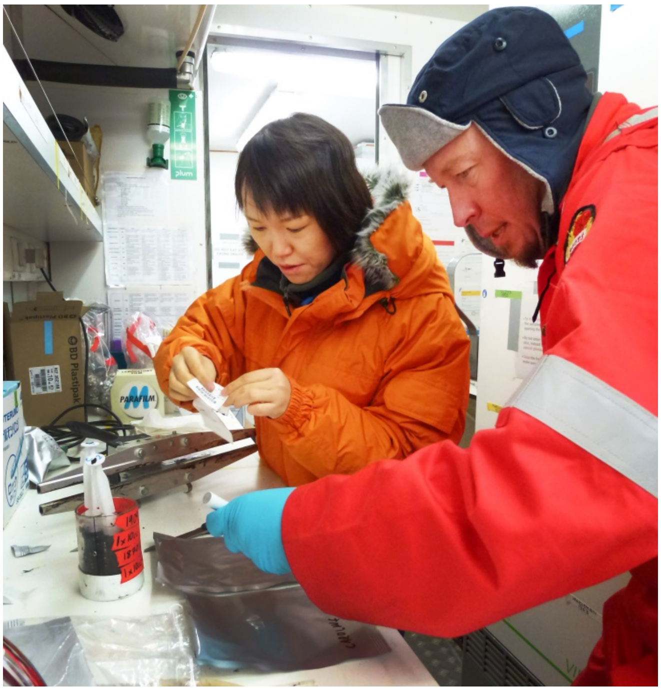


BoBarker@ECORD/IODP: Sediment samples for microbiology are taken by Barry and Michael in the core reception container using sterile syringes with cut-off tips. Each syringe in a core is taken for a different purpose and each sample will be sent to a different scientist.

CarolCotterill@ECORD/IODP: Information about each sample is typed into the ECORD Drilling Information System by Ian or Bo and stored on the on-board server. Nan, Barry and Andrea appear to do quality control. BoBarker@ECORD/IODP: Each sample is given its own label with specifications and a bar code which uniquely identifies it. Here, two 10-cm lengths of core from 15 meters below seafloor are labeled.

When cores land on the deck they are taken immediately to the core curation container and are registered in the Drilling Information System (DIS) before they are brought next door to the petrophysics container and are logged in the "fast-track" MSCL (see Expedition Logbook #6, 30.09.2013). The petrophysicist then brings the cores to the core reception container where a team of three to four people are ready to sample for microbiology and geochemistry. Each sample is typed into the DIS and a label is printed which follows the sample all the way to the recipient somewhere in a laboratory abroad.

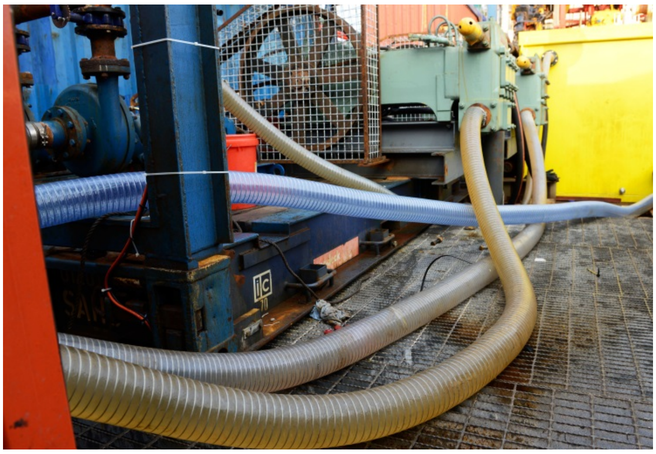
BoBarker@ECORD/IODP: Samples are collected and brought to the microbiology container. Outi is the courier. As the white board shows, we are prepared with a "plan B" and a "plan C".



CarolCotterill@ECORD/IODP: As soon as a core is subsampled, the many syringes and segments of whole core are brought to the microbiology container. It is 12°C in the microbiology container, the ventilation is strong, and the scientists work there for many hours. It gets really cold. Nan and Aarno are well dressed for the job.

There are many challenges and potential errors when sampling the deep biosphere. The samples could be contaminated by bacteria or their DNA from the ocean above. More than hundred liters per minute of seawater are pumped down to the bottom of the bore hole to flush out the loose sediment and cuttings generated when the rotating bit drills down to the next coring depth. This seawater contains more than a billion bacteria per liter, a similar abundance as in the deep sediment that we want to study. How do we know that none of those seawater bacteria have made it into our precious sediment samples?

We use a chemical tracer (PFC, perfluoromethylcyclohexane) that is pumped into the drilling fluid and mixes with it as it flows down to the bottom of the hole where the piston core shoots out to take a new core. We then take syringe samples for PFC analyses from the center and periphery of those core sections where we also take microbiology samples in order to check whether any drill fluid has penetrated into the core. If this is not the case, then it is also unlikely that bacteria have penetrated into the microbiology samples. Later DNA studies may further reveal whether there is contamination from seawater.



BoBarker@ECORD/IODP: From a bottle with pure perfluorocarbon (PFC) and via an HPLC pump a tracer flows directly into the seawater or drilling mud used to flush out sediment and cuttings from the bottom of the bore hole.

While we microbiologists take samples and prepare these for future research in laboratories around the world, we wonder what will be the new discoveries to be made. Who is living down there in sediments that were deposited during different glacial or interglacial stages? Are they all unknown species with unknown functions or will we detect a pattern of adaptations to past and present environmental conditions?

Deep biosphere research remains a young science with many surprises waiting for us. We know that life down there is very different from life on the surface of Earth - very slow and with little food or energy for subsistence over thousands of years. We hope that this expedition will bring us an important step further in our understanding of that hidden, mysterious life.

Info-box

Why are there microbiologists on the drillship when the main objective of this expedition is to understand how the geology and climate of the Baltic Sea region developed during the past glacial and interglacial periods? How can microbiologists contribute to that? The question should be turned around. What does the past climate have to do with the microorganisms living here today?

Bacteria are present everywhere, even deep down in the underground beneath the seafloor, and there are many of them. A recent extrapolation of microbial cell numbers in different global environments has shown that at least half of all microorganisms in the ocean live hidden far down in the sediment. This inhabited underworld occurs worldwide and is called *the deep biosphere*. It constitutes the largest ecosystem and the largest reservoir of organic cabon on Earth, yet it is the least explored by science – and for good reasons. Only when we drill down into the seabed do we enter this microbial world and realize that it is alive. This is what the oil and gas companies do by off-shore drilling. Then they experience how the deep biosphere strikes back with harmful corrosion of steel pipes and strong development of hydrogen sulfide (souring) which deteriorates the oil quality and causes problems to environment and health.

As the seabed accumulates over thousands to millions of years under shifting climatic conditions the freshly deposited sediment becomes buried by new sediment and gradually becomes a stratified archive of past climate. How about the bacteria that live deep down in that archive - are they the last remains of microbial communities that once thrived at the surface and were unfortunate to become entombed? Or is the deep biosphere inhabited by a community of organisms that are indigenous to the underground and perhaps connected worldwide? These fundamental questions have never been answered. The Baltic Sea offers a unique possibility to attack such questions by using the full-scale experiments that nature has done here. The Baltic Sea area has undergone some of the strongest environmental changes of any aquatic ecosystem, alternating between a lake and a sea, cold and temperate, oxic and anoxic.

Now we can drill down through deposits from these shifting environments and study which microorganisms live there and how they subsist. The sediments are sufficiently young and the microbial communities sufficiently active that we can still detect their metabolism and relate this to their abundance and their genes. We will enumerate the microbial cells and extract their DNA to determine their identity, their functional genes, and their use of those genes. We will isolate single cells from the sediment and analyze their entire genomes to understand which catalytic potential those uncultivated organisms may have. We will study their virus and how mortality in the subsurface relates to the turnover of their biomass.

Microbial cells in the deep biosphere are the gate-keepers by the break-down of buried organic matter. They determine how large a fraction of this organic matter returns as carbon dioxide and inorganic nutrients to the ocean and how much is stowed away over geological time. It is the storage of organic carbon in the underground that controls the oxygen level in the atmosphere and thereby determines our ability to breathe the air around us. The deep biosphere is important for long-term global element cycles that interact with ecosystems on the surface of Earth.