

Expedition M70/2a

Antje Boetius

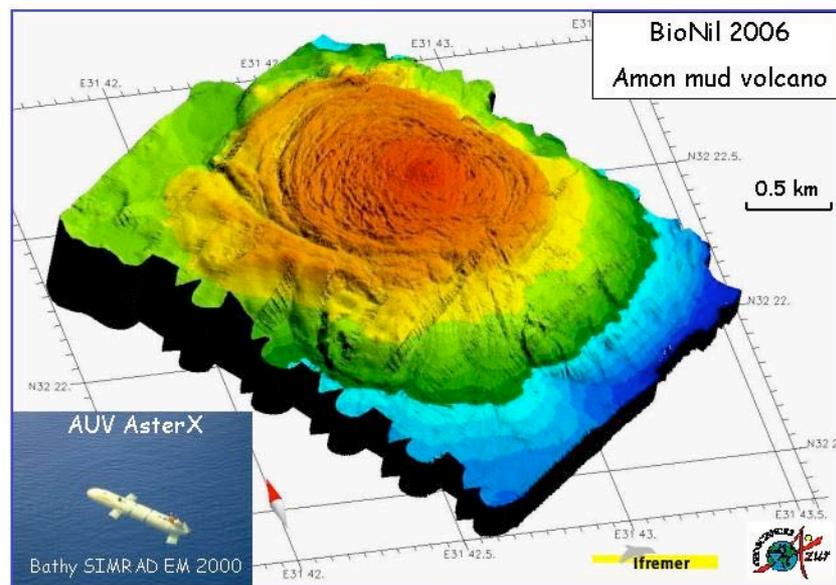
1. Weekly report (21.10.-29.10.06)

RV METEOR

The expedition BIONIL (leg M70/2a) investigates recently discovered seep systems of the deep Nile fan. These consist of numerous fluid-escape structures at water depths between 1000 and 3000 m including pockmarks, mud volcanoes and brine pools. Many of these systems emit hydrocarbons (mostly methane), as well as sulfide. These chemical energy sources build the basis for rich and abundant microbial life and diverse chemosynthetic organisms. Our research combines activities of the ESF EUROCORES EUROMARGIN project MEDIFLUX, the German “Geotechnologien” project MUMM II (funded by BMBF/DFG), and the EU project HERMES. On the basis of geophysical, geochemical and biological data from various seep structures collected during two earlier MEDIFLUX cruises, M 70/2 aims at gaining better understanding of the distribution and functions of the novel seep ecosystems. The objectives are to understand the controls and mechanisms of chemical element transport and breakdown by seep biota, and to obtain insight in the biodiversity and functioning of life at different types of fluid seeps in the Eastern Mediterranean. These goals will be achieved by detailed mapping of selected habitats using the French autonomous underwater vehicle (AUV) AsterX (IFREMER) followed by detailed geochemical in-situ measurements and specific sampling of mud, fluids, carbonates and biota along geochemical gradients. Sampling and in-situ measurements are performed with ROV QUEST of MARUM (University Bremen, Germany).



We used the 19 October to install our equipment on METEOR's deck and labs making sure that every possible place is used well ☺. On the 20 October we had a very nice visit by the scientific steering committee of the Census of Marine Life Program CeDaMar as well as the agency and port authorities of Crete wishing us luck for our mission. We left Heraklion on the 21 October with excellent weather for the first week of work at the East Delta of the Deep Nile fan. Within 4 days we could accomplish 2 ROV and 2 AUV dives, focusing on the highly gassy mud volcanoes "Amon" and "Isis". The "Amon" mud volcano has a very unusual morphology, with a pointed center instead of the typical crater. We could visualize details of "Amon"'s structure with the AUV AsterX carrying the multibeam echosounder Simrad EM2000 (CNRS Geosciences-Azur). AsterX was navigated at a fixed altitude of 70 m above the seafloor, allowing for Digital Terrain Models to be computed with data gridded at a space interval of 1 m, achieving a much higher resolution than allowed by conventional ship-borne bathymetric echosounders. Maps were produced after each AsterX run in less than two hours after the recovery of the AUV on deck and represent an invaluable help to plan the ROV dives.



Our strategy is to use bathymetric maps, gas flare imaging and visual inspection of the seafloor to select representative habitats for detailed analyses including in situ chemical and biological measurements. For the geochemical and microbiological analyses of the different seep structures of the deep East Mediterranean, the ROV QUEST is packed with an array of sampling tools.

Our first analysis of “Amon” mud volcano shows that the tip of “Amon” has a very rough morphology indicating recent gas and mud eruptions. The sediments are laden with gas and emit gas bubbles when touched with sampling tools. We detected diverse patches of giant sulfur oxidizing microorganisms as the main inhabitants of the central area. The central zone is surrounded by a vast area of large biogenic mounds produced by a yet unknown animal. Other unexpected findings with regard to the morpho-structures of the two volcanoes of the East Delta include the presence of three active centres of mud emission on “Isis”, and of old mudflows covered with densely populated carbonate crusts to the south-west of “Amon”.



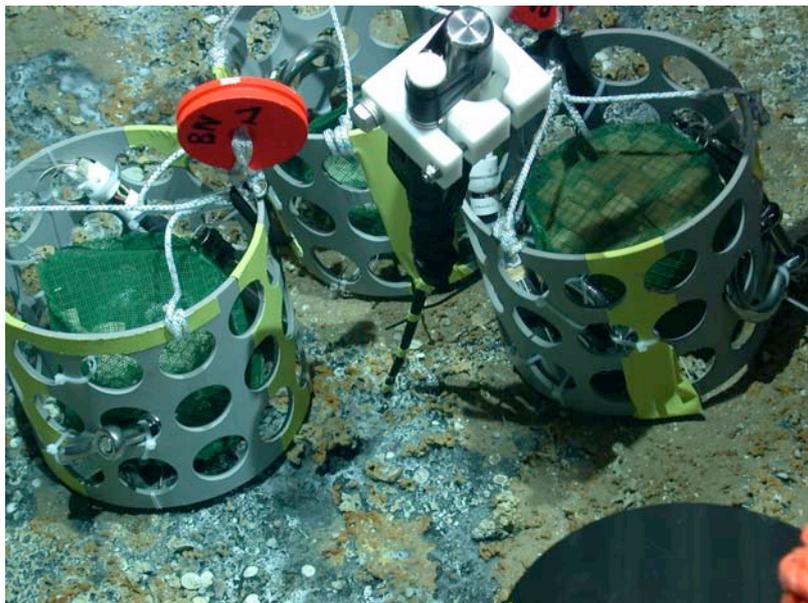
The beauty of ecosystem research in deep waters is that every dive results in unexpected findings questioning our understanding of geosphere-biosphere interactions. At the moment on of our favourite discoveries at “Amon” is a fresh mudflow forming a riverbed between the inner mud volcano and its southern rim (shown above). This highly sulfidic mud flow shows a succession of microbial mats from uncovered black sediments with first small spots of *Arcobacter* mats, then filamentous giant sulfide oxidizers, and finally a dense carpet of an unknown type of bacterium looking like the small sister of the famous “sulfur pearl” *Thiomargarita*. Currently we have moved to the Central Delta and are investigating a flat area of the margin with numerous small pockmarks, large carbonate crusts and spots of highly reduced gassy sediments. Unfortunately, the wind is sometimes too strong to allow AUV and ROV operations, but we are hoping to have a few more dives in this area before moving on to the Caldera in the West of the Nile Fan.

With best greetings to all colleagues, friends and families on land
– the BIONIL team

2. Weekly report (30.10.-05.11.06)

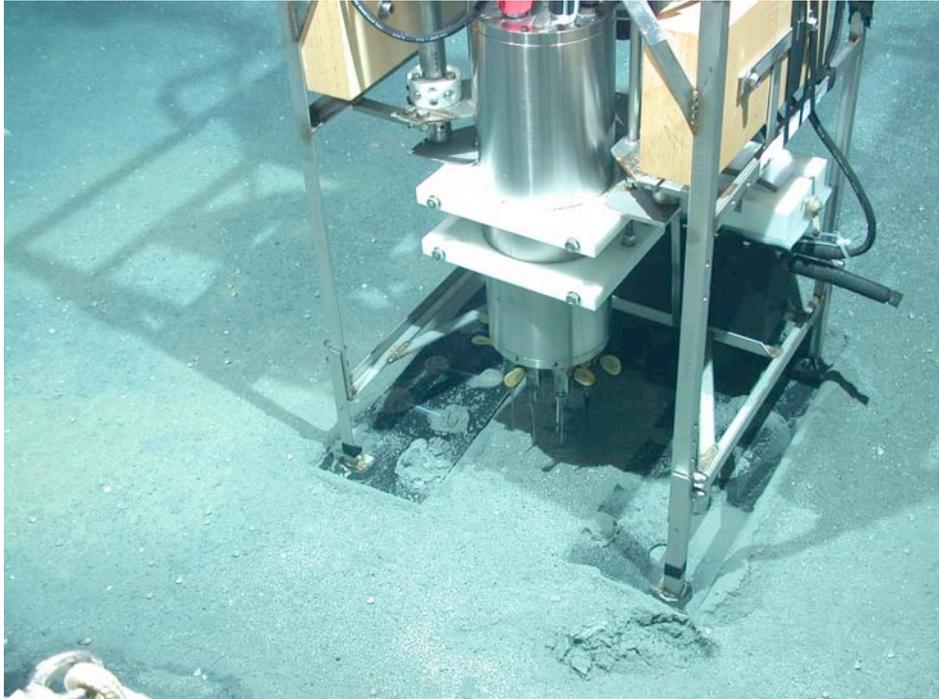
The second week of the expedition BIONIL (leg M70/2a) was dedicated to the study of seeps in the central area of the Nile fan, and to the exploration of the Western Delta. The central area of the deep Nile Fan investigated by us is relatively flat and hosts numerous pockmarks. The results from two previous MEDIFLUX cruises indicate considerable geological and biological activity, hence we selected two sites at 1700 and 2100 m to explore them with the AUV, with alternating use of the multibeam and the echosounder for gas detection, and also study them in detail by ROV-based operations. Unfortunately, Neptune turned against us, and wind speeds and waves rose to the operational limit of both robots, forcing us to be very flexible with the planning of station work. Furthermore, an accident during one deployment kept the ROV team busy with repairs for two days. Nevertheless we managed a total of 3 ROV dives and 3 AUV dives in the central area during leg M70/2a.

In contrast to most known seeps, which are actively emitting gas to the hydrosphere, the area we investigated at the 1700 m site shows no distinct topographic feature but a striking backscatter signal. In the eastern part that is a bit more elevated than the rest of the central area we found vast carbonate pavements and highly porous crusts outcropping from the seafloor (called “soufflé” by our French colleagues).



Deployment of colonization trays and measurement of sulfide emission on carbonate-covered seeps of the central zone.

The carbonates rest on an active fluid-flow impacted area of the seafloor, as shown by the dark patches of sediment between carbonate plates, an abundance of bacterial mats and detectable hydrogen sulfide concentrations in the bottom water. The carbonates appear to be a barn for bivalves and snails as well as for a variety of polychaetes and tubeworms. However, all large shells found on the seafloor were empty, suggesting fluctuations in the activity of the seeps.



In situ Profiler measuring oxygen, sulfide, pH and temperature with microelectrodes in seep sediments of the central area

For the friends of sulfide-oxidizing bacteria we found a large zone of about 60x100 m of exposed, very flat sediments, which are blackish and highly gassy. This area of the seafloor is partially covered by sulfur precipitates. Looking at them under the microscope we recognized the typical morphology of sulfur filaments produced by *Arcobacter*, a tiny bacterial cell, which produces enormous masses of sulfur by oxidation of the sulfide, which seeps from the gassy sediments. Another area with high backscatter further west was not active, but displayed numerous pockmarks of 1-5 m diameter, often showing outcropping carbonate crusts at their bottom (unfortunately also very often plastic debris and other signs of human traffic).

Finally, we used one dive to explore the western region of the Nile delta, namely one of the highly active mud volcanoes of a huge caldera of more than 8 km diameter. The so-called Chefren mud volcano hosts a large and very deep brine pool in its center, which leaks sulfidic brines laterally at the rim of the mud volcano at 3030 m water depth and forms interesting and highly colourful microbial mats at the surface of the seafloor. We were surprised to see major changes in the morphology of the brine pool within the 3 years between the last visit with RV L'Atalante and the French submersible Nautilie. Unfortunately, pushcore sampling with the ROV was impossible due to the very fluidic muds and the association of our target sites with very steep slopes.

Meanwhile we are steaming back to Heraklion (Crete) against wind and waves, to exchange part of the scientific crew. The second leg of BIONIL will be dedicated to geological and biological sampling, hence we will say good-bye to our AUV team. We are very proud of the excellent results obtained with Aster^x, which had completed its deepest dive at 2100 m almost flawlessly (if there had not been this moment of accelerated heart beats when the acoustic contact stopped for 30 min).

Looking forward to a day and night in the old port of Heraklion, with best greetings to all colleagues, friends and families on land

– the BIONIL team



Expedition M70/2b

3. Weekly report (06.11.-12.11.06)

After a day and night in Heraklion, Crete, for the exchange of scientific crew and equipment, we started the second leg of the BIONIL expedition with 14 new scientists on board, including biologists of Ifremer and the University of Paris 6, geochemists of the University Utrecht and NIOZ, and geologists of Ifremer and the National Institute of Oceanography and Fisheries of Egypt. It was quite sad to say goodbye to the fabulous AUV team and our colleagues from Ifremer, GeoAzur, MARUM and MPI. But we have a new goal, to visit the same sites as on the first leg again, this time focusing on biological and geological sampling and in situ geochemical and subsurface temperature measurements. First we had to go around the island of Crete to fuel up, luckily with excellent weather allowing spectacular views on the coastline. The new equipment was installed within a day, and we started the first dive of the second leg on the 9 November at the mud volcano Amon. Everyone agreed to go back to the “sulfur river” at the bottom of the mud volcano to get in situ measurements of sulfide and oxygen fluxes as well as temperature and pH. The second dive was dedicated to deployment of colonizers as well as blade core sampling next to bivalve accumulations and retrieval of carbonate crusts from the western rim of Amon.

Fig. 1 Animals and crusts of the Amon seeps



At first sight, the deep sea of the Eastern Mediterranean looks quite poor in life, even at its cold seep systems, but the diversity is in the detail. The carbonate crusts surrounding the sulfidic sediments of Amon are populated by many different animals: gastropods, anemones, polychaetes, sipunculians, small sponges, and also by chemosynthetic bivalves. Sampling of a few crusts provided 24 small mussels (*Idas* sp) (Fig. 1a) and 6 lucinids (*Lucinoma* aff. *kazani*), both most likely hosting sulfide-dependent symbionts. Interestingly, half of the mussels and one lucinid harboured small white worms (up to 2 cm long, very thin), most likely a parasite. We also collected a beautiful sea urchin (Fig. 1b) named “Benedicte” after an excellent new HERMES student working day and night with smelly muds and crumbly crusts. Furthermore, our biologists were very pleased when a giant shrimp was recovered alive from one of the gravity cores taken at Amon. We suspect that this critter is the cause of zillions of biogenic mounds covering hundreds of square meters around the active center

of Amon, but we certainly have to test this hypothesis with further box core sampling.

But also the geologists got lucky with the first two dives and the gravity coring in between: A spectacular carbonate tower (Fig. 2) was discovered in the southwestern part of Amon mud volcano, in connection with the NW-SE fault visualized with the AUV multibeam dive at

Amon. This massive carbonate deposit could be nicknamed ‘pillow carbonate’, because it exhibits very dark and rounded shapes, which resemble pillow lavas. During the dive, we first thought for a moment that it might correspond to an asphalt deposit, similar to those observed in the Gulf of Mexico. But the analysis of the retrieved sample showed that the entire structure corresponds to numerous small nodule-shaped aragonite chimneys coated by manganese-rich oxides. At Amon we are looking at an enormous variety of shapes and colors of carbonate crusts. Our first results from in situ measurements indicate that part of the area may be influenced by brine and sulfide seepage.



Fig. 2 Carbonate towers on Amon mud volcano

And finally we are proud to report the subsurface temperature record for the Nile delta mud volcanoes of over 70°C at 6 m depth below seafloor in the center of Amon mud volcano. Fig. 3 shows the sampling party of this very hot core. But even more interesting than records are observations helping to understand temporal changes at such deep-water seeps. A gravity core equipped with T-probes taken at the same position as 3 years ago during the NAUTINIL cruise showed a doubling of temperatures from 33° to over 65° , showing that these geo-systems undergo extreme fluctuations. Now, as I write, we are in the middle of the third dive at the Amon sulfur band and have just discovered large patches of giant tubeworms and mytilids associated with a thin, highly sulfidic mud-flow above flat carbonate crusts. This gives a perspective on the time it may take to get a good overview of these highly fractured ecosystems – three 15-hour ROV dives on an area of 100×100 m, and we still find new habitats.

Fig. 3 Sampling gravity cores of Amon mud volcano

Looking forward to the next dives and more discoveries – the BIONIL team



Expedition M70/2b – BIONIL, RV METEOR

4th weekly report

13.11.-19.11.2006

The last dive at the Amon mud volcano brought spectacular findings with regard to cold seep ecosystem studies. By chance we discovered two large patches of living tubeworms on the sediment. Contrary to what is observed in the Central pockmark area, these tubeworms were not encrusted and were associated with highly sulfidic, dark sediment. Several specimens were collected and to our surprise, many were still alive back on board. Four were placed in a tube containing water from the bottom, and the concentration of oxygen and sulphide was monitored for 2 days. During the experiment, worms were shown to uptake sulphide rapidly, probably oxidizing it to inorganic sulfur, and as a result an increase in their oxygen consumption was observed. Thiosulfate and methane were not uptaken. Back to the lab we want to identify the symbionts and their characteristics, and to design experiments for the next cruise!



Fig. 1 Sulfide uptake experiment with living tubeworms.

Fig. 2 Tubeworms growing on sulfidic sediment



The gravity coring program was also continued with highly interesting results. These mud volcanoes are simply puzzling. Whereas the temperatures at Amon were extremely elevated in comparison to earlier studies, Isis showed similar temperature profiles. The investigation of the 3 different centers found with help of the AUV Asterx Bathymetry Map showed that these centers are independent of each other, and temperature gradients decline to background values in between the centers. Presently, pore water of low salinity and of deep origin arrives to close to the sediment surface at Amon mud volcano but is below 2 m at

Isis. Associated to the low salinity deep origin of the water is enhanced temperature, dissolved carbonate, and gas levels, in particular of methane, the latter being well above the sealevel saturation values. Water samples taken at several places above the sediment, all show values that are at least 1000-fold higher than in normal seawater. The surface expression of mud expulsion activity (bacterial mats, pogonophora, shells) seem more intense at Amon than at Isis, whereas the much lower salinity of porewater below 2 meter points either to a higher past mud expulsion activity or to a deeper source for water and mud at Isis compared to Amon. The 8‰ salinity

found at Isis is rather close to that of the Aachener Kaiserquelle mineralwater (5‰) than to ocean water. The upper part of most sediments contain enhanced sulphide levels as observed by the amount of suspension formation during preservation and fixation reactions for on-land quantitative analyses.

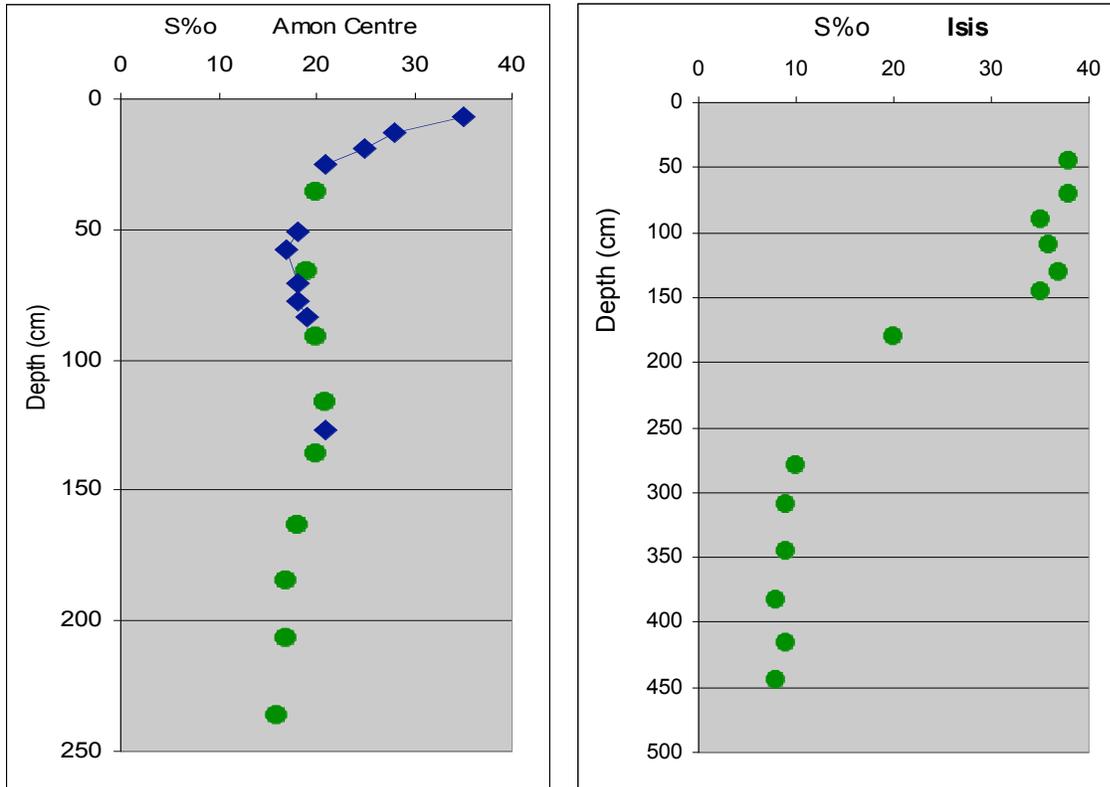


Fig. 3 Salinity versus depth profiles for the Amon and Isis mud expulsion structures; porewater has been extracted by centrifuging (green circles) and by rhizon extraction (blue diamonds)

Most of the second week of the second leg we spent at the central area of the deep Nile Fan, characterized by a flat topography with vast areas of carbonate crusts outcropping on the seafloor associated with highly reduced blackish sediments sometimes covered with whitish bacterial mats in the carbonate free zones. We had a total of 3 dives in this area to do in situ measurements of the biogeochemistry of the seep and to distribute a variety of colonization experiments.

One of the tasks of the in situ work was to investigate the difference in oxygen and sulfur fluxes within and outside the bacterial patches. We deployed the microprofiler with oxygen, hydrogen sulfide, pH and temperature sensors together with the benthic chamber for oxygen respiration measurements and with the planar optode for 2D imaging of oxygen concentration in the sediments (Fig. 4). With the help of the ROV QUEST of MARUM and using the MPI/RCOM elevator system we managed to obtain 2-3 individual measurements for all three of these large instruments within 15 hours bottom time. It was astonishing to see the difference in oxygen consumption with a few meters between the gas fueled benthic community of the black sediment patches and the community in the adjacent sediments not influenced by fluid and gas flow (Fig. 5).

Fig. 4 In situ instruments on bacterial mats. Left: Planar optode with beacon for temperature probe. Right: Benthic chamber. In the front one sees the whitish bacterial mat on blackish sediment, in the background the brownish “reference” sediment

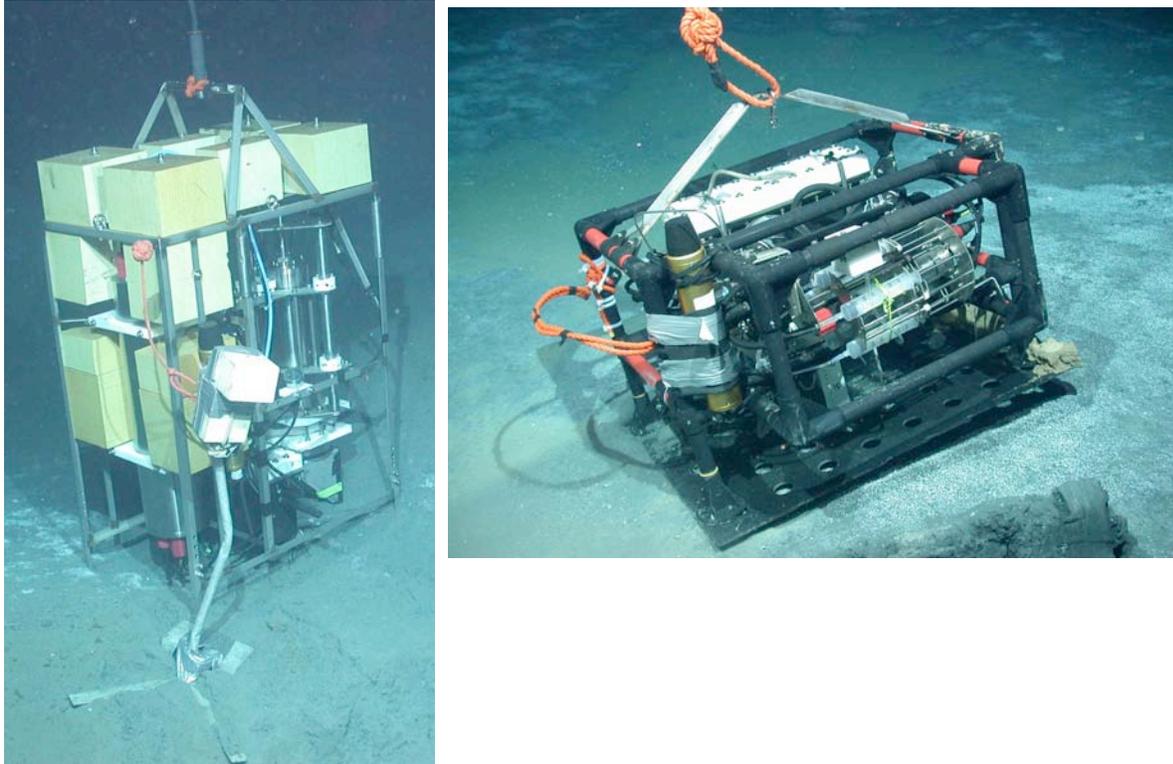
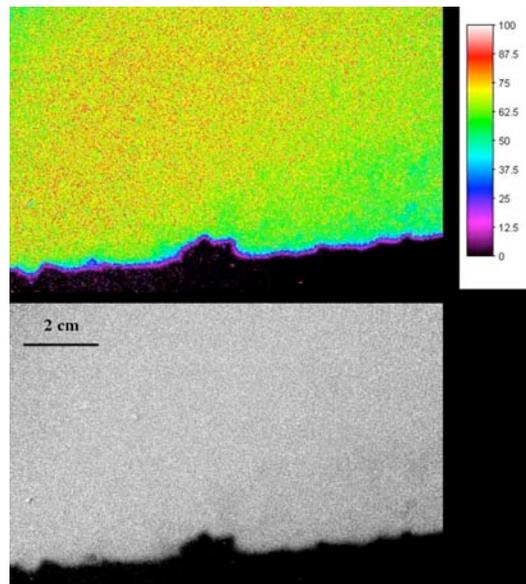
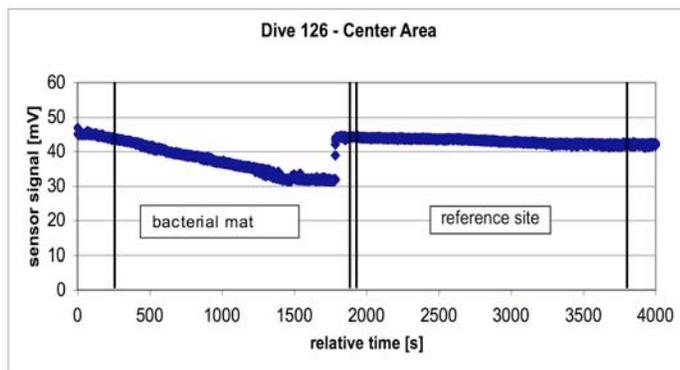


Fig. 5 Oxygen concentration in bacterial mat sediment. Left: Benthic chamber measurements of oxygen concentrations decreasing with time. Right: A planar optode image showing that oxygen does not penetrate into the seafloor below a bacterial mat.



Also the “in situ” chemical profiles within and outside the bacterial mats show a strong change on small scales. Within the bacterial mats, high upward sulfide flux and high consumption of sulfide and oxygen are visible, concurrent with a steep pH decrease (Fig. 6). Oxygen does not penetrate into the sediment inhabited by sulfide oxidizing bacteria. In contrast, oxygen penetrates

deeply in the beige-brown sediments outside of the black patch and sulfide is below detection. The origin of this extreme difference of sediment biogeochemistry is the presence/absence of free gas below 15 cm sediment depth in the black patches, attracting a community of anaerobic methanotrophs and sulfide oxidizers. These achievements made our two “in situ” PhD students Anna and Janine of the project MUMM very happy (Fig. 7).

Fig. 6 Chemical profiles obtained with the in situ microprofiler within and outside bacterial mats

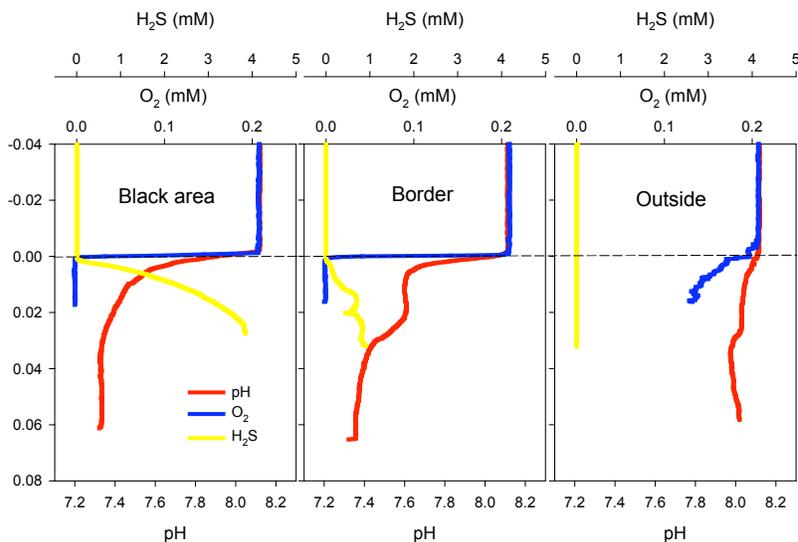


Fig. 7 “In situ” PhD students working hard on board METEOR. Above: Janine Felden programming the planar optode waiting for deployment with the MPI/RCOM elevator. Below: Anna Lichtschlag discussing technical improvements of an instrument with the ship’s blacksmith



The question remains as to which organisms are responsible for transport processes and fluxes leaving such visible imprints on the sediment chemistry. We have collected highly interesting samples from the seafloor, which will allow us to investigate the diversity of benthic microorganisms and animals in great detail, focusing on differences in community structure with regard to small-scale niches in cold seep ecosystems. We are especially fascinated with the immense diversity of sulfide oxidizing bacteria (Fig. 8), and also the impressive adaptation of chemosynthetic bivalves and tubeworms (Fig. 1) to utilize unusual sources of energy in the deep sea, such as methane and sulfide.



Fig. 8 Image of different species of giant sulfide oxidizing bacteria recovered from a black sediment patch. The largest filaments have widths of about 0.1 mm. This patch has been discovered by Alina Stadnitskaia of NIOZ in one of the geochemistry cores – to our surprise at 5 cm sediment depth.

Currently we have started our very last dive of the BIONIL cruise (QUEST dive 128) at the central pockmarks. It is a bit sad to stop diving, because we have discovered a highly active fault system east of the working area (again, thanks to the excellent Asterx map and the good nose for seafloor structures of Stephanie Dupre of IFREMER and Univ. Amsterdam), which shows a beautiful stratification of carbonate cements cracked open by seafloor expansion. Also, the crusts are densely populated by tubeworms, so we put some extra colonization experiments near this area for the future HERMES cruises to the Eastern Mediterranean (Next year we will be back with the MEDECO cruise!). The last 2 working days of the cruise will be dedicated to coring, and measuring the temperature in the enigmatic deep brine pool of the Chefred mud volcano before we steam back to Heraklion.

With our best greetings from board of the RV METEOR

The BIONIL team