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 specimen 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.

![Graph showing salinity versus depth profiles for the Amon and Isis mud expulsion structures](image)

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 Chefren mud volcano before we steam back to Heraklion.

With our best greetings from board of the RV METEOR

The BIONIL team