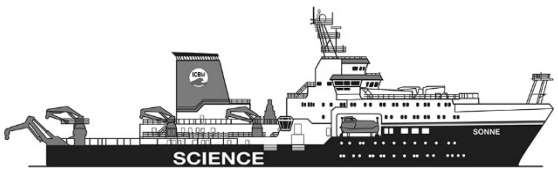


SO268/2

4th Weekly Report

21–27 April 2019

After finalising the baseline studies in the Belgian contract area on Tuesday evening, we headed back to the German contract area. The transit of 540 nautical miles (about 1000 kilometres) took us roughly two days, during which we charted the seafloor below the ship in high resolution using the ship's multibeam echosounder system. Scientific participants used this time to further process the collected samples and data, to repair and maintain equipment and to plan the work of the remaining 3 weeks in detail.

Before we returned to the site of our eddy-dredge experiment, we stopped at a site, where polymetallic nodules are absent at the seabed. Here, we deployed a second set of the restoration experiment of the colleagues from NIOZ (see 3rd report of leg1). This "no-nodule" site will serve as a control site for the recolonization experiment deployed in the German trial area. One more set of twenty-six nodule frames will be deployed at the dredge impact site next week in order to complement the experimental setup. The experiment is laid out to be revisited over the coming decades to investigate, if the chosen artificial hard substrates are suitable to aid recolonization by fauna associated to manganese nodules.

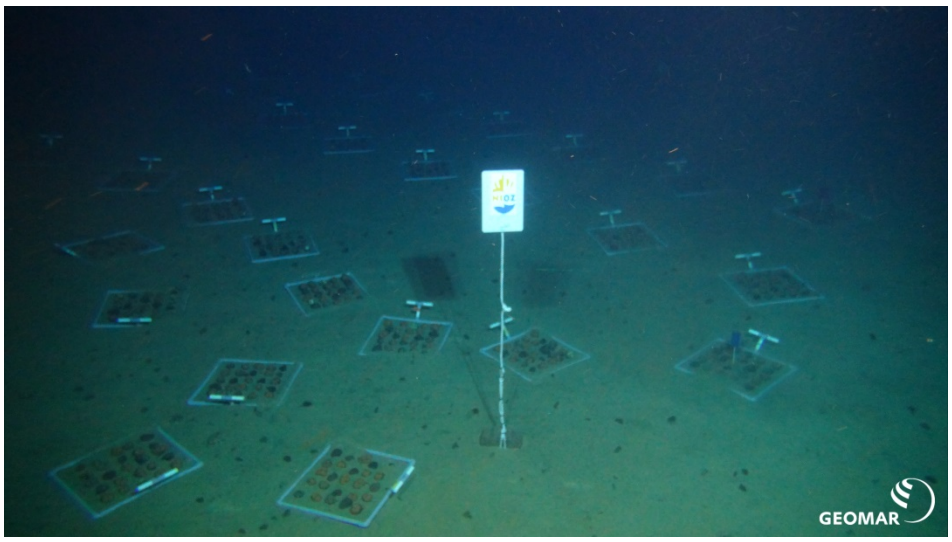
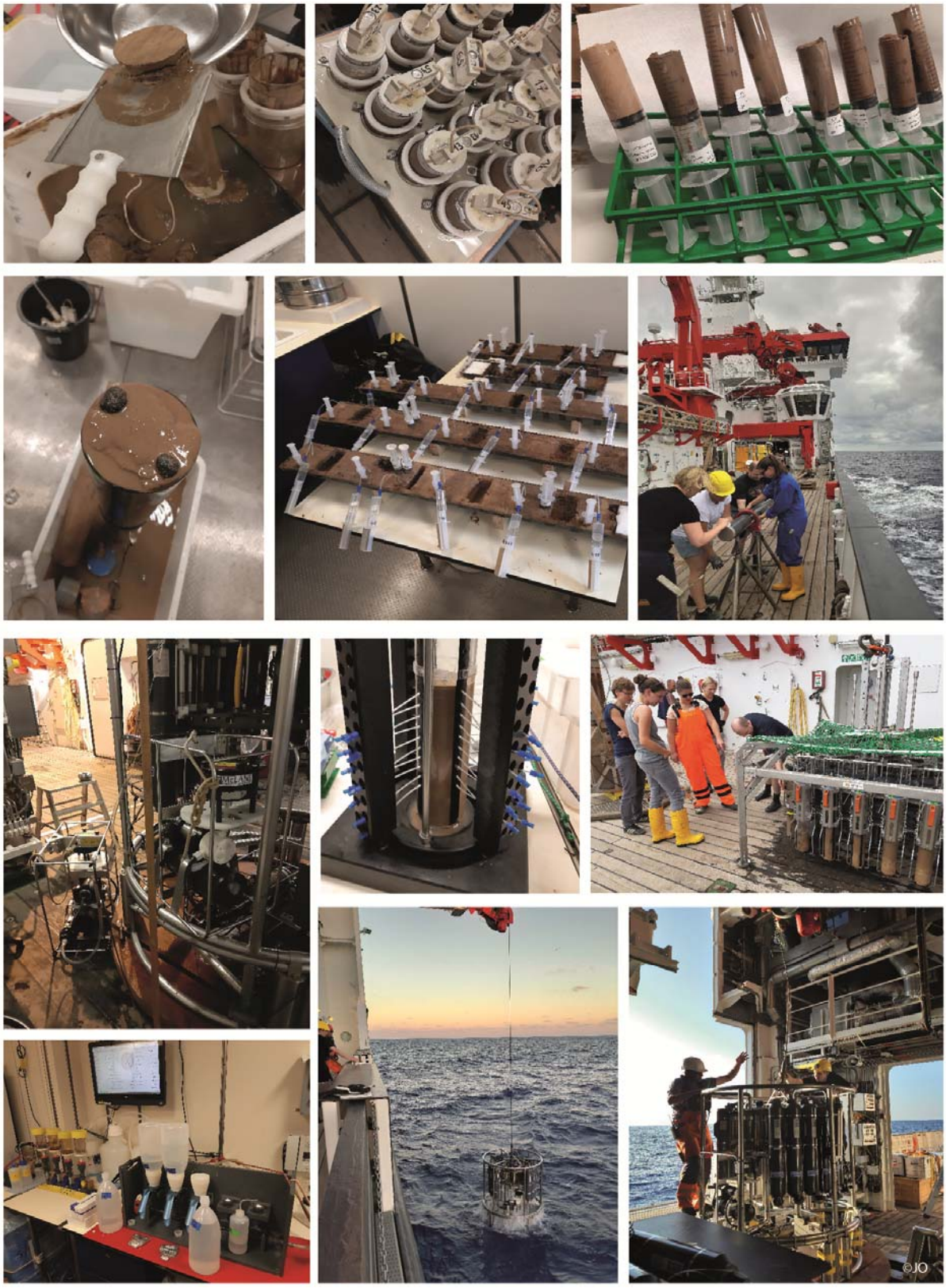


Photo (ROV Kiel 6000): Deployed nodule frames of the NIOZ recolonization experiment in the "no-nodule" area.

On this voyage, the colleagues from MPI Bremen collect seawater, sediment and nodules from the study sites to analyse them for microbial communities and associated metabolic variables. Seawater is sampled with 12-L Niskin bottles from CTD rosette casts. These water samples are processed for cell counting (DAPI and fluorescent *in-situ* hybridizations (FISH)), DNA extraction (for molecular ecology studies), characterization of dissolved organic carbon, and quantification of particulate organic carbon. Extracellular enzymatic activity and oxygen measurements are performed on board. To retrieve large amounts of microbial cells for transcriptomic and genomic analysis, CTD rosette casts are equipped with pre-programmed

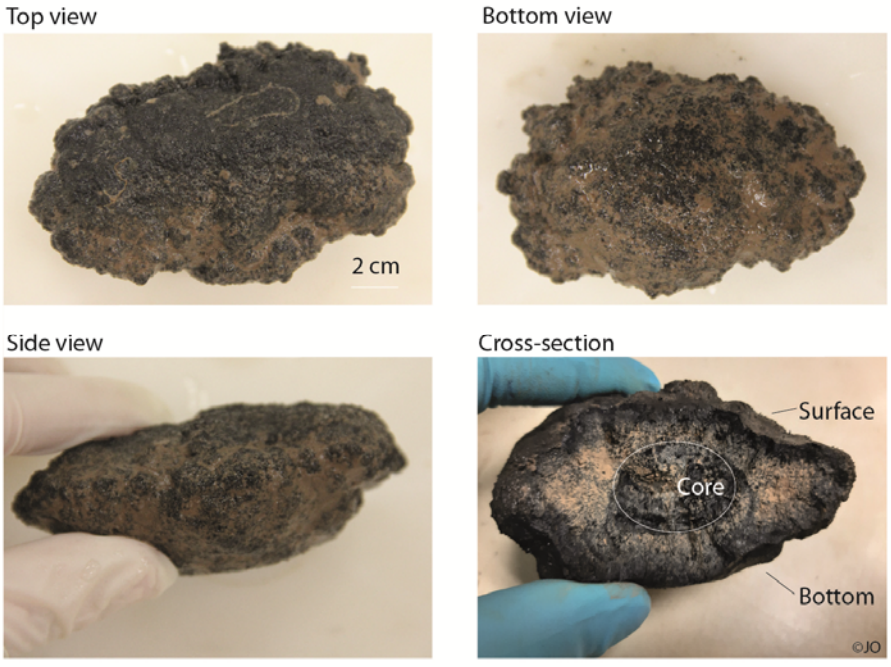
in-situ pumps, which are equipped with 0.2 µm polycarbonate filters. The pumps are operated at two water depths, 5 m and 20 m above the seafloor.



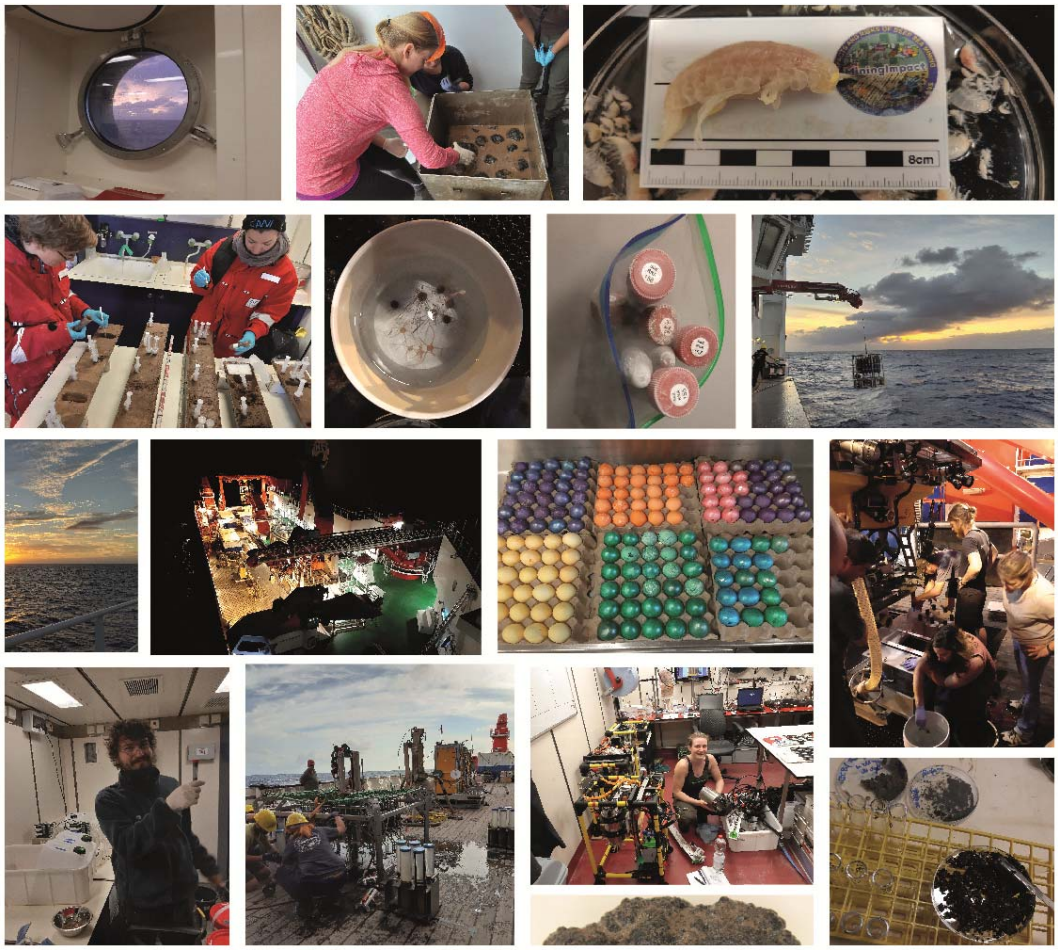
Photos (Julia Otte): Sediment sampling from MUC deployments, gravity core sampling and seawater sampling with CTD casts.

Sediment samples are retrieved by 20-cm long push cores, taken by the ROV, and 50-cm long multiple cores. The sediment cores are sliced into layers at 0-1 cm, 1-2 cm, 2-3 cm, 3-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm depth. These subsamples are then preserved for later measurements (at MPI laboratories) for phytopigments, porosity, phospholipid composition, biochemical composition of organic matter, viral abundance and production, as well as molecular analyses of extracellular DNA. Additional samples are preserved for DNA and RNA extractions to investigate microbial community composition in the deep-sea sediment. Further samples are fixed with formaldehyde for total cell counts via the acridine orange direct cell count method and FISH. Sediment aliquots are also taken for analyses of extracellular enzymatic activities like Aminopeptidase and β-Glucosidase. Deeper sediment layers are sampled from gravity cores. First results show that 150 cm below the surface, substrate conversion is not measurable anymore with these methods indicating an organic poor sediment and lower abundance of sedimentary microbial community.

In addition to water and sediments, the MPI colleagues are also interested in the microbial community living on and within the manganese nodules. A total of 11 nodules were collected this week by box coring and during ROV dives to determine microbial diversity and potential organic matter degradation. The nodules are washed gently with 0.2-µm filtered bottom seawater at *in situ* temperature and are stored in sterile plastic bags at -20°C after subsampling. For subsampling the nodules are divided into three layers: the upper layer exposed to seawater, the bottom layer buried in the sediments and the inner core (after cutting the nodules). From each layer fragments are taken for DNA, RNA, and FISH analyses. Extracellular enzymatic activities of β-glucosidase, chitobiase, aminopeptidase, and esterase are measured onboard via assays with fluorescent substrates. First results show that extracellular enzymatic activities are higher in the upper nodule layer and similar to that measured in the sediments.



Photos (Julia Otte): Manganese nodule collected for characterization of associated microbial communities.



Photos (Julia Otte): Some impressions of this week's work: Nodule sampling, Amphipods characterization, gravity coring, Fauna sampling by ROV Kiel6000, sampling for RNA and DNA, CTD deployment, SONNE by night, Easter holiday, MUC deployment, Rebuilding sensors, and manganese nodule subsampling.

Today we arrived at the dredge impact site again, where we first deployed a CTD to continue characterizing the eddy, which is now already passing over the German contract area with its central part (50-100 kilometers in diameter). In the coming weeks, we will now sample the dredge experiment and finalize the baseline work in the German area.

On behalf of all SO268 participants,

Matthias Haeckel