



SO268/2

5th Weekly Report

28 April – 4 May 2019



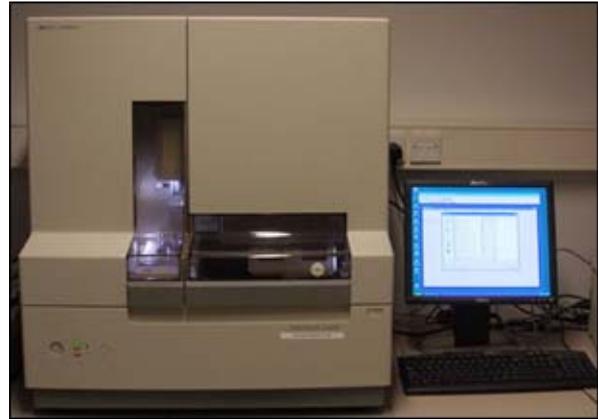
This week started with a ROV dive to finish up the remaining sampling and in situ measurements of benthic oxygen consumption in the German trial area. Subsequently, the seafloor habitat was mapped by the OFOS video sled and a further ROV dive. At the end of this dive the elevator, a lander system used to bring down our in situ equipment to the seafloor, was planned to be released, i.e. the weight keeping it down at 4200 m is dropped upon an acoustic signal from the ship or manual operation by the ROV, after which the lift starts to ascent to the sea surface due to its buoyant floats. Unfortunately, due to currently unknown reasons these manoeuvres failed repeatedly. Another ROV deployment is now necessary to connect a second cable lowered from the ship to the elevator and heave it back on deck. However, this dive has to be postponed by a week, because we need to return to Manzanillo due to a medical emergency.

For the duration of the ROV dives (and sometimes also between two dives) amphipod traps (baited to attract deep-sea scavengers) are placed on the seafloor. Despite the short deployment during the dive in the German trial area, it was a very successful catch with the model species *Eurythenes sigmiferus*, *Paralicella caperesca* and *Abyssorchomene gerulicorbis* present. Onboard, these were sorted, photographed, measured and dissected for later heavy metal (cadmium, mercury and lead) analysis and DNA extraction using the Qiagen Protinase K enzyme.



Photos (Tasnim Patel): (left) Elevator that is used to deploy the amphipod traps on the seafloor; (right top) amphipods sorted in different size classes; (right bottom) amphipod model species *Eurythenes sigmiferus*.

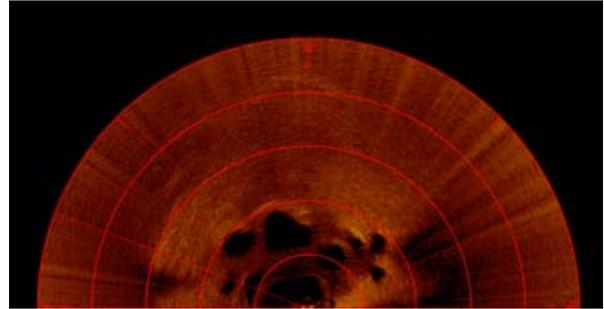
During DNA analysis, the target Cytochrome Oxidase I region of the mitochondrial gene is exponentially amplified to create billions of copies. This process is called Polymerase Chain Reaction (PCR). PCR amplifications are conducted in a T personal Thermoblock (Biometra) in 25µl volumes with the Qiagen HotStar Mastermix (1.5–2 mM MgCl₂, 200µM dNTP, Tris·Cl, KCl, (NH₄)₂SO₄, 1.25 U Taq) and 0.1µM of each primer. Primers are keys which bind to the locks on the 3' and 5' ends of the DNA strands, thus kick-starting the amplification process. These amplified PCR products are then purified enzymatically with Exo-SAP IT™ (Affymatrix™) and sequenced directly in both directions using an ABI 3130x1 capillary DNA sequencer.



Photos (Tasnim Patel): (left) Thermoblock and (right) DNA sequencer.

As part of the Mining Impact project, the genetic connectivity of the benthic amphipods in the Clarion-Clipperton Fracture Zone (CCZ) in the NE Pacific are being investigated and compared to populations in the Peru Basin. The DisCOL Experimental Area (DEA) is an area of the Peru Basin which was artificially disturbed in 1989, and now serves as a proxy for the future effects of deep-sea mining on benthic biodiversity. It is being used to predict how these benthic populations would be affected in the long-term, and what measures need to be taken to protect them, especially important with the impending manganese nodule mining activities planned for the CCZ. Morphological approaches combined with mitochondrial COI and 2b-RAD-Seq DNA analysis will be used to estimate the diversity and genetic connectivity of the different populations within these two oceanic basins. The results thus far, show that the amphipod diversity in the DEA has possibly been negatively affected.





Photos (ROV Kiel6000): The morning of 29th was an ROV dive dedicated to video transects (4.3 km in length, flying 1 m above the seabed). Here, we observed the Bolocera anemone (top left) and outstanding basalt features (top right) and depressions (bottom left), which look particularly striking on the sonar image of the ROV (bottom right).

The transit time was used to plan the sampling of the dredge experiment in detail and to process our samples and data. At the moment we are back on our way from Manzanillo to the German working area for recovery of the elevator and continuing our tasks.

On behalf of all SO268 participants,

Matthias Haeckel