JPI-Oceans

MiningImpact 2

“Environmental impacts and risks of deep-sea mining”

Final report

August 2018 – February 2022

Table of Contents

Acknowledgements

The European collaborative project MiningImpact is funded under the framework of the Joint Programmining Initiative Healthy and Productive Seas and Oceans (JPI Oceans) by the national research ministries from Belgium, Germany, Norway, the Netherlands and Portugal.

Summary

While the first project phase of MiningImpact (2015-2017) could investigate only rather small-scale disturbances of the seafloor from benthic impact experiments conducted in the 1990s, the second project phase (2018-2022) delivered a comprehensive and independent scientific monitoring of the first industrial trial of a pre-prototype collector vehicle for mining of polymetallic nodules by the Belgian contractor DEME-GSR.

The main research topics that addressed with the project were to understand better (1) the larger scale environmental impact caused by the suspended sediment plume, (2) the regional connectivity of species and the biodiversity of biological assemblages and their resilience to impacts, and (3) the integrated effects on ecosystem functions, such as the benthic foodweb and biogeochemical processes. The project specifically worked towards transferring scientific results into usable policy recommendations, particularly for the Mining Code drafted by the International Seabed Authority (ISA). In this context, key objectives were:

- to develop and test monitoring concepts and strategies for deep-sea mining operations;
- to develop standardization procedures for monitoring and definitions for indicators of a good environmental status;
- to investigate potential mitigation measures, such as spatial management plans of mining operations and means to facilitate ecosystem recovery;
- to develop sound methodologies to assess the environmental risks and estimate benefits, costs and risks;
- to explore how uncertainties in the knowledge of impacts can be implemented into appropriate regulatory frameworks.

These overall aims were reflected in the project structure: the 3 main work packages addressed (WP1) the biodiversity, connectivity and resilience of biological assemblages, (WP2) the impact and behaviour of the sediment plume, and (WP3) benthic ecosystem functions and processes. The 3 cross-cutting themes ensured integration of (CCT1) the different aspects into a coherent work flow at sea to accomplish an effective monitoring of the collector trial, (CCT2) of the scientific results into a comprehensive assessment of the environmental impacts, and (CCT3) into joint policy recommendations on risks and best practices of deep-sea mining operations. WP4 facilitated data exchange and archival in the project and develop advanced video/photo annotation technology and WP5 coordinated the project activities and facilitated effective communication and dissemination of project results.

As in the first project phase, MiningImpact 2 has demonstrated the strength of joint European actions. The comprehensiveness and effectiveness of the conducted work at sea was only possible by bringing together the state-of-the-art technology, infrastructure, and expertise that exists in the European marine research institutions. This was key to gaining a better understanding of the complex interactions and process couplings in the deep sea, which in turn is a pre-requisite for developing useful recommendations for decision-makers.

The field work comprised of two expeditions in 2019 and 2021, with a total of more than 150 days at sea. During the first expedition, SO268, the project conducted comprehensive baseline investigations in two exploration contract areas for polymetallic nodules in the Clarion-Clipperton Zone (CCZ), where the first industrial pre-prototype of a nodule collector vehicle was going to be tested by the Belgian company DEME-GSR. In each area, the BGR and the GSR contract area (in the following abbreviated as GER and BEL), a trial site and a reference site were chosen based on data available from both contactors and our own investigations in the first project phase (SO239). Originally, GSR had planned to test its Patania II collector system in parallel to the SO268 expedition, but they had to call off the test due to unresolvable technical problems with the umbilical cable, while SO268 was already ongoing. This unforeseen incident made a second expedition necessary in order to achieve the main project goals. BGR was able to allocate own resources for exploration work to charter a commercial vessel and invited MiningImpact partners to join the expedition and deliver an independent scientific monitoring of the collector trials. This cruise, IP21, finally took place in spring 2021

after further delays related to the world-wide Covid-19 pandemic. The post-impact monitoring will continue with a third expedition, SO295, in October-December 2022.

With the successful campaigns SO268 and IP21, MiningImpact 2 has demonstrated the fundamental suitability of available monitoring technology and the feasibility of executing an efficient monitoring strategy for deep-sea mining operations in the relevant industrial context and in the deep-sea environment (equivalent to a technical readiness level of 6), thus setting the standard for future projects and contractor work. This has provided, for example, the first quantitative data on the removal of seafloor habitat, the spreading of the suspended sediment cloud and its re-deposition on the seabed. In addition, the first measurements of noise pollution of a collector system in the deep-sea environment have been recorded. The project has also documented the spatial variability of environmental conditions and the ecosystem from small spatial (10-100 m distance) to medium (several km) and regional (hundreds of km) scale. This variability needs to be accounted for in baseline and monitoring strategies as well as environmental management of future mining operations, e.g. when defining meaningful preservation reference areas (PRZ) and conservation areas. New methodologies for investigating abyssal ecosystems and their functions have been developed, e.g., rapid assessment tools for biodiversity analysis, such as metabarcoding and proteome fingerprinting, or new in situ analytical methods for determining speciation and bioavailability of metals, such as DGT (diffusive gradient thin-films) passive samplers, have been applied that allow an assessment of toxicity. In situ experiments and modelling have generated first data on carbon flow and food-web interactions and how they are impacted by the seafloor disturbance. Just to give a few arbitrary examples.

The leading expertise of MiningImpact researchers and our novel results received further global recognition from all relevant stakeholder groups (ISA, ISA-LTC, contractors, national authorities, NGOs, mining-interested industry). Through regular information events, we have ensured the necessary transparency that acquired acceptance of our work. This also helped us to achieve one of the main project goals, i.e. an effective transfer of our results into recommendations for the ISA Mining Code. In fact, the ISA-LTC nominated several researchers of the project into an expert group drafting the Guidelines for Baseline Investigations (ISBA 27/C/5) and the Guidelines for Environmental Impact Statements and Assessments (ISBA 27/C/11). In addition, our results also contributed to the establishment of four new APEIs (Areas of Particular Environmental Interest) in the REMP (Regional Environmental Management Plan) of the CCZ (ISBA 26/C/43).

In the following sections, the key results of the work packages and some research highlights are presented, and in the cross-cutting-theme chapters integrated scientific conclusions are provided with a specific focus towards policy recommendations.

WP 1 – Biodiversity, connectivity, resilience

M1.1 Sampling and survey activities completed, and metadata supplied to data archive (IMAR)

Information on all sampling actions and surveys carried out during SO268 and IP21 within the framework of WP1 were compiled. Metadata was standardized and completed as well as information on the sharing on samples and which institute got which sample.

M1.2 Sequencing and imaging data submitted to public data archives and shared among project participants (UAveiro)

Submission of molecular data is completed for SO268, but still ongoing for IP21 due to the delayed collector trials.

M1.3 Benthic assemblage biodiversity data across size classes disseminated to CCT2 for integrated analysis (UGhent)

UGhent was responsible for providing abiotic parameters (TOC, TN, granulometry, pigment) and meiofaunal data (morphology- and DNA-based) within the context of WP1. The abiotic data were analyzed for both BGR and GSR while only the meiofaunal data of the latter were processed by UGhent. The morphological data consisted of meiofauna counts at higher taxon level while the DNA-based data were Nematoda Amplicon Sequence Variants (ASVs) derived through metabarcoding of the 18S v1-v2 region.

D1.1 Connectivity workshop and report (NIOZ)

NIOZ and UGhent organized the connectivity workshop online on 15-17 February 2021 and collated a report of it. The topic of best DNA (meta)barcoding practices was combined with that of connectivity. Barcoding and metabarcoding protocols as performed by different partner institutions were presented as well as their respective genetic inventories for the taxonomic groups Nematoda and Copepoda. Additional High Throughput Sequencing methodologies such as 2bRAD were also presented. Finally, the means by which sequence data can be shared amongst partners was illustrated with the use of Barcode of Life Database (BOLD) taxon-specific datasets. The connectivity of different faunal groups (e.g., Nematoda, Amphipoda, Copepoda, Isopoda), as assessed by these HTS methodologies, were presented. Overall, the overarching conclusion was that connectivity, in the form of shared genetic units, is very limited, thus making each sampling location unique with respect to its genetic diversity.

D1.2 Comprehensive check list for the study area, including data on taxonomy, distribution, biogeography, ecology and life history traits (UAveiro),

This deliverable could not be achieved yet because the integrated taxonomic work on the specimens collected during the project was severely affected by the delayed collector trials and sampling activities.

D1.3 Report on the usefulness of molecular methods and protocols for biodiversity assessments and environmental monitoring, including metabarcoding, eDNA and ddPCR methods, and proteome fingerprinting for rapid biodiversity assessment (SGN)

The report was integrated in D1.1.

D1.4 List of sensitive versus persistent species, report on the analysis of Plenaster as monitoring model species (UResearch)

This deliverable was not accomplished so far. Partner UResearch is still working on it.

D1.5 Report on connectivity of selected key-species Report on connectivity of selected key-species (UGhent)

The report was integrated into D1.1.

Summary of Achievements

The seafloor sampling and imaging activities during the cruise SO268 in spring 2019 were successfully carried out and provided samples for a baseline assessment of the benthic assemblages (mega-, macro-, meiofaunal, microbial and prokaryotic) of the two study areas (BGR and GSR). The baseline study showed relevant variability in the benthic assemblages (e.g., overall faunal densities, taxonomic composition) at different spatial scales (between the study areas, between the reference and trial sites, and between replicate sampling locations). The dredge disturbance experiment that was carried out because of the collector failure allowed sampling and imagery of the seafloor pre- and post- disturbance and enabled the study of direct seafloor and plume-related impacts on the benthic assemblages at a scale smaller than planned with the collector trials. Although some alterations in the benthic habitat and biological assemblages were observed, the results from this small-scale experiment did not allow to adequately scale-up and quantify impacts for more realistic, industrial-scale scenarios.

The collector impact study was carried out during cruise IP21 in spring 2021 and samples were collected to understand the direct and indirect (sediment plume) impacts on the different faunal compartments. Samples of the fauna collected for assessment of ecological impact are still being analyzed, and additional sampling for short term impact and early recovery assessment will take place during the SO295 cruise (end of October to end of December 2022). Preliminary results indicate that benthic assemblages (microbial, meio- and macrofaunal) in the study areas show important spatial and temporal variability. The results also suggest that the plume produced by the collector trial causes a displacement of the fauna, but further investigations are needed to understand re-settlement, survival, and succession and ultimately long-term recovery of the assemblages after disturbance. There is also a need of more replication to increase statistical power and address variability. This will be addressed during SO295.

Overall, the collection and analysis of seafloor images for megafauna biodiversity assessment during the project were an opportunity for the development, improvement, and extension of the web-based marine image annotation software BIIGLE (https://biigle.de; Langenkämper et al. 2017). Software such as BIIGLE is required to make this time-consuming process as fast as possible through collaboration and computer assistance.

We have also shown that molecular methods (e.g. barcoding, metabarcoding proteome fingerprinting) are powerful and promising tools for rapid assessments of biodiversity. However, some technical issues still need to be resolved including species-level identifications, especially because comprehensive and taxonomically reliable barcode databases for deep-sea fauna are currently poorly developed or lacking.

Task 1.1 Megafauna communities and their connection to physical habitat characteristics addressing natural variabilities, disturbance effects, and their temporal evolution (MPI, IMAR)

UBielefeld improved and extended the web-based marine image annotation software BIIGLE (https://biigle.de). BIIGLE now supports the deployment of multiple application instances that can be maintained by different research institutes or during research cruises. Instances can be connected with a "federated search" and data can be transferred between instances using downloadable files. Also, the MAIA (Zurowietz et al., 2018) and UnKnoT (Zurowietz and Nattkemper, 2020a) methods for automated annotation assistance were developed to speed up the annotation process. In collaboration with IMAR/OKEANOS, an annotation study was conducted which found that 86% of interesting objects could be detected using MAIA and UnKnoT, requiring only 57% of the time compared to manual annotation. The collaboration will continue to investigate how the manual effort for training an accurate machine learning algorithm can be reduced. Furthermore, BIIGLE was extended with the first web-based and fully-featured video annotation tool for marine imaging (Zurowietz and Nattkemper, 2021), which, as of February 2022, already supported several annotation studies with more than 1,300 hours of video material and almost 500,000 video annotations. Development will proceed with live-video annotation support. Finally, BIIGLE was extended with an application interface to geographic information systems (GIS). Using the GIS, advanced visualizations can be created, e.g. bathymetric maps overlaid with image locations that highlight the abundance of certain annotated species. Investigations on the compatibility of BIIGLE and the GIS interface with high-resolution bathymetric maps and photomosaics produced by CCT1 is ongoing.

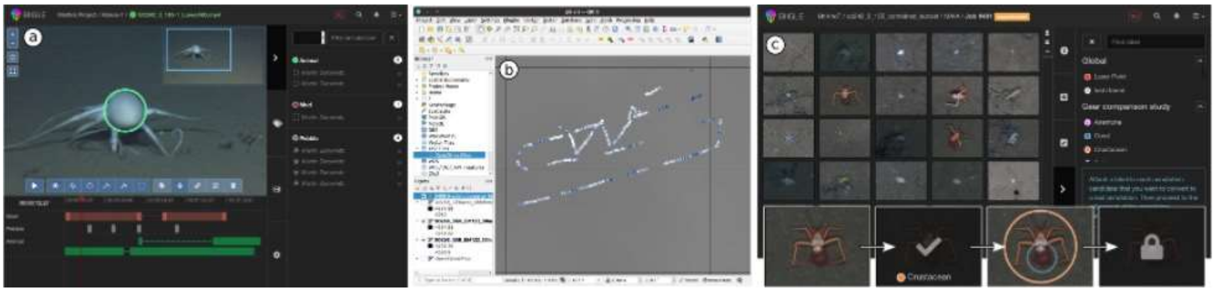

Figure 1.1: A selection of the new features of the BIIGLE annotation software: a) the video annotation tool, b) the application interface to the GIS software, visualizing annotated species abundance, c) the MAIA and UnKnoT methods for automated image annotation assistance.

Baseline study

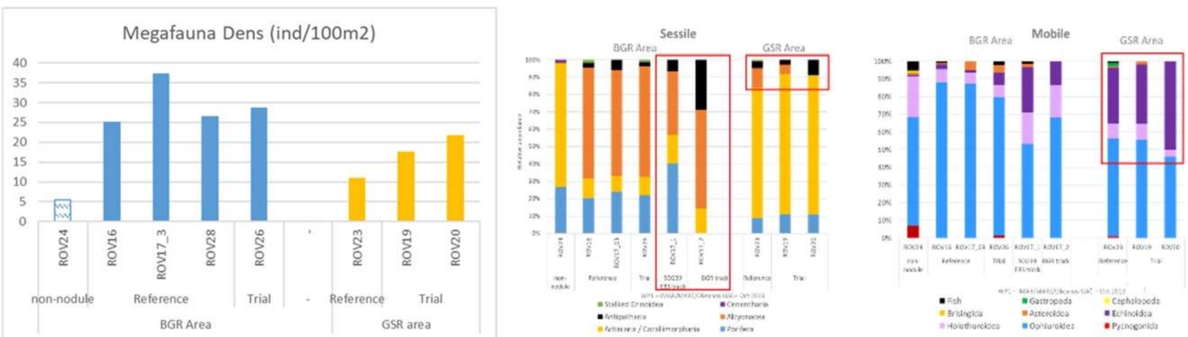

Figure 1.2: (left) Overall faunal densities for systematic ROV transects. Relative taxonomic composition of the systematic ROV transects for sessile (centre) and mobile (right) fauna, grouped per study area (GER and BEL) and study sites (trial and reference, no-nodule) within each area.

Spatial variation. During SO268, 11h of systematic imagery transects were collected by the ROV. Differences and high spatial variation was observed between transects. Taxonomical analyses of the ROV imagery showed variations between the contract areas in overall faunal densities and in taxonomic composition (Fig. 1.2). Overall, the no-nodule transect had the lowest overall densities and a distinctly different taxonomic composition. Furthermore, a distinction could be made in taxonomic composition between GER and BEL transects (Fig. 1.2).

Dredge disturbance experiment

Quantification of impacts on fauna. OFOS transects from the SO268 dredge experiment in the GER area were assessed pre- and post-disturbance in order to learn for the larger-scale nodule collector test. Taxa were annotated as well as the different disturbance regimes and the qualitative sediment cover: i.e., before dredge, undisturbed post dredge, faint cover, thick covered, dredge tracks and old EBS track (Fig. 1.3). With increasing disturbance (faint cover towards dredge track) taxa tend to disappear, both for sessile and mobile fauna. In the 4-year-old EBS track taxa tend to re-appear but in different proportions (Fig. 1.3).

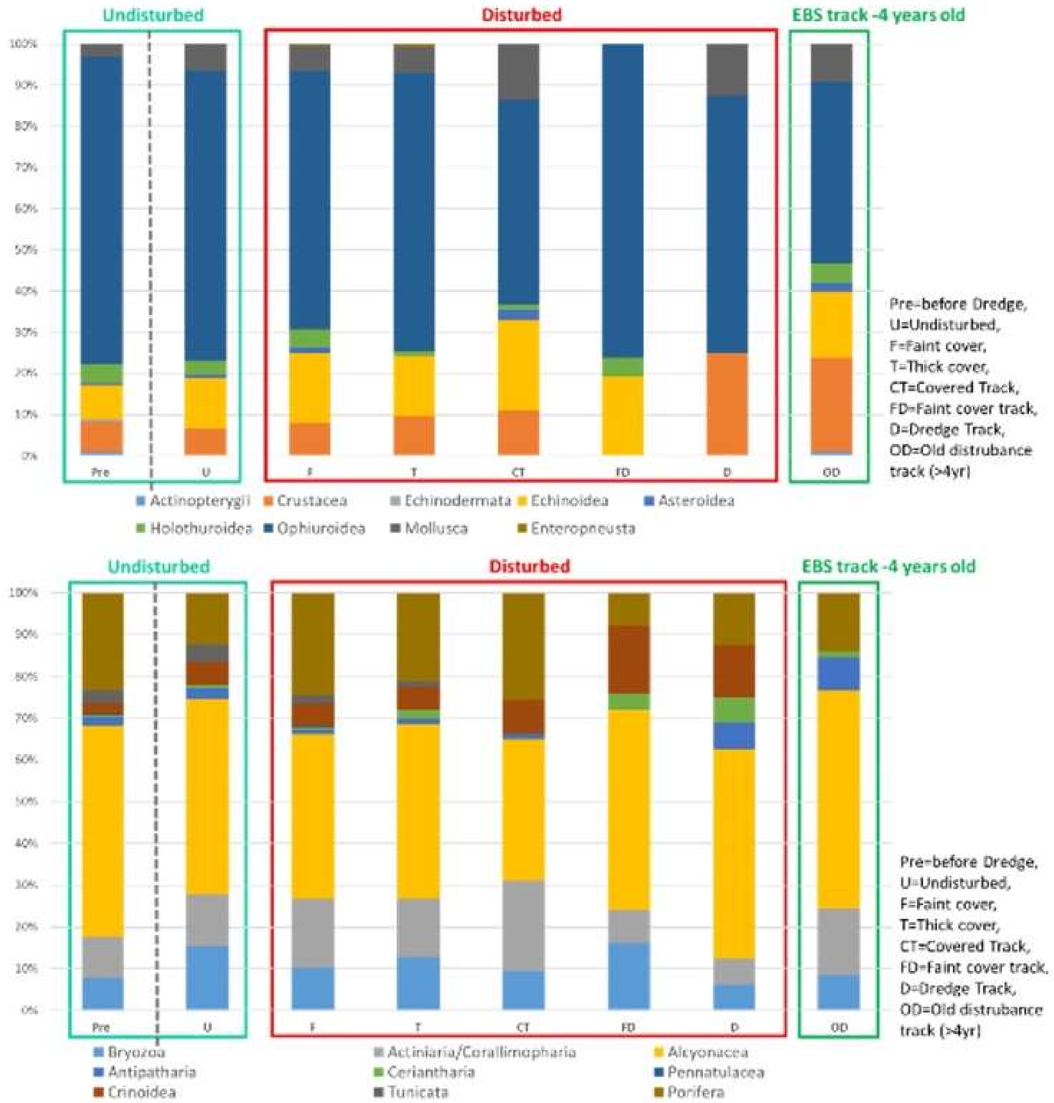

Figure 1.3: Relative taxonomic composition for mobile (top) and sessile (bottom) fauna according to the different disturbance regimes observed pre and post dredge experiment.

The imagery of the dredge experiment also showed that this type of small-scale experiment allowed for, at least short term, fauna survival (Fig. 1.4). The fauna is still present, though disturbed, but it is unclear if they will survive on the long term. This small-scale experiment did not allow to adequately quantify the real impact, because the follow-up time was too short (3 weeks) and the mechanical impact was too small (the dredge disturbance was too shallow).

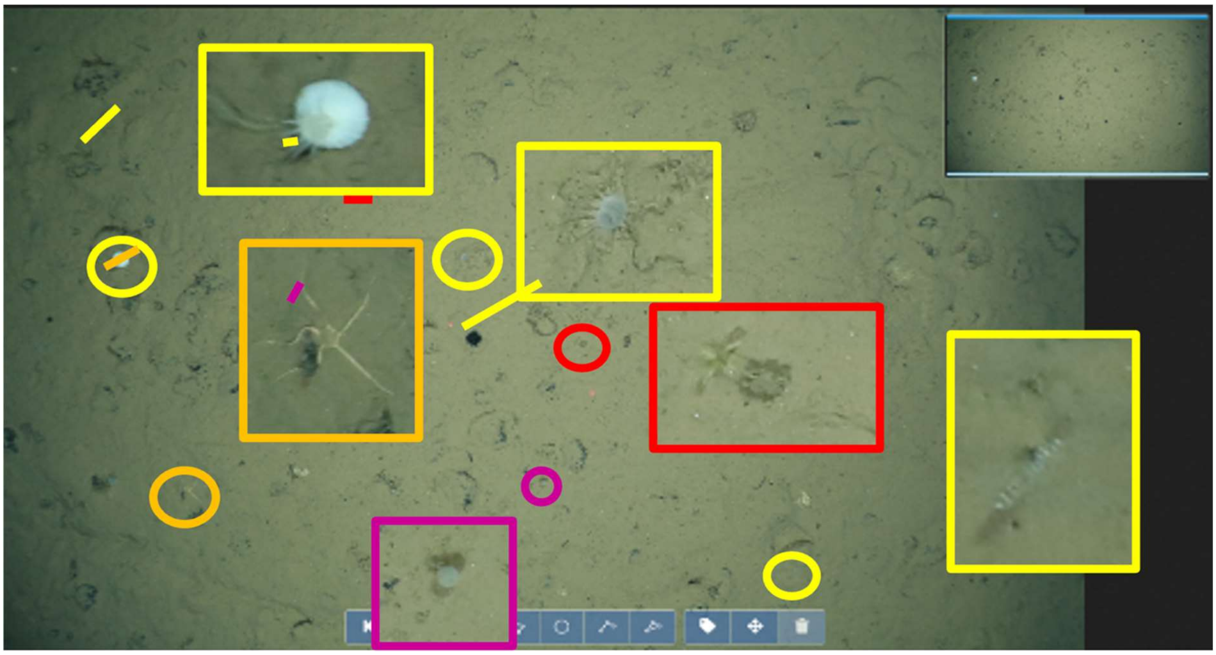

Figure 1.4: Fauna presence 3 weeks post dredge experiment. Yellow: Antozoa (including Actiniaria, Alcyonacea); orange: Ophiuroidea; red: stalked Crinoidea; pink: Porifera.

Recovery. The imagery from 3 OFOS transects from SO268 featuring the dredge experiment (OFOS05 pre-dredge, OFOS11 and OFOS12 post-dredge) were annotated for a specific trace fossil, namely *Paleodictyon* and used to evaluate the recovery of *Paleodictyon* patterns post-disturbance. *Paleodictyon* is a lebenspuren or animal trace which was observed to recover in the actual tracks shortly after disturbance. These interesting observations were included in a paper by Boehringer et al. (2021).

Image quality in fauna assessment. The variability in megafauna and seafloor structures which can be successfully imaged varies by deployment height above seafloor of a sensor platform or device, the speed of movement, illumination, camera type, camera angle etc. To investigate how this may play a role in determining which megafauna can be imaged across disturbed and undisturbed polymetallic nodule areas with currently used remote sensing platforms, such as AUVs, various towed camera sleds, and following different deployment protocols, a study was conducted with data collected from the DISCOL site during SO242. This study demonstrated image resolution and flight height have a very marked influence on megafauna community assessment (Schoening et al., 2020).

Impact of the collector Patania II. During the IP21 cruise, at each station, detailed seafloor imaging was collected by the towed camera platform OFOBS to characterize habitat features and megafauna communities present at the time of survey. In addition, a time-lapse camera unit was deployed on the seafloor prior to the collector trial adjacent to the test site, i.e. in the plume impact site, successfully recording visual responses of epifauna to the disturbance, i.e. the blanketing of seafloor from the suspended sediment cloud.

Task 1.2 Meio- and macrofaunal assemblages and their connection to physical habitat characteristics addressing natural variabilities, disturbance effects, and their temporal evolution (UAveiro, IFREMER, UGhent, RBINS, SGN, UResearch, ULodz, NIOZ)

Meiofauna (UGhent, IFREMER, SGN)

Baseline study. During SO268, the GER and BEL study areas were sampled. In both areas, baseline samples were collected from a reference site and the designated collector trial site. Nematode ASVs were highest in the BEL reference site (331 ± 101) compared to the BEL trial site (242 ±86), although both sites showed a high degree of variability and no statistically significant differences were observed between them. Meiofaunal densities within the BEL reference site also showed considerable spatial variation, with average densities of 89 ± 44 ind./10 cm^2 and meiofauna samples from the BEL trial site are currently being processed.

Dredge disturbance experiment. During SO268 also sampling of the small-scale dredge disturbance took place before/after and at different sediment plume deposition thicknesses. Samples were collected from 4 categories: (1) Pre-dredge, (2) Post-dredge_track, (3) Post-dredge_thick, (4) Post-dredge_thin. Nematode ASV was highest in the Post-dredge_thin samples (149 ± 56), followed by Post-dredge_thick (121± 87), Post-dredge_track (103 ± 66) and lowest in the Pre-dredge samples (92 ± 30) (Fig. 1.5). Statistically significant differences were not supported, indicating that the scale of these experiments was too small to detect the impact. No indication of a disturbance effect was observed for average meiofaunal densities which were comparable for all categories. The absence of a significant effect proved to be a general finding for the majority of measured variables during this experiment which can be attributed to the scale of impact (i.e. small size and weight of the dredge) and the low and unbalanced replication. Subsequently, a detailed analysis was carried out in collaboration with MARUM to link MUC deployments with their exact position and modeled sediment deposition. The modelled simulation revealed that the level of actual sediment deposition was quite low (<10 mm). These findings, together with results from an older nematode sediment deposition experiment were used to develop impact threshold values. These indicate that a deposition level of less than 1 cm might be suitable to indicate “low risk” for infauna (nematodes), whereas higher deposition levels would pose a considerable risk leading to significantly increased mortalities.

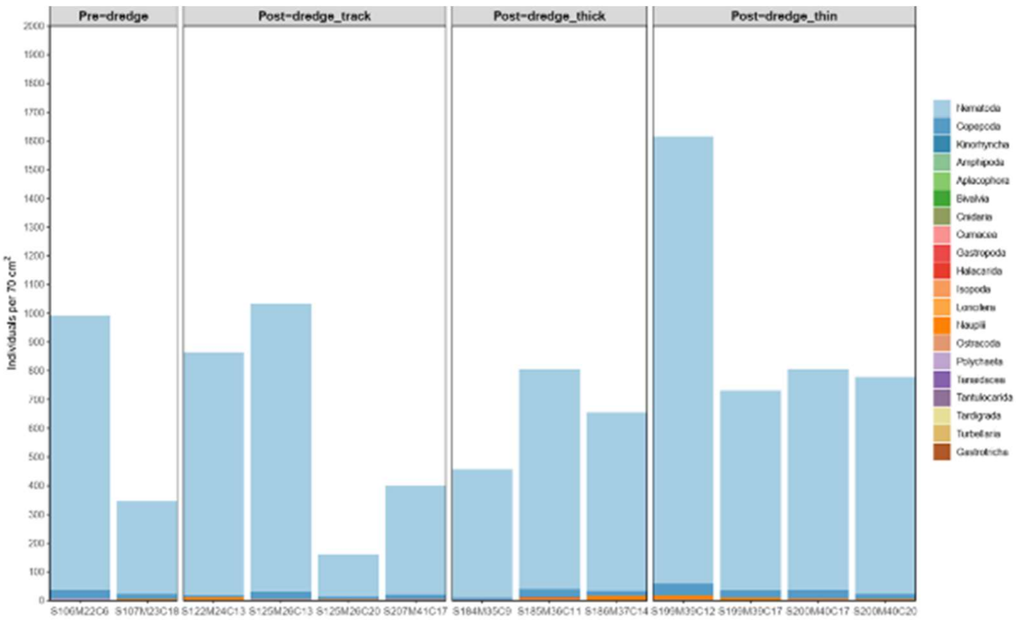

Figure 1.5: Observed meiofauna densities in the GER pre-impact, track, thick_cover and thin_cover sites from sediment samples collected during the dredge-experiment (SO268).

Impact of the collector trial. During the IP21 campaign, the Patania II nodule collector completed a realistic, small-scale harvesting test in the GER and BEL area. However, post-impact sampling of meiofauna was only possible in the BEL area due to the time contraints after the trial in the GER area. Sediment samples were obtained before and after the deployment within 2 different impact types, i.e. (1) pre-impact, (2) track, (3) thick_cover. Similar to the results from the small-scale dredge-experiment during SO268, high spatial variability was observed in total meiofaunal densities within and between impact types. Densities were lower in the track (50 ± 21 ind./10 cm^2, n=6) compared to the pre-impact sample, whereas thick_cover samples showed increased densities (125 ± 37 ind./10 cm^2, n=6). Nematode ASV richness was highest values in the pre-impact samples (176 ± 100), followed by thick_cover (143 ± 83) and lowest in the track (108 ± 73) (Fig. 1.6). This pattern in both datasets demonstrates the removal of biota from the track and its subsequent deposition in the thick_cover site. Assemblages of the pre-impact samples exhibited relatedness similar to a randomly assembled community, while the latter exhibited relatedness that was higher than expected at random (i.e. phylogenetic clustering), an indication that environmental filtering was defining community assembly. Overall, both research methods detected similar results, indicating that metabarcoding may be a suitable alternative to morphological assessments. Increased replication is needed to incorporate the natural variability and to increase statistical power. Finally, these results are thought to represent relatively short-term responses to the disturbance and follow-up studies at different points in time will be necessary to investigate longer term effects. H

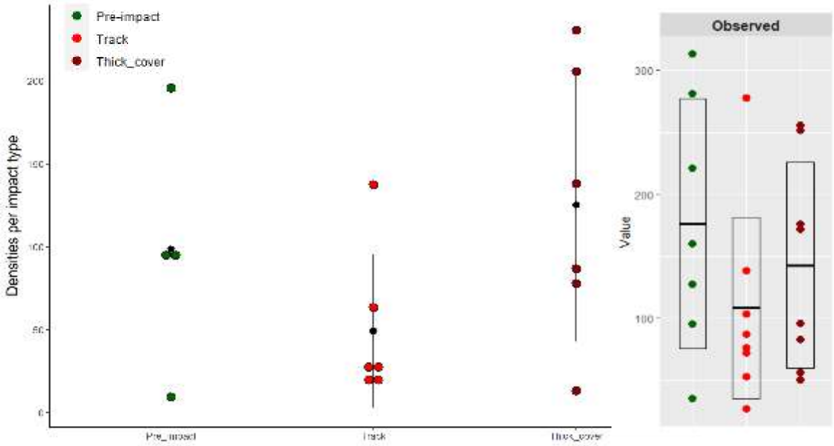

Figure 1.6: Meiofaunal densities (left) and observed Nematoda ASVs (right) in the BEL area. Pre-impact (green), track (red) and thick_cover (dark red) sites from sediment samples collected during IP21.

Abundance and diversity of nodule infauna. NIOZ collected nodules to analyze the nodule infauna, or also termed nodule crevice fauna. More than 4100 individuals were counted, and on average about 20 animals were inhabiting one nodule. Higher taxa included nematodes, copepodes, ostracods, kinorhynchs, tardigrades, polychaetes, tanaids and foraminiferans. In addition, nauplii and eggs were observed. Nematodes dominated the communities with ~78%, followed by copepods with ~11%, and diverse eggs with ~4%. Next steps include identification to species level and comparison of species diversity to sediment infauna, to determine if nodule infauna is nodule obligate.

Macrofauna (UAveiro, IFREMER, RBINS, UResearch)

Sampling operations. Macrofaunal studies included a baseline study and two disturbance studies, a small-scale dredge disturbance experiment and the collector trial impact study carried out during SO268 (2019) and IP21 (2021). The boxcore (surface area of 0.25 m^2) sampling effort allocated to these studies in the GER and BEL areas are shown in Table 1.1. In addition, for the collector trial impact study three sets of two passive samplers (surface area of 0.01 m²) were deployed at three stations along a predicted gradient of plume intensity in each study area in order to collect suspended fauna resettling together with the sediments.

Table 1.1: Number of box cores (n; area of 0.25 m²), number of specimens (ind; total abundance) and provisional number of taxa (T; family level for malacostracan Crustacea and Polychaeta and class or higher level for the remainder) collected at different sites in each study area (Ger-BGR; Bel-GSR)

<table>
<tr><th colspan="4"></th><th colspan="3">Ger-BGR</th><th colspan="3">Bel-GSR</th></tr>
<tr><th colspan="4">A) Baseline Study – SO268 (2019)</th><th>n</th><th>ind</th><th>T</th><th>n</th><th>ind</th><th>T</th></tr>
<tr><td colspan="4">Reference Site</td><td>6</td><td>775</td><td>80</td><td>7</td><td>588</td><td>66</td></tr>
<tr><td colspan="4">Trial Site</td><td>7</td><td>995</td><td>97</td><td>7</td><td>480</td><td>70</td></tr>
<tr><th colspan="4">B) Disturbance Experiment – SO268 (2019)</th><th>n</th><th>ind</th><th>T</th><th>n</th><th>ind</th><th>T</th></tr>
<tr><td rowspan="2">Dredge Site</td><td colspan="3">Pre-Dredge</td><td>5</td><td>526</td><td>69</td><td>---</td><td>---</td><td>---</td></tr>
<tr><td colspan="3">Post-Dredge</td><td>3</td><td>265</td><td>63</td><td>---</td><td>---</td><td>---</td></tr>
<tr><th colspan="4">C) Collector Trial - IP21 (2021)</th><th>n</th><th>ind</th><th>T</th><th>n</th><th>ind</th><th>T</th></tr>
<tr><td rowspan="4">Trial Site</td><td colspan="3">Pre-impact</td><td>3</td><td>822</td><td>74</td><td>2</td><td>260</td><td>53</td></tr>
<tr><td rowspan="3">Post-impact</td><td colspan="2">Collector impact</td><td>---</td><td>---</td><td>---</td><td>3</td><td>196</td><td>46</td></tr>
<tr><td rowspan="2">Plume impact</td><td>Thick</td><td>---</td><td>---</td><td>---</td><td>3</td><td>510</td><td>55</td></tr>
<tr><td>Thin</td><td>---</td><td>---</td><td>---</td><td>3</td><td>314</td><td>50</td></tr>
<tr><td colspan="4">Reference N</td><td>---</td><td>---</td><td>---</td><td>1</td><td>81</td><td>22</td></tr>
</table>

Biodiversity overview. A total of 5812 specimens were collected (SO268: 3629; IP21: 2183). From those specimens, 42% belong to Nematoda (28.9%), Copepoda (10.5%) and Ostracoda (2.7%) and the remainder are from typical macrofaunal groups. The taxonomical analysis is still ongoing. Tissue vouchers for DNA barcoding were collected for all possible specimens and up to now DNA has been extracted from a total of 1307 specimens collected during SO268 cruise. The GER and BEL areas share 21% of polychaete MOTUs, but only 9% of isopod MOTUs. This difference between isopods and polychaete distributions has already been reported in the Clarion Clipperton Zone (Janssen et al., 2015; Janssen et al., 2019) and interpreted as a result of the putative weaker dispersal abilities of isopods. Further work using an integrated molecular and morphological approach is being carried out. Macrofaunal size nematodes (12 families) are also being processed, and preliminary molecular work is ongoing focusing on the dominant families, Phanodermatidae and Oncholaimidae.

Dredge Disturbance Experiment. The dredge disturbance experiment carried out during SO268, enabled to simulate small-scale but direct disturbance at the seafloor and to create a sediment plume and monitor the blanketing by resettled sediment. Macrofauna was sampled pre- and post-disturbance (Tab. 1) for the short-term study of the direct and plume-related impacts on the benthic assemblages. Provisional results using morpho-species data for malacostracan crustaceans and polychaetes, and family or higher level for the remainder taxa, suggests similar densities but lower number of taxa and similar diversity but higher evenness after the disturbance (Post-Dredge) compared to before (Pre-Dredge).

Impact of the Patania II collector trial. The preliminary analysis shows a clear temporal segregation between 2019 and 2021 that may be partially explained by an important overall increase in macrofaunal densities (Fig. 1.7). This interannual variability can make interpretations of impacts more complex. The post-impact results show a decrease in densities in the "collector impact" stations that is somehow lower than expected and an expressive increase in densities in the plume impact stations with thick blanketing when compared with the pre-impact samples. (Fig. 1.8). Differences in total abundances between pre-Impact and collector impact as well as between collector impact and plume impact-thick are significant

(KW p values: 0.031 and 0.004, respectively). These variations in densities are apparently occurring at different rates for different taxa and are likely explained by the occurrence of living specimens in the plume that settle together with the sediments, especially in the areas closer to the collector impact. Further investigations are needed to understand the condition of these specimens and their ability to survive at short to long-term. Nevertheless, our pilot experiments with passive samplers confirmed the occurrence of living specimens settling together with the plume sediments, in decreasing abundances with the increasing distance to the area of direct collector impact (Fig. 1.9).

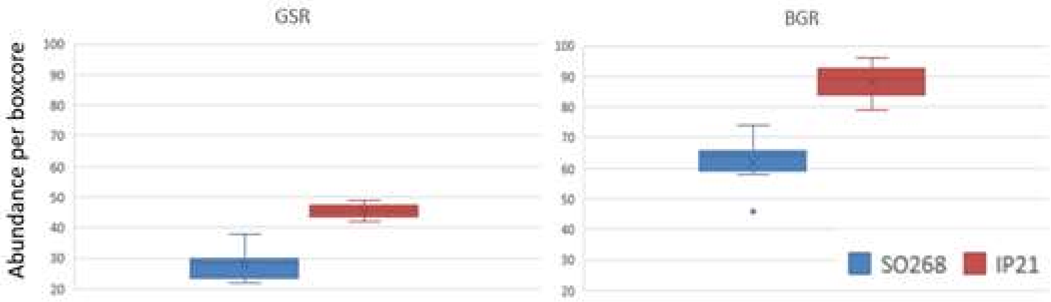

Figure 1.7: Comparison of average abundances in the two study areas (Bel-GSR and Ger-BGR) between 2019 (SO268) and 2021 (IP21).

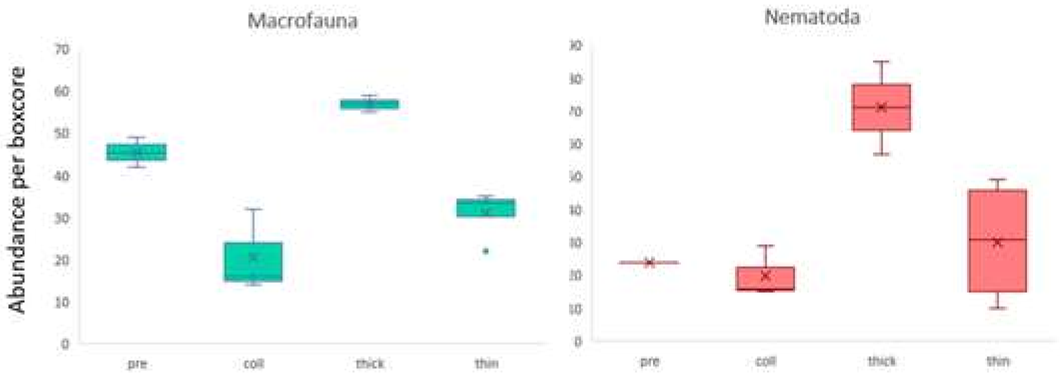

Figure 1.8: Comparison of average abundances of macrofauna and large nematodes (macrofaunal size) in the Trial Site at Bel-GSR showing the variations in the Collector impact and Plume Impact (Thick and Thin sediment blanketing) stations (respectively col, thick and thin) in relation to the Pre-impact stations (pre).

A high variability at different spatial scales was observed both in taxa richness, abundance and community structure of the macrofaunal assemblages sampled during the baseline study in 2019 (SO268). The sampling carried out in 2021 (IP21) revealed an also relevant inter-annual variability. These combined sources of variability can only be rigorously tackled in future monitoring activities by extensive baseline studies and an adequate number of replicates immediately before and after impact.

We have also shown that the DNA barcode is a powerful tool for describing the biodiversity of benthic macrofauna. However, the tool is not 100% effective. A combination of genetic markers and morphological criteria is still necessary to describe species diversity, composition, and distribution accurately and quantitatively.

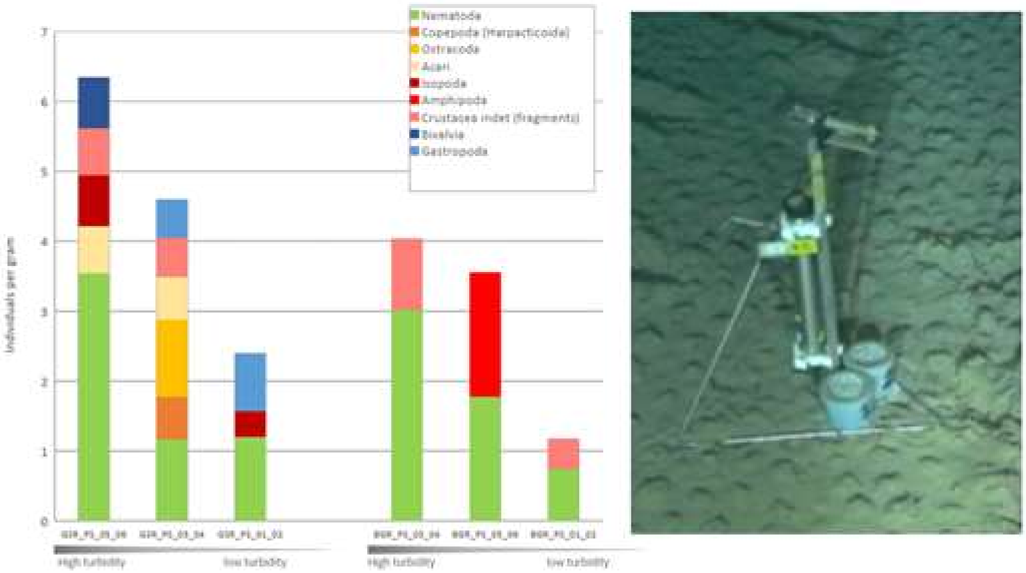

Figure 1.9: Comparison of average abundances (number of specimens per gram of settled sediment) of different faunal groups along a gradient of turbidity (higher turbidity near the collector impact area) at Ger-BGR (left) and Bel-GSR (right) sites. The photo shows a set of two passive samplers deployed with a NIOZ PFM turbidity sensor.

Further investigations are needed to understand re-settlement, survival, and short-term succession (both taxonomic and functional) of the macrofaunal assemblages after disturbance, which are extremely relevant to assess recovery. This issue must be adequately addressed by planning not only medium to long-term sampling but also short-term sampling at a few weeks and months intervals after disturbance.

Faunal traps. During the SO268 cruise six successful stations were sampled in the GER and BEL areas, including post-impact in the dredge track. Sample identifications are complete and DNA barcoding coupled with heavy metals analysis of the amphipod tissue (from P.caperesca and A. gerulicorbis) was performed at RBINS. The dataset produced in the first phase of MiningImpact for Lysianassidae scavenging amphipods was extended by generating additional 157 DNA sequences from part of the 28S nuclear gene. Phylogenetic trees based on combined nuclear and mitochondrial data and applying four different statistical methods support the existence of two genetic species each for both P. caperesca and A. gerulicorbis (Patel et al., in press). These species co-occur in the Belgian and German license areas, evidence that they are connected over hundreds of kilometers. Assessments of biodiversity and connectivity with Next-Generation RAD-sequencing techniques will require further optimisations as standard protocols were not successful. As a first step, estimates of amphipod genome sizes with flow cytometry techniques are recommended.

Task 1.3 Effects of sediment disturbance on microbial and microeukaryote communities (MPI, UNIVPN)

Microbial communities.

MPI completed the extractions of DNA from sediment, polymetallic nodule and bottom seawater samples collected during SO268 (2019), including sediment and seawater samples from areas experimentally disturbed by the dredge experiment. The sediments host the highest microbial diversity, and the bacterial community in water samples has the lowest number of species and evenness (Fig. 1.10a). Substrates host different microbial communities, with those on benthic substrates more similar to each other than with pelagic microbes (Fig. 1.10b).

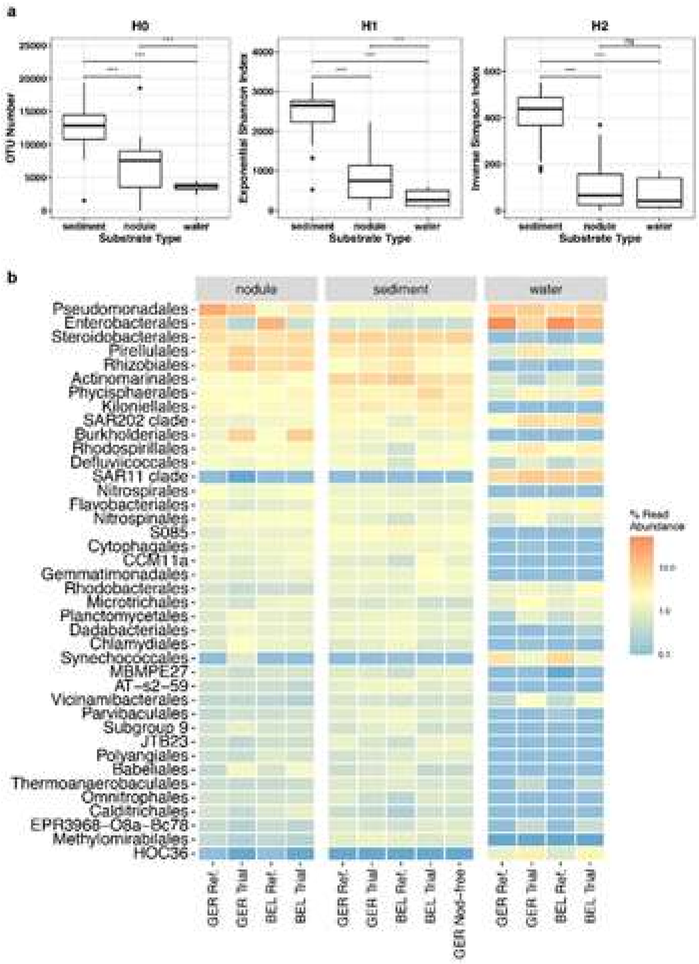

*Figure 1.10: Diversity of bacterial communities associated to different substrates. (a) Bacterial alpha-diversity for three different substrates (i.e. sediments, polymetallic nodules and bottom seawater) sampled in Belgian and German areas. (Kruskal-Wallis Test, (Significance; ns: p> 0.05, *: p<0.05, **: p < 0.01, ***: p<0.001). (b) Bacterial taxa dominating microbial communities in sediments, polymetallic nodules and bottom seawater.*

The bacterial community structure was different between the two areas and between sites within each area (Fig. 1.11a). Differences in bacterial communities were also observed between two investigated layers (0-1 cm and 1-2 cm). Yet, the BEL and GER areas shared a large proportion of Bacterial species (74%), with 17% of observed species unique for the GER area and 9% for the BEL area (Fig. 1.11b). These findings suggest that bacterial communities are connected between the two investigated areas. However, regional differences and high local variability in environmental settings (e.g. organic matter availability, nodules coverage, seafloor topography) may be responsible for the variation observed in microbial community structure.

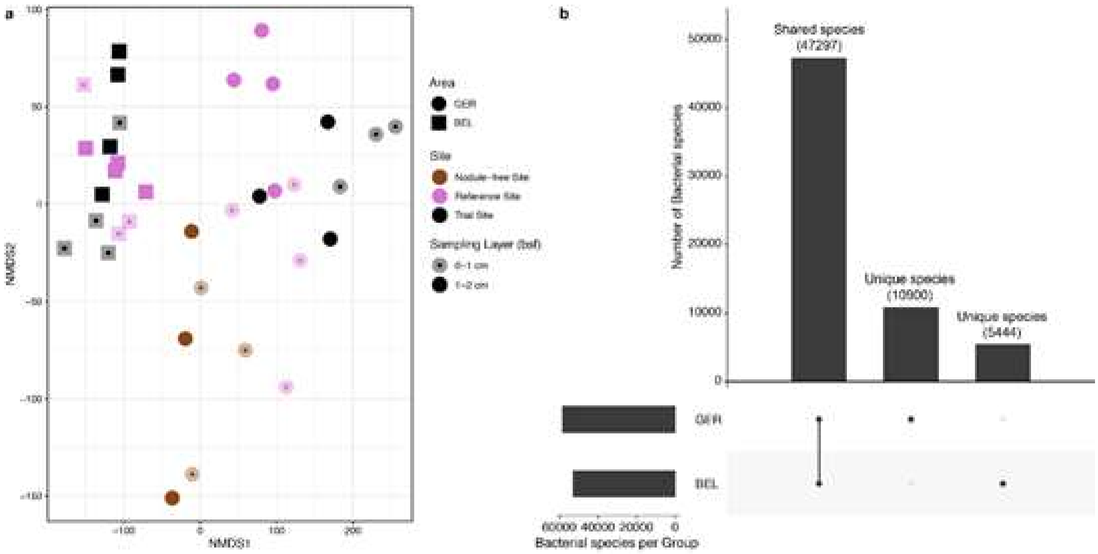

Figure 1.11: Comparison of bacterial communities between BEL and GER areas. (Left) Non-metric multidimensional scaling (nMDS) grouping the stations according to bacterial community similarity. Central log ratio transformation is applied to abundance data, Euclidean distance is used for dissimilarities. (Right) Bacterial connectivity (i.e. shared and unique species) between BEL and GER areas.

Preliminary results show that RNA based active community composition from the Belgian and German areas are highly similar. Differences have been observed in the community structure between the active community (based on RNA) and the DNA based community composition (Fig. 1.12). Transcriptome data indicates that Archaea only play a minor role in CCZ sediments with abundances below 5 % of the active community. An assessment of the transcribed mRNA showed no differences in functional diversity and gene expression between areas and sites (Fig. 1.12b). Further analysis of the transcriptomic data aims to describe active dominant metabolic pathways and metabolic key genes to assess the functional status of benthic microbial communities. This knowledge on functional diversity and metabolic pathways will be applied to identify the effect of PATANIA II tests on microbially-mediated processes

Impact of the Patania-II collector trial. With the knowledge acquired from SO268, it is expected that the disturbance created by PATANIA II operations on physical and biogeochemical conditions, both in bottom water (see WP2) and sediments (see WP3), will cause changes in microbial communities. In order to identify and quantify the extent of impact of the collector on microbial communities, MPI started the analysis of microbial diversity in samples collected during IP21 expedition (2021). Furthermore, comparison between results from samples collected during SO268 (2019) and IP21 (2021) will provide information on inter-annual temporal variability of microbial diversity and community structure, improving our knowledge of baseline variability at BEL and GER areas.

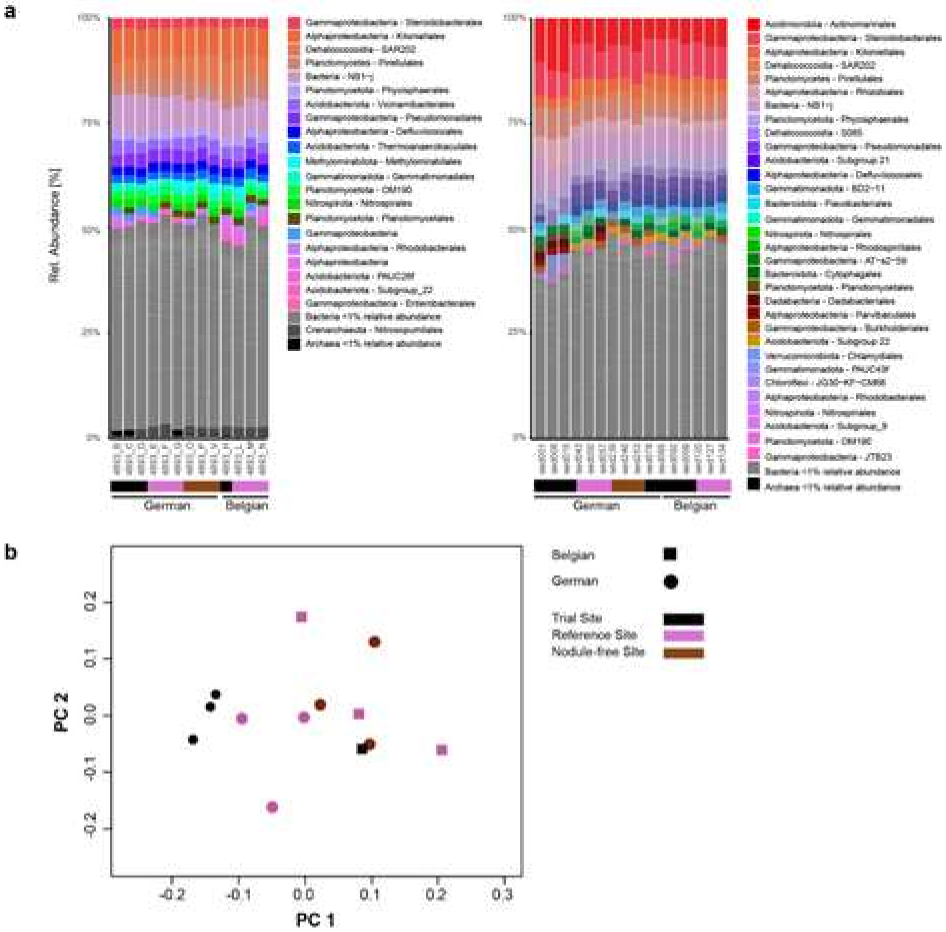

Figure 1.12: Preliminary results of metatranscriptomes from SO268 sediment samples. (a) Comparison between the structure of active microbial community based on 16S rRNA sequences (left panel) and stock microbial community based on 16S rRNA gene sequences (right pannel). Microbial abundances shown as relative abundances (%). (b) Principal Coordinates Analysis (PCoA) showing differences in transcription of functional genes between sites in the BEL and GER areas. Data was normalized to reads per kilobasepare per million (RPKM) and the Bray Curtis distance index applied.

Microeukaryote diversity.

SO268 and Dredge Disturbance Experiment. The microeukaryote diversity was investigated by UNIVPM in the dredge experiment carried out in the GER area during the SO268 cruise. Replicated samples (n=4) have been collected in the four experimental sites: reference, dredge (impact), thick and thin covered, respectively. Our results suggest that microeukaryotes diversity as Amplicon Sequence Variant (ASV) is higher in the thin-covered sediments compared to all other experimental sites that show high variability of ASV diversity (Fig. 1.13a). The Pielou index reveals that the reference site shows a lower equitability in the species distributions compared to the values reported in the sites affected by the dredge experiment (Fig. 1.13b).

The analysis of the microeukaryote composition reveals the presence of different families and genera that show a different pattern in the four experimental plots (Fig. 1.14). Saccharomycetales are the dominant family in the reference site (33%) while their contribution declines in the thin covered sediments (12%). Some genera appear sensitive to the dredge experiment such as *Perkinsea* that is exclusively reported in the reference sediments representing 4% of the overall microeukaryote assemblage composition. Conversely, *Alveolata* increases its contribution in the thick-covered sediments (10%) when compared to the undisturbed reference site (5%).

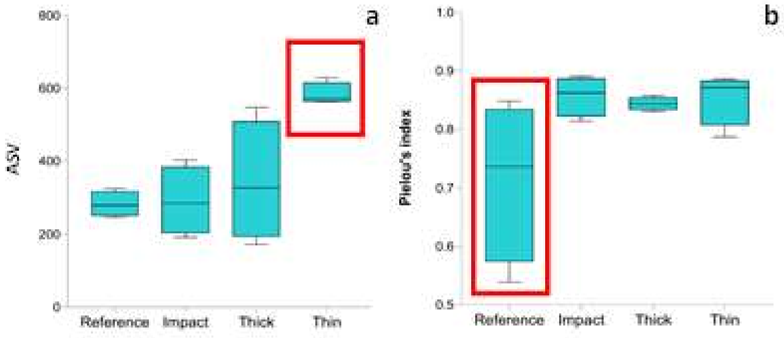

Figure 1.13: Microeukaryote ASVs richness and equitability in the GER Area.

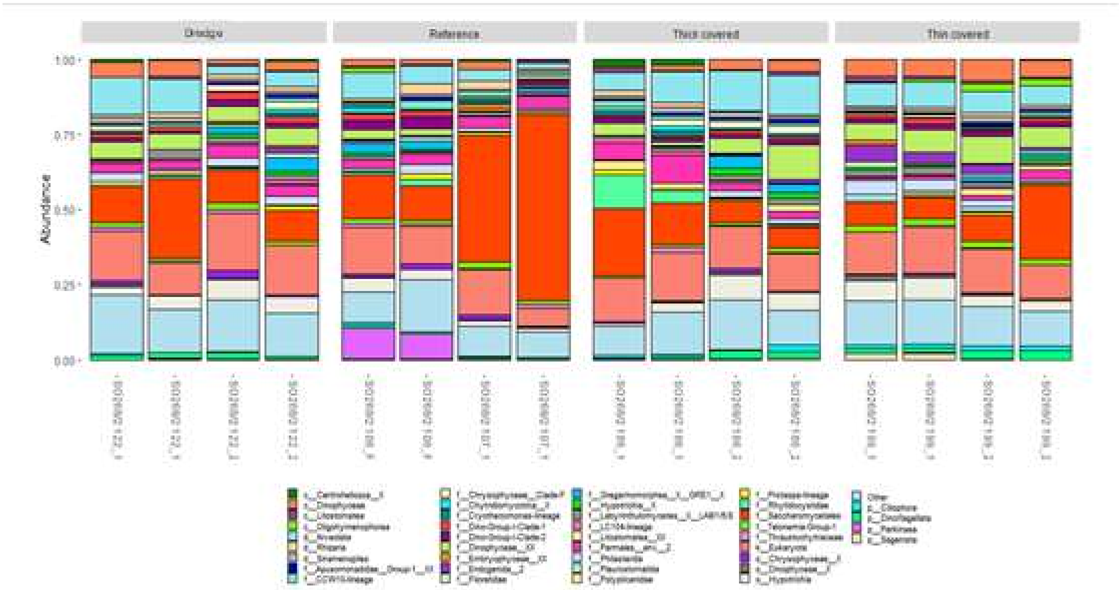

Figure 1.14: Barplot showing the taxonomic composition of the microeukaryote assemblages found in the GER Area at the lowest taxonomic level in terms of sequence contribution to each taxon identified. Taxa contributing less than 1% were summed and indicated as "Other". Taxa names are preceded by a letter according to the maximum depth of taxonomic assignment: "d" for domain, "p" for phylum, "c" for class, "o" for order and "f" for family.

Task 1.4 Development of molecular methods and protocols for rapid biodiversity assessments and environmental monitoring (SGN, UGhent, UResearch)

Rapid biodiversity assessment of benthic Harpacticoida using MALDI-TOF MS (SGN)
Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) measures size and abundances of peptides and proteins of extracts from individual specimens. The resulting proteome fingerprint can be used to identify species based on a reference library. The method is widely used in microbiology for instance to identify certain pathogenic bacteria (Singhal et al., 2015). However, the method was already shown to be a rapid and cost-effective alternative to morphology-based identification as well as specimen identification using DNA methods such as DNA barcoding (Dvorak et al., 2014; Yssouf et al., 2014; Bode et al., 2017; Rossel et al., 2019; Wilke et al., 2020). Until now, one of the major drawbacks of the method for application in deep-sea biodiversity assessments is the lack of suitable reference libraries. To account for this problem, methods for unsupervised identification of species boundaries

using different clustering algorithms have recently been developed (Rossel and Martínez Arbizu, 2020; Renz et al., 2021). These methods allow the assessment of biodiversity without prior knowledge of the species occurring in an area under investigation and were shown to assess biodiversity in high concordance to morphological and genetic species assignments. Within the MiningImpact project the different methods were applied to test the applicability in a deep-sea environment. We used a dataset of benthic copepods including 2115 specimens of different ontogenetic stages as well as from different sampling campaigns in 2018 and 2019 in the eastern BGR contract area in the CCZ; for a total of 727 specimens both a DNA barcode as well as proteome fingerprints were obtained. This resulted in high overlaps between genetic methods and proteome fingerprinting. However, a potential influence of minor quality mass spectra on the results was apparent. Thus, an additional quality control was carried out, further increasing the overlap between genetic methods and proteome fingerprinting.

Comparing the species delimitation based on the biodiversity indices Pielou's Evenness and Shannon Index, results of DNA-based and mass spectrometry-based methods exhibit values within the same range (Fig. 1.15). Variation in the alignment process of MALDI-TOF data in the PAM-clustering pipeline revealed some variabilities but no exceptional differences in the final results (Fig. 1.15). In the end, none of the methods can claim to reveal actual or real species boundaries. Thus, differences in the results need to be accepted but high similarities between different molecular methods show, that there indeed is a high degree of accordance of species boundaries between methods as well as within the processing of mass spectrometry data.

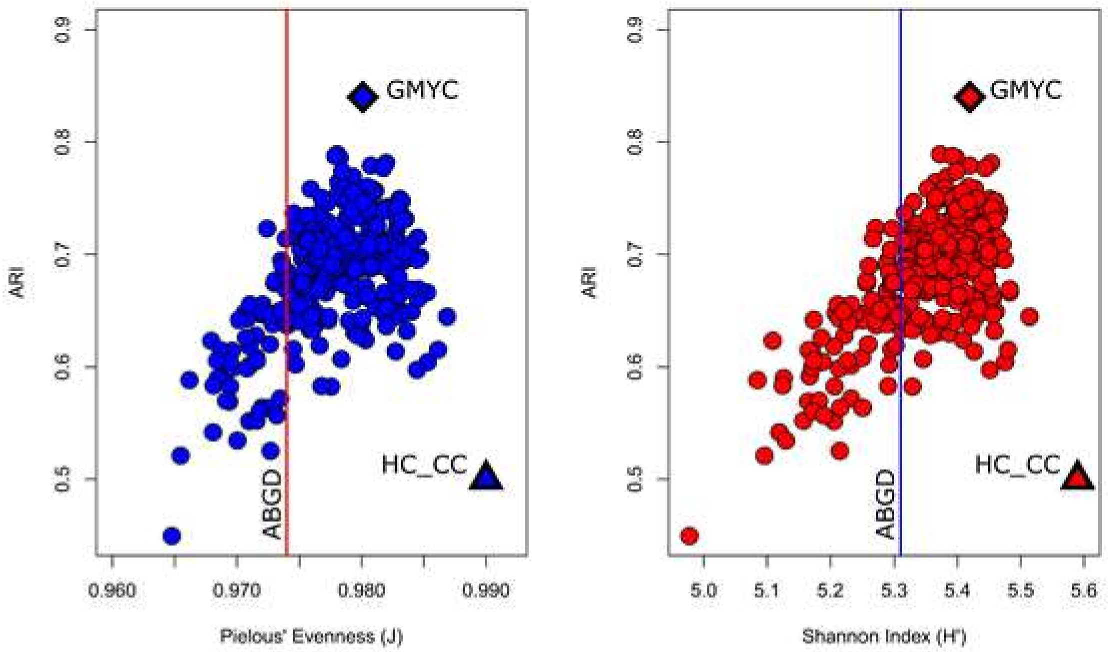

Figure 1.15: Pielou's Evenness and Shannon diversity were calculated for all calculated species delimitations; the processing of mass spectrometry data was varied in the processing pipeline of PAM clustering (circles) to investigate and improve data processing. All results are compared to the species delimitation of ABGD using the Adjusted Rand Index (ARI).

Finally, we used the applied molecular methods to obtain rarefaction curves for the investigated benthic Harpacticoida. Besides the data sets consisting of specimens for which a proteome fingerprint as well as a DNA barcode was available, larger data sets including all specimens for which either a proteome fingerprint (n=1445) or a DNA barcode (n=1298) were analyzed, resulting in rarefaction curves showing, that we are far from having assessed the actual biodiversity in the CCZ (Fig. 1.16). Again, the results show high overlaps between the tested methods, thus making proteome fingerprinting using MALDI-TOF MS an additional, promising tool for biodiversity assessments in a deep-sea environment in the future.

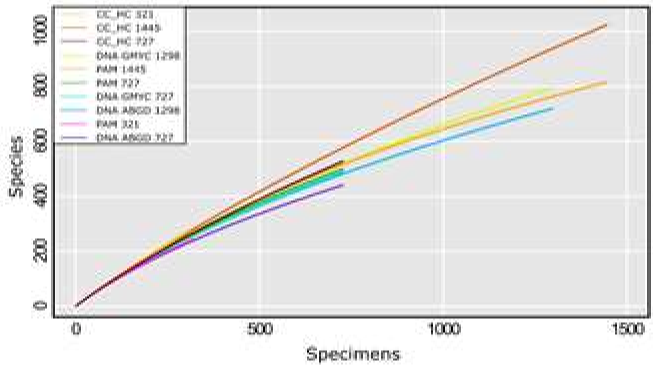

Figure 1.16: Rarefaction curves generated for all tested setups in this study. While curves tend to show slight differences in the number of species by sampled specimens, all agree in not having reached a saturation regarding the number of species to be expected in the CCZ.

Rapid assessment of meiofauna with metabarcoding (SGN**)**

Metabarcoding is a rapid investigation method based on DNA-barcoding. DNA barcoding has already been introduced to the CCZ as an additional tool for species identification and is applied on a large variety of taxa including Crustacea, Annelida, Porifera and Echinodermata (Janssen et al. 2015; Kersken et al. 2018; Christodoulou et al. 2020). The most common barcoding gene for species identification is a fragment of the mitochondrial CO1 gene (Hebert et al. 2003). However, many species of the taxon Nematoda, which is the most abundant meiofauna taxon in benthic sediment of the CCZ (Miljutina et al. 2010; Pape et al. 2017; Hauquier et al. 2019; Uhlenkott et al. 2020), cannot be amplified with standard CO1 primers. In the metabarcoding approach, DNA is extracted from a bulk sample of specimens or in form of environmental DNA (eDNA) from sediment or water and, using next generation sequencing (NGS) multiple sequences (reads) are produced simultaneously with one primer set. Hence, to not exclude a large proportion of the meiofaunal community the V1V2 region of the 18S gene was used as the target gene in this study, although the taxonomic resolution achieved with this DNA-barcode does not reach species level for many taxa (Derycke et al. 2010). The reads derived from the samples are then condensed into amplicon sequence variants (ASVs). These amplicon sequence variants can either be assigned to taxonomic units obtained from a database such as GenBank (Benson et al. 2006) or grouped in operational taxonomic units (OTUs). In the context of deep-sea mining metabarcoding is predestined to play an important role for assessing the natural biodiversity in the CCZ, for assessing the impacts of mining and to monitor recovery.

In the context of the MiningImpact project, we used a specimen-based metabarcoding approach to investigate differences in the meiofaunal composition under different conditions of the dredge experiment. Samples were obtained in 2019 during the cruise SO268 in the BGR-contract area with a 20-core multicorer. In addition to the control samples, that were obtained prior to the impact, samples were divided into three different impact categories. Dredge track samples were obtained directly in the track or very close to the track as the size of the multicorer exceeded the width of the tracks. The other samples were obtained in the vicinity of the track being impacted by the evolving sediment plume which leads to areas with varying degrees of sediment deposition. In this study samples with sediment coverage were divided into thick cover and thin cover according to video images.

Prior to comparisons of different impacts, all ASVs were blasted against the sequences stored in the database GenBank. In order to monitor the accuracy level of GenBank taxonomic assignments for meiofauna community, percentage identity of each species is to the best blast hit is compared to the query coverage of each hit. Setting thresholds of 97% for identity percentage and 90% for query coverage, a significant number of taxonomical assignments fall out of this range indicating the lack of genetic data available from deep-sea meiofauna community in NCBI database.

To assign the ASVs to OTUs the species delimitation tool GMYC (Fujisawa and Barraclough 2013) was used to classify all meiofauna organisms. A total of 2588 ASVs could be classified in 606 GMYC delimited species of target meiofauna taxa encompassing 13 groups of Nematoda, Annelida, Crustacea, Platyhelminthes, Bryozoa, Gastrotricha, Nemertea, Hemichordata, Loricifera, Entoprocta, Scaphopoda, Sipuncula and Tardigrada in respect to their abundancy (Fig. 1.17). Nematodes are the most abundant meiofauna taxa with 267 GMYC species, followed by annelids (140 species), crustaceans (107 species) and Platyhelminthes (36 species). Investigating the most abundant taxon Nematoda in detail, the number of OTUs is highest regarding the control sites and the dredge track, but lowest at sites with faint cover (Fig. 1.18 left). From the second most abundant higher meiofauna taxon Crustacea five orders of Copepoda (Harpacticoida, Cyclopoida, Calanoida, Misophrioida and Siphonostomatoida) were observed in the samples additionally to four species of Ostracoda. The highest relative proportion is presented by Harpacticoida based on 24 OTUs, followed by Cyclopoida (24 OTUs) and Calanoida (14 OTUs) (Fig. 1.18 right).

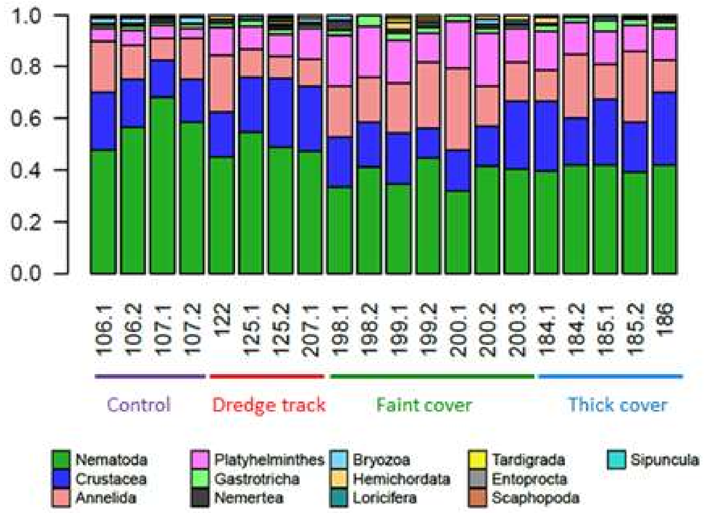

Figure 1.17: Barplot illustrating the relative number of meiofauna OTUs based on GMYC per metabarcoding library and replicate separated to "Control area", "Dredge track", "Faint cover" and "Thick cover". Numbers on the X axis are the stations followed by the replicate numbers.

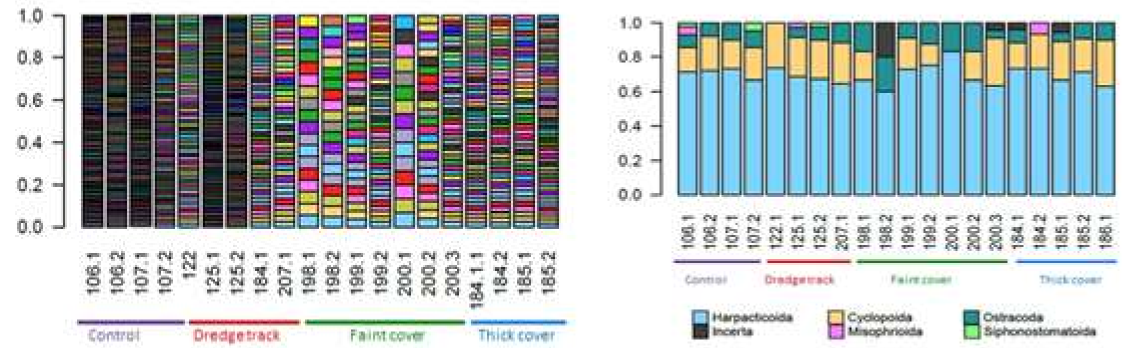

Figure 1.18: (left) Barplot illustrating the relative number of Nematoda OTUs based on GMYC per metabarcoding library and replicate separated to "Control area", "Dredge track", "Faint cover" and "Thick cover". Numbers on the X axis are the stations followed by the replicate numbers. (right) Barplot showing the relative number of Crustacea OTUs based on GMYC per metabarcoding library and replicate separated to "Control area", "Dredge track", "Faint cover" and "Thick cover". Numbers on the x axis are the stations numbers followed by the replicates.

In summary, metabarcoding of bulk meiofauna samples proved to be a promising approach to assess the benthic biodiversity of these small organisms, especially in comparison to the very time consuming traditional morphological methods. However, some technical issues still need to be resolved including species-level identifications, especially in deep-sea fauna, that are currently limited by the lack of a comprehensive and taxonomically reliable barcode database for the high proportion of these taxa. What can be further considered to enhance the accuracy and reliability of the results, would be to prepare and analyses NGS amplicon and reference library for COI loci to compare the results with 18S loci, and help to enhance our understanding of the complexity of meiofauna diversity and composition in the deep-sea and limit the under- or over-estimation of the meiofauna diversity due to specific aspects of distinct gene markers with variable DNA divergence level.

Regarding the observed biodiversity in the light of the impact experiment, biodiversity is observed to be highest directly in the dredge track and decreasing towards lower impacts. Although these findings appear counterintuitive, a potential explanation is the small time-scale that was sampled. The sediment plume settling in the dredge track as well as on the surrounding seafloor does not only contain sediment particles, but also includes the meiobenthic organisms removed within the dredge track. Hence, the organisms are deposited on top of the sediment, which already contains the community described by the control samples. Due to the large size of the multicore, also samples labeled as dredge track sites can be positioned directly outside the track. However, in the metabarcoding approach it is not possible to distinguish living meiofauna from dead specimens. If the dredge site is revisited later, this pattern will probably have changed.

WP 2 – Fate and toxicity of the sediment plume

Milestones and deliverables

M2.1 Plume monitoring, sampling and experiments successfully conducted during SONNE cruise (NIOZ)

Since the DEME-GSR trial of the pre-prototype nodule collector vehicle Patania II scheduled for spring 2019 was cancelled due to technical problems, an improvised dredge plume experiment was carried out during SO268 that was aimed at testing the plume monitoring approach on a small scale. Dispersion of the generated plume was monitored with an array of sensor platforms placed on the seabed, and the collected data were used for validating and calibrating a numerical plume dispersion model. The plume was too limited in extent and intensity, however, to allow a full investigation of its ecological impact.
When the DEME-GSR nodule collector trial finally took place in spring 2021, the produced sediment plume could be successfully monitored during expedition IP21 by deploying sensor platforms on the seabed and conducting AUV surveys, and the physical and chemical characteristics of the plume as well as its ecological impacts could be assessed. Surveys by AUV provided high resolution imagery of the seabed impacted by the nodule collector and by sediment re-deposition from the suspended plume. Assessment of ecological impact will continue during a follow-up cruise SO295 in October-December 2022.

M2.2 Setup of numerical models for near-field and far-field plume dispersion (MARUM, TUDelft)

A far-field plume dispersion model was set up by MARUM using the general circulation model from MIT with a one-way nesting approach for fine and coarse particles. The model was coupled with the sediment-transport module developed in MiningImpact phase 1. The lateral initial conditions for the ocean model were taken from HYCOM and the settling velocity of particles from lab experiments carried out by JUB. To assist planning of plume dispersion and impact monitoring for the DEME-GSR nodule collector trials, a probability forecast analysis of plume dispersion for a relevant time interval in spring was designed from serial simulations using meteorological data and near-bottom current data collected by BGR over a number of years prior to the trials. This exercise was carried out prior to SO268 and was repeated for IP21.

M2.3 Data from plume monitoring array, seabed image analysis, plume particle dynamics and plume trace metal reactions processed and ready for integration in plume dispersal and geochemical models (GEOMAR, JUB)

The data analysis of the delayed collector trials is still ongoing. Information has been transferred to MARUM for integration into the plume dispersal model.

M2.4 Workshop on the spatial and temporal ecological effects of the plume to identify suitable impact indicators (UAlgarve)

This workshop was held online on the 28-30 October 2020 together with the *CCT2 Workshop on quantitative assessment of intensities (including scoring criteria) of pressures and responses of all ecosystem components (M7.1)*. Originally these workshops would have presented and integrated results from the DEME-GSR nodule collector trials but given the known technical issues had to focus on the dredge experiment performed during SO268. Data from different research groups were presented and common patterns and problems were identified and contributed to a better planning of sampling effort of the collector trials during IP21.

D2.1 Workshop and report on the fate of trace metals in the plume and modelling of kinetics of trace metal reactions at the sediment-water interface following the deposition of plume material (JUB, NTNU)

The workshop and report could not yet be completed due to delayed collector trials, because the dredge experiment was too small to produce adequate data. The model itself could not yet be completed.

D2.2 Report on results of numerical simulations of near-field and far-field plume dynamics (MARUM, TUDelft)

The report is currently being prepared.

D.2.3 Report on ecological plume impacts and indicator selection integrated in WOE model and quantification of environmental hazards along spatial & temporal plume impact gradients (UAlgarve)

Given the delayed DEME-GSR nodule collector trials, results from the impact of the plume are still preliminary. The information available so far from the different partners, and beyond these trials, was gathered during the *CCT2 Workshop on integrated analysis of ecosystem responses and environmental impact assessment (M7.3)*. In addition, a chapter in *the CCT3 Guidance document on methodologies for risk assessment of environmental hazards of deep-sea mining (D8.2)*, explains the structure and components of the WOE model, and how it should be applied to assess the risk. We hope that soon the WOE can be implemented to assess the risks of deep-sea mining. As such, the D2.3 deliverable was merged with the M7.3 workshop report and the CCT3 report, to avoid duplication of reports.

Summary of achievements

Since WP2 is strongly focused on field observation and sampling of the sediment plume produced by nodule mining, the 2-year delay of the DEME-GSR nodule collector trial until spring 2021 had serious repercussions for much of the work foreseen in this work package. In addition to that, some of the work suffered from Mexican customs retaining equipment, technical issues with the GEOMAR AUV and MOLAB lander, and scarcity of samples for ecotoxicological assays. Notwithstanding these setbacks, the dredge plume experiment conducted during SO268 provided an excellent opportunity to test the overall setup of the plume monitoring design developed in conjunction with CCT1, and it yielded a valuable dataset for assessment of plume dispersion on a small spatial scale. In the area affected by the dredging and by the associated deposition of a sediment drape, in-situ observations and sampling regarding biogeochemical and ecological impact were carried out. Time during SO268 was used for a very thorough characterization of baseline conditions, including the oceanographic monitoring of a mesoscale eddy passing by the German contract area. Good progress has been made in lab-based research and analysis of data and samples collected during SO268, and a number of publications on this work have already been published. The IP21 expedition for independent scientific monitoring of the DEME-GSR nodule collector trials in spring 2021, despite COVID-19-related organizational challenges, turned out very successful for what concerns the monitoring of the sediment plume and the alteration of the seabed in the collector test areas and adjacent areas affected by the plume. Samples of sediment and fauna collected for assessment of ecological impact are still being analysed, and assessment of the longer-term impact and early recovery will take place during a follow-up cruise SO295 in October-December 2022.

Task 2.1 Plume dispersal and sediment deposition (GEOMAR, BGR, NIOZ, RBINS, MARUM, UDelft, NTNU, JUB, AWI)

For monitoring of the plume produced by the DEME-GSR nodule collector, GEOMAR, NIOZ, RBINS, BGR and JUB brought together a large number of turbidity sensors and current meters as well as a variety of other sensors, which were deployed on static ROV-deployed platforms, a free-fall lander and moorings, and on mobile platforms like CTD, ROV and AUV.

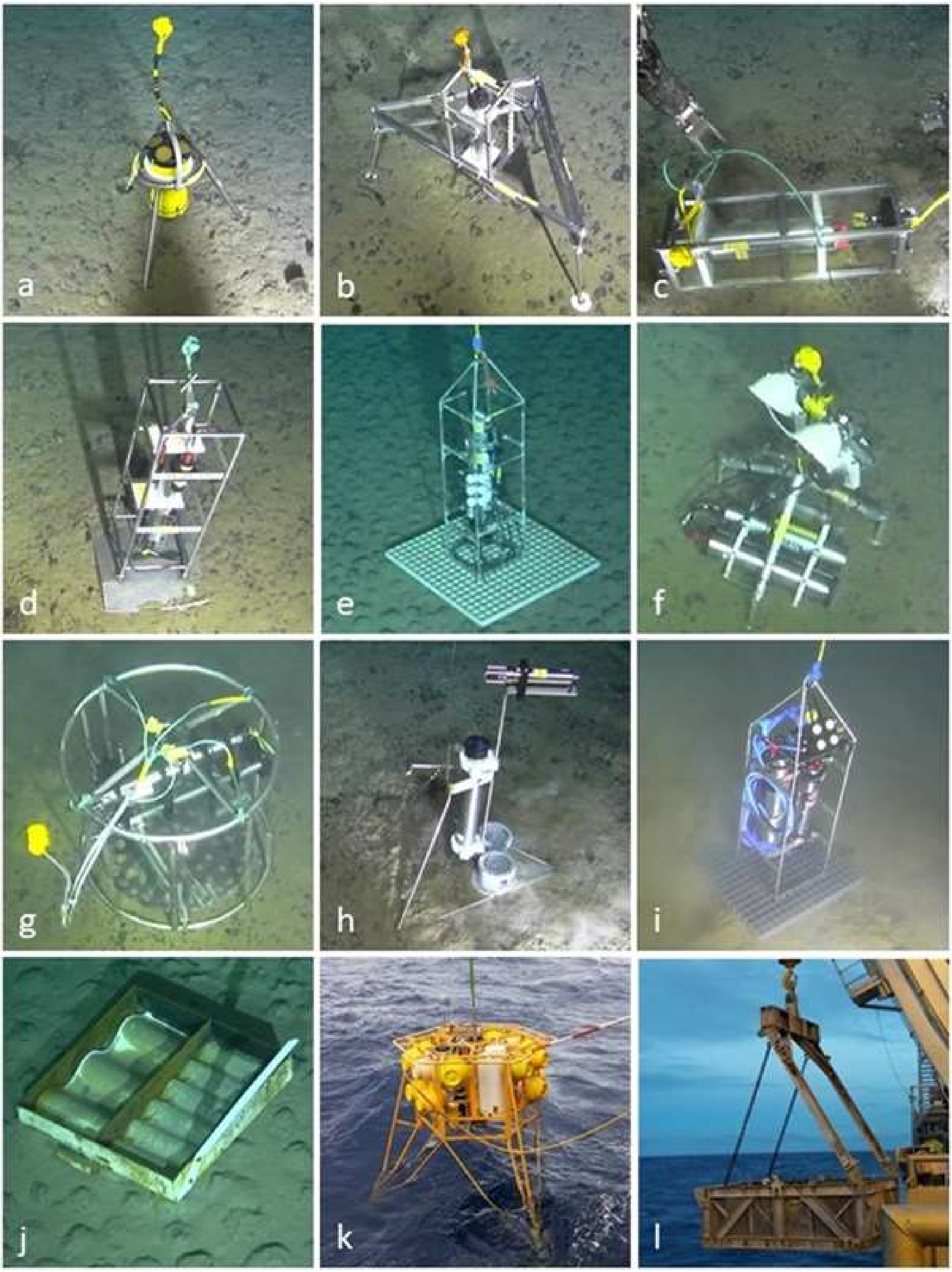

Figure 2.1: Sensor platforms used in the plume monitoring array during IP21. (a) GMR_PFM-41 with 300 kHz ADCP; (b) GMR_PFM-44 with ADV and optical backscatter sensor; (c) GMR_PFM-45 with hydrophone; (d+e) GMR_PFM-48 and RBINS_PFM-01 with CTD and optical backscatter sensors; (f) GMR_PFM-49 with still camera; (g) JUB_PFM-01 with particle camera and optical backscatter sensors; (h) NIOZ_PFM-06 with 2 MHz current profiler and optical backscatter sensor; (i) RBINS_PFM-04 with AquaScat acoustic turbidity sensor; (j) SLIC (Sedimentation Level Indicator) box 08; (k) NIOZ_PFM-01 BOBO lander with 300 kHz upward and 1200 kHz downward looking ADCP, optical backscatter sensor, sediment trap and acoustic recorder; (l) subsea basket of MV Island Pride used for transfer to and from seabed. Photo credits: (a-j) ROV HD14 and HD15, BGR; (k-l) Henko de Stigter, NIOZ.

During the SO268 expedition in spring 2019, most of the collective plume monitoring equipment (Fig. 2.1) was used to monitor the dispersion of a plume generated in a small-scale dredge experiment in the German contract area. 29 sensors were deployed on 14 platforms in a 600 x 200 m^2 array laid out symmetrically around a central lane, in which a plume was produced by dragging a rock dredge 11 times back and forth over the seabed. Some of the equipment was positioned at larger distances for far-field and baseline monitoring. Video surveys made subsequently with the ROV Kiel6000 and the towed video system OFOS of RV SONNE served for visual assessment of the dredge marks on the seabed and the distribution of the sediment drape that had settled from the dredge plume. Sensors in the monitoring array recorded good quality data during their 2-6 weeks deployment, giving insight into the dispersion pattern of the dredge plume. Coordinated within CCT1, calibration of sensors was carried out prior to and during SO268, in order to produce a uniform, intercomparable data set from all the different types of sensors. A paper on the monitoring of the dredge plume and sediment deposition from the plume has been published recently (Haalboom et al., 2022).

During the IP21 expedition in spring 2021, the collective plume monitoring equipment (Fig. 2.1) was deployed again to monitor the plume produced by the DEME-GSR nodule collector trials in the Belgian and German contract areas. At both trial sites, 50 sensors on 20 static platforms were distributed in a broad NW-SE trending swath enclosing the 400 x 400 m^2 large collector test areas, with platforms at 50-100 m, 500 m and 850-1000 m distance from the edge of the collector test area. An AUV rented by BGR from Ocean Infinity was used to monitor the dispersion of the plume at different heights above the seabed and at longer distances than covered by the fixed sensor array on the seabed. The AUV also collected high resolution photo imagery of the seabed before and after the nodule collector tests, from which the spatial distribution of sediment redeposition from the plume can be assessed. Sensors on the seabed and on the AUV recorded good quality data, which give insight into the dispersion of the nodule collector plume in space and time. Optical turbidity sensors were calibrated using suspensions of local seabed sediment to produce intercomparable suspended sediment concentration data for the different types of sensors. Work to also convert acoustic backscatter recorded by current profilers to suspended sediment concentration is ongoing. The outcome of an experiment by NTNU in which local sediment treated with fluorescent dye was placed in the path of the nodule collector (in the GER trial area) in order to trace sediment dispersion in the plume is still uncertain.

To investigate the long-term (seasonal and interannual) variability of near-bottom currents and natural fluxes of sinking particles in the area, BGR has recurrently deployed moorings equipped with current profilers, turbidity sensors and sediment traps in the German contract area. Current meters recorded very low current speeds of mostly < 8 cm s^{-1} that are strongly influenced by semidiurnal tidal variations. Current directions vary cyclically, with a tendency to south-easterly directions. In combination with other data, current data were used by MARUM to produce numerical model predictions of dispersion and redeposition of sediment mobilized by the nodule collector tests (Purkiani et al., 2021; Haalboom et al., 2022). The collected particle flux data reveal a high variability across time and space, with average fluxes varying between 23 and 46 mg m^{-2} d^{-1} during the individual, collected time series. Biogeochemical analyses show that the particle composition is dominated by biogenic components (carbonates, biogenic silica and organic material). X-ray diffraction (XRD) analyses on the lithogenic fraction, which only makes up a few weight percent of the samples, indicate that the fraction is composed of quartz, feldspar including albite, anorthite and clinopyroxene (diopside) as well as amphiboles (hornblende), chlorite-group minerals (clinochlore), micas (muscovite) and clay minerals (kaolinite, smectite). Due to technical defects, the sediment traps that were deployed between 2018 and 2021 only collected particles over relatively short time intervals (several months). Therefore, up to this point, long-term (seasonal) particle flux variability has not been obtained with sufficient resolution. During the IP21 expedition, sediment trap moorings were deployed about 300 m away from the nodule collector test site in the German contract area, prior to the test. These will only be recovered during SO295 in October 2022 and should deliver important information on the effects of plume dispersal on particulate matter flux and composition, as well as complete the time series of background fluxes for long-term analyses. Of particular interest is whether material collected during the nodule collector test might be enriched in nodule fines produced in the nodule collection process.

Given the lack of an industrial scale disturbance in 2019, during SO268 AWI sampled the small-scale disturbance produced with a dredge for the assessment of the thickness of redeposited sediment by means of ^{230}Th. Due to different impact intensities during dredging (e.g. dredge was partly lifted off the seafloor), it proved difficult to determine the sediment removal, which was estimated up to 5-12 cm using ^{230}Th in combination with visual core observations and other biogeochemical variables such as C_{org}, solid-phase Mn and Ca (see Task 3.3). Sediment redeposition could not be quantified. Sediment samples taken during IP21 after the DEME-GSR nodule collector trials are currently being processed in the home laboratory at AWI in Bremerhaven for the analysis of ^{230}Th.

Planning of plume dispersion and impact monitoring activities during the SO268 and IP21 expeditions was guided by a probability forecast analysis of plume dispersion and sediment deposition performed by MARUM. Plume dispersion forecasts were designed from serial model simulations for a period of 8 years (2010-2017) for the month April.

During SO268, as an alternative for supporting plume monitoring efforts with model scenarios, MARUM analyzed satellite sea-surface height data to track an anticyclonic mesoscale eddy that was traveling towards the German contract area at the time of the cruise. This eddy was formed in June 2018 offshore southern Mexico in the Tehuantepec gap wind region. To assess the effect that the eddy might have on the deeper water column and seabed in the study area, CTD water column profiles of temperature, salinity, turbidity, dissolved oxygen, and other variables were recorded during SO268 along the eddy track, in the eddy center as well as outside of the eddy region. The CTD profiles clearly indicated that the eddy had a warm, less saline, and oxygen-enriched core in the subsurface layer, with a recognizable temperature anomaly extending up to a depth of 1500 m (Fig. 2.2). The recently published paper by Purkian et al. (2022) compares field observations with numerical simulations of the eddy.

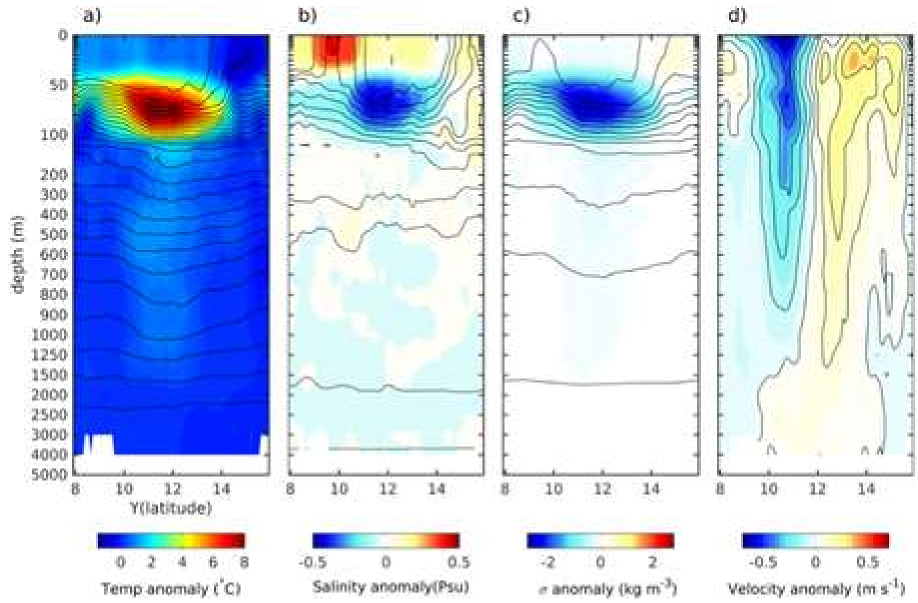

Figure 2.2: Modeled longitude-depth sections of a) temperature, b) salinity, c) density, and d) current velocity on 28 April 2019 compared with climatological mean values (Purkiani et al., 2022).

Field observations of the plume produced during the SO268 dredge experiment served as a basis for validating the plume model developed by MARUM. In this model, ocean hydrodynamics are simulated using the Massachusetts Institute of Technology general circulation model (MITgcm). The model was forced by hydrographic observations on the open boundaries as well as atmospheric parameters on the sea surface obtained from ECMWF reanalysis data. Following results of Gillard et al. (2019), the model assumed aggregate sizes of 70, 340 and 590 µm with settling velocities of 25, 140, and 300 m/d, respectively. An extra sediment class 4 with a settling velocity of 2500 m/d was introduced later to enable the model to simulate redeposition of sediment “lumps” that did not come into resuspension and were only dislocated by the dredging activity. Variation in suspended sediment concentration in space and time observed in the field were very well reproduced by the model. The main modeling challenge was in reproducing the irregular sediment release by the dredging device which part of the time appeared to have been jumping over the seabed. Adopting a variable sediment release rate, the spatial and temporal variation of sediment deposition from the plume was also investigated (Fig. 2.3) and qualitatively compared against images of the seabed with redeposited sediment. Results were published in Purkiani et al. (2021).

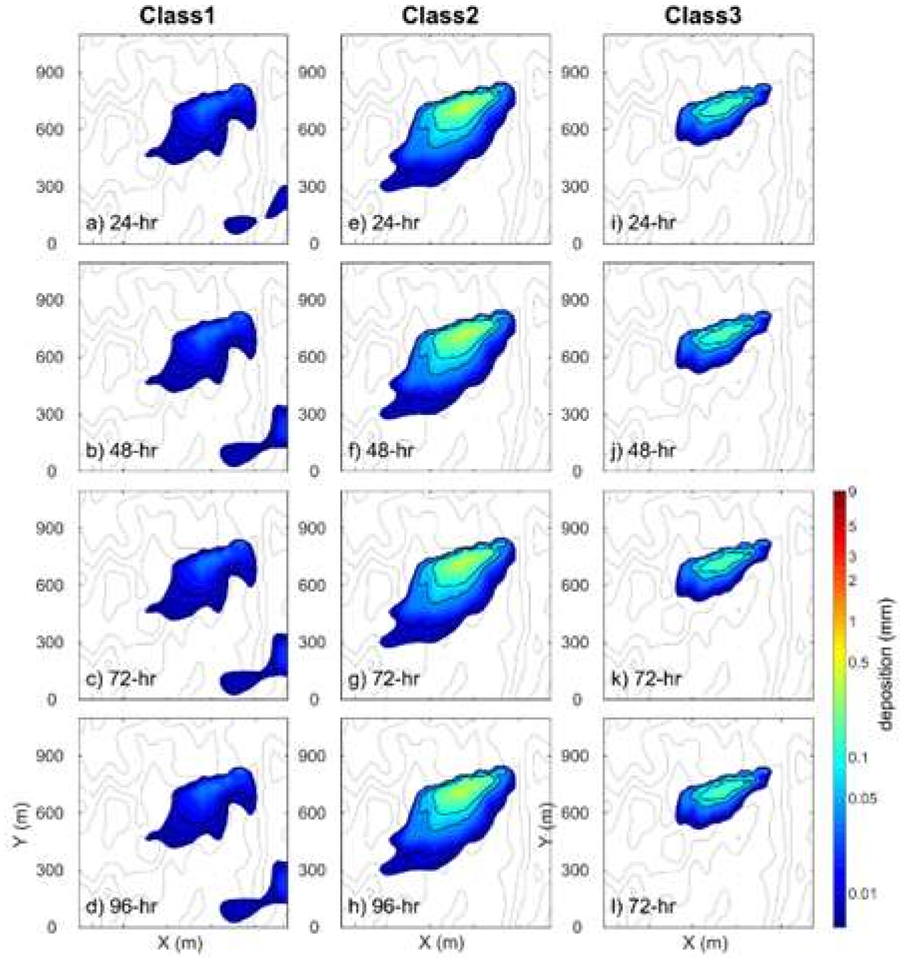

Figure 2.3: Numerical simulation of sediment deposition from the plume generated during the SO268 dredge experiment, for aggregates of 70, 340 and 590 µm, respectively size classes 1, 2 and 3.

A numerical simulation following a similar modeling approach as for the SO268 dredge experiment was also done for a small-scale plume experiment conducted in 2015 during the SO242 expedition to the DISCOL Experimental Area in the Peru Basin. Results are published in Baeye et al. (2022). Numerical simulation of the sediment plumes produced during the DEME-GSR nodule collector trials in spring 2021 is currently ongoing. First results were presented at the MiningImpact 2 final meeting in February 2022.

Arguing that the initial conditions of discharge of sediment from the nodule collector vehicle set the stage for the further dispersion of the plume in the far field, TUDelft investigated the near-field dispersion and settling of sediment released by the nodule collector. Experiments were carried out in the Dredging Engineering Laboratory of TUDelft to investigate the influence of discharge velocity, concentration and geometry on plume behavior in the near-field (Fig. 2.4). Different diffuser designs were 3D printed and tested in a large tank, using different discharge concentrations. It was found that at low discharge concentration, impingement of the plume on the seabed occurs relatively far from the diffuser. The turbidity current that forms after impingement on the seabed spreads laterally, it's velocity decaying only slowly in longitudinal direction. Higher discharge concentration leads to a steeper fall of the plume to the seabed, with higher impact velocity and more vertically directed velocity. The turbidity current that forms after impingement on the seabed spreads radially, it's velocity decaying rapidly away from the discharge location. The experiments thus demonstrated that an optimal design of the diffuser can help to reduce the momentum of the discharge jet, which in turn reduces concentration in the plume and leads to deposition of the sediment nearer to the source.

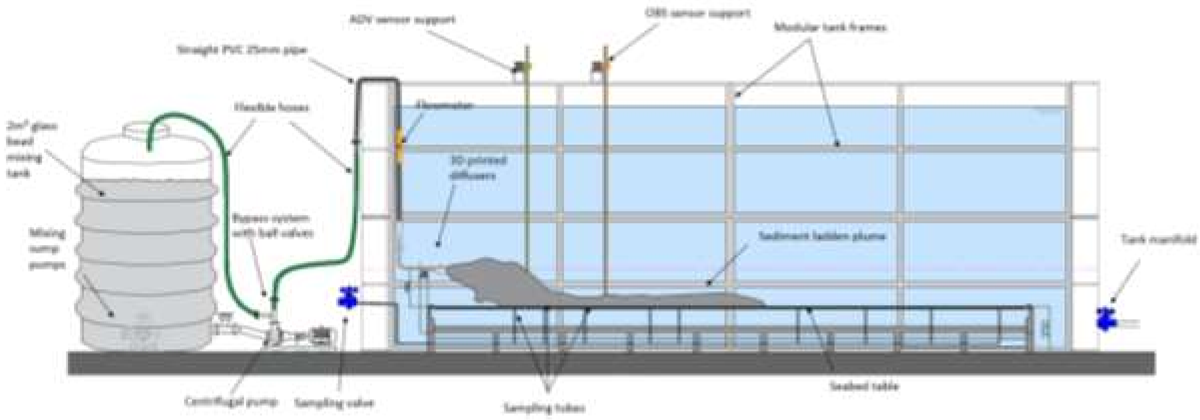

Figure 2.4: Laboratory experiment at UDelft investigating the influence of the shape of the diffuser at the rear of the nodule collector on plume dispersion.

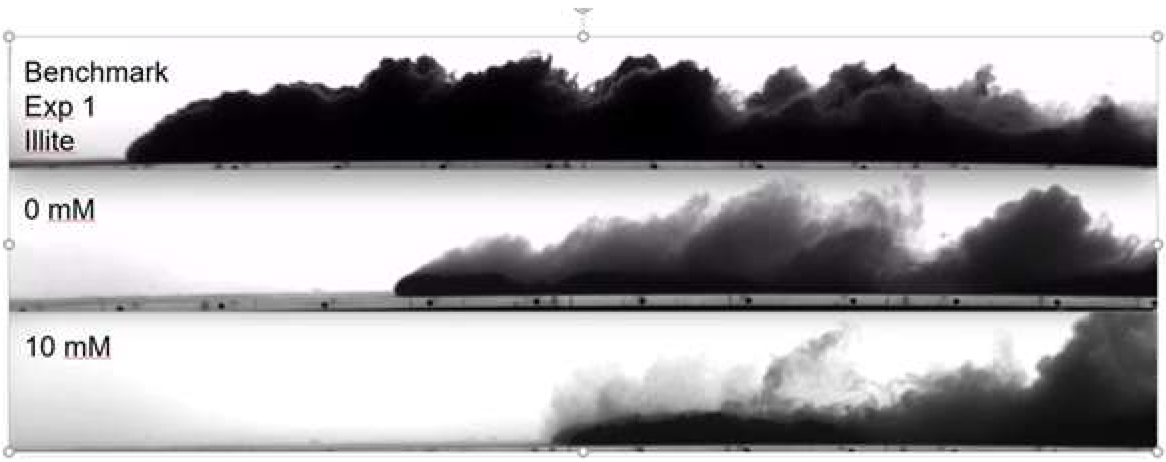

Figure 2.5: Propagation of turbidity currents in lock-exchange experiments. Upper, middle and lower panel show the turbidity current formed with, respectively, illite in fresh water, CCZ sediment in fresh water, and CCZ sediment in salt water, all three at the same time after opening of the lock and with similar initial concentration of suspended solids. Experiments were carried out in a rectangular perspex tank of 3 m long, 0.2 m wide and 0.4 m deep. The gate holding the initial sediment suspension in the mixing section was located at 0.2 m in longitudinal direction. White led strips behind a screen of white paper at the back side of the tank produce homogeneous diffused light. The gray scale of the photographed sediment plume was calibrated to measured sediment concentration, so sediment concentration profiles could be computed.

The propagation of turbidity currents formed after impingement of the sediment plume on the seabed was investigated with so-called lock-exchange experiments (Fig. 2.5). For similar initial concentration of suspended solids, it was found that settling in a turbidity current of CCZ sediment in salt water occurred much faster than in a turbidity current of illite or CCZ sediment in fresh water. The faster settling of CCZ sediment in salt water, significantly reducing the propagation of the turbidity current, can be attributed to flocculation of the suspended sediment. Profiles of suspended solid concentration and speed of the front of the turbidity current determined from photos taken during the experiments showed good agreement with numerical simulations of the density currents. A manuscript on the results of the experiments has been recently submitted by Elerian et al.

Task 2.2 Evolution of physical and chemical characteristics of the plume (JUB, AWI, NIOZ, NTNU)

For in-situ observation of particle aggregation in the sediment plume produced by the nodule collector, JUB developed a deep-sea particle camera (PartiCam, Gillard et al., 2022) with a resolution ranging from 70 µm to a couple of millimeters. During SO268 the PartiCam was lowered with the ship's CTD/Rosette to acquire data on physical properties (size, shape) of background suspended particles in the water column. During IP21 a platform with PartiCam and Aqualogger 310 TY turbidity sensor (Fig. 2.6A) was deployed successfully during the DEME-GSR nodule collector trials in the Belgian and German contract areas. On both occasions the platform was located in the path of the plume at a few hundred meters from the source, resulting in excellent recordings of the passage of the plume (Fig. 2.6C).

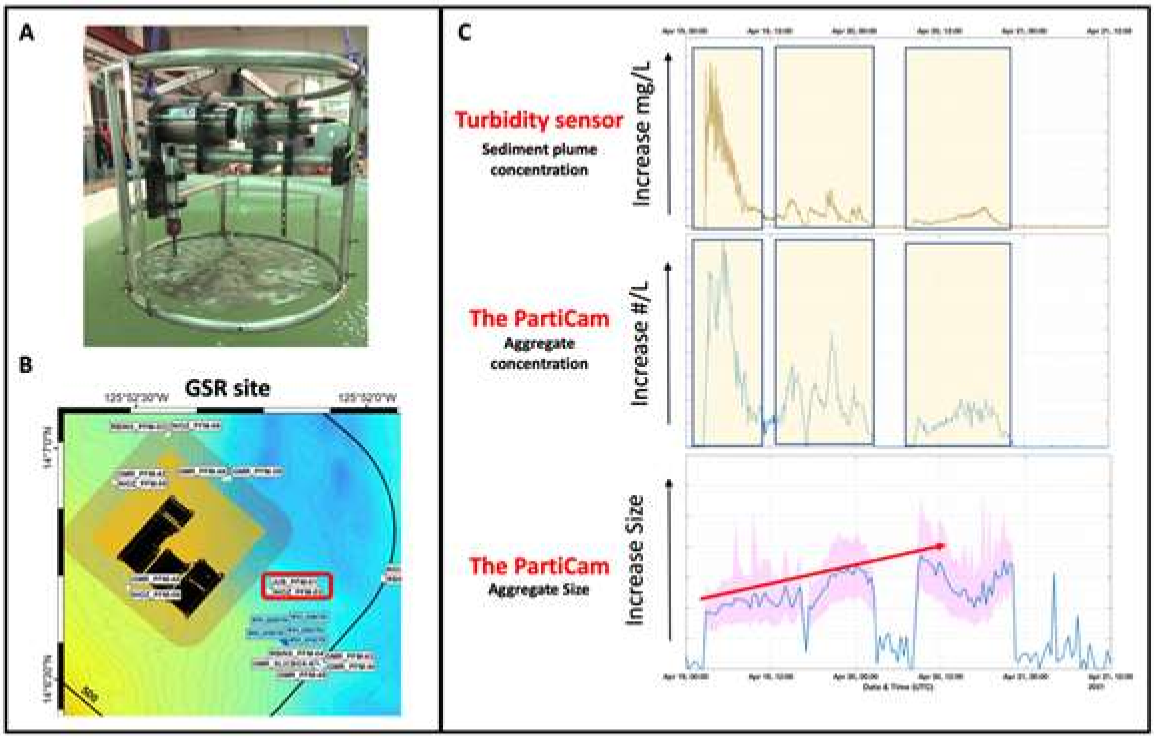

Figure 2.6: JUB plume monitoring platform and overview of results from the collector test in the Belgian contract area. A: Platform with PartiCam and AquaLogger turbidity sensor; B: Platform location in relation to nodule collector tracks in the Belgian area test site; C: Overview of results from turbidity sensor and PartiCam.

In the BEL area, three plume pulses were recorded, representing three consecutive series of runs of the collector vehicle at increasing distance from the platform. The recorded turbidity showed a stronger decrease over time than expected on the basis of recorded particle concentration, suggesting that aggregation occurred during plume dispersion. This was confirmed by the overall increase in median particle size observed with the PartiCam. Physical aggregate characteristics indicate that the sediment plume was in a late aggregation phase (see below and Gillard et al., 2019), suggesting that most of the sediment in the plume had already settled on the seafloor by the time the plume reached the sensor platform. While optical turbidity sensors are very suited to acquire high-resolution data about sediment plumes, they will underestimate suspended sediment concentrations once the aggregation of the plume has started. Further research is needed to address this subject.

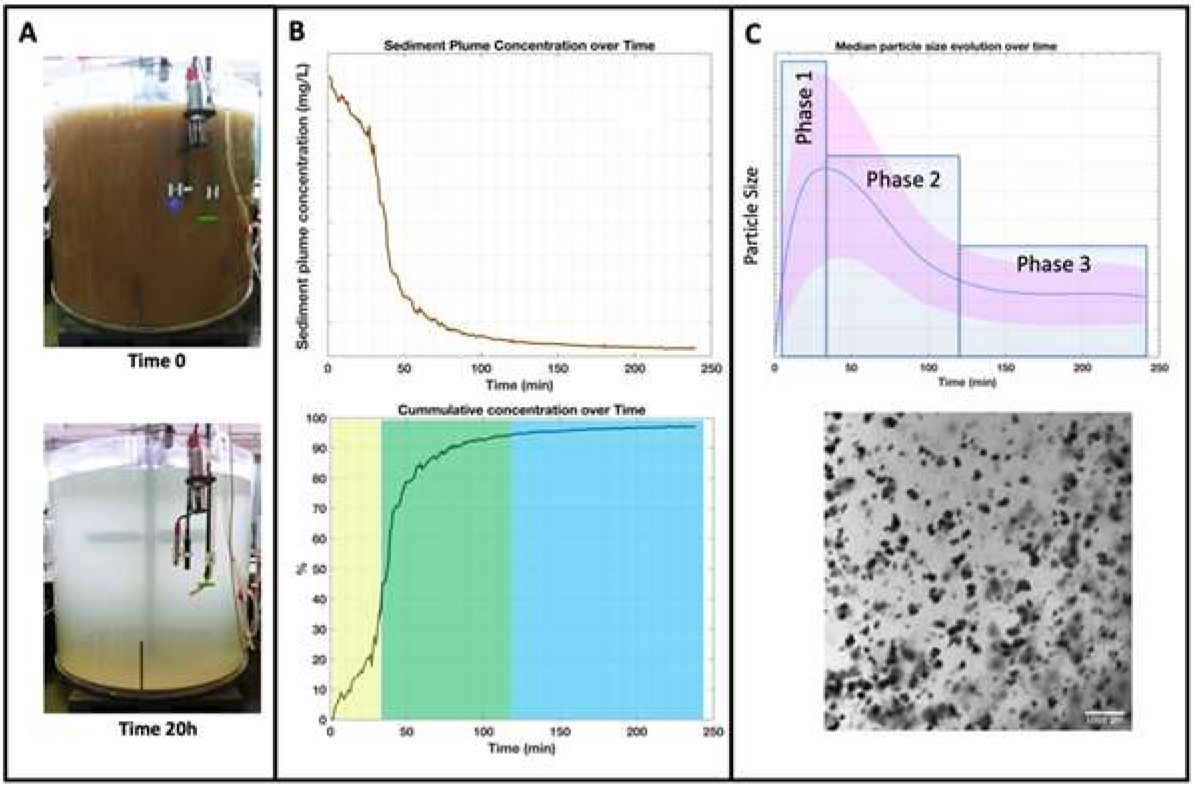

Figure 2.7: Aggregation processes and hydrodynamic behaviour of particles within the plume. A: Water column simulator overview; B: Evolution of plume concentration (top) and cumulative curve (bottom) over time; C: (top) Evolution of median aggregate size over time and (bottom) photo of particle aggregates after 60 min.

JUB also carried out laboratory experiments focusing on aggregation processes and hydrodynamic behaviour of particles within the sediment plume. Experiments were conducted under deep-sea in-situ temperature and salinity conditions using surface sediments from the CCZ. Different particle concentrations (from 10 mg/L to 10 g/L of dry weight) and turbulence regimes were investigated. Figure 2.7 presents the conditions during the first 4 hours within a concentrated plume in a 0.9-m water column (1000 L) under shear rates of 0.1 s^{-1} (normal deep-sea condition). Irrespective of the concentration investigated, three main flocculation phases could be identified: core aggregation, export, late aggregation (Fig. 2.7 B+C). The core aggregation phase, during which aggregates were formed, occurred extremely fast within the first 20 min, confirming results of Gillard et al. (2019). The distribution of primary particle sizes ≤100 µm indicates a general shift towards increasing particle size. The export phase occurred from 30-60 min after plume release. During that time, 85% of the plume was exported to the deeper section of the water column simulator. Doubling the plume height is likely to double the export phase time. The late aggregation phase lasted from 60 min onward. During that time, the fallout slowed down as the remaining concentration drastically decreased. Even after 20 h, the analysis of the remaining suspended particles indicated the presence of aggregates. Those aggregates have been created from the remaining plume particles over a long period and will potentially be exported over a more significant distance.

Studies on the trace element composition (Fe, Mn, Co, Cu, Ni, Zn, Pb, Cd, V, As, rare earth elements) of near-bottom seawater were conducted by BGR in close cooperation with JUB. Applied methods included (1) the size-fractionated determination of metal concentrations in water samples collected by CTD Niskin / GoFlo bottles or a bottom water sampler (BWS), and (2) the in situ determination of labile-bound (and thus potentially bioavailable) trace elements with DGT passive samplers, that were installed near the bottom on moorings for periods of 4 weeks (during SO268) and 2 years (from SO268 until IP21). Passive samplers were also attached to sensor platforms deployed during SO268 and IP21 for monitoring of the small-scale dredge experiment in the German contract area and the DEME-GSR nodule collector trials in both the Belgian and German contract areas, respectively. These investigations have allowed us to obtain: (1) first baseline data sets for trace metals in near-bottom seawater in both areas, including total concentrations, element associations with different size pools, and speciation-dependent potential availability to organisms (Schmidt et al., 2022) and (2) impact data from the IP21 sediment plumes, that provide new insights into the mobilization of trace elements during mining activities including their physical size distribution and their potential bioavailabilities. The results provide essential data for ecotoxicological work and environmental risk assessment being carried out by the UAlgarve and DNV in WP2 and CCT3. Analyses of the trace metal composition of particles sampled from background seawater and the nodule collector plume on filters by NIOZ, BGR, and JUB during SO268 and IP21 are still underway at JUB. These results will complement the results for the dissolved phase (Schmidt et al., 2022). Due to unsuccessful plume sampling during SO268 and the delayed DEME-GSR nodule collector trials, JUB work focused on trace metal dynamics in the pore water and sediment and unfortunately only few results for the water column were obtained that could support modelling of trace metal behavior in the plume. However, this remains an important question and should be addressed in future work.

For lack of an industrial scale disturbance during SO268, AWI sampled the water column mainly for the collection of baseline data of the dissolved radium isotopes ^{226}Ra and ^{228}Ra (see Task 3.1) using large-volume in-situ pumps. Attempts of sampling the plume produced by the small-scale dredge disturbance during SO268/2 remained unsuccessful, i.e. the results are inconclusive. Due to limited personnel onboard IP21, the in-situ pumps could not be deployed for sampling the plume generated by the DEME-GSR nodule collector.

Task 2.3 Ecological impact of the plume (UAlgarve, IPMA, IMAR, CIIMAR, NIVA, UGhent, RBINS, USOU, UUtrecht, SGN, JUB, MPI)

As the DEME-GSR nodule collector trial was delayed, results are still preliminary and many partners are still working on their samples. Although all efforts have been made to mitigate the impact of the delay, it was not possible to fully accomplish the objectives of this task according to the proposed timeline.

Sediments collected during SO268 from the GER area were analysed for metal concentrations by IPMA and for ecotoxicity by UAlgarve using the solid phase Microtox® bioassay. This is a quantitative and functional test measuring the changes in luminescence by non-pathogenic naturally luminescent marine bacteria (*Vibrio fischeri*) upon exposure to a sample containing toxic materials. Levels of V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, and Pb were determined by ICP-MS after acid digestion. Concentrations varied between the two sediments, although both sediments presented high levels of Mn, Cu, and Zn. Comparing concentrations found in both sediments, from deep-sea and coastal areas, the first one presented lower concentrations of all contaminants except Cu (deep-sea levels of 487 and 364 mg/kg). These sediments were also homogenised in artificial seawater and submitted to subsequential dilutions to evaluate the impact of metal release into water and to the biota. Water samples were filtered through 0.45 µm filters and concentrations of V, Cr, Ni, Cu, Zn, As, Cd, and Pb were analysed by ICP-MS after SeaFast pre-concentration and saline matrix elimination. Analyses of metals released into water are presented in Figure 2.8. Vanadium, Cu, Zn, and As presented higher concentrations in the water, suggesting a higher and/or faster transfer from the sediments. An abrupt decrease in dissolved metal concentrations was observed as concentrations of suspended sediments decreased. The remaining elements showed very low, but measurable, dissolved concentrations suggesting minor transfer from the solids to the water.

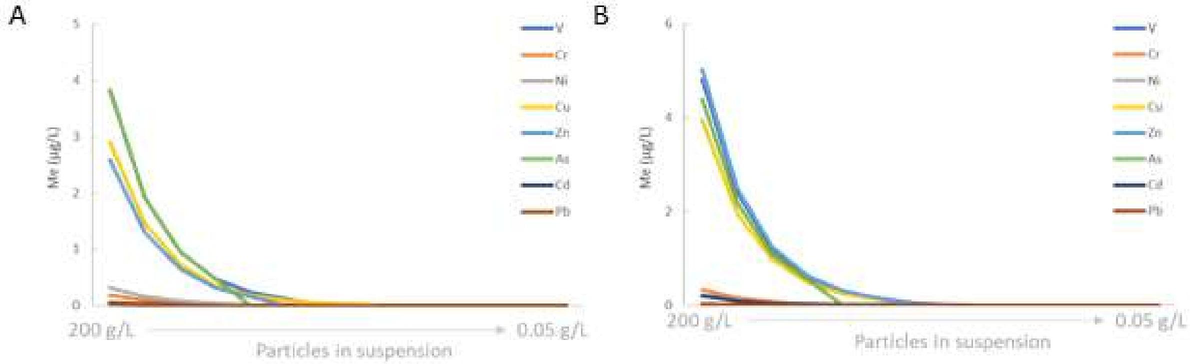

Figure 2.8: Concentrations of V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, and Pb (mg/L) in the water from the Microtox test from sediment 12KG (A) and sediment 19KG (B) collected in the GER area.

As for bulk sediment ecotoxicity of these sediments from the GER area, Microtox results revealed rather similar values for both sediments and nodules, with an EC_{50} (15 min) of 2.2 g/L for BGR12KG, and 3.7 g/L for BGR19KG, while crushed nodules (fraction < 63 µm) EC_{50} (15 min) were 1.6 g/L for NOD16-12, 3.2 g/L for NOD16-19 and 2.1 g/L for NOD16-23. Toxicity bioassays were also applied to nodules and sediment samples from the Area of Particular Environmental Interest (APEI) 6 of the CCZ. Here, the Microtox results revealed that crushed nodules were slightly more toxic in comparison to sediments from different topographic settings. The EC_{50} values obtained with Microtox have similar ranges to those obtained in the GER area. In addition, an embryotoxicity test was performed by UAlgarve using the sea-urchin *Paracentrotus lividus*. In this bioassay, fertilized sea-urchin eggs were exposed for 72 hours to elutriate dilutions of sediment and crushed nodules, after which the percentage of larvae with abnormalities was determined. Toxicity to *P. lividus*, quantified by calculating the EC_{50} reducing embryogenesis success, was higher for the nodule samples as compared to sediment samples.

Trace elements (V, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Ag, Cd, Ba, Pb, and REE) were measured by IPMA in the elutriates by ICP-MS after pre-concentration in a SeaFast. Mn, Cu, Ni, Cd, and the rare earth elements yttrium and europium showed a measurable transfer from the solids to the water column. Figure 2.9 illustrates this for the release of Cu and Eu. The rate of transfer depends on the resuspended material but was always higher in the experiments with higher concentration of particulate material. Overall, sediments and nodules release metals to the seawater that can induce ecotoxicological effects in *Paracentrotus lividus* embryos.

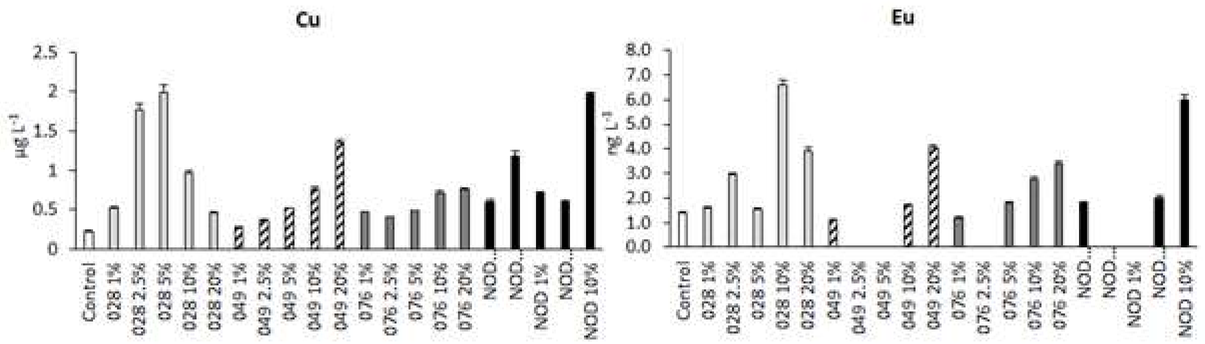

Figure 2.9: Concentrations of Cu and Eu (µg/L) in water from the control, sediments (028, 049, and 076), and nodules (NOD) Microtox experiments.

During the IP21 cruise, a few Actinaria individuals were collected, preserved at -80ºC and processed for the analysis of biomarkers of toxicity. Preliminary results were obtained by UAlgarve for lipid peroxidation (LPO, a biomarker of cell membrane damage). Ongoing work is directed at analysing other oxidative stress biomarkers and the chemical composition of different cellular fractions (the latter in collaboration with IPMA). These are the first results of the natural baseline variability for the GER area. With the limited number of samples available from the pre- and post-impact at the BEL area, it is obviously difficult to demonstrate if there is an effect from the exposure, although the post-impact specimen showed greater LPO levels.

For a study on the impact of the sediment plume on the reproductive output and microbiome of Anthozoan corals carried out by UAveiro and NIVA, coral specimens were collected during SO268 from reference and trial areas of the GER and BEL areas (Fig. 2.10). Microbiome analyses revealed significant differences in bacterial community compositions of three families: the corals Isididae and Primnoidae and the anemone Actinostolidae. Anemones harboured bacterial microbiomes composed mainly of Hyphomicrobiaceae, Parvibaculales and *Pelagibius* members. Core microbiomes of corals were mainly dominated by different Spongiibacteraceae and Terasakiellaceae bacterial members, depending on coral taxonomy. Moreover, the predicted functional profiling suggests that deep-sea corals harbour bacterial communities that allow obtaining additional energy due to the scarce availability of nutrients (Quintanilla et al., in press). This is the first report of microbiomes associated with abyssal gorgonians and anemones and will serve as baseline data and crucial insights to evaluate and provide guidance on the impacts of deep-sea mining on these key abyssal communities. The reproductive studies are currently underway.

For a study by UAveiro and NIVA on the impact of the plume on early-life stages, a McLane Large Volume Water Transfer System LV 30 with a 64 µm filter was deployed during SO268 and IP21. Although not conclusive our results add to the very limited knowledge on larvae diversity and abundance and diversity in the CCZ. Up to now, the only data on larvae from the CCZ are from a single plankton pump sample, which obtained two individual polychaete larvae from the same morphotype (Kersten et al., 2017). We found a large diversity of taxa (Polychaeta, Crustacea, Holothuroidea, Ophiuroidea, Bivalvia and Gastropoda) and different morphotypes (Fig. 2.11) with different abundance at the different sampling sites. All the collected specimens have been photographed and processed for barcoding. Unfortunately, no successful DNA extraction was obtained – further methods are currently being employed.

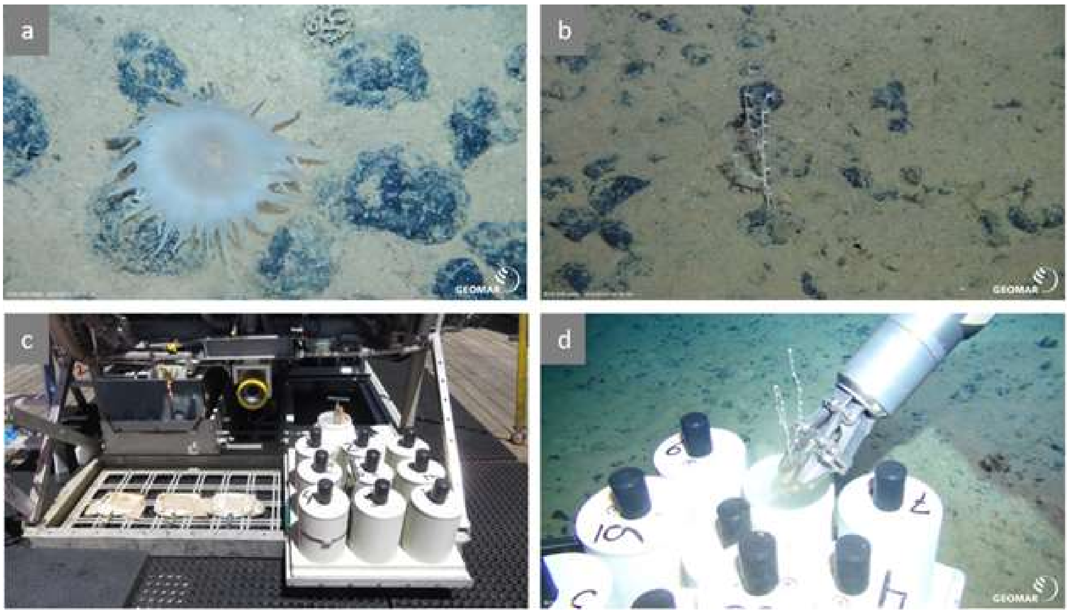

Figure 2.10: Seafloor images of (a) Actiniaria and (b) Alcyonacea; (c) Anthozoan container on deck; (d) collection of an alcyonacean coral. Photo credits: (a,b,d) GEOMAR ROV Kiel 6000; (c) Ana Hilario, UAveiro.

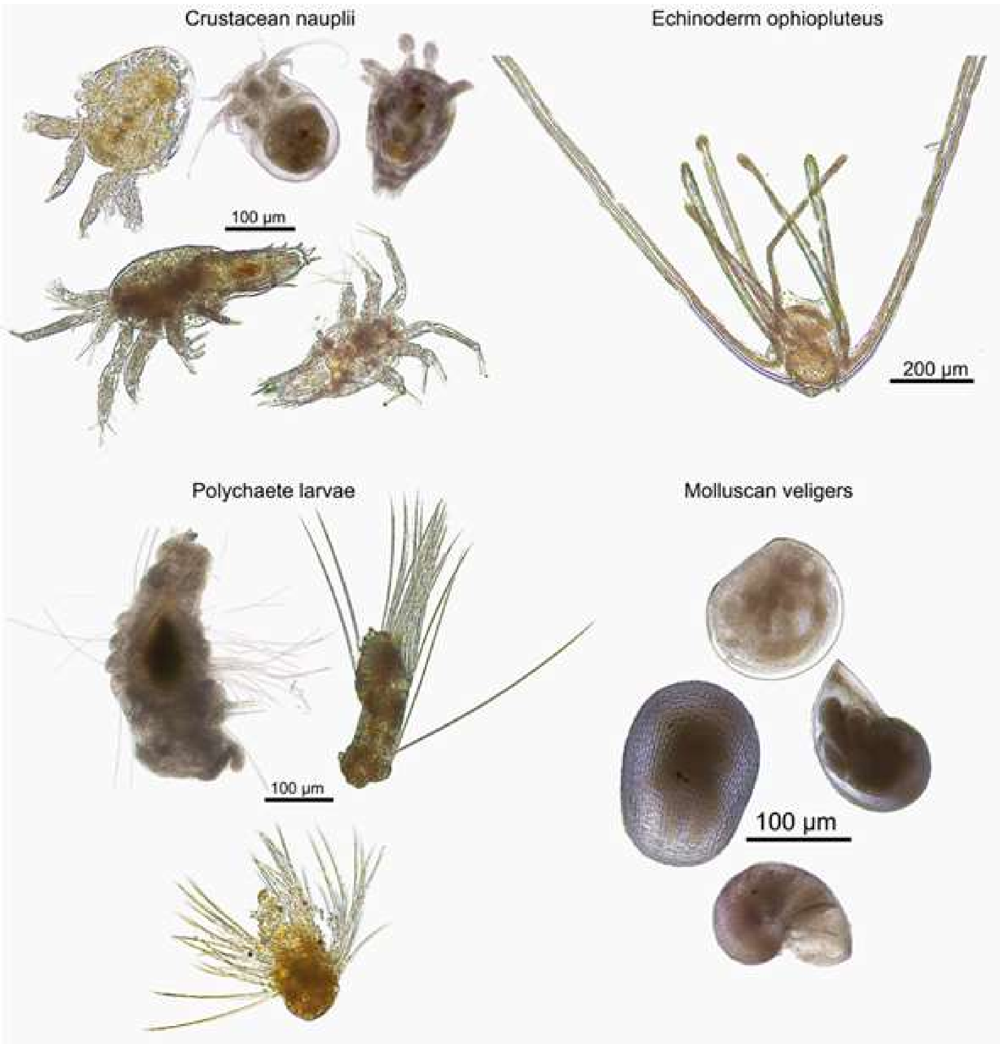

Figure 2.11: Examples of larvae collected during the IP21 cruise.

For sedimentation impact experiments conducted by IMAR, specimens of the cold-water coral *Dentomuricea* cf. *meteor* were collected from Azorian waters at depths of ~200 m and experiments conducted at the IMAR-UAzore's DeepSeaLab aquaria facilities in Horta, Azores. Corals were exposed for a period of four weeks (28 days) to suspended plumes of sediments from nodule fields (NFS) and hydrothermal polymetallic sulfide particles (PMS) at two concentrations (10 and 50 mg L^{-1}) and a control treatment with no sediment addition. Sediment concentrations were selected based on plume dispersal models, as near field (hundreds of meters for 50 mg L^{-1}) and far field (kilometres for 10 mg L^{-1}). Sediments from the CCZ were obtained from abyssal depths of 4000 m in the BEL area. Polymetallic sulfide particles were obtained by grinding broken dead sulfide chimneys collected at the hydrothermal vent field Lucky Strike at 1750 m depth. The grain sizes ranges were 95-87% <63µm for NFS and 80% 0.5-10 µm / 20% 10-70 µm for PMS particles. The physiological effects of exposure to these sediments were measured every week at different levels in the organism: at the whole-organism level (survival, polyp activity, respiratory metabolism, excretion), tissues composition (C:N-ratio, metal bioaccumulation), and at the molecular level (protein expression). Here we report physiological effects only at organism level as other analyses are still under progress. Preliminary results showed high sensitivity of *D. meteor* to polymetallic sulfide particles, with survival for only 4 days at both PMS concentrations, but full survival for 4 weeks under NSF. Tissue condition of *D. meteor* was affected by exposure to suspended particles (Fig. 2.12). Tissue necrosis and loss were evident in treatments with PMS after only 2 days exposure (4-7% tissue loss). Tissue loss of corals exposed to nodule field sediments progressively increased throughout the experiment (from ~1 to 7%).

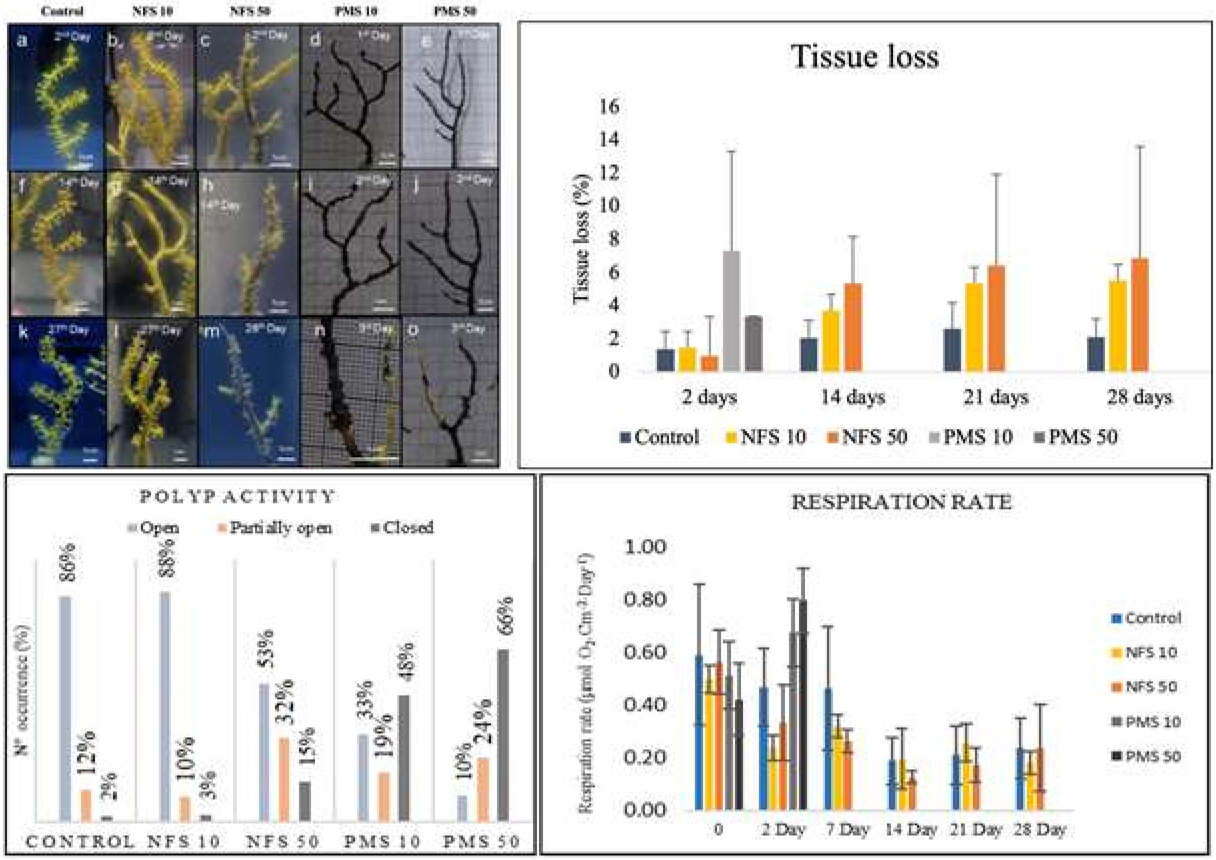

Figure 2.12: (top) General appearance and loss of tissue in Dentomuricea meteor fragments and (bottom) polyp activity and respiration rates of Dentomuricea meteor exposed to experimental treatments with sediments from nodule fields (NFS) and polymetallic sulphide particles (PMS) at concentrations of 10 and 50 mg L^{-1} and a control treatment with no sediment addition during the 28 days of the experiment. Data values as average ± standard deviation.

Water samples were collected at different times, to evaluate the time-scale variation of metal release to water. Bioaccumulation in coral tissues was also determined. Dissolved concentrations of V, Mn, Co, Ni, Cu, Zn, Cd, and Pb were determined by ICP-MS after filtration and pre-concentration in a SeaFast system at IPMA. As an example, variation of Mn, Co, Cu, and Cd concentrations during a 24 h cycle (12 h with and 12 h without particles) are shown in Figure 2.13. All elements showed a clear increase during plume formation (from 0 to 12 hours resuspension) with a subsequent decrease in the period with no particles (T12½ - T24). It is noteworthy that the concentration peak time scale was dependent on the element. For Mn, concentrations peaked after 4 h of the plume and remained similar until particles were removed from the system (T12). During the phase with no plume, concentrations decreased slowly to levels close to the initial conditions (T0). Cobalt concentrations showed a bell shape curve, with a broad peak at 12 h. For Cu, the highest concentration was obtained after only 2 h of the plume with a sharp decrease after 4 h until 12 h (during plume exposure), after that, levels reached a plateau for another 12 h (T12-T24, no plume present in the medium). A less pronounced increase was observed for Cd with a higher level at 12 h and a smooth decrease when particles were removed from the system. Elemental concentrations were analysed during 3 cycles (during 66h) and similar patterns were observed for each element at each cycle.

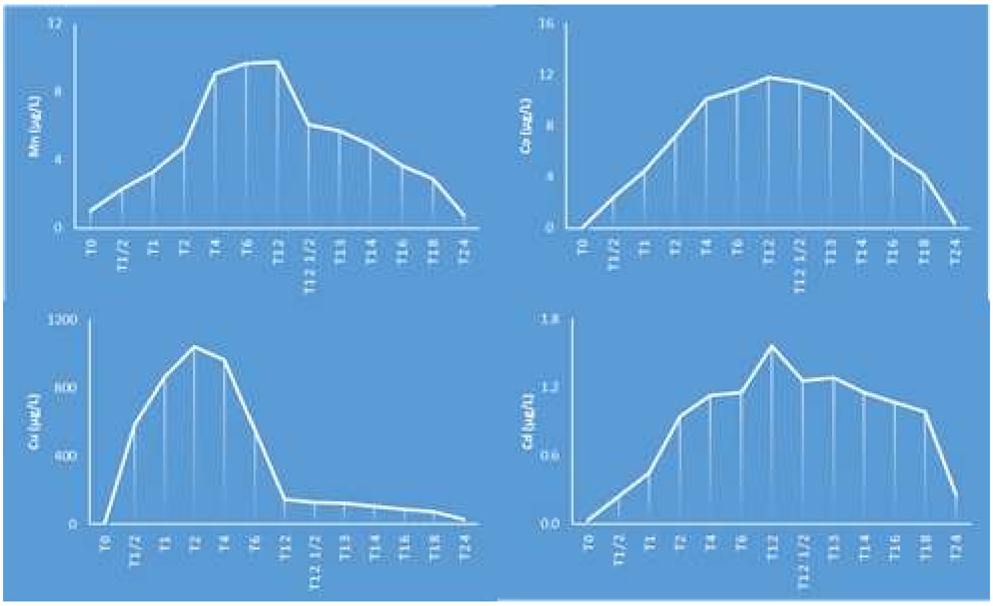

Figure 2.13: Concentrations of Mn, Co, Cu, and Cd (µg/L) in water collected from tanks with particles and corals.

To mitigate the absence of a representative in-situ sediment plume during SO268, MPI investigated the impact of sediment resuspension on pelagic microbial communities through an ex-situ mesocosm experiment carried out on board of RV SONNE. The experiment had two aims: i) to assess the effect of sedimentary organic matter released in the water on microbial activity and ii) to investigate the fate of benthic microbes resuspended together with sediments. Impact on microbial activity was investigated by measuring extracellular enzymatic activity (EEA) as measure of potential organic matter degradation and by oxygen consumption as measure of organic matter remineralization rates. To assess the effect of benthic microbes on pelagic microbial community, DNA was extracted from water samples and microbial diversity was investigated applying Automated Ribosomal Intergenic Spacer Analysis (ARISA), a molecular fingerprinting technique. The results of this experiment, however, seemed to reflect a “bottle effect” induced by the experimental conditions, rather than the suspension of sediment in the seawater. During IP21 the DEME-GSR nodule collector successfully operated on the seafloor, thus MPI could pursue the objectives of the ex-situ experiment by collecting the sediment plume samples created by the collector, as well as sampling bottom seawater before and after the tests. As for the ex-situ experiment, seawater and plume samples were used to measure microbial activity (EEA and oxygen consumption) and describe taxonomic/functional diversity. The latter is investigated with next generation sequencing method, which resolves better microbial diversity compared to molecular fingerprinting technique. Radiotracer incubations were also carried out to measure uptake rates of organic and inorganic substrates, and hence provide additional information on microbial metabolic rates under natural and disturbed conditions. The preliminary results and status of analysis for microbial diversity and activity are described in WP1 (Task 1.3) and WP3 (Task 3.4), respectively.

In-situ ecosystem functioning experiments carried out by CIIMAR during SO268 and IP21 are reported under WP1 and CCT2. Since all equipment which UUtrecht sent to SO268 was withheld by Mexican customs, the planned sedimentation experiments with the NIOZ CUBEs could not be performed during SO268.

WP 3 – Biogeochemistry and ecosystem functioning

Milestones and Deliverables

M3.1 Sampling, in situ measurements and experiments conducted successfully during baseline and impact cruise (MPI)

Sampling, in situ measurements, and experiments have been conducted successfully during SO268 given the custom problems in Mexico and the postponement of the Patania II trials from 2019 to 2021. While MPI's radiotracer-based measurements of microbial activity and biomass production rates and CIIMAR's *in situ* foodweb experiments in the presence of the collector plume had to be postponed to the IP21 expedition in 2021, UUtrecht's *in situ* experiments on plume impacts on filter feeders had to be cancelled completely. However, the ship time available to the project due to the additional expedition allowed for extended replication, more detailed investigations of nodules and deeper sediment layers, as well as investigations of a small-scale dredge disturbance experiment. A detailed account of the achievements is provided in the SO268 cruise report (Haeckel and Linke, 2021) and will be found in the IP21 cruise report currently in preparation.

M3.2 Biogeochemical sediment characteristics, fluxes, and organic matter processing by benthic communities (including baseline variability and effects of impacts) quantified, presented at annual meeting, and provided to project partners for integration in CCT2 (CIIMAR & MPI)

Biogeochemical data have been presented at the annual meetings and two CCT2 workshops held in November 2020 and December 2021 with a focus on the environmental consequences of the dredge experiment and the Patania II trials, respectively. Further, WP3 members shared their data with project partners via the project data space at OSIS Kiel.

M3.3 Setup of food-web and diagenetic models and first simulations results discussed with project partners (NIOZ & GEOMAR)

Due to problems and delays with the Patania II collector tests and the realization of the in situ experimental work, UUtrecht had to cancel plans for food-web modeling based on experimental data and developed - based on literature data - an interaction web model and a carbon-based food web model for the Clarion-Clipperton Fracture Zone (see Task 3.4). Diagenetic modeling of GEOMAR built on data of the first project phase and was updated with data from SO268 and IP21 as they became available. The main focus was on modelling of bioturbation activity in the GER and BEL areas using radiotracer data from the reference and trial sites provided by AWI and on in situ oxygen profiles from the collector trial sites provided by MPI. Annual Meetings and WP3 workshops (D3.2 and D3.3) were used to discuss the biogeochemical data with partners.

D3.1 First results and data from field work, in situ and experimental studies are made available to project partners (MPI)

An online list of data and samples relevant to WP3 has been compiled early on in the project and shared with WP3 partners for guidance. It also served the WP4 team for scheduling of data submission deliverables. Most data have been submitted to OSIS Kiel by now and more (especially from IP21) will continue to flow into OSIS Kiel as they become available. In addition, WP3 data have also been shared with the project consortium by presentations at the annual meetings and two CCT2 workshops.

D3.2 Interim workshop & report on biogeochemical processes, ecosystem functions, and data requirements for diagenetic and food-web models (CIIMAR & NIOZ)

As no data from *in situ* experimental work could be made available in the time frame of the project and modeling at UUtrecht was based on existing data (see M3.3), there was no need for workshops with modelers and data providers. At the WP3 breakout during the final meeting, next steps in food web modeling once data from the *in situ* experiments carried out by CIIMAR (see Task 3.5) become available were planned between UUtrecht and CIIMAR.

D3.3 Data integration & modeling workshop and integrated report on impacts on ecosystem functions (GEOMAR & MPI)

At the WP3 breakout during the 2nd Annual meeting it was decided that due to the delayed collector trials the workshop would need to be postponed. While biogeochemical consequences of mining-related impacts were addressed in a more general manner in Haffert et al. (2020) and Volz et al. (2020), the data obtained from the small-scale dredge experiment was not suitable to progress further with respect to mining impacts. Thus, discussions got delayed to after the IP21 cruise in 2021. Exchange on plans to follow up on diagenetic modeling took place at meetings on data integration and joint publications on biogeochemical baseline conditions and disturbance effects as well as during the WP3 breakout session at the final meeting. The integrated analyses of biogeochemical baseline conditions and disturbance effects is still ongoing after the official end of the project.

Task 3.1 Effects on sediment physical properties and porewater expulsion (GEOMAR, JUB, AWI, BGR)

Physical properties of the sediments and effects of mining-related disturbances with a focus on sediment mechanical properties and compaction were addressed in this task. On-board shear strength measurements during SO268 and IP21 by GEOMAR aimed at determining possible compaction of the underlying sediments by the collector vehicle, which could induce outflow of pore fluids and changes diffusion properties of the sediment by reducing porosity. Increased shear strength could furthermore hinder recolonization by fauna (as results from the 1st project phase suggested). Overall, the profiles show typical patterns and values for deep-sea sediments with microstructures due to components such as minerals or diatoms (Fig. 3.1). In the BEL trial area, pre-impact shear strength values are significantly higher than in the other sites. Comparing the values before and after the collector test, no measurable compaction due to driving of the collector vehicle over the seabed can be detected (Fig. 3.1 bottom). In contrast, the deposition of the suspended sediments resulted in a surface layer with weak shear strength, both in the collector tracks and outside in thick sediment deposits. Measurements in cores from the SO268 dredge experiment did not show any difference between pre- and post-impact due to the shallow disturbance created by the dredge (not shown).

Sediment porosity profiles measured by GEOMAR and BGR were quite similar between the GER and BEL areas and the different sites. However, porosity was clearly reduced near the sediment surface in the dredge tracks due to removal of the top layer and the same was observed for Patania II tracks at sites that did not show much sediment deposition (Fig. 3.5). In contrast sediment porosity was slightly increased at plume impact sites with thick sediment deposition.

During IP21, JUB sampled cores collected pre-trial and in Patania II tracks, to compare fluxes across sediment layers to assess the impact of sediment compaction / reduced pore space on the diffusion of metals as well as sorption/desorption properties. These experiments are still underway at JUB. AWI sampled bottom waters with *in situ* pumps after the dredge experiment in 2021 but the detection of pore-water loss based on the dissolved $^{226}Ra/^{228}Ra$ signature in the water column was unsuccessful. Due to the lack of personnel and limited working days during IP21, in situ pumps for radionuclide studies in bottom waters could not be deployed.

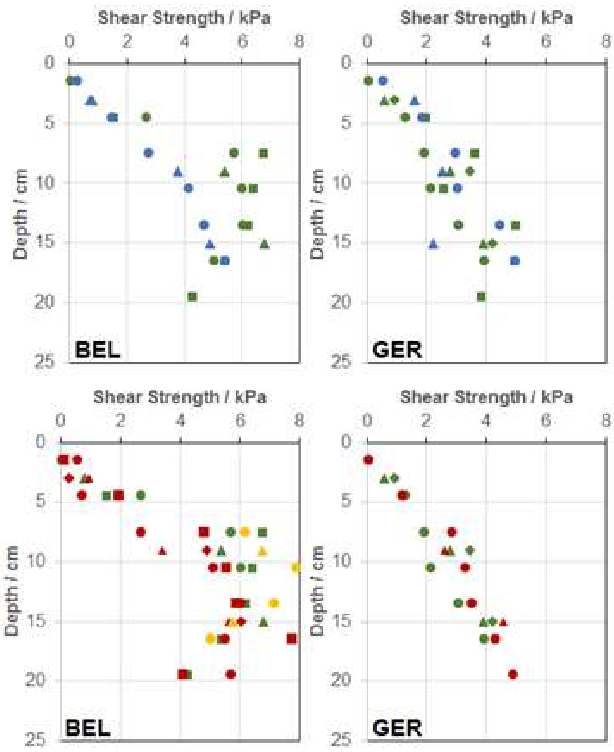

Figure 3.1: Depth-profiles of sediment shear strength in the BEL and GER areas. (top) pre-impact reference (blue) and trial (green) sites; (bottom) post-impact in the collector impact (red) and in the adjacent plume impact sites (yellow).

Task 3.2 Assessment of sedimentation and bioturbation dynamics (AWI, NIOZ, GEOMAR, MARUM)

Sedimentation rates, bioturbation characteristics, and sediment structure were assessed with natural radioisotopes and computed tomography (3D X-ray) core scans. Sedimentation rates were similar in the GER and BEL areas. Bioturbation activity based on ^{230}Th was slightly deeper in the GER than in the BEL area, but showed no significant difference between trial and reference sites. Analyses of ^{230}Th and ^{210}Pb allowed a quantification of sediment removal of ~1-4 cm in the dredge tracks created in 2019. Based on ^{210}Pb the blanketing thickness in the vicinity of the dredge tracks could also be resolved at a site where the deposited layer was several cm thick. Analyses of radionuclides in cores taken after the Patania II trials are currently being processed. Computed tomography (CT) of whole cores proved a powerful tool to visualize disturbance (cracks introduced by the collector, deposition of a homogenized surface layer, loss of biogenic structures). At the same time the scans also show clear signs of sampling artifacts, especially when nodules were captured with the cores. Based on the visualizations it has to be assumed that coring artifacts introduce variability and uncertainty to any core-based observations. Both, radionuclides and CT scans may be used for assessments of longer-term effects on and potential recovery of bioturbation in post-impact studies.

Sedimentation rates and bioturbation activity

AWI and NIOZ took sediment samples before and after the dredge experiment during SO268 and the Patania II test during IP21 in the GER and BEL areas for the determination of sedimentation rates and bioturbation depths using ^{230}Th, ^{210}Pb and ^{226}Ra, respectively. An interval of homogenous $^{230}Th_{ex}$ has typically been observed in undisturbed surface sediments in the CCZ (Volz et al., 2018; Volz et al., in prep.), which can only be achieved by mixing of sediment by burrowing macrofauna. While corresponding mixing activities were quite similar (D_b = 0.14-0.15 cm^2/a), the bioturbation depth is slightly deeper in the GER area (x_{DB} = 7.5 cm) compared to the BEL area (x_{DB} = 5.4 cm) (Fig. 3.2). Based on the exponential decay curve of $^{230}Th_{ex}$ below the bioturbated layer, natural sedimentation rates were calculated. They vary between 0.28 and 0.38 cm kyr^{-1} in the GER area and between 0.18 and 0.38 cm kyr^{-1} in the

BEL area (Volz et al., in prep.). No significant differences in natural bioturbation depths, bioturbation activity and sedimentation rates were observed between trial and reference sites (Fig. 3.2).

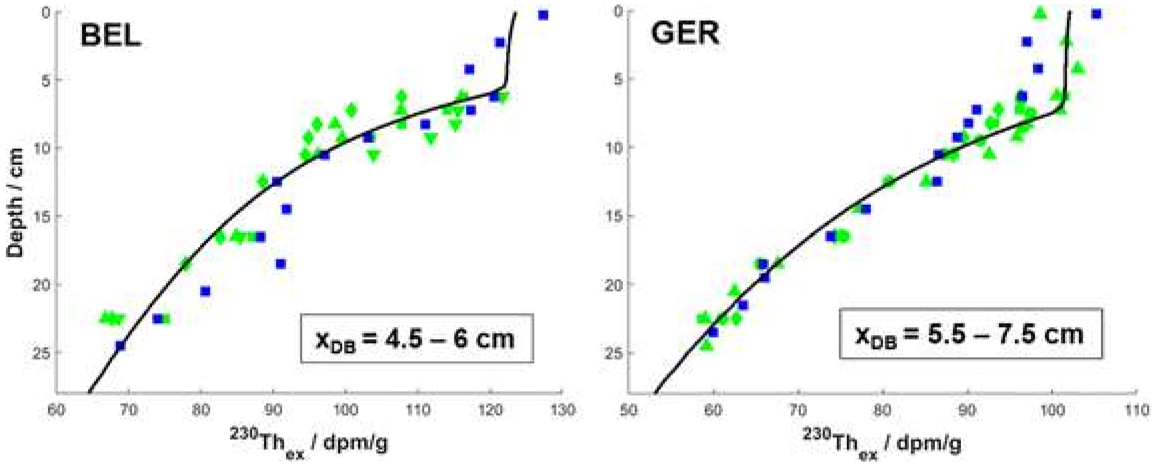

Figure 3.2: Baseline $^{230}Th_{ex}$ data produced by AWI for reference sites (blue) and pre-impact trial sites (green) for the BEL and GER areas and calculated bioturbation activity (black line) by GEOMAR.

In some of the cores from the dredge experiment site the top few centimeters with the highest $^{210}Pb_{ex}$ and $^{230}Th_{ex}$ activity at the sediment surface seemed to be removed by the dredge, and in some cases replaced by a layer of freshly redeposited sediment characterized by homogeneous ^{210}Pb and ^{226}Ra distributions (Fig. 3.3).

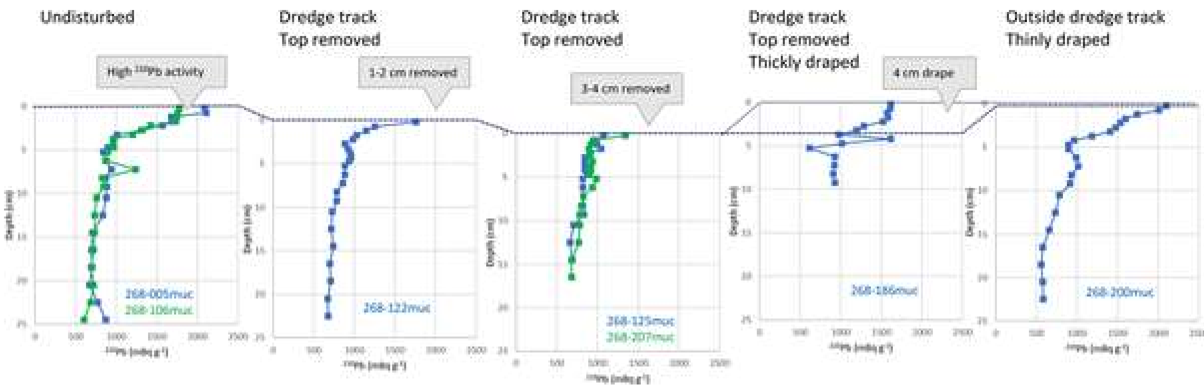

Figure 3.3: Downcore profiles of total ^{210}Pb from multicores collected during SO268 from undisturbed and disturbed sites of the dredge experiment in the German contract area.

Physical and biogenic sediment structures

Marum investigated 47 multicorer and push cores collected in the GER and BEL areas in 2019 and 2021 with CT to study the depositional environment under natural conditions and the effects of the different impact experiments. Sampling included areas with thin and thick sediment cover and within the dredge and the Patania II track. The CT-based investigations allow a 3D quantitative acquisition of manganese nodules, open bioturbation traces and sediment fractures. Bioturbation burrows were well visible in all cores but could not be quantified. The deposits showed a high heterogeneity in replicates. Homogenized sediment layers were identified on top of most cores and showed a reduced density, no burrows and rare open bioturbation traces. These probably represent escape traces suggesting the partial survival of infauna after the impact / sampling. In post-impact cores, these layers were interpreted as sediment plume deposits. Their occurrence in pre-impact cores, especially in cores with large manganese nodules, suggests that core sampling and/or handling caused a comparable homogenization. In contrast to cores from SO268 dredge track, IP21 cores from the Patania II track exhibited sediment fractures, most likely introduced by the weight of the collector (Fig. 3.4). These fractures might alter pore water flow pathways and microbial habitats within the sediment significantly.

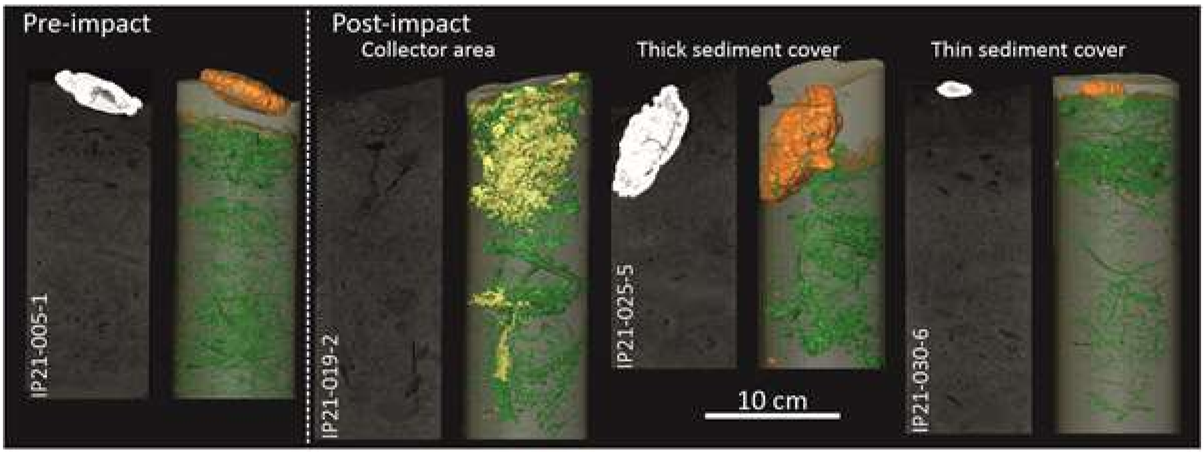

Figure 3.4: Examples of MUC cores from the various depositional environments. Left columns: vertical orthoslices through the MUC core, grey scale colormap shows the x-ray attenuation (low: black, high: white). Right columns: Three-dimensional reconstruction of the core. Orange: Mn nodule, green: open bioturbation traces, yellow: fractures within the sediment, transparent light brown: sediment cover, transparent dark brown: underlying undisturbed sediment.

Task 3.3 Effects on sediment biogeochemistry (redox zonation, diagenetic fluxes, biogeochemical processes) (GEOMAR, AWI, JUB, MPI, CIIMAR, UNIVPM)

Baseline conditions of solid phase and pore water constituents were studied to assess the general biogeochemical setting and processes. As expected from the overall decrease in productivity from SE to NW, many variables show pronounced differences between the GER and the BEL area, for example several proxies for organic matter availability, including TOC, phytopigments, lipids, biogenic carbon, and oxygen consumption. Many biogeochemical variables also show pronounced small-scale variability between replicate samples, but there are only few indications for differences between the reference and trial sites within both study areas.

Several biogeochemical variables were clearly affected by the impacts and could serve as indicators for the assessment of (1) the physical impact and (2) the (immediate) effect on biogeochemical conditions and processes. Using these biogeochemical variables in combination with optical methods (e.g., visual inspection of the cores and CT, see Task 3.2) and physical properties (see Task 3.1) proved powerful for a robust quantification of the impact. For the dredge experiment, results indicate a possible sediment removal of up to 3-12 cm in the tracks, but generally minor redeposition of <1 cm. For the collector impact sites of Patania II in the BEL area, biogeochemical variables indicated net sediment removal in a range of about 3 cm and up to a maximum of 6-8 cm in and between the vehicle tracks. Generally, the quantification of the bulk sediment removal in the collector impact sites (i.e., the thickness of the layer initially removed together with the nodules) is difficult as part of the suspended material settles in the tracks behind the collector vehicle. In the plume impact sites near the collector impact sites (distance of <100 m), biogeochemical variables indicate redeposition of a blanketing layer of maximal 3-4 cm. The characterization of the physical impact based on solid phase variables is generally supported by analyses of pore water constituents (e.g., oxygen, trace metals, nutrients) though interpretation is complicated by the dynamic redistribution of the initial 'disturbance signal' by diffusion. An accumulation of nitrite was observed in the blanketing layers and may serve as a 'redeposition indicator'. For the trace metals, information on physical and chemical speciation was collected to assess the potential toxicity of metals getting released by the collector impact. From the analyses carried out so far the uncertainty in the quantification of the physical impacts (sediment removal and redeposition) appears to be at least 1-2 cm.

Organic matter compounds may serve as indicators of physical disturbance, but also provide crucial information on potential changes in the food available for benthic life. In the dredge experiment, total phytopigment concentrations and most biochemical organic matter compounds decreased in the dredge tracks, as compared to the reference area. For the Patania II collector impact site the same is true for phytopigments that were redistributed along the sediment column and generally reduced in the top 5-10 cm of the sediment. For other organic matter compounds like lipids, the patterns were less evident and differed between the GER and BEL area. Porewater constituents connected to the remineralization of organic matter (e.g., oxygen, nutrients, and trace metals) can be used to quantify, how biogeochemical processes are affected by changes in the amount and distribution of organic matter and other biogeochemical properties. Quantification of rates and fluxes, however, requires consecutive post-impact studies until the initial strong diffusional gradients have been smoothened by diffusion. Biogeochemical modeling of generalized mining-related impacts (Haffert et al., 2020; Volz et al., 2020) predicts centennial time scales of recovery of biogeochemical processes in surface sediments, especially in cases where the active, organic matter-rich surface layer was lost or severely disturbed.

Solid phase and pore water biogeochemical properties

Baseline investigations have been collectively performed by AWI, JUB, GEOMAR, MPI, and BGR, which provide pre-impact SO268 data on the large-scale (between the GER and BEL areas) and small-scale (between reference and trial areas) natural spatial variations of sedimentation rates, bioturbation depth, redox zonation (incl. oxygen penetration depth and nutrient availability) and sediment composition (Volz et al., in prep.). Based on the results from the SO268 Dredge experiment and the IP21 monitoring of the Patania II tests, useful solid phase (Corg, Mn) and pore water (nitrite, Cu, Mn) indicator variables with characteristic depth profiles have been identified for the assessment of sediment removal and sediment plume deposition at the seafloor.

Due to the presence of a carbonate-rich horizon in the German license area, with a maximum at a depth of about 15-17 cm, Ca represents an additional variable in this area that can be used for physical disturbance quantification. Depth profiles of solid phase C_{org}, Mn (and Ca depending on the location) concentrations, ^{230}Th and ^{210}Pb activities, and sediment porosity (see Task 3.1) proved suitable to characterize and quantify the physical impact. Based on the vertical shift observed in the profiles in comparison to baseline conditions, sediment loss can be quantified. Using the same approach to quantify blanketing thickness proved more difficult, also because of the variability in the composition of the redeposited layers. Sediments redeposited at the collector impact sites often show a strong stratification with coarser-grained, Mn-oxide rich layer at the base of the redeposited layer and finer material on top (Fig. 3.5). There is some uncertainty in the assessment of net sediment removal at the collector sites. Besides the fact that the data set is not yet complete, this primarily results from the difficulties in clearly distinguishing signals of redeposition from signals of sediment removal, which impedes the total thickness of the removed layer to be quantified, and from the natural variability of variables.

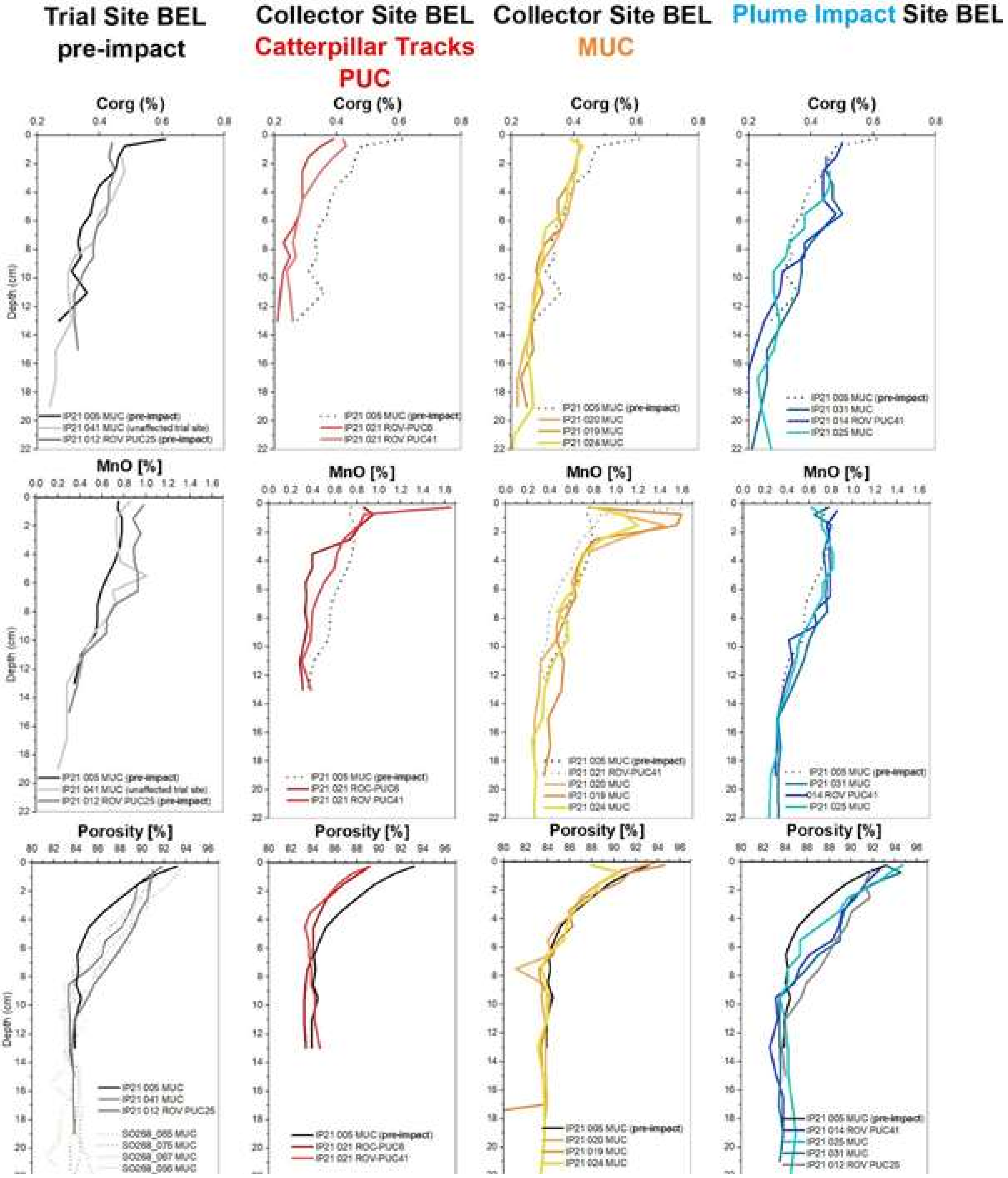

Figure 3.5: Pre- and post-impact depth distribution of Corg, MnO, and porosity in the BEL area visualizing profile shifts due to physical impacts. Track cores taken with PUC are dominated by sediment removal (upward shift), with additional 2-3 cm redeposition. In covered tracks, effects of net removal and net deposition partially level out, the redeposited layer is depleted in Corg but enriched in Mn oxide. Plume impact cores show a downward shift of profiles of several cm.

Extensive baseline investigations of dissolved (<0.2 µm) and soluble (<0.02 µm) trace metals (TM) in pore waters of the GER and BEL areas were conducted by JUB and BGR to establish baseline concentrations, understand TM cycling and potential toxicity. In contrast to V, Mo, U, Cd, and As, which are only present in the soluble phase, the colloidal fraction (> 0.02 µm < 0.2 µm) of Mn, Co, Ni, and Cu increased with depth in the oxic pore waters suggesting a change in chemical metal speciation associated with the relative availability of organic and inorganic ligands and the presence of nanoparticles and colloids (Paul et al., in review). To understand bioavailability of differently speciated metals from different pore water layers that may be introduced to bottom waters upon mining, chemical speciation of Cu was investigated by JUB. The bioavailability and thus toxicity of Cu is strongly influenced by its speciation, with ligand complexes being considered less bioavailable than free Cu^{2+}. Analysis revealed that >99% of Cu was organically complexed in surface pore waters and ligands were present in excess (Paul et al., 2021). A release of toxic Cu^{2+} concentrations seems therefore unlikely during deep-sea mining.

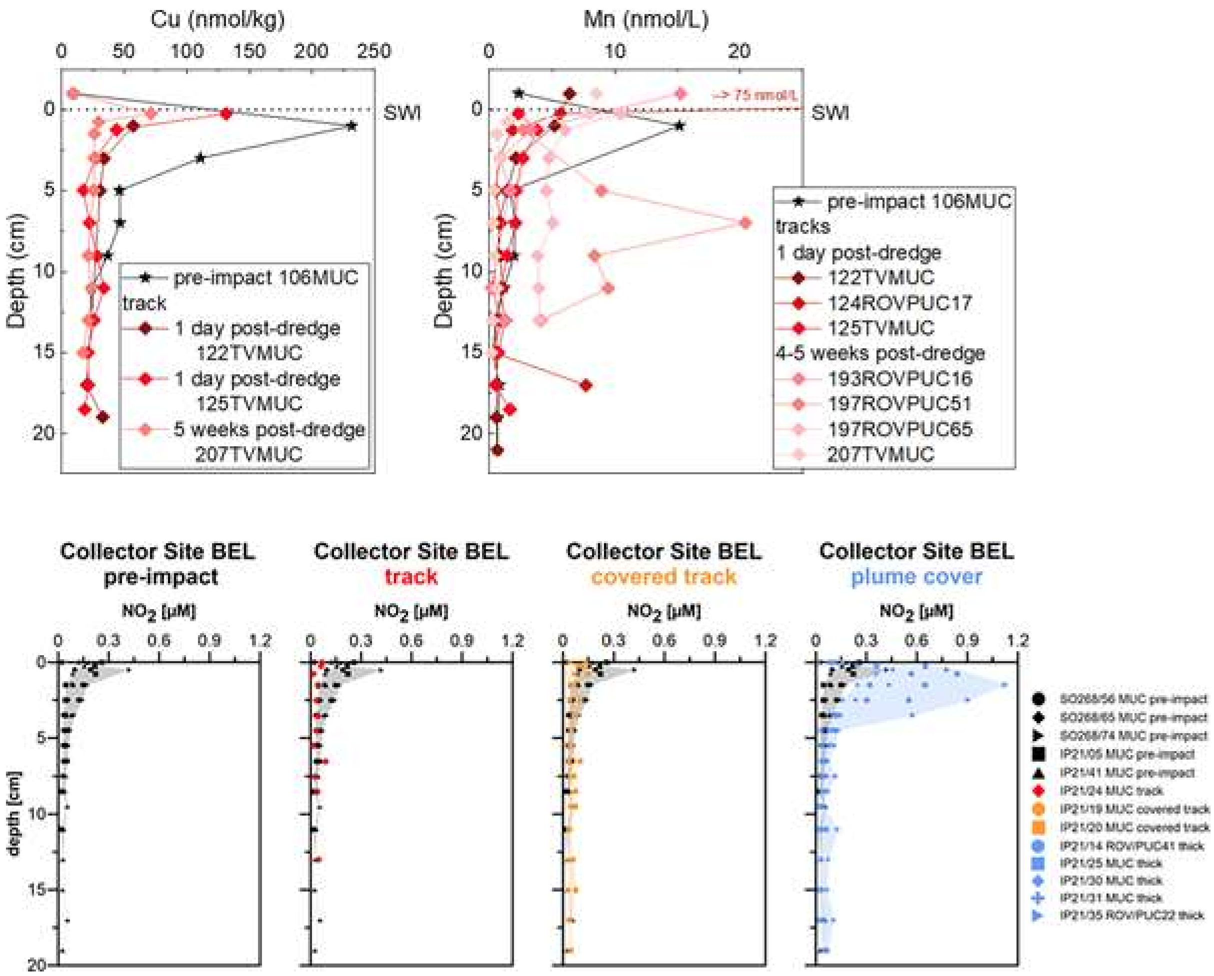

Figure 3.6: (top) Depth profiles of dissolved Cu and Mn pre- and post-impact of the dredge experiment (SO268). SWI: sediment-water-interface. (bottom) Pre- and post-impact depth distribution of porewater nitrite at the collector impact site after the Patania II test (IP21).

The impacts of the dredge experiment and Patania II test on biogeochemical processes were assessed by comparing pre- and post-impact pore-water depth profiles of TM and nitrite. Usually, TM such as Cu, V (and Mn) as well as nitrite show a pronounced peak in the upper 2-4 cm (Paul et al., in review; Volz et al., in prep). These peaks were absent in the dredge and Patania II impact sites (only BEL area so far) after the experiments indicating the loss of the organic-rich surface sediment layer. Samples obtained in impact sites up to 7 days after the Patania II trial and up to 5 weeks after the dredge experiment showed no peak re-establishment within this time (Fig. 3.6 top). In contrast to these findings, an increase in nitrite has been observed in the upper centimeters of the sediment in the plume impact sites, right away within hours to days after the Patania II test (Fig. 3.6 bottom).

***In situ* studies of oxygen distribution and fluxes**

Approx. 80 *in situ* oxygen measurements were carried out by MPI at all project expeditions directly at the seafloor ('*in situ*') by means of microprofilers and benthic chambers manipulated by ROV. Microprofilers were primarily used to record oxygen depth profiles from which seafloor diffusive oxygen uptake was calculated. For comparison, some measurements with chambers assessed total oxygen uptake. Baseline observations showed clear differences between the two contract areas with steeper oxygen gradients and approx. 1.8 times higher uptake rates in the GER area as compared to the BEL area and pronounced small-scale variability.

There were no indications of strong differences between the respective reference and trial areas. Immediate effects on oxygen distribution were assessed after the Patania II trials in the collector- and plume impact sites. Profiles show strong indications of the physical disturbance. A comparison to baseline profiles results in estimates of ~3-5 cm net sediment removal in collector impact areas and blanketing thicknesses of up to 3 cm in plume impact sites. Oxygenation increases in the upper sediment layer upon sediment removal by the collector while it decreases in response to blanketing.

Organic matter compounds

In sediments collected during SO268 and IP21, UNIVPM assessed biogeochemical organic matter compounds (i.e., proteins, carbohydrates, lipids), biopolymeric C and total phytopigments as proxies for the fraction of organic C available to benthic consumers. All individual organic matter compounds and biopolymeric C showed significantly higher concentrations in the GER as compared to the BEL area. Concentrations were significantly higher in the reference than in trial sites of the BEL area, whereas the differences were not significant in the GER area (Fig. 3.7).

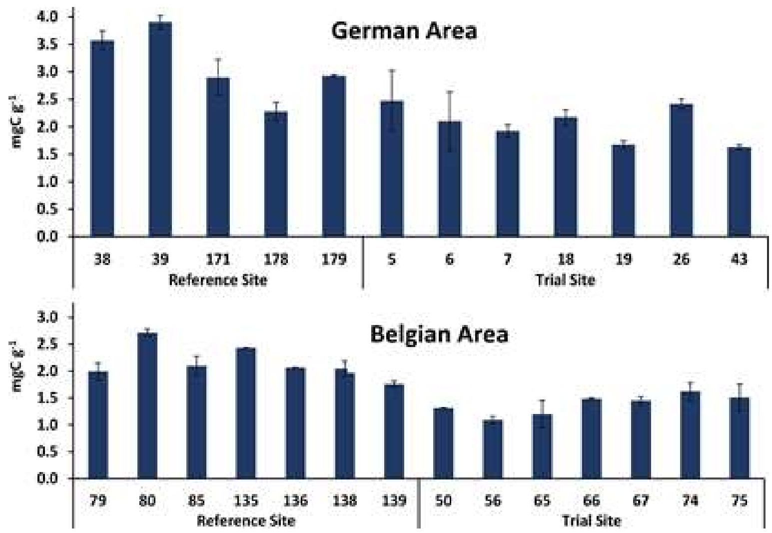

Figure 3.7: Spatial variability of biopolymeric C in the Reference and Trial sites in the GER and BEL areas (SO268).

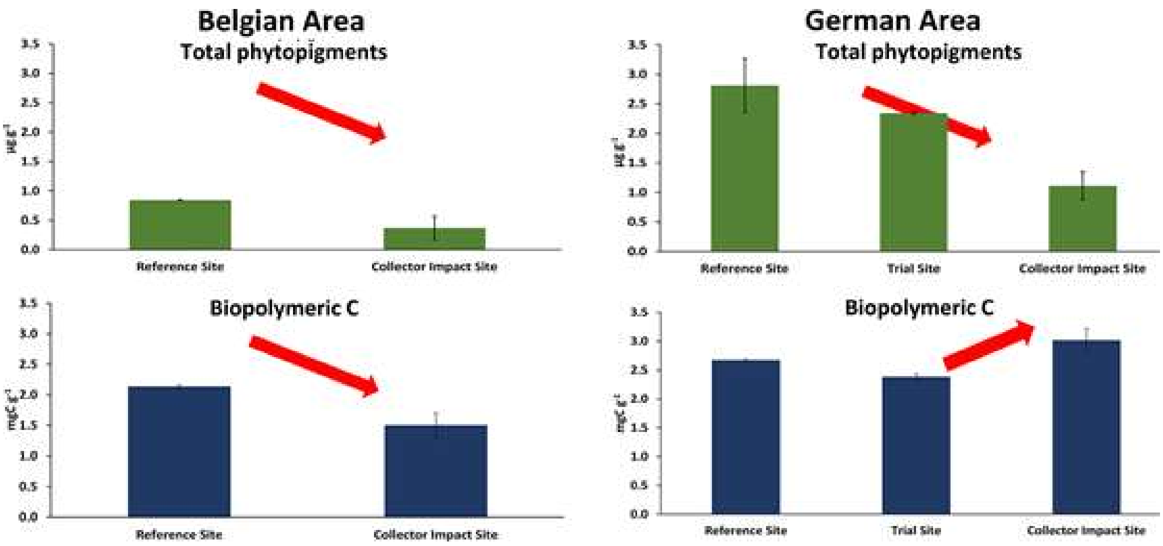

Figure 3.8: Total phytopigments and biopolymeric C in the Reference, Trial and Collector impact sites in the BEL and GER areas (IP21).

Analyses of samples collected during the dredge experiment provided evidence that total phytopigment concentrations and all biochemical components of organic matter (with the only exception of lipids) decreased significantly in the dredge as compared to reference sites. The dredge experiment thus resulted in significant changes in the food availability associated with the deposition of a thick sediment layer. During IP21, only total phytopigments showed a lower concentration in the collector impact sites in both areas (Fig. 3.8).

In the BEL area the concentrations of biopolymeric C were lower in the sediments impacted by the collector while in the GER area concentrations were higher after the collector impact as compared to samples taken before (i.e., in the trial site, Fig. 3.8). Solid-phase amino acid (AA) analyses of undisturbed and disturbed sites by JUB from the dredge experiment and the Patania II collector test indicated no significant differences in the AA total concentration and composition between the GER and BEL areas, but the impact of the dredge was clearly visible. AA concentrations were considerably lower in dredge tracks – both after 1 day and 35 days – as well as in the plume blanketed area. This suggests that the organic-rich upper layer had been removed and the organic matter degradation processes were impacted by the removal of the surface layer. Solid-phase amino acids could therefore serve as qualitative indicators of sediment loss.

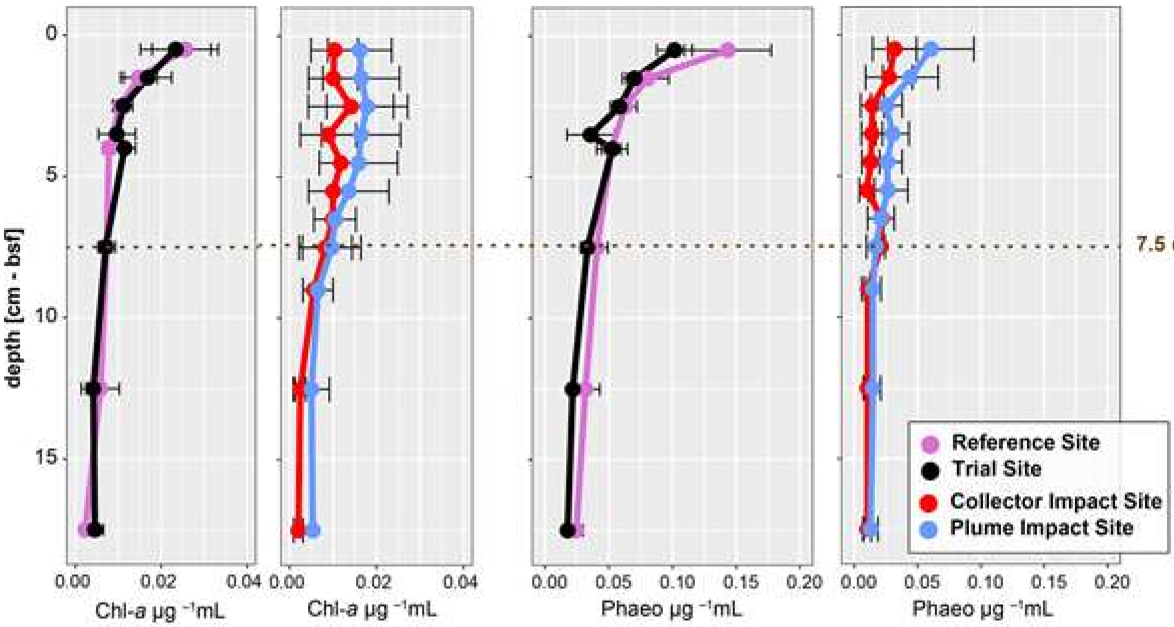

Figure 3.9: Phytopigment sedimentary profiles in the Belgian area before and after Patania II tests. Average Chlorophyll-a (Chl-a) and Phaeopigments (Phaeo) profiles in sediments from stations sampled during IP21 (2021) and SO268 (2019) expeditions, respectively. Error bar show 95% confidence intervals. The dashed line indicates the sediment depth until post-impact concentrations differ significantly from pre-impact conditions (Analysis of variance: Kruskal Wallis test).

MPI quantified Chlorophyll a (Chl-*a*) and Phaeopigments (Phaeo) in sediments collected before (n=425) and after (n=317) the Patania II trials. The collector trial partially changed the typical depth distribution of phytopigments (Fig. 3.9) leading to a significantly lower phytopigment content in the upper 7.5 cm of the sediment at the collector impact site compared to pre-impact samples. While the effects of the collector trial generally can be recognized in changes of phytopigment distribution, no unique pattern emerged. This suggests that different mechanisms of disturbance are taking place simultaneously. While removal of sediment is expected to reduce Chl-*a* in the top cm, mixing or re-deposition of the sediments may increase the variability in phytopigment distribution. However, a loss of Chl-*a* in the top cm of the sediment observed in the collector impact sites suggests a reduction in trophic resources that is expected to affect the benthic food web.

Using lipid biochemistry, CIIMAR quantified the organic matter (OM) quality and quantity in sediment and holothurian samples for both reference and impacted areas in the BEL and GER areas. Little Polyunsaturated Fatty Acids (PUFAs) were found in the sediment samples, indicating a low lipid quality. In the BEL area, lipid composition in the sediments with a presence of sterols appeared more bioavailable as compared to the GER area. Lipid biochemistry in the tissue of the holothurians showed similar patterns as in the sediments. In the BEL area, lipid concentration was 10 times higher than the GER area where more PUFAs and sterols were encountered. In holothurians from both areas, bacterial biomarkers were present in the fatty acids, suggesting that these organisms are feeding OM of low quality. As far as samples have been analyzed, the data indicate that OM quality and quantity is generally small and that a higher lipid quality is found in the BEL area that is also reflected in the tissue of the holothurians analyzed.

Task 3.4 Effects on microbial ecology and functions (MPI, UNIVPM)

Microbial communities and their functions were investigated as key components of benthic ecosystems with essential contributions to biogeochemical processes. Bacterial and archaeal abundances showed no significant differences, neither between the GER and BEL areas nor between the respective reference and trial sites and did not decrease significantly with sediment depth. Hence, microbial biomass does not reflect differences in organic matter availability or rates of biogeochemical processes (see Task 3.3) and cannot be expected to show immediate effects of physical disturbances. Disturbances seemed to generally increase virus-induced microbial mortality (observed in the dredge experiment as well as in the Patania II trial in the BEL area) but effects on virus productivity do not appear consistently. Enzyme-based studies of the microbial organic matter degradation showed higher potential degradation rates in the GER as compared to the BEL area and a general preference for the degradation of proteins although local variability was high. There are strong indications that the Patania II trial led to changes (both reduction and elevation) in potential rates of organic matter degradation, but patterns differed between areas and the specific organic compounds. Studies of microbial metabolic activity and biomass production with radiotracers could only be performed on IP21 before and after the collector test and analyses are still ongoing.

Microbial abundances, viral production and virus-induced prokaryotic mortality

MPI has quantified cell numbers in a large number of sediment and (n= 172) and nodule (n=14) samples collected in the BEL and GER area as microbial abundances in the CCZ were largely unknown. Microbial abundances did not show significant differences among the sites and between the areas (Fig. 3.10). The microbes associated with the nodules represent 10% of total benthic microbial abundance. Surprisingly, microbial abundances in the CCZ sediments did not decline significantly with sediment depth at least in the upper 10 cm. Although IP21 samples are so far only partly analyzed, preliminary results do not indicate significant differences between before and after the collector trial.

Analyses of samples obtained from the baseline and dredge experiment during SO268 in the GER area were carried out by UNIVPM and revealed a reduction in viral production in the impacted sediments. The virus-induced prokaryotic mortality, on the other hand, was higher in the sediments impacted by the dredge experiment than in the baseline samples obtained in the reference and in the trial sites before the impact (Fig. 3.11). The viral production and virus induced prokaryotic mortality in sediments of the BEL area were reduced by the Patania II trial when compared to the reference sites. In the GER area, viral variables showed a higher variability between sampling sites and no significant differences were observed between reference, trial and collector impact sites.

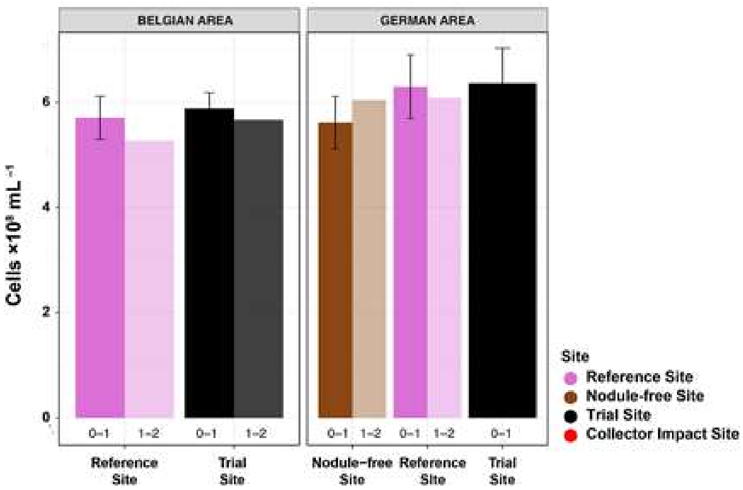

Figure 3.10: Average microbial abundances in the top 2 cm of sediments collected during the SO268 expedition as determined with the acridine orange direct count (AODC) method.

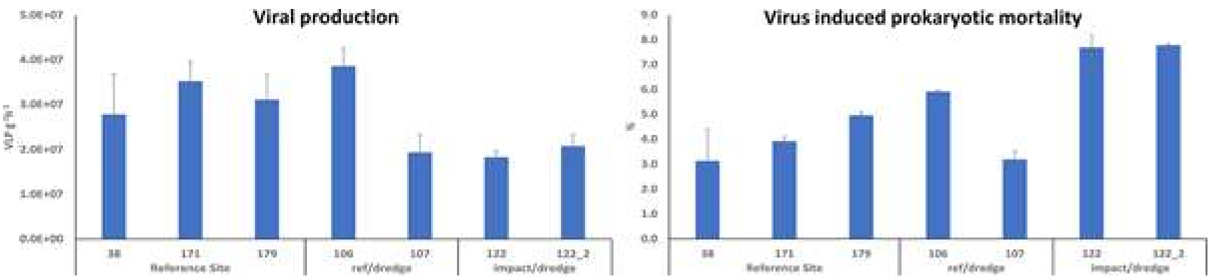

Figure 3.11: Viral production and virus-induced mortality in the dredge experiment in the GER area (SO268).

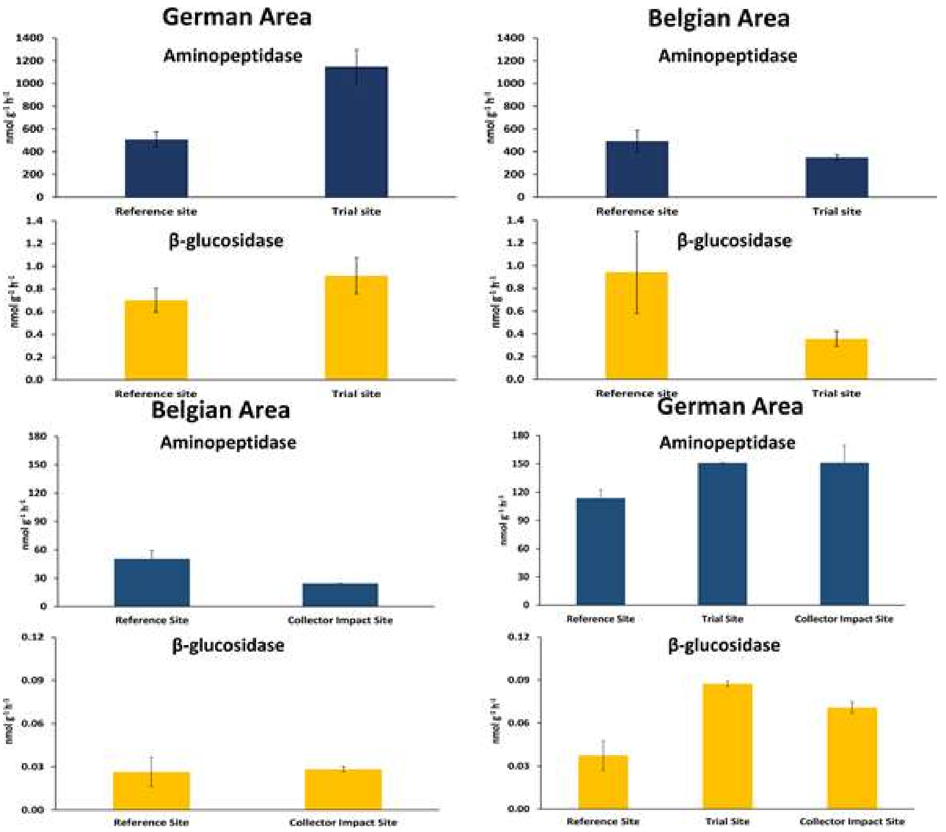

Figure 3.12: Extracellular enzymatic activity as a proxy for ecosystem functioning in (top) reference and trial sites (SO268) and (bottom) reference, trial, and collector impact in the GER and BEL areas (IP21).

Microbial metabolic activity

Extracellular enzymatic activities have been studied by UNIVPM to investigate the potential of microorganisms for the degradation of organic matter in the reference and trial sediments during SO268 in the GER and BEL areas (Fig. 3.12 top). Aminopeptidase activities were up to 4 orders of magnitude higher than β-glucosidase suggesting a preferential degradation of proteins over carbohydrates. All enzymatic activities are generally lower in the reference sediments compared to the trial sites in the GER area while in the BEL area lower values were observed in the trial site. Prokaryotic-mediated OM degradation shows high variability in the GER and BEL reference (baseline) area. Aminopeptidase and beta-glucosidase analyses in the reference and the collector impact sites in the BEL and GER areas (Fig. 3.12 bottom) showed reduced aminopeptidase activities in sediments impacted by Patania II in the BEL area while no differences are evident for β-glucosidase. In the GER area, on the contrary, values of both activities are lower in the reference sites.

MPI measured extracellular enzymatic activities (EEA) in bottom water before and after the collector test, as well as within the sediment plume while Patania II was operating in the BEL as well as the GER area. EEA were substantially elevated in the sediment plume in the GER area (Fig. 3.13). This could indicate higher organic matter availability but may also be interpreted as a stress response. In order to assess the activity of pelagic and benthic microbes, MPI studies the incorporation rates of organic (^{3}H-leucine) and inorganic (^{14}C-bicarbonate) substrates. Because of the large number of on-board incubations (n=1278) carried out during the IP21, analyses are still ongoing. The measurements serve to quantify the effect of the collector trial on primary and secondary microbial production and will greatly contribute to our understanding of the microbial response to mining-related impacts.

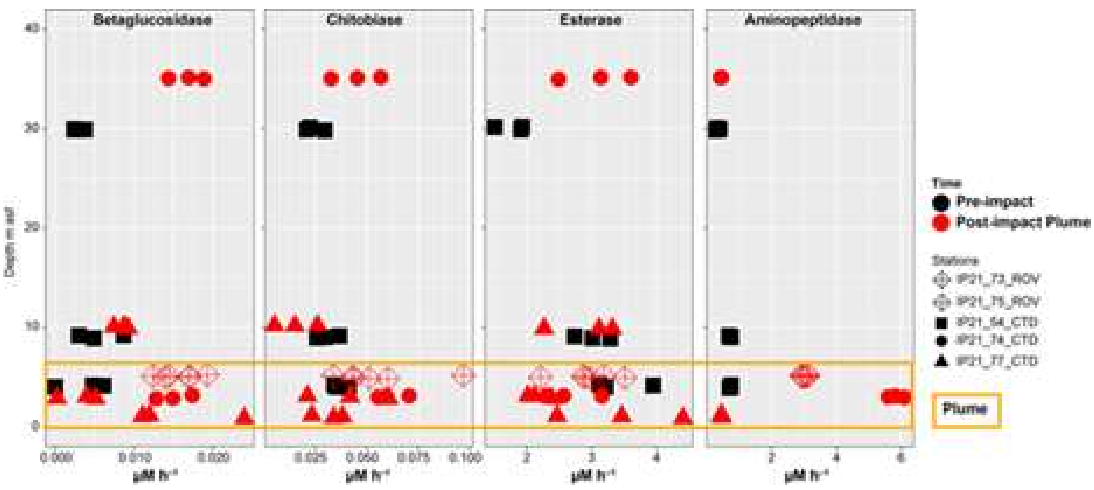

Figure 3.13: Extracellular enzymatic activities measured in bottom waters before Patania II test and in the sediment plume generated by collector operation in GER area. m asf: meters above the seafloor.

Task 3.5 Effects on ecosystem functioning (CIIMAR, NIOZ, UGhent, UNIVPM)

While benthic ecosystem functioning at the abyssal seafloor and the effects of nodule collection were supposed to be studied by *in situ* experiments followed by subsequent food web modeling, a series of delays required an adjustment of plans. Experiments with benthic exclosures focusing on plume effects on filter feeders had to be abandoned completely due to issues with equipment delivery. First *in situ* experiments with open mesocosms addressing infauna and deposit-feeder food webs could be conducted on SO268, but experimental runs assessing effects of the sediment plume had to be postponed to IP21 and are still being analyzed. Hence, modeling had to be based on existing data and could show that the removal of nodules and nodule-attached fauna is expected to dramatically reduce benthic trophic and non-trophic interactions and the stability of the benthic ecosystem. Exclusion of taxa with high numbers of interactions and high susceptibility to nodule removal from a food web model did reduce the number of food web compartments and the recycling of carbon inside the food web but did not affect - or even increase - the total carbon flow in the system.

In-situ experiments

CIIMAR has performed *in situ* food web experiments in the BEL area prior to impact for periods of 5 and 20 days. Labeled particulate organic material (^{13}C algal 'POM') was added to the experimental devices designed by USouthampton. From the analysis of the results after 5 days, several taxa (nematodes and copepods) showed significant uptake of labeled POM with a stronger uptake observed after 20 days. Most nematodes and copepods show significant uptake, and Foraminifera, Nemertea, and Tanaidacea (Akanthophoreidae) started showing significant enrichment. UGhent has helped with identification of the meiofauna and designed a new experimental chamber to be used on the IP21 cruise. Analyses of the collector impacts on the food web are not yet finalized, due to delays in the delivery of samples to CIIMAR. Nevertheless, from the data obtained so far, we can already suggest the feasibility of the experimental approach to detect differences in the sediment food web in response to the Patania II trial, especially after 20 days.

Ecosystem modeling

Due to problems and delays with the Patania II trials and the realization of the *in situ* experimental work, experimental data on the effects of the sediment plume on the benthic ecosystem were not available to perform food-web modeling as originally planned. Instead, UUtrecht compiled existing data and developed an interaction web model for the CCZ to study the role of polymetallic nodules on food-web integrity. Subsequently, carbon-based food-web models were developed for two sites in the GSR license area (B4S03, B6S02) to assess the importance of specific taxa on the overall carbon cycle (Fig. 3.14). The trophic-non-trophic interaction web model included 1,018 faunal interaction-web compartments that were linked with 59,793 trophic links and 386 non-trophic links. When the nodule compartment was removed from the interaction web, 18% of all faunal compartments were lost due to non-trophic interactions; only the fish *Pachycara* sp. was lost due to the loss of its prey. Most compartments were lost due to obligatory dependence on nodules as substrate and the obligatory and facultative dependence on other faunal compartments. The megabenthic hexactinellid sponge *Hyalonema* sp. was the taxon with the highest impact, i.e. its removal had the largest impact on food-web properties and resulted in the loss of 4% of all interaction-web compartments. To test the importance of taxa that - according to the interaction web model - would be lost when polymetallic nodules are removed, these were excluded from the carbon-based food web model for the B4S03 and B6S02 sites. The model showed that the total carbon flow in the system increased by 1% at B4S03 and by 29% at B6S02. The number of food-web compartments was reduced by 6% (Fig. 3.14) and Finn's cycling index, a proxy for the recycling of carbon inside the food web, decreased by 0% at B4S03 and by 21% at B6S02.

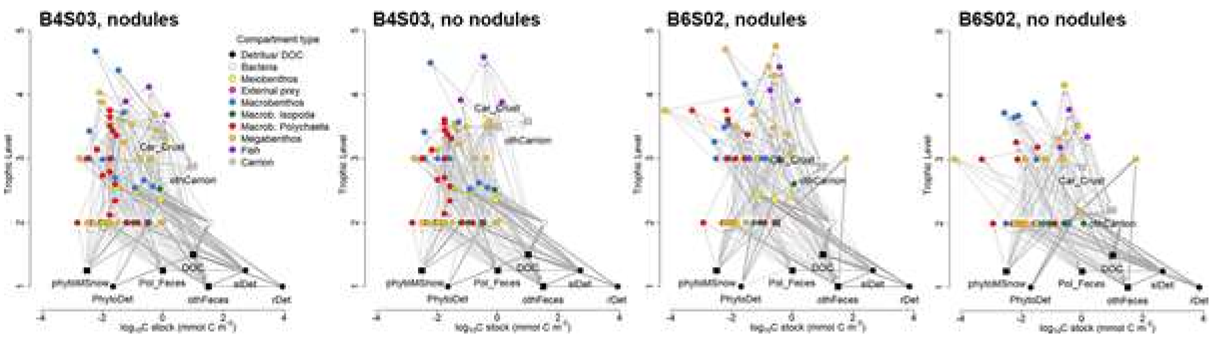

Figure 3.14: Structure of food-web models developed for the GSR exploration contract area in the presence (a, c) and absence (b, d) of polymetallic nodules for the B4S03 and the B6S02 site. The thickness of the arrows corresponds to the magnitude of carbon flow (mmol C m^{-2} d^{-1}) between two food-web compartments after double square-root transformation.

WP 4 – Data and sample management

Milestones and deliverables

M4.1 Data policy, including schedule for data sharing, archival, and open access, is agreed on by all partners (GEOMAR)

At the kick-off meeting the data sharing and archival schedules were agreed on. All project data will be publicly available latest two years after the end of the project.

M4.2 Shared datasets are made available to all project partners via OSIS-Kiel, PANGAEA, BIIGLE, Vidlib and internal project website (GEOMAR)

Shared datasets were uploaded by the scientists and are available in different database systems, depending on their status and type. Around 90 data files to be shared between project partners were uploaded to the Ocean Science Information System (OSIS-Kiel) (https://portal.geomar.de/osis) hosted at GEOMAR. About 100 TB of video and images were stored in the ELEMENTS media database hosted at GEOMAR. All images were made available to the scientists via local instances of the image annotation software BIIGLE. Raw and processed datasets are stored in PANGAEA with an optional moratorium period. A collection of links to datasets in BOLD is available on the internal website of the project.

All UGhent sequence data that was generated during the MiningImpact project phases have been deposited in a Barcode of Life Database (BoLD) dataset, with access given to individuals from each partner institute that expressed interest. This includes 18S rRNA (v1-v2, n=608) and COI-3P (n=370) sequences generated using Sanger sequencing during the process of Nematoda barcoding at UGhent. As such, this dataset also includes sequences from other areas of the world which can be useful for downstream analysis of metabarcoding data. A total of 978 sequences is available for shared use through this dataset.

M4.3 Geospatial browsing and video annotation tool have been tested successfully (UBielefeld)

BIIGLE now allows the deployment of multiple application instances that can be maintained by research institutes or during research cruises. The Machine learning Assisted Image Annotation (MAIA; Zurowietz et al., 2018) and Unsupervised Knowledge Transfer (UnKnoT; Zurowietz and Nattkemper, 2020a) methods were developed to speed up the annotation process. The methods automate the detection of interesting objects in the images, so the human observer only needs to select appropriate class labels for the objects. BIIGLE was, furthermore, extended with an application interface to geographic information systems (GIS). Geospatial browsing and video annotation have been successfully tested.

D4.1 Cruise Reports and meta-data published; photo/video data uploaded to BIIGLE/Vidlib (GEOMAR, BGR)

The SO268 cruise report is available as GEOMAR report and citable with a DOI:
https://doi.org/10.3289/GEOMAR_REP_NS_59_20
The IP21 cruise report will become available as BGR report with a citable DOI.
All cruise underway datasets of the DSHIP System are available from the land-based DSHIP system at BSH: https://dship.bsh.de
Further information about the cruises (station list, data files, links to publications, published datasets and other databases) are publicly available in OSIS:
SO268/1: https://portal.geomar.de/metadata/leg/show/348623
SO268/2: https://portal.geomar.de/metadata/leg/show/348628
IP21: https://portal.geomar.de/metadata/leg/show/358959

D4.2 All project data, specimens, and molecular data are archived for long-term accessibility (GEOMAR, SGN, MPI)

Datasets from the SO268 cruise are already available at the World Data Center PANGAEA: https://www.pangaea.de/?q=campaign%3A%22SO268*%22.
For both project phases together almost 500 datasets have been archived up to now: https://www.pangaea.de/?q=project%3AJPIO-MiningImpact.
Data archiving is still in progress, due to the delayed Patania II trials and ongoing data processing and analysis, particularly with respect to IP21 data. All datasets are citable with a digital object identifier (DOI) and are long-term available according to the FAIR principles (Findable, Accessible, Interoperable, Reusable). The project partners have agreed that all project data will become publicly available latest two years after the end of the project (February 2024).

Task 4.1 Data Management

Microbial samples and sequencing data
All material (sediment, nodules, particulate material filtered from waters) collected by MPI for microbial analyses during expeditions SO268 and IP21 are long-term stored at MPI except for aliquots used for the extraction of DNA and RNA. The same is true for remaining DNA and RNA extracts after sequencing. While the necessary capacities for the storage of further microbial samples is available at MPI, UNIVPM, the other project partner addressing microbial communities, decided for sample storage at their facility in Italy. Basic metadata regarding the collection of samples for microbial studies have been made available for integration in cruise reports (Haeckel and Linke 2021; IP21 report in prep.). More detailed sequencing metadata are stored at MPI's data server and are successively transferred to OSIS at GEOMAR (https://portal.geomar.de/metadata/leg/list) for later transfer to PANGAEA. This is the case for all sequencing metadata from SO268 samples. Metadata for the sequencing of samples from IP21 are currently checked and will be uploaded to OSIS in the coming months. Once all sequencing data have been provided by sequencing facilities and data validation and quality checks have been performed, the sequences and the related metadata will be submitted to the European Nucleotide Archive (ENA) for open access after a moratorium period of 12 months. This assures long-term data availability, contributing to D4.2. Data submission to ENA is facilitated by brokering services provided by the German Federation for Biological Data (GFBio).

OFOS imagery archiving
Surveys with the Towed Ocean Floor Observation System (OFOS) were carried out during expedition SO268 while the lack of suitable fiberoptical cables and ship time restricted seafloor imagery during expedition IP21 to AUV surveys. 38,000 high resolution digital still images of the seafloor and video footage were collected during SO268 with OFOS. Space constraints of RV SONNE restricted the involvement of additional technical personnel that would have been required to collect bathymetric data with the Ocean Floor Observation and Bathymetry System (OFOBS). MPI therefore decided to focus investigations during SO268 on high resolution imaging surveys with the OFOS system provided on board and maintained by SONNE's technical staff. Image data were submitted to PANGAEA by MPI and metadata were provided for integration in the SO268 cruise report (Haeckel and Linke, 2021) thereby contributing to D4.1. For joint analyses, the images are shared through GEOMAR with project partners via UBielefeld's online annotation environment BIIGLE. This joint analysis approach was used successfully for analyses of impacts of simulated mining activities conducted on the seafloor, e.g. regarding impacts on *Paleodictyon* (Boehriger et al., 2021). OFOS video data are stored at the MPI/AWI video platform and can be made available on request. Currently, this video data is being used to test the new MPI/AWI online video browsing system, which is envisioned to go live in late 2022 and will allow remote access viewing. The video data have been used

to construct 3D seafloor models to provide rugosity input into particle transport models developed at JUB (Gillard et al., in prep).

Task 4.2 Sample Management

OSIS-Kiel serves as a central information hub for the generated datasets within the project. This includes the MiningImpact cruises, with station lists, shared datasets, links to internal data repositories, publications and published datasets in PANGAEA as well as data deliverables and sample storage locations. Time schedules for data sharing and final publication of datasets were defined at the beginning of the project. After the cruises data deliverables were defined and listed within OSIS-Kiel. These lists acted as a continuous status report on the availability of datasets. Datasets had to be uploaded to OSIS six months after the end of the cruise or after finalizing the laboratory work and quality processing. To ensure the data delivery, quarterly reminders were sent to the responsible scientists. Also, during the annual meetings scientists were regularly reminded to upload missing datasets. Time schedules of data deliverables were continuously adjusted according to the status of data processing and analysis. As soon as datasets were finalised they were published at the World Data Centre PANGAEA (Data Publisher for Earth & Environmental Science) according to the FAIR principles (Findable, Accessible, Interoperable, Reusable). A moratorium of up to two years after the end of the project can be set to each dataset. All datasets are citable with a digital object identifier (DOI) and are distributed to international data portals through the harvesting technique OAI-PMH and standard metadata exchange formats as ISO19115 and Dublin-Core.

In total the data exchange and publication workflow was successful, about 90 shared data files are available in OSIS-Kiel and for both MiningImpact project phases a total of almost 500 datasets has been published in PANGAEA so far (Fig. 4.1). Some of the datasets are still under moratorium. Published and publicly accessible datasets are linked to their corresponding paper publications. There is a complete list of scientific articles with the linked datasets available on the project's website (https://miningimpact.geomar.de/publications). Furthermore all datasets published in PANGAEA from both project phases are available via a central data portal.

Figure 4.1: Geo-referenced datasets archived in PANGAEA (SO239, SO242, SO268, IP21).

Task 4.3 Video Annotation Software BIIGLE

One methodological and technical contribution was the development, improvement, and extension of the web-based marine image annotation software BIIGLE (https://biigle.de; Zurowietz and Nattkemper, 2021). Image annotation is the primary method for the analysis of marine imaging data, e.g. in the context of megafauna community and biodiversity assessment. Software tools such as BIIGLE are required to make this time-consuming process as fast as possible through collaboration and computer assistance. The development and extension of BIIGLE as part of this project was split into three topics: data fusion, video annotation, and geospatial browsing.

Data fusion
BIIGLE now allows the deployment of multiple application instances that can be maintained by research institutes or during research cruises. To avoid risks for data fragmentation of user accounts, annotation projects or annotations across multiple application instances, a "federated search" feature was implemented in this project. This feature allows users to search for resources across all connected BIIGLE instances through the same query interface. Hierarchical annotation labels referred to as "label trees" were substantially improved as to support high quality collaborative annotation in impact assessment or biodiversity studies. It is now possible to create multiple versions of the label trees that can be modified, merged, stored, shared and exported as files to facilitate archival, publication or definition of species catalogues.

Purely manual image annotation is too time-consuming for a comprehensive analysis of the large volumes of imaging data that are generated in environmental exploration, impact assessment and monitoring. The Machine learning Assisted Image Annotation (MAIA; Zurowietz et al., 2018) and Unsupervised Knowledge Transfer (UnKnoT; Zurowietz and Nattkemper, 2020a) methods were developed to speed up the annotation process. The methods automate the detection of interesting objects in the images, so the human observer only needs to select appropriate class labels for the objects (Fig. 4.2c). In collaboration with IMAR, we have conducted an annotation study and found that 86% of interesting objects could be detected in a realistic scenario using MAIA and UnKnoT. In addition, the assisted annotation was two times faster than the median speed observed for purely manual annotation and four times faster than the average speed. The collaboration will continue until the end of this project to investigate how the manual effort for training an accurate machine learning algorithm can be further reduced.

Video annotation
Underwater videos are routinely captured (e.g., for in-situ impact assessment) by AUV, ROV or fixed platforms. Video is well suited for instance to investigate dynamical processes like non-sessile animals or particle clouds. BIIGLE was extended with the first web-based and fully featured video annotation tool for marine imaging (Fig. 4.2a; Zurowietz and Nattkemper, 2021). The video annotation tool already supported several annotation studies, and, as of February 2022, more than 1,300 hours of video material were annotated with almost 500,000 video annotations. Development will proceed with live-video annotation support until the end of this project.

Geospatial browsing
Geospatial visualization and browsing is essential for the evaluation of marine imaging data in the context of MiningImpact2. For this purpose, BIIGLE was extended with an application interface to geographic information systems (GIS). Using the GIS, advanced visualizations can be created, e.g. bathymetric maps overlayed with image locations that highlight the abundance of certain annotated species (Fig. 4.2b). Until the end of this project, the compatibility of BIIGLE and the GIS interface with high-resolution bathymetric maps and photomosaics produced by CCT1 will be investigated.

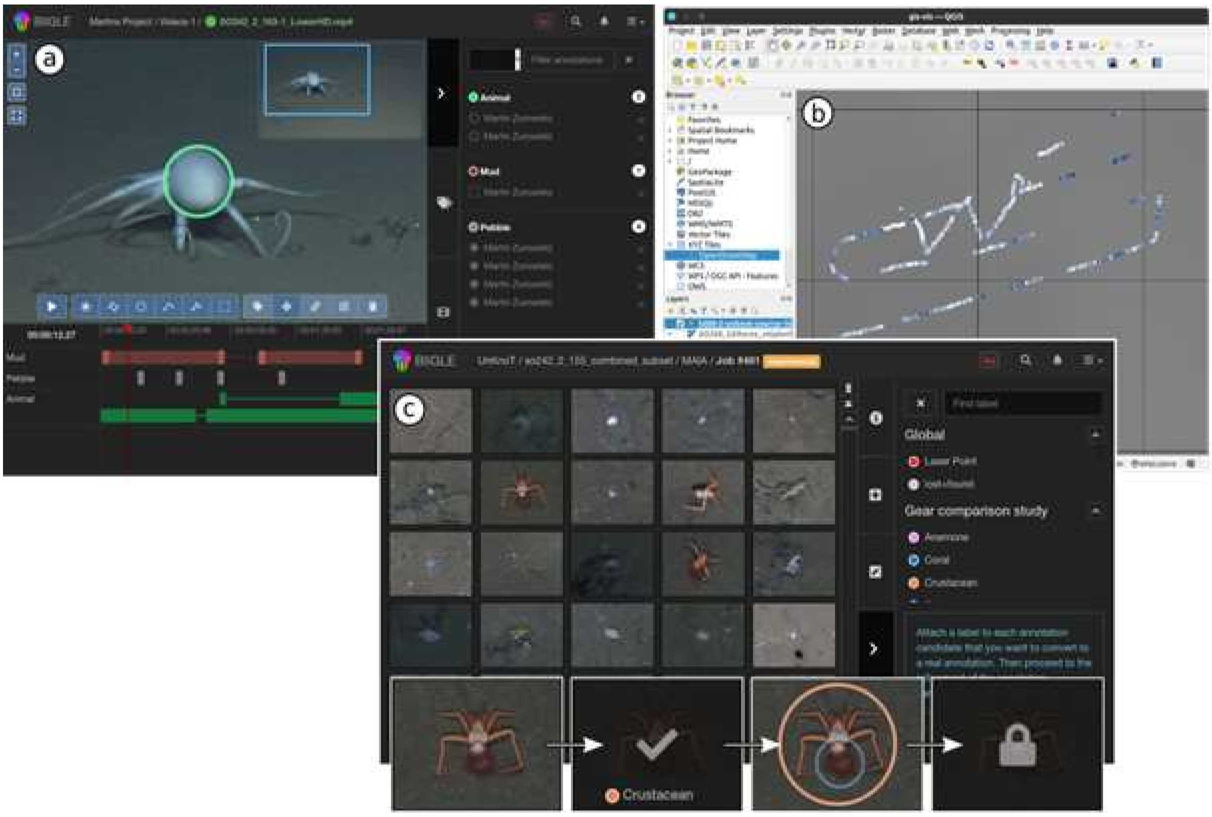

Figure 4.2: A selection of new features of the BIIGLE annotation software that were implemented as part of MiningImpact2. a) The video annotation tool. b) The application interface to GIS software, visualizing annotated species abundance. c) The MAIA and UnKnoT methods for automated image annotation assistance.

WP 5 – Project dissemination and coordination

Milestones and deliverables

M5.1 Regular updates of project website

The project website has been frequently updated over the duration of the project. It consists of a publically accessible and an internal part.

M5.2 Annual project meetings

Four annual meetings have been carried out: the kick-off meeting in September 2018 at RBINS in Brussels and the meeting in October 2019 at UAveiro were held in person, while the following meetings in October 2020 and the final meeting in February 2022 had to be carried out online due to the Covid-19 pandemic. Stakeholders from authorities, industry, NGOs, and science were invited to all Annual meetings, and dedicated stakeholder information events were conducted in conjunction with these meetings to discuss project results and the independent scientific monitoring campaigns of GSR's Patania II trials. The virtual events allowed to attention of more than 100 people from all around the world, during the final meeting in February 2022 on average 180 persons were participating.

D5.1 ISA workshops and project side events at an ISA annual meeting

Due to the postponed collector test, no side event was organized for the ISA Annual Meeting in 2019. It was decided to do this after a successful monitoring of the Patania II trials. Instead, the invitation from The Pew Charitable Trusts to present the project results at their side event in July 2019 was accepted. Unfortunately, in the following years the ISA has not allowed any side events with their annual meetings, also not for the last two meetings that were held in person again. However, we managed to convince the ISA to have a virtual side event during their UN World Ocean Day workshop in June 2022. We also participated in the ISA workshops on "Deep CCZ Biodiversity Synthesis" in 2019, "Deep-sea taxonomic standardization" in 2020, and "Enhanced image-based biodiversity assessment to advance deep-sea taxonomy" in 2021.

D5.2 Publication of focused project reports on WP and CCT topics

Several reports focusing on the results of the WP and CCT topics are currently being prepared for publication. Draft documents have been incorporated in this final report document.

D5.3 Publication of final project results in an internationally peer-reviewed special issue

A special issue in the EGU journal Biogeosciences on "Assessing environmental impacts of deep-sea mining – revisiting decade-old benthic disturbances in Pacific nodule areas" with results of the first MiningImpact phase was completed in 2020. For the second project phase a special issue in Frontiers of Marine Science has been initiated. Deadline for submissions is July 2023.

Task 5.1 Dissemination activities

MiningImpact website

The project website (https://miningimpact.geomar.de) has been updated frequently over the duration of the project. It provides information about the project (both phases), the research expeditions, about past, present and future events, different outreach materials such as interviews, videos, presentations, blogs, and brochures, as well as a list of published scientific peer-reviewed articles (ordered by publication year). The internal project website is the central access point to OSIS-Kiel and Pangaea, and contains all presentations from the annual meetings and project workshops.

GRID-Arendal/ UNEP website on deep-sea mining

A team at GRID-Arendal consisting of two geologists, a 3D animator and web designer, a web developer, and a cartographer, have started developing a website on the topic of deep-sea mining. The website is intended to serve as a general source for information on deep-sea mining that can guide stakeholders, decision makers and journalists to critically follow the results and recommendations of the scientific community. The website intends to bring the audience to the seabed, show the life there as documented by scientists, and visualize the known and potential environmental impacts. In addition to this, the website will share information about legal and economic considerations and examine the role of the companies involved in the activity. The website should inform the reader about the pros and cons of deep-sea mining. Although connected to and informed by the MiningImpact results, the website acts independently from it.

Stakeholder information events

Besides the stakeholder information events in conjunction with annual project meetings (see M5.2), additional events were carried out to inform stakeholders about the Patania II trials by DEME-GSR and the independent scientific monitoring campaign by MiningImpact. The first event was conducted in October 2018 at the BGR in Hannover to inform about the SO268 programme, a second one online in January 2021 with about 150 participants to inform about the IP21 campaign, and a third event was conducted online in conjunction with the final meeting in February 2022 to present results from the expeditions and the project. This last one attracted about 180 participants from various stakeholder groups from around the world. In addition, together with DEME-GSR we conducted another event at the UN World Ocean Day workshop of ISA in June 2022. The main purpose of these events and also our open annual meetings was to offer as much transparency into our plans and work and results as possible. This seems particularly appropriate given how controversial the topic of deep-sea mining and its induced environmental consequences is.

In addition, we engaged in several public panel discussions, organized by NGOs (e.g., The Pew Charitable Trust, DOSI, WWF, Fair Oceans), the World Economic Forum, the Norwegian Academy of Sciences (VISTA), and had frequent exchange with several stakeholder groups, members of the ISA-LTC, delegates of the ISA Council, and national authorities.

Outreach activities

During the reporting period, there was a very high level of interest in the project and the general topic of “deep-sea mining” from the national and international media as well as different stakeholders during the entire project phase. This resulted in numerous newspaper and online articles, podcasts, radio interviews, TV documentaries and news pieces, exhibitions and art projects, and information material of NGOs and industry.

A highlight was certainly the Arte documentary “Gier nach Meer”, which was filmed by Michael Stocks and Thomas Aigner from the German TV channel ARD, who accompanied the IP21 expedition. During the SO268 expedition we had the media student Steffen Niemann on board of RV SONNE, who recorded video and interview material for the project that is available on the MiningImpact website and was used for a news piece in the ZDF Morgenmagazin and the BMBF-FONA forum "Marine biodiversity - What protection does the high seas need" in 2019.

Also in this project phase we collaborated with artists. The Belgian theatre artists Silke Huysman and Hannes Dereere (http://silkehuysmanshannesdereere.com) used recorded interviews from IP21 for their project „Out of the Blue“, which premiered in May 2022 in Brussels and is now on tour through Europe until late 2023.

In addition to several press releases, GEOMAR has also produced a brochure informing on "Mineral Resources from the Deep Sea: Formation, Potential and Risks" (https://oceanrep.geomar.de/id/eprint/50433).

Transfer of results into policy recommendations

Overall, our activities have led to effective transfer of results into policy recommendations. Several colleagues were nominated by the ISA-LTC into expert groups to draft the Guidelines for Baseline Assessments (ISBA 27/C/5) and Environmental Impact Assessments / Statements (ISBA 27/C/11) of the ISA Mining Code. As a result of the Friday Harbour workshop of ISA on "Deep CCZ Biodiversity Synthesis", where project partners contributed to, 4 new protected areas (APEI = Areas of Particular Environmental Interest) were defined in the REMP (Regional Environmental Management Plan) for the nodule license areas in the Clarion-Clipperton Zone (ISBA 26/C/43).

In addition, we also contributed to an online hearing on deep-sea mining of the Belgian parliament in 2020 and to international policy documents, such as the White Paper of the High Level Panel for a Sustainable Ocean Economy (https://oceanpanel.org/publication/what-role-for-ocean-based-renewable-energy-and-deep-seabed-minerals-in-a-sustainable-future/) and the report “Decision-Making on Deep-Sea Mineral Stewardship: A Supply Chain Perspective” of the World Economic Forum (https://www.weforum.org/whitepapers/decision-making-on-deep-sea-mineral-stewardship-a-supply-chain-perspective).

Overview on dissemination and outreach activities:

Art projects & exhibitions

- Silke Huysman & Hannes Dereere theatre play “Out of the Blue”, premiere May 2022 in Brussels, Europe tour 2022 and 2023, http://silkehuysmanshannesdereere.com
- Armin Linke video installation "Broken Nature" in the German Pavilion of the Triennale 2019 in Milan, https://www.triennale.org/en/events/carceri-dinvenzione/
- DEEPWAVE Film Festival for the Protection of the Oceans 2019, https://www.deepwave.org/projekte/deepwave-filmfestival/
- Fast Forward Science 2019 video competition by Wissenschaft im Dialog, https://www.youtube.com/watch?v=YiJOUBdi4J0
- Helmholtz Association Science Picture of the Month in September 2019
- Helmholtz Association Science Picture of the Month in March 2020
- Interactive posters for the Ocean Academy onboard the Hapag Lloyd ships HANSEATIC nature, HANSEATIC inspiration and HANSEATIC spirit, https://www.hl-cruises.de/blog/hanseatic-nature-the-study-wall-of-the-ocean-academy
- Exhibition "Deep Sea and Marine Research" at the Senckenberg Museum Frankfurt, incl. public talk (online) on 27 January 2021, https://museumfrankfurt.senckenberg.de/en/exhibition/permanent-exhibitions/deep-sea/
- Exhibition "Station Tiefseeexploration" at the Deutsches Museum in Nuremberg, https://www.deutsches-museum.de/museumsinsel/ausstellung/meeresforschung
- Material for a touch table of the BMBF
- Autun Purser, 2nd prize at JPI-Oceans Photo and Art Awards 2021
- Sarah Marie Kröger, participation at ISA Art Competition 2022

Print and online newspapers and journals

- https://www.nature.com/articles/d41586-019-00757-y
- https://www.nature.com/articles/d41586-019-02242-y
- https://science.sciencemag.org/content/363/6432/1129.full
- https://www.sciencemag.org/news/2019/03/bus-size-robot-set-vacuum-valuable-metals-deep-sea
- https://www.bbc.co.uk/news/resources/idt-sh/deep_sea_mining
- http://www.bbc.com/future/article/20201202-deep-sea-mining-tracks-on-the-ocean-floor
- https://www.scientificamerican.com/article/deep-sea-mining-how-to-balance-need-for-metals-with-ecological-impacts/
- https://www.nationalgeographic.de/umwelt/2021/05/goldrausch-in-der-tiefe-der-meeresgrund-als-rohstoffquelle
- https://www.nationalgeographic.com/environment/article/proposed-deep-sea-mining-would-kill-animals-not-yet-discovered
- https://www.newscientist.com/article/2192495-deep-sea-mining-could-wreck-the-last-unexplored-ecosystem-on-earth/
- https://www.the-scientist.com/news-opinion/proposed-deep-sea-mining-zone-harbors-previously-unknown-species-66584
- https://www.nzz.ch/wochenende/schwerpunkt/tiefseebergbau-die-letzte-landnahme-ld.1512041
- https://www.zeit.de/2019/48/tiefseebergbau-tiefsee-rohstoffe-meeresboden
- https://www.zeit.de/2019/23/kobalt-manganknollen-rohstoff-smartphones-elektroautos
- https://www.sueddeutsche.de/wissen/rohstoffe-das-rennen-um-die-tiefseeknoedel-1.4536105?reduced=true
- https://www.sueddeutsche.de/wissen/tiefseebergbau-moratorium-nautilus-1.4631999
- https://www.spiegel.de/wissenschaft/natur/abbau-von-manganknollen-ressourcenrausch-in-der-tiefsee-a-1262034.html
- https://www.spiegel.de/wissenschaft/natur/tiefsee-forscher-finden-hinweise-auf-zahllose-unbekannte-arten-a-0993d6a8-9c85-4108-bbbf-901bf4a89f5d
- https://www.spektrum.de/news/tiefseebergbau-projekt-komplett-gescheitert/1675750
- https://www.spektrum.de/magazin/interview-abbau-von-manganknollen-in-greifbarer-naehe/1681218
- https://www.noz.de/deutschland-welt/politik/artikel/1870856/im-rausch-der-tiefe-maritimer-bergbau-zwischen-chance-und-risiko
- https://www.apache.be/?p=101148
- https://www.levif.be/actualite/magazine/ruee-miniere-sur-les-abysses/article-normal-1189241.html
- https://www.bazonline.ch/wissen/natur/das-rennen-um-einen-tiefseeschatz/story/15764059
- Regionale und überregionale Tageszeitungen, RND (Redaktions Netzwerk Deutschland) im September 2019
- https://magazin-forum.de/de/node/16330
- https://www.wissenschaft.de/magazin/natur-archiv/geister-der-meere/
- https://www.riffreporter.de/anthropozaen/tiefsee-bergbau-umwelt/
- https://krautreporter.de/3358-die-menschheit-mochte-rohstoffe-im-meer-fordern-und-hat-keine-ahnung-was-sie-da-tut
- P.M. Magazin 08/2020 “Alle wollen diese Knollen“
- Stuttgarter Zeitung, 18. September 2020
- https://www.mo.be/fr/longread/la-belgique-en-eaux-profondes
- https://www.republik.ch/2020/11/13/goldrausch-in-der-blauen-welt
- https://www.morgenbladet.no/ideer/kronikk/2022/02/10/gruvedrift-pa-havbunnen-er-irreversibelt-tap-av-natur/
- https://www.derstandard.de/story/2000134317544/meeresforscher-haeckel-wir-finden-bei-jeder-forschungsfahrt-100-neue-arten
- https://www.focus.de/magazin/archiv/rohstoffe-caspers-knoellchen_id_77058596.html

- https://issuu.com/greenpeace-switzerland/docs/gp_magazin_04_2021_de_es/1?ff
- https://www.geo.de/heftreihen/geolino-magazin/geolino--tiefsee-in-gefahr-32617840.html
- https://taz.de/Meere-als-Bergbaureviere/!5822108&SuchRahmen=Print/
- https://www.lalibre.be/planete/environnement/face-a-l-entreprise-belge-gsr-en-pleinpacifique-greenpeace-proteste-contre-les-projets-miniers-dans-les-fonds-marins-607058a19978e2410f5bb08b
- https://news.mongabay.com/2021/10/antithetical-to-science-when-deep-sea-research-meets-mining-interests/

TV, radio, videos, podcasts

- https://www.arte.tv/de/videos/098421-000-A/die-gier-nach-meer/
- https://www.zdf.de/nachrichten/zdf-morgenmagazin/moma-future-bergbau-am-meeresgrund-100.html
- https://www.cbsnews.com/news/rare-earth-elements-u-s-on-sidelines-in-race-for-metals-sitting-on-ocean-floor-60-minutes-60-minutes-2019-11-17/
- Documentary „From the Abyss to the Moon“ for Netflix, Quentin Noirfalisse & Laura Cole
- https://www.br.de/radio/bayern2/rohstoffabbau-in-der-tiefsee-waere-umweltzerstoerung-
- 100.html
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- https://www.ardaudiothek.de/episode/wdr-5-quarks-wissenschaft-und-mehr/10-09-2018-tiefseebergbau-kaese-wltp-teilchenbeschleuniger/wdr-5/10057289/
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- https://www.ardaudiothek.de/episode/wdr-5-quarks-hintergrund/tiefseebergbau-hat-ungewisse-folgen/wdr-5/59862464/
- https://www.ardaudiothek.de/episode/wdr-5-quarks-wissenschaft-und-mehr/forschen-in-computerspielen-zucker-schaetze-aus-der-tiefsee/wdr-5/10058423/
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- https://www.swr.de/swr2/wissen/mangan-knollen-ernte-in-der-tiefsee-stoert-das-oekosystem-100.html
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- https://www.tagesschau.de/wirtschaft/technologie/tiefsee-bergbau-manganknollen-101.html
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- https://www.deutschlandfunkkultur.de/tiefseebergbau-im-pazifik-wettrennen-um-die-letzten.979.de.html?dram:article_id=465529
- https://www.deutschlandfunk.de/schaetze-am-meeresgrund-ist-tiefseebergbau-die-zukunft.724.de.html?dram:article_id=481319
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- https://www.deutschlandfunk.de/meeresschutz-forum-der-meeresregionen-will-ozeane-schuetzen-100.html
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- https://www.butenunbinnen.de/videos/tiefsee-bergbau-forschung-meer-100.html
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- https://www.dw.com/de/abbau-auf-dem-meeresboden-tiefsee-in-gefahr/av-62818870
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- Mineral deposits - Manganese Potatoes from our Deep-Sea garden (in danish Mineralforekomster - Mangankartofler fra vores dybhavshave), Aktuel Naturvidenskab, Aarhus, Denmark, Magazine 15th March 2022
- https://scilogs.spektrum.de/klartext/elektro-hard-rock-und-heavy-metal/
- Being at home all over the world – Microbiologist explores the deep sea (28th January 2021), in cooperation with Auswandererhaus, Nordsee-Zeitung, Bremerhaven, Germany
- https://www.levif.be/actualite/sciences/course-a-la-science-dans-les-abysses-quid-du-deep-sea-mining-enquete/article-normal-1427327.html?cookie_check=1646297941
- https://www.youtube.com/watch?v=9iy5jEHWykQ
- https://www.youtube.com/watch?v=9iy5jEHWykQ&ab_channel=DWPlanetA
- https://podcasts.apple.com/us/podcast/academic-dating-and-the-deep-sea/id1530011202?i=1000527460810
- https://youtu.be/bUCk_THAPNQ (In Too Deep - The True Cost of Deep Sea Mining, Deep-Sea Conservation Coalition)
- https://detektor.fm/wissen/zurueck-zum-thema-tiefseebergbau
- https://themenspezial.eskp.de/fileadmin/user_upload/PDFs/rohstoffe/ESKP-Rohstoffe-in-Tiefsee.pdf

Panel discussions, conferences, workshops

- JPI-Oceans Conference "Increasing the impact of European investments in marine and maritime research - What's on the Horizon?", Brussels, 17 January 2019.
- UBA expert meeting "Assessment benchmarks for the impact of mining projects on the deep seabed on marine ecosystems", Hamburg, 29 January 2019.
- BMBF FONA Conference "Marine biodiversity - What protection does the high seas need? Ocean Governance in the Conflict between Protection and Utilization", Berlin, 14 May 2019.
- BMBF exchange meeting "Integration of deep-sea research into regulatory processes at the Seabed Authority", Bremerhaven, 24 June 2019.
- IASS Workshop "Developing regional environmental management plans in the field: an approach to legal and substantive issues", Potsdam, 2 July 2019.
- IASS International Expert Workshop “Towards a standardized approach to Regional Environmental Management Plans in the Area”, Hamburg, 11-13 November 2019.
- ISA Annual Meeting Side Event, The Pew Charitable Trusts & DOSI, Kingston, 18 July 2019.
- ISA Workshop "Deep CCZ Biodiversity Synthesis", Friday Harbor, 1-4 October 2019.
- ISA Workshop “DeepData: focusing on data management strategy”, online, 15-20 September 2020.
- ISA Workshop "Image Analysis Biodiversity", online, October 2021.
- ISA Workshop ”Regional Environmental Management Plan for the Area of the Northern Mid-Atlantic Ridge with a focus on PMS Deposits”
- ISA UN World Ocean Day, Panel Discussion, online, 8 June 2022.
- World Economic Forum Virtual Ocean Dialogues "Deep Dive Session", online, 3 June 2020.
- Hearing in the Belgian Parliament on deep-sea mining, online, 24 June 2020.
- Fair Oceans discussion event "The Deep Sea in Distress? Deep-sea mining and the International Seabed Authority between resource, climate and Marine Conservation Policy", Bremen, 10 December 2019.
- Fair Oceans discussion event "Tiefseebergbau - Die Zeit drängt - Über seine möglichen ökologischen und ökonomischen Kosten", Bremen, 7 October 2022.
- 40th DIN meeting, Presentation and discussion on deep sea mining, online, 1 December 2020.
- DOSI Panel Discussion “Treasures of the deep: life and rocks” (https://www.dosi-project.org/deep-sea-mining-webinar), online, 21 January 2021.

- International VISTA Seminar “Subsea floor processes and a sustainable ocean” panel discussion, Oslo, 17 November 2021.
- Workshop “Informal Working Group on Cumulative Impact Modelling”, ISA and Atlantic REMP project (funded by the EC)
- BMBF, UNESCO “Ocean Decade Laboratories”, episode “A clean ocean”, https://www.oceandecade-conference.com/en/a-clean-ocean.html
- Workshop „Runder Tisch der Bundesregierung“, „Internationalisierung von Bildung, Wissenschaft und Forschung“, Themenzyklus „Meere und Ozeane“, expert team „Mineralische Ressourcen der Tiefsee“. Several meetings in 2018, 2019, 2020.
- Workshop “Deep-sea mining – an opportunity to do things right”, The European Centre for Information on Marine Science and Technology, Lisbon, 7 May 2018.
- EGU 2020 Poster by Volz J. et al.
- Goldschmidt 2021, Session “Mineral Ressources”, convener Otte J., Volz J.; Talk by Otte et al. „Spatial distribution of metal-cycling microbial communities along geochemical gradients in sediments of polymetallic nodule fields of the Eastern Pacific Ocean”.
- Netherlands Annual Ecology Meeting 2020, Session “Marine Benthic Ecology”, convener Stratmann T.
- Underwater Mining Conference 2022, Session “Current State of the Art Technology Trials & Learnings”, 2-7 October 2022.

Public Talks and lectures

- Boetius A., Talk “Fremder Planet Tiefsee“. Rhetorik und Wissen - Seminar für Allgemeine Rhetorik, Universität Tübingen, 2019. https://www.youtube.com/watch?v=BburMxUN8iA
- Haeckel M., Talk “Umweltauswirkungen von Tiefseebergbau”, GEOMAR „Wissen Schaffen“ Seminar, 29 September 2021.
- Haeckel M., Talk “Environmental impacts and risks of deep-sea mining”, Lunchtime Seminars, Department of Geology, Trinity College Dublin, 3 December 2021.
- Haeckel M., Talk “Umweltauswirkungen von Tiefseebergbau”, Naturwissenschaftliche Gesellschaft Winterthur, 21 January 2022.
- Haeckel M., Lecture mageoMaSus: Marine Geosciences & Sustainability, Master Program Geosciences and Marine Geosciences, CAU Kiel, Sommersemester 2020, 2021, 2022.
- Hilario A., Talk “Missão mar profundo”, Escola Basica da Amaia - Portagem, Marvão, 14 June 2021.
- Hilario A., Talk “Encontro com o cientista”, Fábrica da Ciência – centro de ciência viva de Aveiro, 28 May 2021.
- Otte J., Talk “Be Ok – career orientation and life planning without clichés”, Oberschule an der Egge, Bremen, 15 April 2021.
- Otte J., Talk „WGs auf dem Tiefseeboden – welche mikrobiellen Gemeinschaften finden sich in Manganknollen“, Five AWI Open house lectures, Bremerhaven, January 2021.
- Otte J., Talk “Job Speed Dating: Marine microbiologist working on marine manganese nodules”, Bremen. https://www.theaterbremen.de/de_DE/programm/oikos-welt-wirtschaft-1-das-meer.1307607
- Van Doorn E., Talk „20.000 Maßnahmen unter dem Meer: Schutz & Nutzung von Bodenschätze der Tiefsee“, 'Spätschicht trifft Wissenschaft' CAU Kiel.

Task 5.2 Project coordination

During the second phase of MiningImpact, four annual meetings were carried out and several WP/CCT workshops to discuss preliminary results and plan for joint work and publications. Planning of the research expeditions SO268 and IP21 also involved several preparatory meetings before the cruises, among project partners and participants, but also with DEME-GSR to coordinate the joint campaigns. Until the outbreak of the worldwide Covid-19 pandemic in 2020 these meetings were held in person, but afterwards all meetings were conducted as webmeetings.

Due to the technical failure of Patania II in spring 2019, while the SO268 expedition was already ongoing, and the postponement of the trials, a large part of the project coordination work had to be spent on re-organizing the additional research expedition IP21 and adjusting the overall project work plan. This expedition was only possible, because BGR offered to use their funding for exploration work to charter a commercial vessel, MV Island Pride, and invite MiningImpact partners to join the cruise. The organization of this expedition was complicated by the pandemic, which resulted in several delays of the campaign until spring 2021 and additional logistics work, such as devising a detailed hygiene plan for the expedition, including two weeks of hotel isolation and defining the necessary facilities of lab containers and basic onboard equipment (winches, cables, ROV and AUV tools). In the end a successful monitoring campaign of the first industrial nodule collection test could be delivered.

CCT 1 – Plume monitoring and habitat/disturbance characterization

Milestones and deliverables

M6.1 Planning of SONNE cruise and EMMP layout (GEOMAR)

The partners jointly organized and discussed the sensor array layout, sensor intercalibration and sensor data management issues prior to SO268 and IP21. Further all partners supplied sensors and equipment which made it a complex logistical challenge to get all needed sensors to the respective cruises. Due to the malfunction of Patania II in 2019 the entire exercise needed to be repeated, but with a very successful result in 2021. The planning of the sensor layout also included remote consultation with colleagues onshore updating the sediment plume modelling based on recent current data recordings from recovered moorings.

M6.2 Evaluation of employed EMMP concept and monitoring technology (JUB)

The partners developed an EMMP including the necessary technology during the first year of the project, verified it in 2019 during the dredge experiment (Purkiani et al., 2021; Haalboom et al., 2022) and executed it during the IP21 cruise when the collector trial took place. The lessons learned from the research cruises pointed out that an adaptive management allows adjustments to effective environmental monitoring concepts which result in improved EMMPs.

D6.1 Report about recommended workflow for planning of monitoring of Mn-nodule mining operations (GEOMAR)

A recommended workflow about the planning of sediment plume monitoring and the use of different sensor types and specific sensor models has been published in Haalboom et al. (2022). An important precondition is a good knowledge about the most prominent direction and extent of plume dispersion in the area. Figure 6.1 gives an example of how the plume dispersion was predicted for the monitoring efforts in the BEL and GER areas. Stationary sensor platforms were deployed accordingly. Results from the IP21 cruise show that mobile platforms as an AUV are important additions to stationary and very accurate lander platforms, as they allow adaptive monitoring even during changing/not predicted current and settling conditions. A high-ranked paper summarizing the results from the IP21 cruise is currently in preparation and will elaborate more on the ‘best possible’ workflow and approach for monitoring Mn-nodule mining operations.

D6.2 Guidance document on suitability of different monitoring technologies (JUB)

This guidance document on suitable plume monitoring technologies is the paper by Haalboom et al. (2022).

Summary of achievements

WP2 investigated the dispersal of mining plumes in great detail, complemented by in-situ and ex-situ sediment exposure studies. All onsite data were fed into a near-field plume model which was used both, for ground truthing of the model and to carry out prognostic plume dispersal simulations under varying hydrodynamic conditions. In this context, CCT1 focused its efforts on the planning and execution of the monitoring of the sediment plume created by the nodule collector trial. A primary goal of CCT1 was to develop a guidance document on how plume monitoring of the seabed around mining operations should be performed.

Task 6.1 Exchange with DEME-GSR on the nodule collector trial

Essential for planning an appropriate experimental sensor layout was a detailed knowledge of the technical specifications of the collector vehicle and its nodule removal and sediment dispersion concept. Technical details, such as the collector size, sediment uptake and dilution, volume flow at the disperser, the vehicle's speed and track were gained, the locations of the test mining sites were defined and the consecutive dataset including results from mesocosm laboratory experiments on aggregation and particle transport were fed into numerical models. During annual workshops the discussions with engineers of DEME-GSR allowed to further improve the EMMP (environmental management & monitoring plan) for the trial which finally took place in 2021. A lively exchange between the project partners and with DEME-GSR on the final location of the test sites in both the BEL and GER areas ultimately enabled a successful test.

Task 6.2 Planning of the experiment and the SONNE cruise station time

The planning of SO268 and IP21 sediment plume monitoring included modeling of the predominant bottom water currents (Purkiani et al., 2021; Haalboom et al., 2022), detailed sensor selection including laboratory-based intercalibration and in depth discussions about pros and cons of various sensor layouts. The planning occurred prior to the cruises, with onshore support during the cruises.

Numerical oceanographic and sediment plume modeling prior the cruise
For both areas particle transport modelling was conducted which required information on bathymetry, particle properties, aggregation/disaggregation under differing turbulence and sediment loadings, expected blanketing as well as information from existing long-term ADCP deployments. The data were provided by DEME-GSR and BGR for the respective areas. The data were used to forecast the dispersal of suspended sediment following its mobilisation by the Patania II nodule collector vehicle. During SO268, the plume dispersion forecast was used instead in the monitoring of a sediment plume produced with a dredge experiment in the GER area. The observations of current properties and suspended sediment concentration from the sensor array were first used to identify the dominant bottom currents and later to validate the model results at two sensor locations (Purkiani et al., 2021).

The initial sensor layout for monitoring the plume generated by Patania II was based on a plume dispersion probability map, produced on the basis of numerical simulations using the MIT-gcm hydrodynamic model combined with a sediment transport module (for a detailed description see WP2 and Purkiani et al., 2021). The numerical simulation was driven by 10 years of wind data (2009-2019) and was validated with long-term current data acquired through oceanographic mooring deployments by BGR (Haalboom et al., 2022).

Planning of EMMP layout
Based on information from Tasks 6.1 and 6.2, plume impact, a collector impact and an unaffected reference zone was defined. The location of the different zones were determined based on the plume modeling results and habitat mapping efforts using ship- and AUV-based bathymetric and optical information. For the layout see Figure 6.4. The sensor array consisted of stationary landers (BoBo of NIOZ, equipped with CTD, ADCPs, sediment trap) and short moorings of BGR with ADCP and CTDs as well as seabed platforms equipped with e.g. upward-looking ADCPs, CTDs, optical turbidity sensors and particle cameras (JUB, and on Patania II provided by MIT). Based on the results of Task 6.1, pre-impact studies, and the bottom current conditions at the time of the collector trial, the EMMP layout was adjusted during the cruise.

Cruise station time planning

In cooperation with all partners and based on results from Task 6.2, a detailed cruise plan was developed accounting for AUV survey times, ROV work, biological, geological and biogeochemical sampling and OFOS surveys as well as deployment times for moorings and the plume monitoring array. Technical pre-conditions of the collector system and safety issues for the two-vessel operation as well as real-time communication with DEME-GSR were included as well. Monitoring technologies as outlined above and described in WP2, as well as a large amount of monitoring equipment and sensors were deployed and intercalibrated. Two workshops on intercalibration of turbidity sensors were carried out at JUB´s OceanLab prior to and after the cruises. Details are described in the cruise reports of SO268 and IP21.

Task 6.3 Onsite pre-impact assessment

Several workshops and webmeetings were held to plan the pre-impact assessment. The recommendations are summarized in CCT2. The report includes improved habitat characterization using statistical methods which made biological/optical sampling more effective in regards to the habitat distribution. CCT2 concluded that "the need of at least one but better several control areas in environmental impact studies in the CCZ is evidenced by the combination of high small scale spatial and the temporal variability in benthic assemblages. Under these circumstances, reference areas are needed to disentangle spatial and temporal changes produced by natural environmental variability from changes derived from mining-related impact and the recovery of benthic populations".

Task 6.4 Small scale in-situ experiments related to plume behavior

During SO268 a small-scale, disturbance experiment in the GER area using a chain dredge was conducted. Sediment plume dispersion and deposition was monitored using an array of optical and acoustic turbidity sensors and current meters placed on platforms on the seafloor, and by visual inspection of the seafloor before and after dredge deployment. We found that seafloor imagery could be used to qualitatively visualize the redeposited sediment up to a distance of 100 m from the source, and that sensors recording optical and acoustic backscatter are sensitive and adequate tools to monitor the horizontal and vertical dispersion of the generated sediment plume. The experiment went well and allowed to improve the preparation of the Patania II nodule collector vehicle test in 2021. The results are published in Haalboom et al. (2022) and particularly recommend the use of AUVs for repeated seafloor imaging and water column plume mapping. Data on particle behavior were ground truthed by a particle camera in 2022, after intensive laboratory testing and intercalibration with turbidity sensors.

Task 6.5 In-situ monitoring of the collector plume experiment

CCT1 oversaw a coordinated experiment to ground-truth and validate the plume behavior using stationary and mobile observations with ADCPs, OBS, cameras, particle cameras, tracer particle cameras. Important was the use of a water column imaging multibeam echo sounder and parallel downward looking ADCP on both a ROV and AUV to map the distribution of the plume from 30 to 50 m above the seafloor. Figure 6.1 shows the tools used for the plume monitoring and habitat mapping during the cruises in 2019 and 2021.

Figure 6.1: Tools used for the plume monitoring and habitat mapping during SO268 and IP21.

Task 6.6 Ex-situ experiments related to plume behavior

Laboratory experiments provided data for modeling using results from local 10 cm surface sediment-layers sampled from the CCZ. JUB carried out laboratory experiments focusing on simulating aggregation processes and hydrodynamic behaviour of particles within the sediment plume. Experiments were conducted under in-situ temperature and salinity conditions. Different particle concentrations (from 10 mg/L to 10 g/L dry weight) and turbulence regimes were investigated.

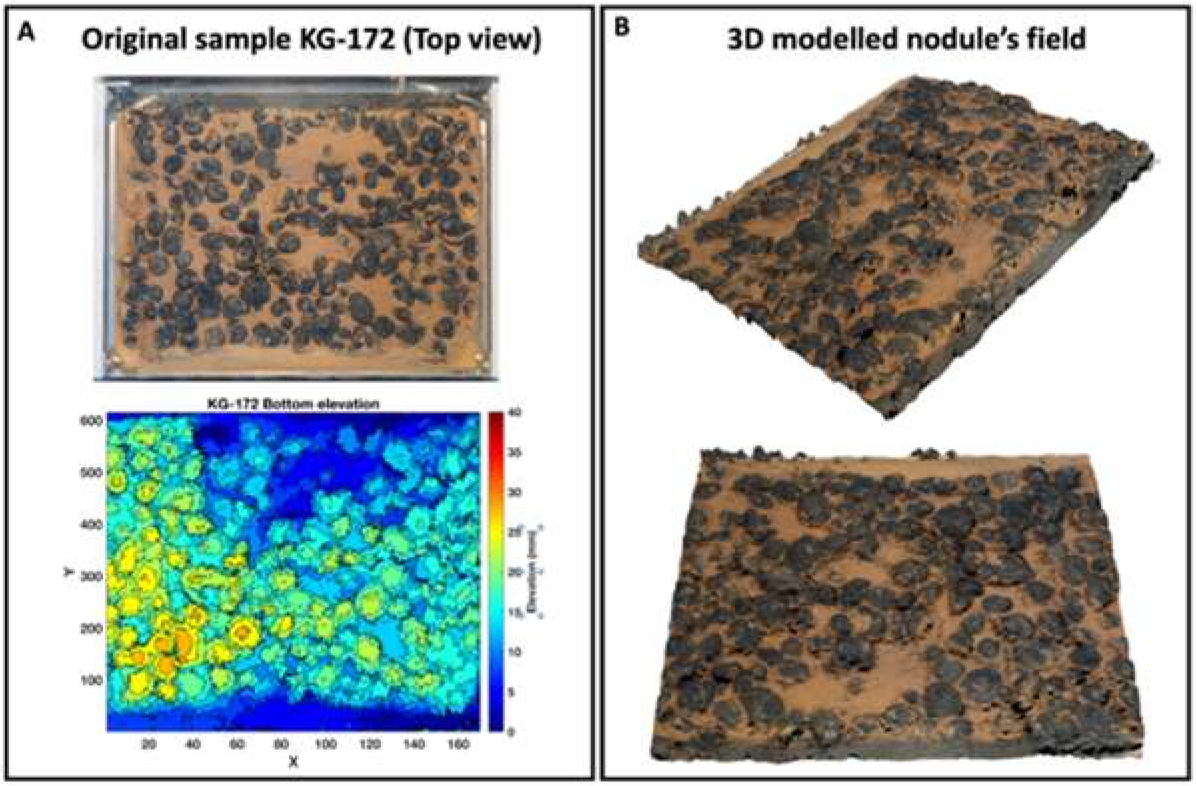

Figure 6.2: (A-top) Photo of original box core sample KG-172 from the top view; (A-bottom) Bottom elevation express in mm; (B) 3D modeled nodule field output result from two different points of view.

Three main flocculation phases of sediment plume could be identified irrespective of the starting sediment concentration: the core aggregation, the export, and the late aggregation (Fig. 2.7). In addition to experiments on particle aggregation and settling behaviour, flume experiments were conducted to investigate the redeposition of sediment from the plume on seabed with different nodule size and density and under different flow conditions. Decreasing nodule height leads to an elevated blanketing. Sedimentation dominates between the nodules, which results in a change of the general flow field around the nodules. The newly formed sediment surface under the tested plume concentrations will not resuspended under typical flow conditions found at the study site. Even higher flow of >15 cm/s does not result in strong resuspension. We observed a small erosion on top of nodules. From an ecological point of view, the habitat forming environment of manganese nodules which favors passive sedimentation of food particles is transforming into a rather smooth surface of accumulated plume sediments which reduces the passive fallout of food particles. A publication is in preparation.

Task 6.7 Near real-time modeling to predict fallout areas

Results from in-situ observations and from the laboratory experiments on particle transport were used to predict particle fallout areas on the seafloor and particle dispersion above the sediment. Using particle aggregation and settling data from Gillard et al. (2019) determined in laboratory experiments, Purkiani et al. (2021) performed modelling of plume dispersion for the dredge experiment conducted on SO268 and concluded that the developed numerical model was capable to reproduce the observed spread and deposition of the sediment plume with some smaller uncertainties. This was confirmed via in-situ sampling after a small scale sediment erosion experiment. While the spatial pattern of sediment deposition was satisfactorily simulated in accordance with photos taken during the experiment, the deposition height in the dredge tracks remains uncertain. It was inferred that a significantly greater sediment release during industrial mining would lead to a higher sediment deposition of up to a few centimeters in the near-field area and an expanded far-field low sedimentation area up to several kilometers away from the source. Good quantification of deep sea currents, the sediment settling velocity, and the sediment release rate are the main factors for a reliable simulation of the sediment transport in the deep sea.

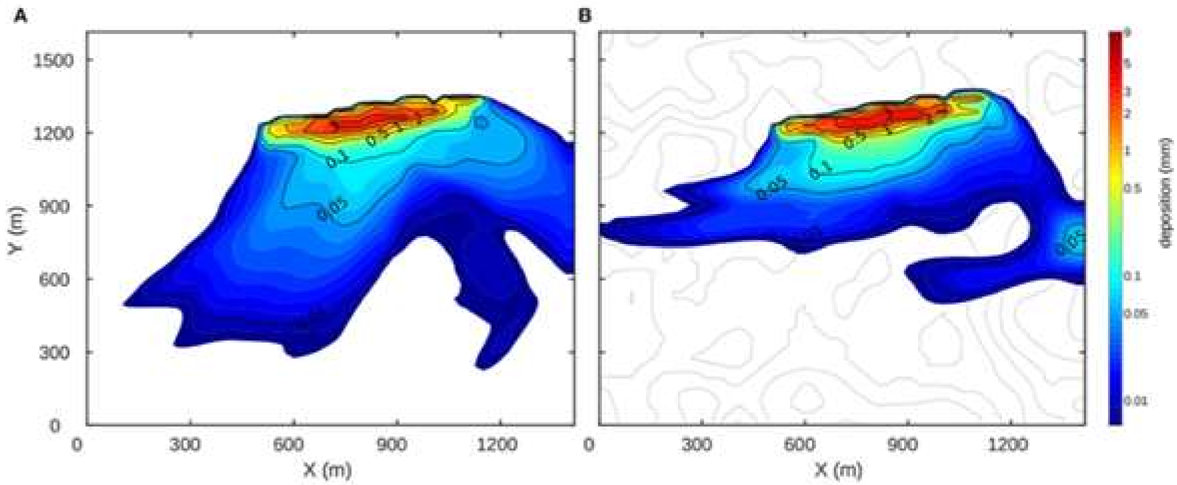

Figure 6.3: Numerical simulation of the deposition pattern for (A) a flat seabed and (B) higher release height above the sea floor (Purkiani et al., 2021).

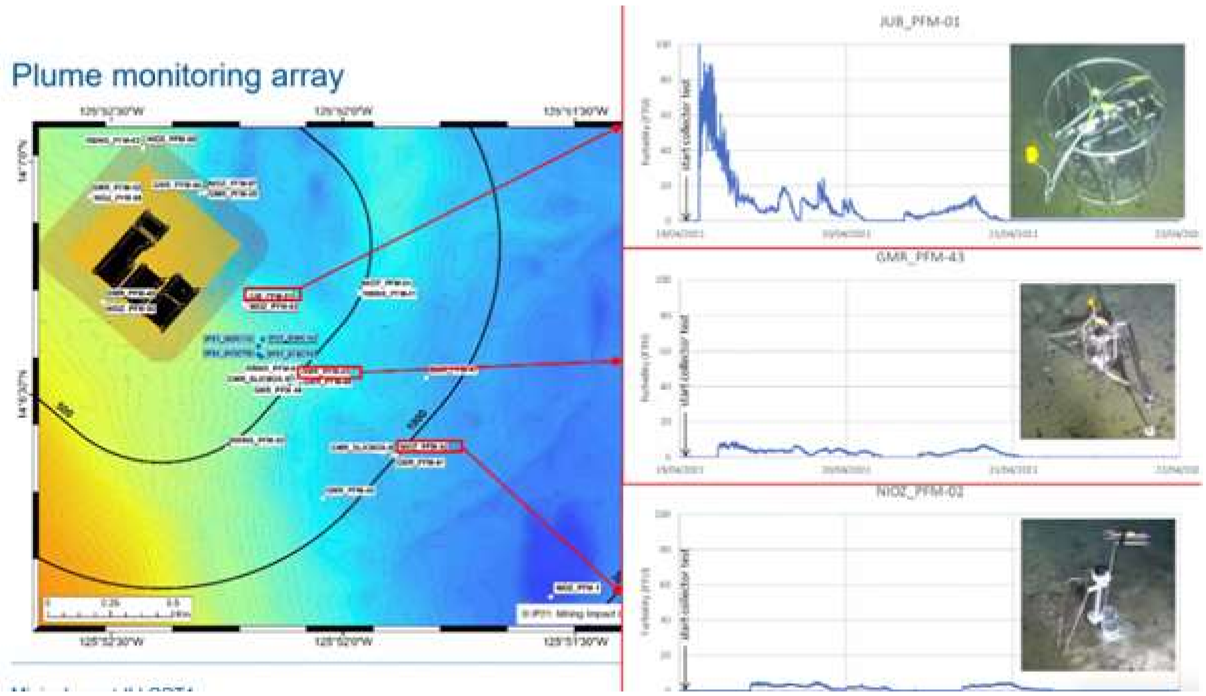

Figure 6.4: Distribution of plume monitoring platforms in the BEL area during the Patania II collector trial in 2021, and recorded time series of near-bottom turbidity showing a strong decrease in turbidity away from the source as a result of aggregation and subsequent fallout and dilution of the plume with ambient clear water.

Task 6.8 On-site post-impact assessment

After the collector trial in 2021 all monitoring activities of the pre-impact assessment were repeated to determine the extent of the fallout area and the thickness of sediment blanketing as well as the impact on fauna, microbial activity and biogeochemical conditions/processes. This included the determination of blanketing effect/layer thickness by AUV based imagery, changes in multibeam backscatter intensity, and measurement by ROV. Preliminary results from the post-impact assessment revealed a relatively contained area where plume fallout could visually be established and where sediment was mainly deposited in form of aggregates (Fig. 6.4). AUV surveys around the collector test showed that the plume traveled in accordance with the prevailing current direction measured with current meters on the seabed. In the GER area this turned out to be in a direction opposite to what had been forecast by the probability map. Data analyses are ongoing and will be published.

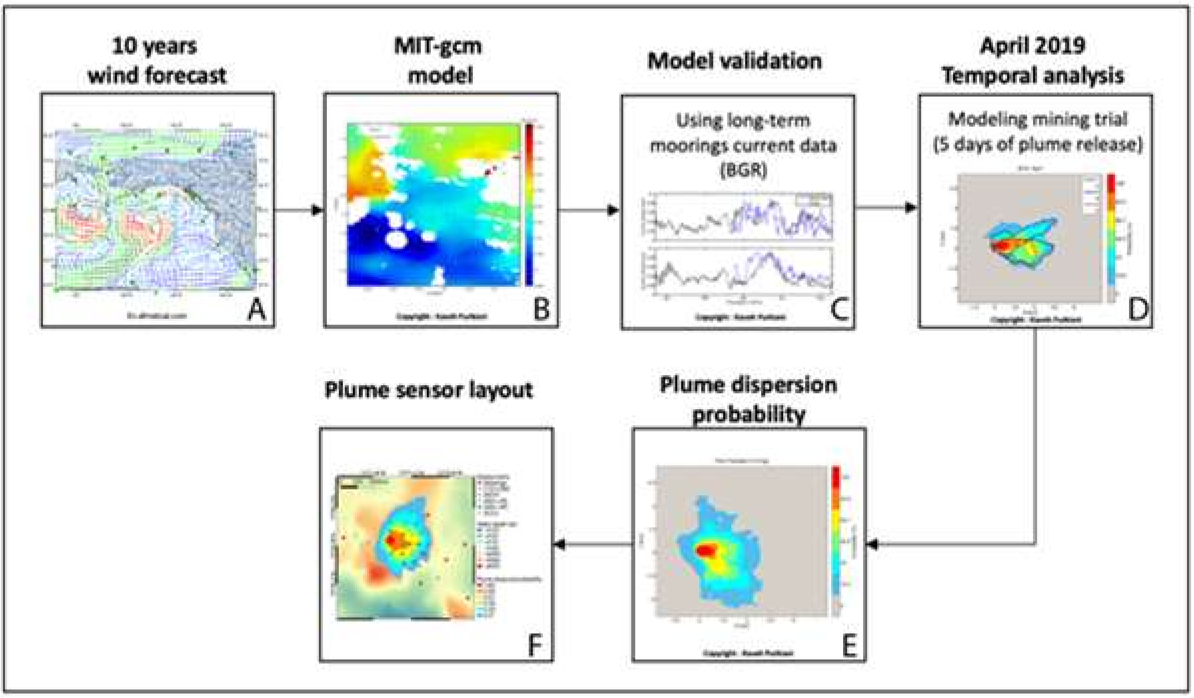

Figure 6.5: Exemplary workflow for the prediction of extent and direction of plume dispersion for defining the 'best possible' deployment position for autonomous sensor platforms (from Haalboom et al., 2022).

Task 6.9 Evaluating the effectiveness of the CCT1 workflow and used monitoring technologies

A best practice guidance document on monitoring technology, layout scheme and workflow (Fig. 6.5) was published in Haalboom et al. (2022). The main recommendations are: A monitoring array should cover both near-field (few km^2) to far-field areas (300 km^2). Although this will not occur at once, the scale to monitor a one-week operation period, for example, needs to encompass at least a radius of several kilometres. Based on probability maps for plume dispersion and deposition, sensors can be distributed along the main axes or gradients of plume transport and suspended particle mass concentration. This should be accompanied by sensor deployments in the less probable fallout areas, thus taking the spatial and temporal variability of the collector site into account. A dynamic sensor layout should improve the quality of environmental monitoring, as also suggested by Aguzzi et al. (2019), where residential robots are used for monitoring and are supported by stationary sensors for regular ground trothing (Fig. 6.6).

Seafloor imagery obtained by both ROV and OFOS deployments proved to be useful to visualise plume-related sediment deposition on the seafloor and AUVs for visual monitoring are recommended, as larger distances can be more easily covered. Optical backscatter sensors are a good choice for monitoring of the suspended particle mass concentration, as the recorded signal of these sensors is more easily quantified. Extensive sensor calibration prior to and after deployment is essential to guarantee high data quality. This can be accompanied by upward-looking acoustic profilers providing information on plume height and turbidity gradients. In-situ particle sizers such as LISST (Laser In-Situ Scattering and Transmissometry) and particle cameras are necessary to understand the aggregation behaviour of plumes and improve the determination of actual fallout areas.

Figure 6.6: Illustration of a variety of cabled observatories providing the sea bed infrastructure to control and coordinate mobile benthic and pelagic platforms such as docked crawlers, rovers, and AUVs. Platform monitoring is assisted by vessels and satellite-based technologies (modified after Aguzzi et al., 2019).

CCT 2 – Disturbance effects in time and space

Milestones and deliverables

M7.1 Workshop on quantitative assessment of intensities (including scoring criteria) of pressures and responses of all ecosystem components (UGhent, AWI)

A three-day online workshop was organized by UGhent in September 2020 prior to the Patania II test and was exclusively based on the dredge experiment. During the first day, preliminary data was presented by the different institutes that participated, resulting in a comprehensive overview of the studied variables of several ecosystem components. Throughout the following days of the online workshop, several common problems were discussed, and it was concluded that potential reasons for the absence of significant trends could be attributed to three major causes namely, 1) baseline conditions (natural variability), 2) strength of disturbance impact and 3) sampling strategy issues.

M7.2 Workshop on scales, indicators and tresholds (SGN, IFREMER)

M7.3 Workshop on integrated analysis of ecosystem responses and environmental impact assessment (UAlgarve)

In December 2021, a workshop was organized by UAlgarve to perform an integrated analysis of ecosystem responses and environmental impact assessment. The results of this workshop contributed to CCT3 objectives to develop policy recommendations.

D7.1 Report on different ecosystem component responses and sensitivities to specific pressures (interactions and intensities) from mining activities (SGN)

D7.2 Report with recommendations on scales, indicators & thresholds for EIA (UGhent)

D7.3 Report on recolonization by fauna and microbiota, and adjacent sediment biogeochemistry (month 41, AWI)

Task 7.1 Linking mining activities to pressures and effects on ecosystem components (all partners)

Integrate the spatial and temporal variability in (bio)geochemistry, element fluxes, bioturbation, sediment and pore water characteristics, and relate the observed changes after disturbance to specific pressure (and combinations) and their intensity against the observed baseline variability in environmental conditions.

A comprehensive integrated dataset of biogeochemical variables has been established from sediment and pore-water samples and in-situ measurements taken and performed before and after a small dredge disturbance experiment in 2019 and the Patania II trials in 2021 in the GER and BEL areas in the CCZ. Our observations are based on variables on (1) the availability and freshness of organic matter (phytopigments, TOC, C/N, amino acids, biochemical composition of organic matter and ‘biopolymeric C’), (2) the availability and fluxes of electron acceptors used for organic matter degradation (oxygen uptake, vertical oxygen distribution and penetration depth, nitrate), (3) proxies for microbial activity and organic matter degradation rates (extracellular enzymatic activity (EEA), leucine, CO_2 uptake), (4) pore-water constituents released during organic matter degradation and reductive oxide mineral dissolution (dissolved (trace) metals, nitrite), (5) sedimentation rate and bioturbation depth ($^{230}Th_{ex}$, ^{210}Pb), (5) sediment elemental composition (e.g. Ca, Mn) and (6) physical properties of the sediment (porosity, shear strength).

Most biogeochemical variables differ between contract areas (on scales >100 km), which is largely explained by the trophic difference of organic matter input to the seafloor. For many variables, baseline conditions show a strong variability on local scales within exploration areas (1-10 km) and even between replicates taken at the same location. Some pore-water variables, EEA and phytopigments also indicate a natural temporal variability when comparing samples obtained in 2019 and 2021. In order to settle solid baselines that allow to identify and quantify disturbance effects despite the natural spatial and temporal variability, sampling of pore water and sediment for the analysis of biogeochemical variables has to be properly replicated and should cover different seasons and years. Where analyses complement each other, they should be performed on the same sample (i.e. same sediment core, homogenized sediments). Biogeochemical variables that are suitable to assess impacts on the ecosystem need to be sensitive to physical changes caused by the disturbance and / or to the resulting changes in biogeochemical processes and rates.

During the investigations that were carried out shortly after the Patania II tests and the dredge experiment, several biogeochemical variables proved to be clearly affected by the impact and, hence, may be suitable as indicators for the assessment of (1) the physical impact and (2) the (immediate) effect on biogeochemistry. In order to assess sediment removal and plume deposition as key pressures, a multi-proxy approach (i.e. using several variables in combination) proved most powerful. After the Patania II test, a range of net sediment removal between 3 and 8 cm in the collector impact sites (where Patania II operated) and a plume deposition layer of max. 3 cm in the adjacent plume impact sites were determined based on the combination of biogeochemical variables (e.g. visual core observation, oxygen concentrations, porosity, solid-phase Mn). Currently, the approach has some limitations: material deposited right away on the fresh tracks impedes the total thickness of the removed layer to be quantified, while a deposition of less than 0.5 cm cannot be resolved so far due to sampling resolution. The immediate effects of the impact on biogeochemical conditions (e.g. organic matter availability, oxygen distribution) differ between the collector impact and plume impact sites. Changes of biogeochemical processes resulting from the disturbance could only partly be assessed as some variables (e.g. oxygen fluxes as a proxy for organic matter remineralization rates) show steep gradients in the upper sediment layer after the impact that need to adjust first (takes a few months) until the alteration of biogeochemical processes can be quantified. Only then it will be possible to fully address the effects on biogeochemical processes and to reliably determine threshold values for the physical impact (e.g. sediment removal and plume deposition thickness). These observations will also serve as a starting point for the assessment of long-term trajectories of ecosystem deterioration and a possible recovery of biogeochemical processes.

Integrate the spatial and temporal variability in benthic communities (microorganisms, meio-, macro-, and megafauna) with respect to biodiversity, abundances, biomass as identified in WP1 and relate the observed changes after disturbance to specific pressure (and combinations) and their intensity against the observed baseline variability in benthic communities

Most of the variables that were identified to characterize the different functional, taxonomical and size groups of the benthic communities showed a high natural variability. This observation confirmed the need for sufficient replication for baseline studies as well as for future impact studies. Consequently the two impact experiments that were performed respectively small scale (dredge deployment) and medium scale (Patania II) did not result in overall statistically supported evidence for changes in the communities. Some of the Patania II test samples are still being processed and given the high natural variation in the control and before impact data, it is too early to draw quantitative conclusions on the impact. Clear is that the removal of sediments and nodules in the tracks was followed by thick sediment redeposition, not only in the tracks but also in adjacent areas near the track. The redeposition of sediments together with originally removed benthic biota further hampers to identify the true impact since possibly no differentiation can be made yet between dead and living biota at the time of sampling which was only a few days after the disturbance. The planned revisit to the area in late 2022 (SO295) is therefore crucial to identify the true impact of the Patania II experiment in combination with sufficient replication post impact.

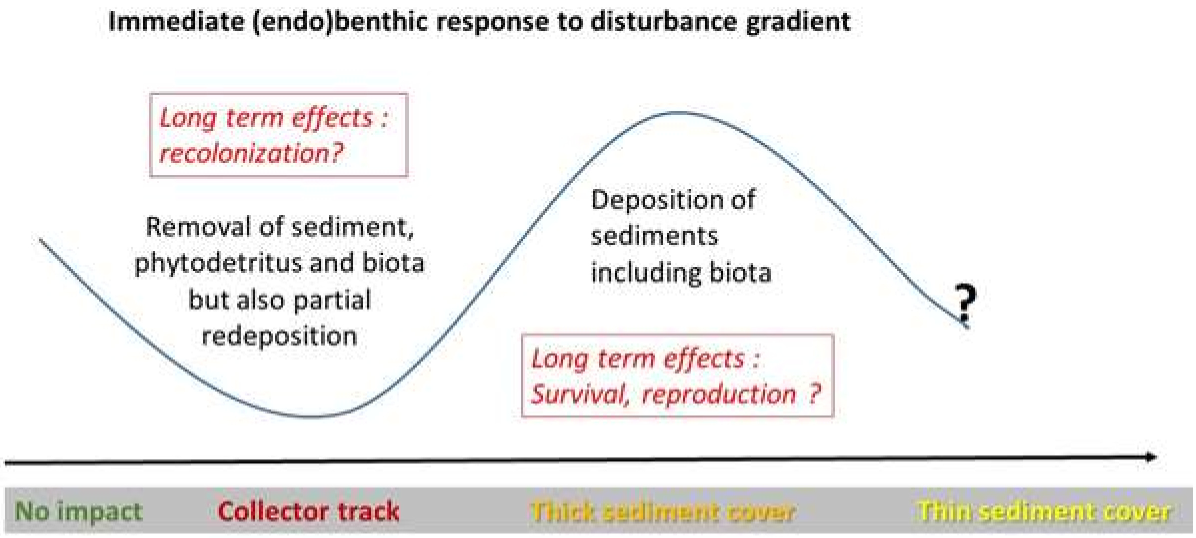

Figure 7.1: Schematic visualization of changes in densities and diversity of benthic communities including meio-and macrofauna based on samples collected before and after the Patania II test.

The removal of the upper sediment layer by Patania II is estimated to reach up to 8 cm sediment depth, which is equivalent to the biologically active, bioturbated layer, but the tracks are immediately after passing by the collector covered with resettling material. This blanketing layer has a variable thickness up to a maximum of 3 cm not only in the tracks, but also adjacent to the track. There are indications of changes in benthic biota abundances, diversity and composition as visualised in Fig. 7.1, but differences in response variables between sites with different degrees of impact including no-impact were only occasionally statistically supported. Despite its small impact range and the limited number of replicates reducing the statistical power, this experiment proved to be valuable due to the applied sampling strategy (gradual impact categorization), the use of extensive sensor deployments (sediment plume monitoring) and the wide array of biophysical, biogeochemical and biological samples. It also allowed to identify recommendations for future realistic scale experiment in order to apply sufficient replication pre- and post in trial and reference sites, while taking temporal variability into account in the design for pre- and post-impact.

Integrate the spatial and temporal variability in benthic ecosystem functions (e.g., organic matter processing, microbial growth, food web) as identified in WP 3 and relate the observed changes after disturbance to specific pressure (and combinations) and their intensity against the observed baseline variability in benthic communities

Extracellular enzymatic activities provide information on the degradation potential of organic matter. Data were collected in the reference and trial sediments during the SO268 cruise in 2019 in the GER and BEL areas. Aminopeptidase activities were up to 4 orders of magnitude higher than β-glucosidase suggesting a preferential degradation of proteins over carbohydrates. All enzymatic activities are generally lower in the reference sediments compared to the trial sites in the GER area while in the BEL area lower values were observed in the trial site. Prokaryotic-mediated OM degradation shows high variability in the GER and BEL reference area. Data collected during the IP21 cruise show that aminopeptidase activities are lower in sediments impacted by the Patania II collector while no differences are evident for the β-glucosidase in the BEL area. A different pattern is observed in the GER area where values of both activities are lower in the reference sites. In the BEL area only, aminopeptidase is lower in the Patania II collector impact site; in the GER area, overall lower values in the reference site. Ecosystem functions show a different response to the collector impact in the BEL and GER area, respectively. Overall, OM degradation rates changed among experimental plots when different extracellular enzymatic activities and areas are investigated.

From the analyses of the lipid biochemistry in the sediment samples of both GER and BEL areas, we can suggest that lipid concentrations are comparable between the two different areas, however in the BEL area, we encountered the presence of sterols, indicating that the sediment has a more bioavailable lipid composition than in the GER area. For both areas, lipid quality was generally low, as little Polyunsaturated Fatty Acids (PUFAs) were found within the samples. When looking at the lipid biochemistry in deep-sea holothurians, in the BEL area, these organisms have lipid concentrations 10 times higher than the GER area, where we encountered more PUFAs and sterols. For both areas, there was the presence of bacterial biomarkers in the fatty acids. Although we still need to analyze all the samples to make a general conclusion, we can already suggest that in both areas, the organic matter quality and quantity is little, but the higher lipid quality in the BEL area is reflected in the holothurians analyzed.

As for food web trophic studies, an in-situ experiment was performed in the BEL area during 5 and 20 days, to assess the mining impacts on benthic ecosystem functioning at the abyssal seafloor. The objective of the study was to look at the effects of the settling and resuspension of plume material on the trophic food web. From the results already analyzed, we can suggest that after 5 days, several taxa (nematodes and copepods) showed significant uptake of labeled organic material. However, after 20 days, a stronger uptake is observed. Most nematodes and copepods samples show significant uptake, and Foraminifera, Nemertea, and Tanaidacea (Akanthophoreidae) started showing significant enrichment. Although the results are still not complete, we can suggest that phytodetritus has been assimilated into various taxa at different times (different pathways and trophic links). Thus, mining activities may have an impact on the sediment food web, since they may interfere with the remineralization of settling phytoplankton detritus, which follows by a few weeks the surface phytoplankton bloom period.

Trophic and non-trophic interaction webs allow to quantify the importance of non-trophic interactions, i.e. interactions that are not related to prey-predator interactions, in deep-sea food webs. In this particular case, the importance of polymetallic nodules for food-web integrity in the Clarion-Clipperton Fracture was investigated and the taxon whose removal had the largest impact on food-web properties was determined. Removing the nodules led to a decrease in food-web compartments of 18% and to a loss of 31% of all network links. Stalked glass sponges living attached to the nodules were key structural species that provided a habitat for Antipatharia, Alcyonacea, Pennatulacea, Ophiuroida, and Porifera. Therefore, the removal of nodules and the subsequent loss of these stalked sponges caused a loss of 4% of all food-web compartments.

Task 7.2 Tools for integrated (cumulative) environmental impact assessment (all partners)

Perform a multiple-scale analysis to test the importance of the observation scale (both spatial and temporal) for impact assessment

Environmental variables investigated within the project show small-scale (in the order of meters) spatial variability that is sometimes as high as medium (km) or large scale (whole contractor areas) variability. This includes biogeochemical variables as well as differences in the faunal composition. Regarding solid phase of the sediment, highest spatial variability has been observed in extracellular enzymatic activity, and in the contents of phytopigments and calcium (Ca). Porewater analyses revealed highest variability in oxygen uptake, trace metal and nutrient concentrations. Regarding the benthic fauna, variability in meiofauna abundance between the cores of one multicore deployment can be on the same range as regional differences (Uhlenkott et al., 2020). We need to keep in mind, that coring devices (multicorer and box-corer) retrieve only a very small area of the seafloor (70 cm² to 0.25 m²) while results derived from these samples are extrapolated to much larger areas. In the dredge experiment, these high levels of variability in combination to the low number of replicates hindered the identification of significant responses to the impact. For baseline studies and impact studies following the BACI (Before-After-Control-Impact) approach a sufficient number of replicates will be needed (traditional 3 replicates per site is far too low!) to confidently enable the differentiation of natural variability compared to the effect of anthropogenic impacts and to monitor recovery to stable sates.

Temporal differences could only be investigated annually comparing the two cruises conducted in 2019 and 2021 and most pronounced differences were observed in pore water and microbial variables. However, to reliably address temporal variability, focused sampling would be needed for the different variables. Regarding a time-series of meiofauna abundances from the reference site in the BGR area, annual differences were reported (Uhlenkott et al., 2021). Results from metabarcoding of meiofauna in the BGR time-series sampled in 2010, 2013, 2014, 2016, 2018 and 2019 showed significant differences in assemblages between years.

The need of at least one but better several control areas in environmental impact studies in the CCZ is evidenced by the combination of high small scale spatial and the temporal variability in benthic assemblages. Under these circumstances, control areas are needed to disentangle spatial and temporal changes produced by natural environmental variability from changes derived from anthropogenic impact or the recovery of benthic populations.

Identify the types of impacts (compaction, nodule or surface sediment removal, blanketing, particle concentration and shape in the water column, toxicity, etc) that have the largest effects on benthic communities and functions and determine the relevant 'intensity thresholds' (thickness of surface sediment mixed or lost, thickness of blanketing layer, etc)

These results follow primarily from the M7.1 workshop in 2020 that discussed observations from the small dredge experiment conducted during SO268. As reported in Task 7.1, most of the abiotic -and biotic variables exhibited strong variability within the pre -and post category samples. While some of the abiotic variables (geochemistry -and biogeochemistry components) showed slight differences between pre-dredge and track samples, no clear changes were detected within the sediment plume deposition impact categories (thick/thin cover) compared to baseline conditions. This general lack of conclusive findings was also found for most of the biological variables under study, which showed "no effect" for all the post-dredge impact categories. Hence, during the M7.1 workshop several common problems were discussed, and it was concluded that potential reasons for the absence of significant trends could be attributed to three major causes namely, 1) baseline conditions (natural variability), 2) strength of disturbance impact and 3) sampling strategy issues.

Also, the Patania II experimental design suffered from time constraints and limited replication, but results confirmed the trends already observed in the dredge experiment such as the reduced densities and biodiversity in the track (see Task 7.1). Based on the results of these experiments and other evidence from previous experiments in nodule areas, Figure 7.2 summarizes the expected mining impact over time on different benthic variables in relation to different disturbance intensities such as the sediment reworking, nodule removal and sediment deposition in the collector track as well as different degrees of sediment deposition near the track. However, the identification of intensity thresholds is still ongoing, and further evidence will be collected in a follow up cruise SO295 in late 2022.

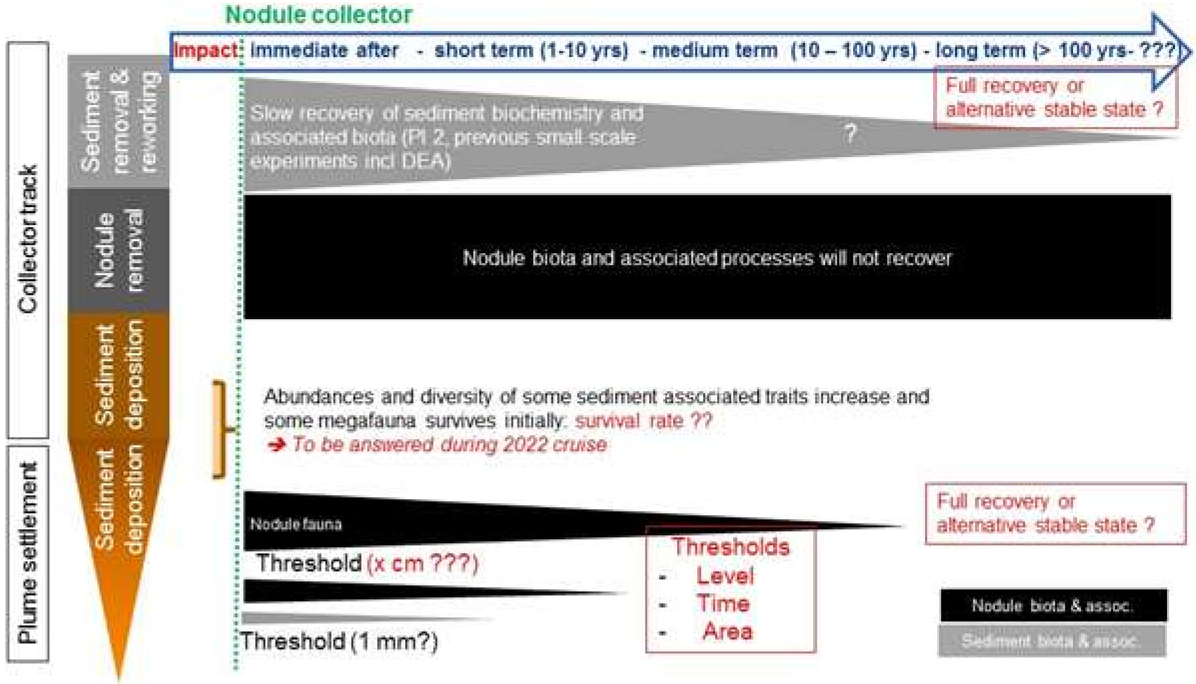

Figure 7.2: Summary of the evidence based response of benthic variables to different degrees of impact including recovery over time. The text blocks in red indicate aspects for which evidence is still lacking.

Identify 'indicator species' / 'indicator groups' / 'indicator functions' as a proxy for effects on specific parts of the benthic ecosystem (e.g., distinct taxonomic or functional groups, size classes, or distinct functions like organic matter remineralization, bioturbation, element and energy transfer in food webs) or the ecosystem in general (sensu 'seafloor integrity')

Due to the high number of undescribed species and the on-going use of morphotypes or operational taxonomic units instead of validated species, no species has been identified as an indicator for mining impacts. Furthermore, biodiversity in the benthic environment of the CCZ is very high, but in combination with the very low abundances in the deep sea, the use of individual species for the evaluation of impacts remains generally questionable. Even using next generation sequencing based methods such as metabarcoding of benthic samples were not able to show the occurrence of certain ASV (species equivalences) during specific stages of disturbance. However, metabarcoding data from benthic nematode communities imply possible relations of disturbance stages and phylogenetic relatedness of occurring nematodes, changing from random relatedness before an impact to higher relatedness after mining. This may indicate the prevalence of certain ecological groups (e.g. scavengers) which would profit from a critical disturbance of the environment. However, re-achieving a random relatedness of species over time could likewise be the result of a recovering community. Nonetheless, this was not demonstrated on nematodes in morphological studies such as carried out on 26-year old tracks before (Miljutin et al. 2011).

Furthermore, benthic communities highly rely on certain key groups. In a polymetallic-nodule environment, stalked glass sponges were found to be a major component structuring benthic communities (Stratmann et al., 2021). Because nodules are the main hard substrata usable for sessile organisms in this environment, a loss of nodules directly causes a loss of food-web integrity and thus can subsequently cause a depreciation of biodiversity (Stratmann et al., 2021). The presence or absence of stalked glass sponges can subsequently be considered a good indicator for both food-web integrity and impact of deep-sea mining in an area such as the polymetallic nodule fields in the CCZ.

Identify robust approaches for ecological impact assessment (e.g. WOE)

The results to assess the ecological impact of the plume generated during the DEME collector trial are very preliminary. As such, during the M7.3 workshop on integrated analysis of ecosystem responses and environmental impact assessment, additional data available from other experiments and considered useful for this assay, and available from consortium partners was also gathered. This information was compiled with the aim to identify the importance, sensitivity and indicator value per response variable and related to the different impacts and pressures, as well as timescales. For each impact response variable, the different experts scored its importance for ecosystem functioning, biodiversity, biomass, and indicator potential. The practicality in conducting the analysis as well as the estimated confidence in the results from their response variable to be able to correctly assess the impact was also assessed. Although all the limitations due to an overall limitation of data available, it is already possible to distinguish that some response variables are more promising impact indicators than others, per type of variable. It is, however, very clear that the available scientific data is not sufficient to determine thresholds per pressure gradients for most of the variables analysed. Still this exercise enabled the development of a conceptual framework for assessing impacts per type of variable and physical disturbance, in the short and long terms. Given the data limitations it was not possible to run the WOE yet, but we hope that soon this tool can be used to assess the ecological risks of deep-sea mining.

Task 7.3 Colonization experiment (NIOZ, UGhent, SGN, MPI, UAveiro, NIVA, GEOMAR)

Test the feasibility of artificial hard substrates for restoration action through time and space and explore the role of substrate type for settlement success of biota, including early formation of microbial biofilms, and impact on sediment biogeochemistry

One tool to manage biodiversity risks is the mitigation hierarchy, including avoidance, minimization of impacts, rehabilitation and/or restoration, and offset. We initiated long-term restoration experiments at sites in polymetallic nodule exploration contract areas in the Clarion-Clipperton Zone (CCZ) that were (i) cleared of nodules by a collector vehicle, (ii) disturbed by dredge or sled, (iii) undisturbed, and (iv) naturally devoid of nodules. To accommodate for habitat loss, we deployed artificial ceramic nodules to study the possible effect of substrate provision on the recovery of biota and its impact on sediment biogeochemistry. Ceramic nodules were made from commercial clay and from deep-sea clay collected in the CCZ. The clay nodules were fired in a kiln (oven) at 800-1200 °C, resulting in ceramic nodules. Mineral content was evaluated for ceramic nodules and compared to real nodules. Nineteen ceramic nodules of different size/shape and six natural nodules as scientific control were mounted on a frame. A total of 116 experimental frames with a total of 2900 nodules (2204 ceramic nodules + 697 natural nodules) were deployed in the CCZ on the expeditions in 2019 and 2021 (Fig. 7.3 A-H). Seventy-five nodules were recovered after eight weeks and had not been colonized by any sessile epifauna. No data could be collected on formation of biofilms and impact of ceramic nodules on sediment biogeochemistry. The next recovery of frames with nodules is planned on SO295 in late 2022, and will be accompanied by collection of sediments underneath the nodules. Data analyses will include sediment biogeochemistry, microbial and faunal diversity in sediments and on nodules. The majority of nodules will remain on the seafloor for several years before recovery. Considering the slow natural recovery rates of deep-sea communities, these experiments represent the beginning of a ~30-year study during which we expect to gain insights into the nature and timing of the development of hard-substrate communities and the influence of nodules on the recovery of disturbed sediment communities. Results will help us to understand adverse long-term effects of nodule removal, providing an evidence base for setting criteria for the definition of “serious harm” to the environment.

As an additional experiment, and to account for habitat modification of the top sediment layer, sediment in an epibenthic sled track was loosened by a metal rake to test the feasibility of sediment decompaction to facilitate soft-sediment recovery (Fig. 7.3 I-J). Analyses of granulometry and nutrients one month after sediment decompaction revealed that sand fractions are proportionally lower within the decompacted samples, whereas total organic carbon values are higher. Diversity of fauna is not analyzed yet. Similar to the ceramic nodule experiment, also the decompaction experiment represents the beginning of a ~30-year study during which we expect to gain insights into the nature and timing of recovery of disturbed sediment communities after decompaction.

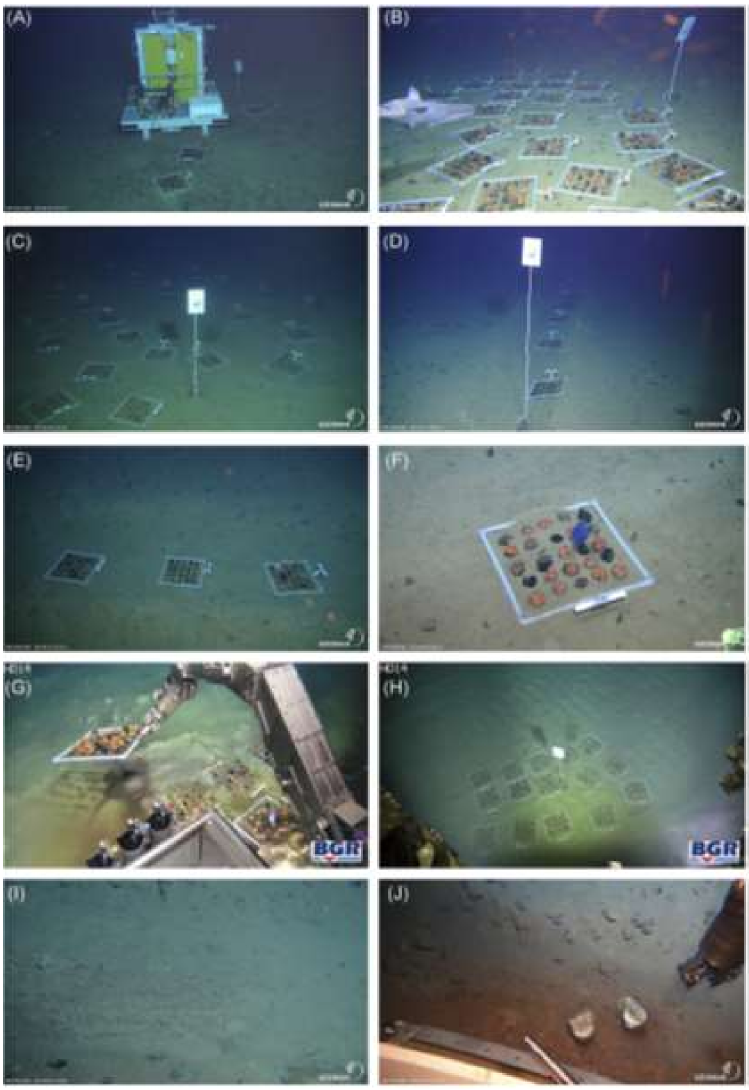

Figure 7.3: Photographs of deployed restoration experiments in disturbed polymetallic nodule areas in the Clarion-Clipperton Zone in ~4100 and ~4500 m depth. (A–H) Deployed nodule frames with ceramic artificial nodules and natural nodules as control. (A) GER trial site before the trials, (B) GER reference site, (C) GER no-nodule site, (D) GER dredge site, (E) GER SO239 EBS track after decompaction, (F) close-up of a nodule frame with an artificial sponge, (G) GER collector impact site, (H) BEL collector impact site. (I–J) Decompacted sediment. (I) Decompacted sediment patch in the GER SO268 decompaction experiment site, and (J) push core sampling in one of the decompacted sediment patches. Gollner et al. (2021).

Explore the role of ecosystem engineers for biodiversity in manganese nodule fields

In the framework of colonization experiment, eleven frames were equipped with a single artificial plastic kitchen sponge, mimicking natural sponges (Fig. 7.3 F). Considering the slow natural recovery rates of deep-sea communities, these experiments shall be recovered in ~5 to 10 years. We expect to gain insights into the role of ecosystem engineers for recovery of biodiversity in nodule fields. Thus far, sessile epifauna has not been observed to settle on ceramic nodules within a period of 8 weeks. For more information see Gollner et al. (2021).

CCT 3 – Environmental risk assessment and policy recommendations

Milestones and deliverables

M8.1 Description of the structure of the models showing how the data from the collector trial will be integrated into the models (UAlgarve)

M8.2 Validation of WOE model achieved, recommendation on this methodology ready to be incorporated into guidance document (DNV)

D8.1 Report on outline of WOE and ENVID model (DNV)

D8.2 Guidance document on methodologies for risk assessment of environmental hazards of deep-sea mining (DNV)

Task 8.1 Description of the structure of the models (UAlgarve, DNV)

Task 8.2 Report on outline of WOE and ENVID model (UAlgarve, DNV)

Task 8.3 Guidance document for methodologies for risk assessment of environmental hazards (DNV, UKiel, SNF, BGR, GEOMAR)

Instead of reporting on individual milestones, deliverables and tasks, an abbreviated version of the draft of the CCT3 guidance document is presented.

OVERVIEW

The aim of the MiningImpact project was to investigate the expected environmental impacts of deep-sea mining. Impacts from mining activities on the marine environment will differ between resource types, but However, two consequences appear to be common for the currently discussed mining technologies, (1) the removal of the surface of the seafloor, including its epifauna and endofauna, and the creation of a plume consisting of mineral debris and/or sediment that will blanket also some untouched seafloor in the vicinity of the mining area. MiningImpact focused on the impacts related to the harvesting of polymetallic nodules.
While the first project phase revisited and investigated only experimental and rather small-scale disturbances of the seafloor that were up to almost 4 decades old, the second project phase designed a comprehensive, independent scientific monitoring programme around the first industrial test of a pre-prototype nodule collector system in the deep-sea. The collector vehicle Patania II, built by the Belgian contractor DEME-GSR with an undercarriage at a scale of 1:4 compared to the planned full-scale mining vehicle, was tested in 2021 in the GSR and the BGR contract areas of the CCZ. The trial areas where the polymetallic nodules were removed from the seabed were approximately 37.000 m^2 and 22.000 m^2 large, respectively. MiningImpact 2 provided an independent scientific investigation and assessment of the environmental impacts of this operation, in particular studying the spatial and temporal dynamics and footprint of the suspended sediment plume and the redeposited sediment blanket, as well as the induced consequences for the abyssal ecosystem and its functions. Furthermore, MiningImpact 2 specifically worked towards developing policy recommendations for international regulations, such as the Mining Code developed by the ISA. For exploitation, the ISA working document "Draft Regulations on Exploitation of Mineral Resources in the Area" (ISBA/25/C/WP.1) is currently still in review and under adaptation.

The current draft regulations on exploitation do not provide details on environmental thresholds and standards. In Annex IV, as part of the template for an Environmental Impact Statement, it is however stated that thresholds may be developed as standards and guidelines to support the regulations. The outcome of MiningImpact and the work in CCT3 can be a first step towards the thresholds needed and will provide guidance in determining those thresholds. Furthermore, regarding the environmental hazards of mining-induced sediment plumes, methodologies like Weight of Evidence (WoE) are proposed by CCT3. Also methodology for risk assessment for the exploitation of marine mineral resources that takes into consideration the state of knowledge and evolving research on deep-sea ecosystems and their environment forms part of the outcome. In this CCT3 report descriptions of the environmental risks are listed, followed by the private and social risks from an economic perspective. Finally, the focus lies on how these outcomes translate into the future regulations of deep-seabed mining.

WEIGHT OF EVIDENCE APPROACH FOR ENVIRONMENTAL RISK ASSESSMENTS

The investigation of the environmental impact of sediment plumes formed an important part of the project. For specific risk assessment of the environmental hazard of sediment plumes, the Weight of Evidence Approach (WoE) could be used. This has also been done in the tests of GSR's pre-prototype nodule collector vehicle Patania II within the project. The WoE was hereby used to, first, classify the environmental hazard of the trial at the test sites and, second, estimate the degree of confidence in the defined model. WoE is an instrument often used in environmental risk assessments (ERAs) in general, in spite of deep-seabed mining, to characterise the hazard. This procedure is commonly applied when different types of science-based evidence are needed to draw a conclusion and present a recommendation to decision makers. There are three major components in a WoE procedure: (1) it is necessary to assemble the evidence; (2) this is followed by weighting the gathered evidence; and (3) the body of lines of evidence (LoE) are finally weighted. With regard to deep-seabed mining, the WoE framework should be incorporated in the initial stage of the risk assessment (problem formulation) and in the final stage (risk characterisation), with reassessment during the process of decision-making.

ENVIRONMENTAL RISKS AND THRESHOLD LEVELS FOR DEEP-SEABED MINING

Environmental risks

The main environmental impacts related to deep-seabed mining will typically relate to:

- **Size and distribution of mining areas**. Long-lasting change of seafloor integrity by the mining activity (i.e. removal of the upper active benthic surface layer, which consists of the colonised hard substrate (mineral resource) and, in the case of polymetallic nodules, its associated soft sediment habitat; possible compaction by mining gear) can impact ecosystem functions and services on local and possibly also regional scale and potentially interrupt the connectivity of species if suitable management tools are not in place.
- **Spreading of particles**. The mining activities will lead to sediment disturbance and plume discharges, which can lead to negative impacts like smothering of benthic (seabed) habitats outside the mined areas. In addition, strongly elevated suspended particle concentrations are induced in the water that can impact benthic and pelagic organisms.
- **Spreading of contaminants**. The sediment plume deriving from the mining area may contain particles with a high concentration of metals and other contaminants like reduced substances, which can have toxic or detrimental effects on organisms in the surrounding areas.
- **Light pollution**. The light used during mining activities at the surface platform (e.g. vessel) but also the mining vehicle at the seafloor can deter and disturb the activities and natural rhythms of algae, fish and other fauna.
- **Noise and vibrations**. Noise and vibrations can disturb the acoustic communication of marine mammals and can influence the natural behaviour of fish and invertebrate populations in the water column (e.g. reduction in foraging ability) as well as the benthos.
- **Discharges to water**. The surface vessel could discharge cooling water, which often contains antifouling chemicals and corrosion inhibitors and other chemicals, as well as any other utility- or process-related discharges. In addition, the potential releases of ballast water could lead to spreading of invasive species. These alien species can put the existing ecosystems under severe stress and overgrow the native species or introduce exotic diseases.

Furthermore the impacts of deep-seabed mining on the marine environment differ depending on the resources being exploited (Levin et al., 2016) but many impact types are quite similar (Boetius and Haeckel, 2018). Threshold levels for deep-seabed mining do not have to and, more importantly, should not differ between the different mineral resources (polymetallic nodules, polymetallic sulphides and cobalt-rich crusts). This is based on the principle that there should be the same level of environmental protection irrespective of which type of ore is being mined.

Environmental threshold levels for deep-seabed mining projects
With regard to the Clarion-Clipperton-Zone (CCZ), it appears not possible so far to identify an indicator species because there are, at the moment, no known dominant species in the CCZ. To remedy this, it has been proposed to identify indicators for functional change and for connectivity. It might be possible in the future to define an indicator group, for example deposit feeders or suspension feeders or structural fauna (e.g. stalked sponges). It also seems sensible to determine indicators of change: e.g. an indicator for the pristine environment, for disturbance or for recovery. It is recommended to apply the concept of connectivity for spatial management, i.e. Areas of Particular Environmental Interest (APEIs) and Preservation Reference Zones (PRZs), something that has been considered to some extent when defining the four new APEIs in the CCZ (ISBA 26/C/43). This should not only include genetic connectivity but also demographic connectivity, such as reproduction, larval development, recruitment and mechanisms of dispersal, amongst others. Although demographic connectivity will not be able to state anything about the connection between populations, it will allow for estimations of resilience.

In general, environmental threshold levels should be based on the local conditions at the actual site. Existing environmental threshold levels that have already been developed for offshore activities can therefore not be directly applied to deep-seabed mining but they can be of help in defining a way forward.

Spreading of particles
Spreading of particles from deep-seabed mining operations has two main sources:

- Spreading caused by the mining vehicle/mining equipment. The mining vehicle will cause spreading of particles while moving on the seabed and while the mining equipment is collecting/cutting the ore and the host sediment.

- Spreading caused by return water with particles from the mining support vessel. In many mining concepts, the ore is pumped through a riser to the mining support vessel (e.g., Oebius et al., 2001; Weaver et al., 2022). Sediments will be pumped up together with the ore and these can be separated on the mining support vessel and returned through a pipe back to the seabed. This will also cause spreading of particles. Injection of this waste water into shallower water depths should be avoided to minimize the spread of the plume. In addition, accidental failure or rupture of the riser pipe may cause a particle plume in the water column.

Manganese nodules lie on soft deep-sea sediment, which are expected to be removed and suspended during the mining of nodules, irrespective of the type of collection technology used. Many components of the collector, the riser system and the platform vessels will be new and have yet to be tested under real conditions. However, it is undisputable that plumes of resuspended sediments will develop, which, depending on the amount of sediment mobilised, may represent one of the major sources of impacts on the faunal communities that live at, on, in or close to the seabed. Such plumes drastically add to the natural sedimentation rate, which normally lies in the order of a few millimetres per thousand years only. The sinking sediments will cover the organisms that live on the seafloor sediment or on nodules in the immediate vicinity of the mining area. Furthermore, increased particle load in the water column near the ocean floor, which may additionally be contaminated by released heavy metals, is likely to affect suspension feeders that extract their food from the water column, such as sponges, corals and some fish species or larvae.

Overall, it is necessary to understand how fast the suspended sediment will be redeposited and how it will be distributed spatially due to topographic irregularities (e.g., seamounts) and with the bottom currents. The scale of the impacts and the subsequent environmental risks involved will be strongly dependent on the type of mining technology used. Important questions are: How do deep-sea sediments behave when they are suspended? How quickly do aggregates form and resettle? What quantity of sediment is redeposited, and what is the thickness of and how does blanketing thickness relate to distance from the mined area? How much labile organic matter is contained in the suspended and resettling sediments and how does this affect the availability of food for the fauna? In order to answer these questions, oceanographic conditions, such as bottom current velocity and direction at the seafloor, as well as the local bathymetry, must be studied over a period of several years (Aleynik et al., 2017; Peukert et al., 2018; Purkiani et al., 2020; Purkiani et al., 2022). Furthermore, realistic laboratory and field experiments provide important insights into sediment properties such as aggregate formation and sinking rates (Gillard et al., 2019). Finally, such information is important to model the dispersion of sediment plumes (Purkiani et al., 2021), and model results must be validated by sub-industrial mining tests.

Suggested threshold levels for spreading of particles from deep-seabed mining.

It is suggested that preliminary threshold levels for plumes are based on ongoing research from deep-seabed mining and from experiences in the oil and gas sector.

The threshold levels should be related to the environmental impact area which is the area outside the mining area (Fig. 8.1). The mining area will have elevated sediment particle concentrations during the operation, but this should be accepted as long as the threshold levels for the environmental impact area are not exceeded and a limit for the rise height of the plume above the seafloor is not exceeded.

It is suggested that threshold levels are set with respect to the total deposition thickness caused by the mining activity.

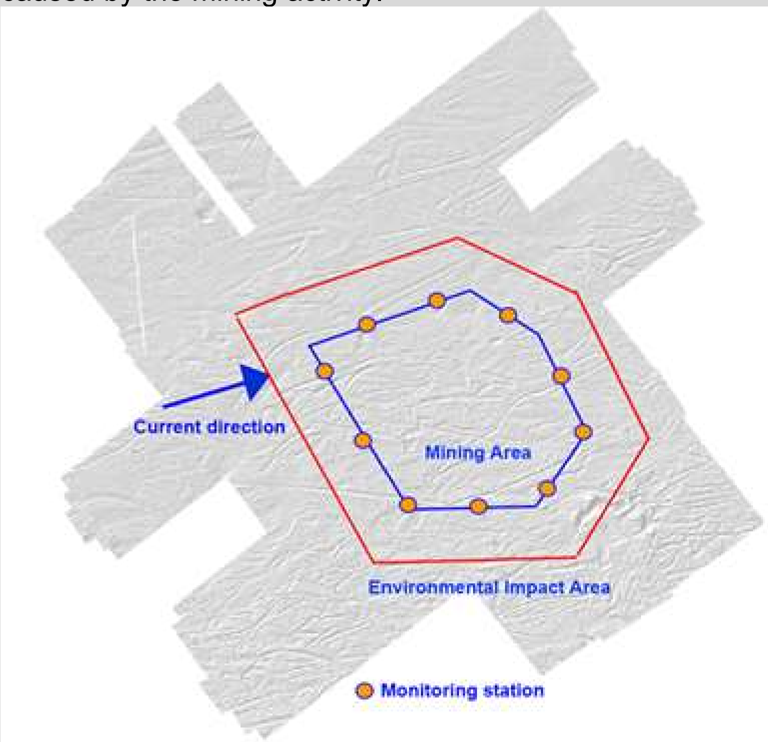

Figure 8.1: Suggested threshold levels for the total deposition thickness caused by the mining activity:
Within the Mining Area (blue line): No threshold levels
Within the Environmental Impact Area (red line, outside the Mining Area): Maximum total deposition thickness of 10 mm.
Outside the Environmental Impact Area: Maximum total deposition thickness of one mm.
The size of the Environmental Impact Area (distance from the Mining Area) will vary from case to case.

Spreading of contaminants
During mining, particles and debris from the mined mineral ore will likely spread to surrounding areas together with sediments from the seabed. The ore itself will have relatively high levels of metals and these could cause negative effects on the pelagic and benthic fauna (organisms in the water column or on the seabed). Trace metals in bottom seawater and their cycling at the sediment-seawater interface may be affected by mobilisation in the sediment plume and changed fluxes from surface sediment into bottom seawater, with potential impacts on metal toxicity and regional biogeochemical cycles. The specific effects strongly depend on the environmental conditions in the area of interest. To define threshold values for trace metal concentrations (e.g. Mn, Fe, Co, Cu, Ni, Zn, V, As, Pb, Cd, U, Mo, REYs) in bottom seawater and surface pore water, above or below which an impact is proven, we need to define the natural concentration ranges. However, the baseline variability sums up from natural variability and the uncertainty derived from sampling and analysis, and especially for the natural variability, we do not have enough data yet. Especially bioavailability data is missing for different metal size fractions. We can indicate average trace metal concentrations in natural bottom seawater that are available for bio-uptake through cell membranes but effects of mining-like disturbances are not yet quantifiable with our available data. In order to define threshold values, trace metal baseline concentrations and speciation need to be combined with ecotoxicological assessments for various species (levels) (micro, meio, macro, megafauna) and data for this is lacking as well.

Due to the limited data that so far is available, we cannot yet present robust baseline ranges or threshold values, e.g. for trace metal concentrations in bottom seawater and pore water but we can indicate suitable variables to for 1) quantification of sediment removal (based on small-scale impact experiments), 2) indicators for ecosystem health.

There are no international threshold levels for contaminants in seawater. However, the EU has published a technical guidance for deriving environmental quality standards (EC, 2017). Based on this, several European states have developed quality standards for their coastal waters. One example is Norway where the Norwegian Environment Agency (2016, rev. 2020) has set quality standards for coastal waters based on the EU Guidance. The quality standards are divided into five categories, Class I to Class V, starting from “Background” (Class I), over “Good”, “Moderate”, “Bad” and, in the end, “Very bad”, which would be Class V. The category depends on the effects that can be expected on the organisms in the water column and in the sediment.

Suggested threshold levels for the spreading of contaminants from deep-seabed mining
There are no international threshold levels for contaminants in seawater. We suggest that such threshold levels should be developed specifically for deep waters.
Existing quality standards for coastal waters as shown above provide an example of how threshold levels can be developed for the deep sea.
While waiting for such international threshold levels existing quality standards for coastal waters could be used as a first approach, but need to be related or scaled to baseline concentrations measured in the deep sea. The Norwegian Quality standards for good water quality are more recently revised (and stricter) than the Australian and New Zealand guidelines. **It is suggested that the water quality is monitored at the Environmental Impact Area perimeter (red line in Fig. 8.1) and should not exceed:**

Arsenic	0.6 µg/L
Lead	1.3 µg/L
Cadmium	0.2 µg/L
Copper	2.6 µg/L
Chromium	3.4 µg/L
Mercury	0.047 µg/L
Nickel	8.6 µg/L
Zinc	3.4 µg/L

Noise and vibrations

Noise and vibrations can disturb the acoustic communication of marine mammals and can influence the natural behaviour of fish and invertebrate populations in the water column (e.g. reduction in foraging ability) and dwelling on and in the seabed (e.g., reduction in nursing/brooding ability/time). Guidelines for offshore activities have suggested threshold levels for noise and vibrations. Such threshold levels are mainly based on studies related to the different effects on larvae, fish and mammals, which show that high noise levels can be stressful, harmful or even mortal.

There is some data for noise in more shallow waters. As an example, a literature study carried out for underwater noise related to an offshore wind turbine project (Xodus Group, 2015) found that the behavioural disturbance threshold for marine mammals is around 120 dB re 1 µPa (rms), (rms = root-mean-square pressure).

Suggested threshold levels for noise and vibrations generated from deep-seabed mining

There are no international threshold levels for noise and vibrations in the deep ocean.

We suggest that such threshold levels should be developed. While waiting for such threshold levels, existing levels from guidelines for offshore activities could be used as a starting point.

Based on this a threshold level for noise (sound exposure level) ≤ 120 dB re 1 µPa2 (rms) at the Environmental Impact Area perimeter (red line in the Fig. 8.1) is suggested.

Other discharges to water

Discharges of cooling water from the surface vessel could contain different types of chemicals that are harmful to the marine environment. Such chemicals could for example be antifouling chemicals, corrosion inhibitors or other utility- or process-related discharges. Biofouling is the accumulation of microorganisms, plants, algae or small animals on the hull of the vessel. It is assumed that about half of the problematic species that are brought around the world by ships are related to the hull and to biofouling. In addition, the potential releases of ballast water could lead to spreading of invasive species. These alien species can put the existing ecosystems under severe stress and overgrow the native species or introduce exotic diseases.

Suggested threshold levels for other discharges to water from deep-seabed mining

The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (BWM Convention) entered into force on 8 September 2017 (IMO 2019). The Convention requires that ships treat/manage their ballast water to ensure that the ballast water satisfies the requirements for maximum organism densities when discharged.

This is controlled by installing and using ballast water management systems (BWMS) with a valid Type Approval Certificate (TAC) on board. During Type Approval testing, the BWMS must document consistent performance in a series of full-scale test cycles. Several different technological solutions are available. Most BWMS are based on a two-step technology, where filtration or use of cyclone will be the first step that removes larger particles, followed by a disinfection step (typically UV-irradiation or chemical oxidants) to kill the remaining, smaller organisms.

Based on this it is suggested to set no specific threshold levels for discharges from the surface vessel but instead use the existing rules of the BWMS Convention.

For biofouling, there are so far no IMO requirements. It is recommended to follow the IMO biofouling guidelines (IMO 2011).

PRIVATE AND SOCIETAL RISK FOR DEEP-SEABED MINING

Also, private and societal risk have been addressed within the project. While private risks refer to the risks facing the contractors undertaking the project, societal risks are carried by all stakeholders impacted by a deep-seabed mining project. In addition, private contractors focus on profits, revenues and costs accruing to the project owner. This contrasts to societal economic analyses, where emphasis is on overall benefits and costs to all relevant stakeholders to deep-seabed mining projects. The background for this distinction is that private evaluations of deep-seabed mining projects cannot be expected to fully include all impacts from deep-seabed mining activities, nor impacts on all stakeholders affected by the mining activities. At least two aspects of deep-seabed mining projects cause private evaluations of these activities to differ from societal economic analyses. First, as noted above, mining activities on the deep-seabed may well last for decades, hence the impacts from deep-seabed mining activities will last longer than what one may expect private entities to consider. Second, private entities do not fully internalise impacts on e.g. other industries like tourism and fisheries.

MINIMIZING HARMFUL IMPACTS ON THE ENVIRONMENT (SPATIAL PLANNING)

Reference zones and habitat conservation and preservation areas must closely match ecosystem characteristics of mined areas (e.g. ocean productivity, nodule density, faunal densities and composition) to safeguard abyssal biodiversity and protect specific vulnerable and important ecosystems as well as their functions and services. One impact reference zone per mined area might not be enough. Just APEIs alone cannot be expected to compensate for biological diversity and ecosystem functions and services lost through mining operations: additional marine protected areas are needed. That it is not clear to what extent an ecosystem approach is applied in the currently existing collection of APEIs emphasises this point. Minimising large-scale impacts requires careful and adaptive spatial planning of mining operations, establishment of a network of representative protected areas, and development of low-impact mining equipment. Science as well as technological research and development are critical to all of these requirements.

In the final report from CCT3 *"Guidance document on the Assessment of Environmental Impacts and Risks of Deep-Sea Mining"* it will be pointed out in more detail, where the current APEI-network leads to a lack of protection. It further shows possibilities, also from a legal point of view, to adapt the current APEI-network and relocate or propose further new APEIs.

There is still a need for comprehensive research in order to provide scientifically sound recommendations on suitable mining strategies. It is not yet possible to predict the extent to which spatial management options may mitigate environmental impacts of deep-seabed mining and prevent major irreversible damage. In order to strike a balance between the need for raw materials on the one hand and a strict interpretation of the precautionary principle on the other, mining operations should develop gradually from small to large spatial scales. These include component trials, pilot mining tests and small commercial test production sites, and need to be accompanied by thorough, comprehensive and independent scientific environmental investigations and risk assessments.

CLOSING REMARKS

The list of environmental and economic risks provided by CCT3 might not be exhaustive but aims to give a good indication of the risks associated with mining activities in the deep ocean. The WoE approach can help to identify and characterise the risks ahead, both qualitatively and quantitatively. This study attempted to gather threshold values for a variety of environmental risks based on other regulatory frameworks (mainly in coastal seas) that still need to be adapted to the environmental conditions in the deep sea. Yet to identify threshold values for the economic risks is something that might be done differently in different parts of the world and the outcomes may depend on particular circumstances, both economically and geopolitically.

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