Impacts of deep-sea mining sediment plumes on cold-water corals

Dissertação de Mestrado

Sandra Carvalho Marques

Mestrado em

Estudos Integrados dos Oceanos

Horta

[2021]

Impacts of deep-sea mining sediment plumes on cold-water corals

Dissertação de Mestrado

Sandra Carvalho Marques

Orientadores

Doutora Marina Carreiro Silva

Prof.ª Doutora Manuela Juliano

Dissertação de Mestrado submetida como requisito parcial para obtenção do grau de Mestre em Estudos Integrados dos Oceanos

Abstract

Mining activities for deep-sea mineral resource extraction, such as seafloor massive sulphides and ferromanganese nodules, are expected to impact deep-sea fauna by generating sediment plumes that disperse over large areas of the ocean. Benthic suspension feeders, such as cold-water corals, are likely to be particularly sensitive. Exposure to suspended sediments can mechanically damage corals by smothering and clogging their tissues and by containing toxic substances that affect coral physiological processes. This study elucidates the impacts caused by sediment plumes potentially generated during mining activities for the extraction of nodules in the Clarion-Clipperton Fracture Zone (CCZ), northeastern equatorial Pacific Ocean and seafloor massive sulphides from a hydrothermal vent field in the Mid-Atlantic Ridge region of the Azores (NE Atlantic) on the octocoral *Dentomuricea* aff. *meteor* using an aquaria-based experiment. For 28 days, corals were exposed to two concentrations (10 and 50 $mg.L^{-1}$) of suspended plumes of sediments from nodule fields, hydrothermal polymetallic sulphide particles, and a control treatment with no sediment addition. Sediment concentrations were selected based on plume dispersal models, as close-field and far-field plume dispersal scenarios. The effects of sediments were assessed on the survival, tissue condition, polyp activity and respiration rates. Results of the study confirmed the hypotheses put forward in this dissertation that *D. meteor* responds differentially to sediment plumes generated by the extraction of seafloor massive sulphides (PMS) and ferromanganese nodules (NFS) and that this response is different for the two sediment concentrations tested (~10 and ~50 $mg.L^{-1}$). The main results of this study showed the high sensitivity of *D. meteor* to PMS since the survival of all corals nubbins was only four days of the experiment under both concentrations tested. On the contrary, coral fragments exposed to NFS treatments survived until the experiment's end (28 days). Polyp activity was lowest in both PMS treatments and was also reduced under NFS sediments at 50 $mg.L^{-1}$ compared with NFS sediments at 10 $mg.L^{-1}$ and control levels. Tissue necrosis and loss were evident in treatments with PMS after only two days' exposure and in NFS 50 after seventeen days of the experiment but not in NSF 10 and control treatments. Histological analysis did not reveal any detectable changes in tissue integrity or the presence of sediment particles. Respiration rates significantly increased under PMS exposure after two days and decreased in the NFS 50 treatment during the first week. Nonetheless, further analysis

of toxicological effects caused by metals associated with PMS particles, such as metal bioaccumulation on coral tissues and antioxidant stress biomarkers, are necessary to clarify the causes of coral *D. meteor* mortality in the PMS treatment. This research contributes to broadening our knowledge on the effects of deep-sea mining on benthic fauna. The information presented here can aid the creation of standards, guidelines, and monitoring programs of deep-sea mining activities by the International Seabed Authority Mining Code (ISA). It is desirable to extend this study to other cold-water coral species in inactive hydrothermal vents in the Azores and the CCZ region.

Keywords: Sedimentation; octocorals; *Dentomuricea* aff. *meteor*; Pacific Ocean; ferromanganese nodules; Atlantic Ocean; massive sulphide deposits.

Resumo

As atividades de mineração para extração de recursos minerais no mar profundo, como sulfetos maciços e nódulos de ferromanganês, podem causar impactos na fauna do mar profundo, gerando plumas de sedimentos que se dispersam por vastas áreas do oceano. Os organismos bentónicos suspensívoros, como os corais de águas frias, são provavelmente particularmente sensíveis. A exposição a sedimentos suspensos pode danificar mecanicamente os corais ao sufocar e obstruir os seus tecidos, podendo também conter substâncias tóxicas que afetam os processos fisiológicos dos corais. Este estudo elucida os impactos causados por plumas de sedimentos potencialmente geradas durante as atividades de mineração para a extração de nódulos na região Clarion-Clipperton Fracture Zone (CCZ), Oceano Pacífico equatorial Nordeste e sulfetos maciços de um campo hidrotermal na Crista Média Atlântica (MAR), região dos Açores, Atlântico Nordeste no octocoral *Dentomuricea* aff. *meteor* numa experiência realizada em aquários. Durante 28 dias, os corais foram expostos a duas concentrações (~10 e ~50 $mg.L^{-1}$) de plumas de sedimentos suspensos geradas num campo de nódulos, partículas de sulfetos polimetálicos hidrotermais e um tratamento controlo sem adição de sedimento. As concentrações de sedimentos foram selecionadas com base em modelos de dispersão de plumas de sedimento, como cenários de dispersão em curta e longa distantância. Os efeitos dos sedimentos foram avaliados na sobrevivência, condição do tecido, atividade dos pólipos e taxas de respiração dos corais. Os resultados do estudo confirmaram as hipóteses apresentadas nesta dissertação de que *D. meteor* responde diferencialmente às plumas de sedimento geradas pela extração de sulfetos maciços (PMS) e nódulos de ferromanganês (NFS) e que esta resposta é diferente para as duas concentrações de sedimento testadas (~ 10 e ~ 50 $mg.L^{-1}$). Os principais resultados deste estudo demostram elevada sensibilidade de *D. meteor* às partículas de sulfetos polimetálicos (PMS), uma vez que a sobrevivência de todos os fragmentos de corais foi de apenas quatro dias, nas duas concentrações testadas na experiência em aquário. Pelo contrário, os fragmentos de coral expostos a sedimentos de campos de nódulos (NFS) sobreviveram até o final da experiência (28 dias). A atividade dos pólipos dos corais foi menor em ambos os tratamentos com PMS e também foi reduzida sob sedimentos de NFS a 50 $mg.L^{-1}$ em comparação com sedimentos NFS a 10 $mg.L^{-1}$ e o tratamento controlo. A necrose e perda de tecido foram evidentes para os tratamentos PMS após apenas dois dias de exposição e em NFS 50 após dezassete dias da experiência, mas não nos tratamentos NSF 10 e controlo. A análise histológica não revelou alterações detectáveis na integridade do tecido ou a presença de partículas de sedimento. As taxas de respiração aumentaram significativamente sob a exposição a PMS após dois dias e diminuíram no tratamento com NFS 50 durante a primeira semana. No entanto, uma análise mais profunda dos efeitos toxicológicos causados por metais associados a partículas de PMS, como bioacumulação de metais nos tecidos dos corais e biomarcadores de stress oxidativo, são necessários para esclarecer as causas da mortalidade de coral *D. meteor* no tratamento de PMS. Este estudo contribui para alargar o conhecimento sobre os efeitos da mineração do mar profundo na fauna bentónica. As informações aqui apresentadas podem auxiliar na criação de normas, diretrizes e programas de monitorização de atividades de mineração no mar profundo pelo International Seabed Authority Mining Code (ISA). É desejável estender este estudo a outras espécies de corais de águas frias que habitam tanto em fontes hidrotermais inativas nos Açores como na região do CCZ.

Palavras-chave: Sedimentação; octocorais; *Dentomuricea* aff. *meteor*; Oceano Pacífico; nódulos de ferromanganês; Oceano Atlântico; depósitos maciços de sulfureto.

Acknowledgements

I am grateful to Dr Marina Carreiro Silva and Prof. Dr Manuela Juliano for acting as my supervisors and for all the effort in thesis preparation and revision.

I would also express my gratitude to António Godinho for all the knowledge and monitoring during the practical experience.

A particular word of thanks goes to Mariana Cruz and Dr Inês Martins for helping perform my experiments and Dr Ana Colaço for managing the MiningImpact2 project.

I would also like to thank Dr Miguel Caetano for providing the data on PMS particles metal concentration and Dr Benjamin Guillard (Jacobs University) for helpful discussions on sediment concentrations to use in the experiment based on plume dispersal scenarios.

Sincere thanks are also extended to Domitília Rosa for guidance while performing histology.

I want to express my deep gratitude to Miguel Matinho, that was always helpful to answer my questions.

Funding for this research was supported by the following: projects JPIOceans2/ MiningImpact2 (Mining2/0004/2017 and Mining2/0005/2017) funded by the Foundation for Science and Technology (FCT) and by the Direção-Geral de Politica do Mar (DGPM) and by H2020 iAtlantic (Grant Agreement No 818123). This research was also supported through a Fellowship under project JPIOceans2/MiningImpact2Mining2/0005/2017. This support is greatly acknowledged.

The MiningImpact cruise with RV Sonne is also acknowledged for the collection of sediments used in this dissertation. The cruise was financed by the German Ministry of Education and Research (BMBF) as a contribution to the European project JPI Oceans, MiningImpact (grant nos. 03F0812A-H).

I am also very thankful to the staff members of the Department of Oceanography and IMAR-Okeanos UAç for allowing me to use facilities and equipment.

I want to thank all my Master colleagues with whom I have shared many important moments. Their companionship was crucial in the process.

Some particular words of gratitude go to my friends, who have always been a significant source of support when things get a bit discouraging.

Most importantly, I would like to thank all my family, especially my mother, Cristina Carvalho, sister Catarina Marques, and my boyfriend Bruno Gomes, for their constant encouragement and guidance. I could not have made it without your love.

Finally, I would like to leave a special note of gratitude to the jury members present at the oral defence of my thesis for having dedicated their precious time to reading this manuscript and attending my oral presentation. I am truly grateful for your effort.

Contents

List of figures

List of tables

List of abbreviations

ATJ: Azores triple junction

CCZ: Clarion-Clipperton Fracture Zone

CeGUL: Center of Geology, University of Lisbon, Portugal

CIR: Central Indian Ridge

CWC: Cold-water corals

DW: Dry weight

EDAX: Energy Dispersive X-Ray Analysis

EPR: East Pacific Rise

EU: Eurasian plate

IMAR: Instituto do Mar (Horta, Portugal)

MAR: Mid Atlantic Ridge

NA: North American plate

NE: Northeast

NFS: Nodules fields sediments

NU: African/ Nubia plate

OKEANOS: Institute of Marine Research, DOP, University of the Azores (Portugal)

OMZ: Oxygen minimum zones

PE: Polystyrene

PMS: Polymetallic sulphide particles

PP: Polypropylene

REEs: Rare Earth Elements

ROV: Remotely Operated Vehicle

SE: Southeast

SMS: Seafloor massive sulphides

SR: Sedimentation rate

1. Introduction

The deep-sea includes all ocean waters below 200 m in depth and is characterized by low temperatures, high pressure and low to no light (Ramirez-Llodra et al., 2020). Previous studies have shown that the deep-sea harbours ecosystems of high diversity of species adapted to these extreme conditions, including hydrothermal vents, cold-water coral reefs and gardens and sponge aggregations.

Deep-sea ecosystems are threatened by human activities such as trawling, hydrocarbon exploitation (Ramirez-Llodra et al., 2011) and climate change (Levin and LeBris., 2015). Cold-water ecosystems can be vulnerable to these impacts, with deep-sea mining as an important threat expected to impact these ecosystems in the near future (Levin et al., 2016a; Miller et al., 2018; Van Dover., 2019).

1.1. Cold-water coral ecosystems

Cold-water corals (CWC) are marine invertebrates that belong to the Phylum Cnidaria and comprise colonial stony corals (Scleractinia), sea fans and soft corals (Octocorallia), black corals (Antipatharia) and stylasterid lace corals (Hydrozoa) (Cairns, 2007). They are commonly found in all oceans and can occur in a wide variety of different habitats including continental shelves, continental slope settings, seamounts, fjords, canyons, island slopes and along the flanks of oceanic banks (Roberts et al., 2006, 2009; Cordes et al., 2017) Most of the CWC species can be found between 200 m and 1000 m deep (Cordes et al., 2017). However, there are several coral taxa (mainly octocorals and black corals) that can be found at the base of continental slopes at depths shallower than 3000 m (Yesson et al., 2012). Cold-water coral ecosystems are recognized as important biodiversity hotspots in the deep sea, since corals provides essential habitats and niches for many species, whether for food, spawning or nursery, (Roberts et al., 2006; Buhl-Mortensen et al., 2010; D'Onghia et al., 2010; Carreiro-Silva et al., 2017; Rossi et al., 2017), including many species of important economic interest (Baillon et al., 2012). Cold-water corals have high longevity, slow growth rates, (e.g. Watling., 2011; Carreiro-Silva et al., 2013), long reproductive cycles, low rates of recruitment and high juvenile mortality in the early benthic stages, probably due the cold temperatures and limited food resources in the deep-sea (Lacharite and Metaxas, 2012). These factors and the expected environmental stability at the sea bottom imply that corals can be vulnerable to anthropogenic changes and have reduced ability to recover from disturbance events, such as trawling and oil and gas exploration (Ragnarsson et al., 2017). Therefore, CWC ecosystems may be one of the ecosystems to be highly affected by mining activities.

The Azores region is considered one of the areas in the NE Atlantic with high density of CWC assemblages which contain several species belonging to the scleractinian, antipatharian and octocoral taxa (Braga-Henriques et al., 2013; Tempera et al., 2013). Octocoral diversity in the Azores is the highest in the North Atlantic (Sampaio et al., 2012) and coral gardens formed by octocorals are the main type of CWC communities in the island slopes and seamounts (Tempera et al., 2013). Octocorals colonize inactive vent fields and nodule fields where there is interest in deep-sea mining exploration (Cairns, 2016; Amon et al., 2016; Van Dover, 2019).

1.2. Deep-sea mining

With land-based minerals sources becoming depleted of deposits that are abundant at the seabed and the consumption increase of these metals, it drew several countries' attention to mineral resources in the deep ocean seafloor (Hein et al., 2013), being the deep-sea mining defined as the process of retrieving mineral deposits from the sea-floor.

The exploration interests cover an ample range of resources and four ecosystems associated: seafloor massive sulphides (SMS) or polymetallic sulphides found at active and inactive hydrothermal vents, manganese nodules or polymetallic nodules found at abyssal plains, cobalt-rich ferromanganese crusts on seamounts, (Levin et al., 2016b; Cuvelier et al., 2018) and phosphorites nodules found along continental margins (Levin et al., 2016b).

The commercial exploitation of deep-sea mineral resources has not started yet. However, in the last 15 years, interest in exploration for and exploitation of these resources has greatly increased (Ramirez-Llodra., 2020).

1.2.1. Deep-sea mining techniques

Several evaluations of deep-sea mining technologies suggested different engineering approaches, however hydraulic dredging is considered the most effective system for commercial mining regarding to the larger economic recovery (Jones et al., 2017). In the near-future, will have seafloor crawlers for polymetallic sulphides mining and auxiliary rock cutters to grind mineral deposits plus collecting machines to recover the massive sulphide fragments. The resulting rock mixture will be pumped from the collecting machine into a container that rises to the ship transporting the mining material mixed with seawater, as slurry, to the surface. (Fig. 1a).

Regarding to polymetallic nodule mining (Fig. 1b), machines are suggested to plow a depth between 5 and 15 cm, depending on the thickness of the seabed soft top layer (Oebius et al., 2001; BMWi, 2015). The nodule collection system will separate the nodules from water and sediment due to gravity where sediments and very fine abraded nodule fragments mixed with water will pass through the diffuser-exhaust. This sediment discharge occurs 3 m above seabed behind the nodule collector, in which is expected a 500 mg L^{-1} plume concentration (BMWi, 2015).

A mining support vessel will be used as base for the associated mining activities where the resulting unused ore and the sediment and water separated during dewatering will be discharged and pumped back to the seafloor, (Gwyther, 2008; Lange et al., 2014). According to Gwyther, (2008) at the Solwara 1 project for the potential recovery of minerals in the SMS deposits at Bismarck Sea, a discharged sediments are expected to be release in depths between 25 to 50 m above the seabed. The discharge plume of the sediment in the return water according to Nautilus mining operations plan is expected to contain a concentration of suspended solids smaller than 8 µm in diameter of approximately 6000 $mg \cdot L^{-1}$.

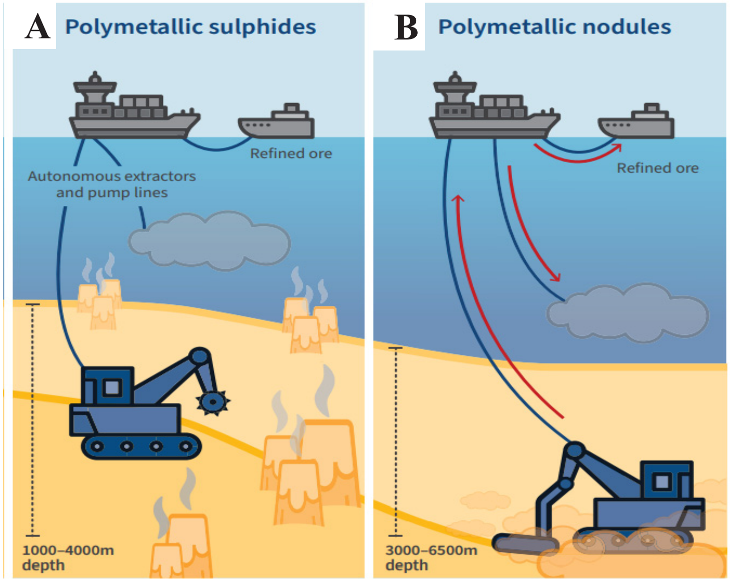

Figure 1: (A) Polymetallic sulphides mining scenario at hydrothermal vents; (B) Polymetallic nodules mining scenario on abyssal plains (Source: Haugan and Levin, 2020)

1.2.1. Polymetallic nodules in abyssal plains

Polymetallic nodules also called as ferromanganese nodules are mineral concretions (Petersen et al., 2016) characterized by discoidal and sub-spherical shapes with a diameter range of 3-12 cm (Kim et al., 2021) at most 15 cm, with grow average rates of 10-20 mm per million years (Gollner et al., 2017). Nodules are composed mainly by Manganese Mn(II) and Iron Fe(II) which, during the nodule growth, oxidation reactions occur into the respective oxyhydroxides (Wang and Müller, 2009). During this process, those dissolved metals present in the seawater are attracted to each other electrostatically (Petersen et al., 2016), where other metals are integrated by scavenge or ionic bonds interactions (Wang and Müller, 2009).

Although iron and manganese are the major metals in polymetallic nodules, the main economic interest metals are nickel, copper and cobalt. Other metals as manganese, titanium, lithium, REEs and molybdenum, among others, can also have industrial importance in several high-tech, emerging-tech, green-tech and other energy applications (Hein et al., 2013; Petersen et al., 2016).

Polymetallic nodules occur widely across the global ocean, over vast areas on the ocean floor abyssal plains at water depths around 3500–6500 m (Hein et al., 2013) where environment is very stable, with low bottom-current velocities (3.8 ± 2.0 cm s^{-1}) (Gollner et al., 2017) and sedimentation rates lower than 20 mm per thousand years (mm ky^{-1}) (Petersen et al., 2016).

Nodules occur on the sediment surface (up to ~50 cm in the sediment layer) and can be partially or fully buried in the sediment (Fig. 2) (Somayajulu, 2000; Hein et al., 2013) which provide a hard substratum habitat on ecosystems dominated by sediment (Gollner et al., 2017). The surface sediments around nodules consist predominantly of mixture of fine-grain carbonate clays, siliceous sediment oozes and red clays, at the first 60 cm of sediment column, and present low organic matter content and are rich in oxygen (Mewes et al., 2014; BMWi, 2015). The topmost sediment layer usually contains a 3 to 10 cm thickness of semiliquid (BMWi, 2015).

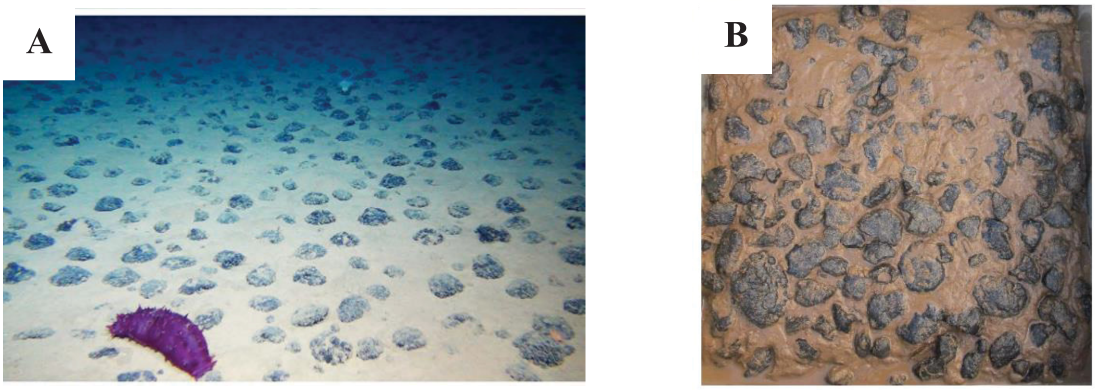

Figure 2: (A) Photography of nodule field ecosystem (IFREMER license area) (Source: Vanreusel et al., 2016; Copyright: ROV Kiel 6000 Team/ GEOMAR Kiel). (B) Photo of the sediment surface (0.25 m^2) with nodules (Source: BMWi, 2015).

In the Clarion-Clipperton Fracture Zone (CCZ) region, two types of nodules can be found: Type 1 present a size range of 9–12 cm and are more porous and fragile (Figs. 3a and c), while type 2 nodules have a size range of 3–6 cm and present a smoother surface with a dense internal structure (Figs. 3b and d) (Kim et al., 2021).

The most extensive occurrences of manganese nodules are known from the Pacific Ocean, especially in the Clarion-Clipperton Fracture Zone (CCZ), the Peru Basin and Penrhyn-Samoa Basins. Large nodules fields, although less explored, can be found in the Central Indian Ocean Basin and the in the Cook Island region. (Hein et al., 2013; Gollner et al., 2017). The CCZ are the most significant economic areas of interest. Presently there are 17 exploration contracts with 75,000 km^2 each according to the International Seabed Authority (ISA).

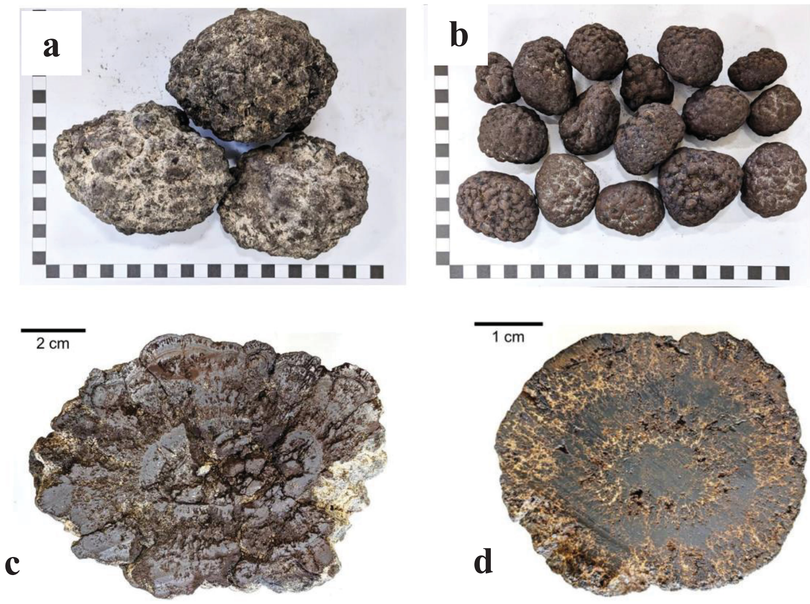

Figure 3: Samples of (a) Type 1 nodules and (b) Type 2 nodules and representative cross-section of (c) Type 1 nodules and (d) Type 2 nodules. (Source: Kim et al., 2021).

1.2.2. Polymetallic sulphides at hydrothermal vents

Polymetallic sulphides, also called as seafloor massive sulphides (SMS) (Fig. 4a) occur over ocean ridges on active and inactive hydrothermal vents, (Miller et al., 2018). Massive sulphides are formed by high temperature hydrothermal fluids emitted by active volcanic centres, where, during this process the cold seawater penetrates through cracks on the seabed and reach high depths bellow the seafloor surface, which is heated to temperatures above 400 °C. The chemical reactions between the seawater with metals and minerals that are leached from the surrounding rock, result in a mineral-rich hot fluid that is slightly acidic, reduced, and enriched in dissolved metals and sulphur. Due to the lower density, the fluid rises quickly to the seafloor, where is expelled into the overlying water column at “chimney-like” vents (Fig. 4b). A high proportion of the metals are expelled as “smoke” from the vent, forming a dispersing hydrothermal plume. The hot fluid interacts with cold seawater causing precipitation of the dissolved metals, where some metals precipitates as sulphides and sulphates producing black and white smoker chimneys and mounds of accumulated massive sulphide. (Petersen and Hein, 2013; Petersen et al., 2016). Black smoke vents contains suspended sulphide minerals and have temperature above 300 °C while white smoke contains sulphates and carbonates minerals and present temperature below 200 °C (Sharma., 2017).

The amount and composition of SMS differs according to the spreading ridges speed. At fast spreading ridges, lavas generally interrupt the flow of hydrothermal fluids, burying the sulphide deposits on a time scale of decades, such as in East Pacific Rise (EPR), while at slow spreading ridges, eruptions occur on intervals of thousands of years presenting large scale deposits, such as in Mid Atlantic Ridge (MAR) and Central Indian Ridge (CIR) (Boschen et al., 2013; Petersen and Hein, 2013; Gollner et al., 2017). Most SMS deposits occur in the mid-ocean ridges (65%), but also at back arc basins (22%) and along volcanic arcs (12%) (Hannington et al., 2011).

Hydrothermal chimneys and mounds have a high sulphide amount but are also rich in several minerals of economic interest, as iron sulphides (pyrite), chalcopyrite (copper sulphide) and sphalerite (zinc sulphide). Precious metals as gold, silver, barium and lead may also occur in large concentrations (Hein et al., 2013; Petersen and Hein, 2013; Petersen et al., 2016; Miller et al., 2018; Petersen et al., 2018;).

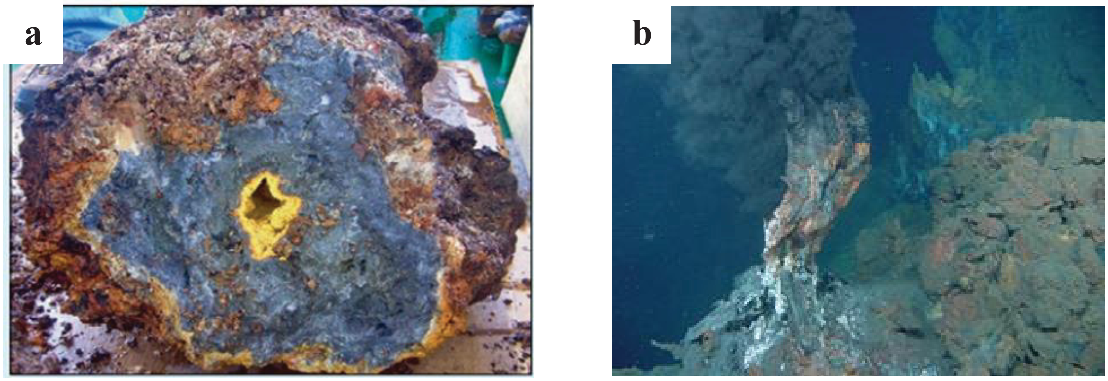

Figure 4: (a) Photo of SMS mineral resource (Source: Levin, Amon, et al., 2020; Copyright: James Hein, USGS; (b) Black smoker at the Lucky Strike hydrothermal vent field at 1600 m depth; (Copyright: MoMARETO2006 IFREMER)

1.3. Potential mining impacts on benthic communities

Mining for mineral extraction may be the largest-scale human activity to directly impact the deep-sea ecosystems (Glover and Smith., 2003), with the intensity of impacts expected to be higher at the local scale. Potential impacts of mining activities can cause environmental physical and chemical changes that result into a biological response. The main impacts on sessile benthic fauna are expected to be physical impacts such as habitat loss and degradation through removal of ferromanganese nodules and seafloor massive sulphides where several organisms are associated (Boschen et al., 2013; Van Dover, 2014; Gollner et al., 2017; Van Dover, 2019) and sediment plumes generated during mining activities (Boschen et al., 2013; Van Dover, 2014; Gollner et al., 2017; Miller et al., 2018). Other impacts include increased noise and light that may affect the behaviours of benthic fish and other mobile organisms, that may avoid or be attracted to the region for potential mining (reviewed by Christiansen et al., 2020).

1.3.1. Physical impacts caused by polymetallic nodules mining

The mining techniques aforementioned will probably impact most of the organisms at the area (Jones et al., 2017, 2020), which in the case of polymetallic nodules mining, the nodules provide the only hard substrate present at the abyssal seabed for the sessile biota such as xenophyophores, actiniarians, alcyonacean corals, antipatharian corals, sponges (Fig. 5) and several meiofaunal and microbial taxa that inhabits inside the crevices of sediment-filled nodules (Amon et al., 2016; Vanreusel et al., 2016). More than half of the megafauna found at the CCFZ region depend on the nodules as a hard substrate and the highest epifaunal densities associated with nodules are practically absent from nodule-free areas (Amon et al., 2016; Vanreusel et al., 2016). The removal of nodules will cause extinction of the nodule associated fauna, which differs from the biota of the surrounding sediment (Oebius et al., 2001; Thiel, 2003; Kaiser et al., 2017; Gillard et al., 2019). Species with small geographic distribution will be particularly vulnerable to extinction caused by nodule removal (Levin et al., 2016b). Ferromanganese nodules will require millions of years to grow, due to the low natural sedimentation rates and near-bottom current velocities, by which the changes of these structures will persist for a long period of time (Christiansen et al., 2020).

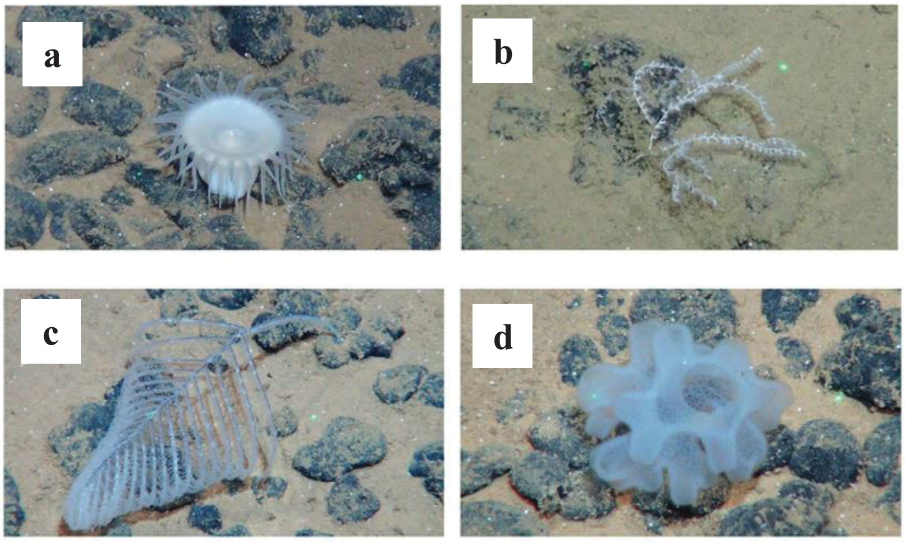

Figure 5: Fauna associated to nodules on the CCZ: (a) actiniarians; (b) alcyonacean corals; (c) antipatharian coral; (d) hexactinellid sponges. (Source: Vanreusel et al., 2016; Copyright: ROV Kiel 6000 Team/ GEOMAR Kiel).

1.3.2. Physical impacts caused by polymetallic sulphides mining

The main potential targets for seafloor massive sulphide (SMS) mining are inactive hydrothermal vents, since the proposed mining equipment can only endure temperatures lower than 35ºC (Gwyther, 2008). Inactive hydrothermal vents support a lower community's biomass than active hydrothermal vents, however, the SMS removal will cause complete destruction of the local fauna (Glover and Smith., 2003), since they are less used to environmental changes in water and sediment regimes than active hydrothermal vents (Gwyther, 2008). The loss of habitat quality, chemical modifications, changes of sediment and mineral composition, topography and fluid flux regimes for inactive hydrothermal vents may cause local populations and organisms losses and can result in a potential extinction of endemic and unique species (Fig. 6) (Van Dover, 2014; Gollner et al., 2017) such as, shrimps, urchins, hydroids, echiurans, barnacles, squat lobsters, cladorhizid sponges, scleractinian and bamboo corals, mussels, among others (Van Dover., 2019).

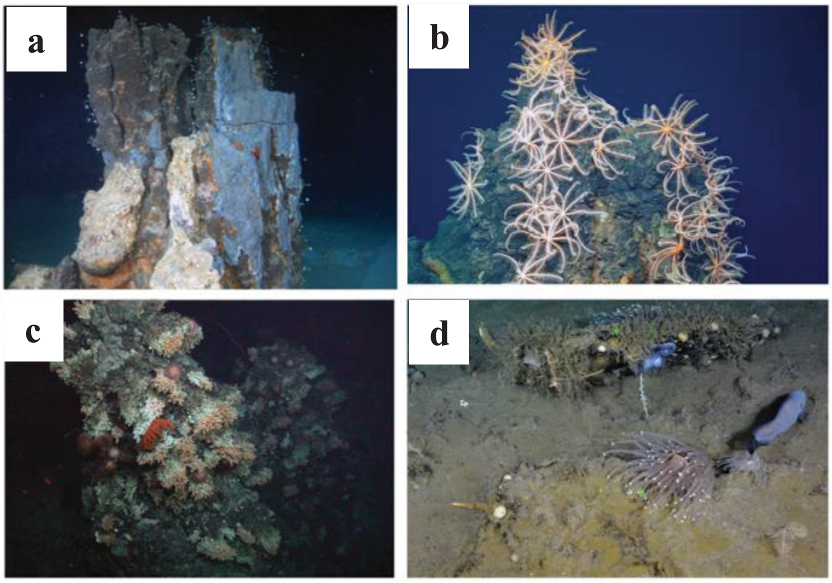

Figure 6: Fauna associated with inactive hydrothermal vents, as stated at Van Dover, 2019: (a) Manus Basin (SE Pacific; Collins et al., 2012) inactive sulphide chimney with "lollipop sponges" (Cladorhizidae), courtesy Nautilus Minerals Niuguini Ltd. (b) Pito Seamount (SE Pacific): inactive sulphide chimney with brisingid seastars, courtesy Mike Cheadle, Barbara John, University of Wyoming. (c) Kermadec Arc (modified from Boschen et al., 2016): inactive sulphide with corals and urchins assemblage, courtesy of Rachel Boschen-Rose (Seascape Consultants), Malcolm Clark (NIWA), Ashley Rowden, (NIWA) and Neptune Minerals Inc. (d) Middle Valley (Juan de Fuca Ridge): inactive sulphide chimney with invertebrate assemblage including stalked barnacles, brooding octopods, corals, anemones, sponges, tunicates, hydroids, fish, courtesy Verena Tunnicliffe, University of Victoria; (source: Van Dover, 2019).

1.3.3. Sediment plumes

The mechanical and hydraulic action of the mining equipment at the seafloor and the remaining rejected mining products (mixture of bottom water, sediment, benthic biota, and unrecovered nodule or massive sulphide fragments) that are released to the water column after the separation treatment at the support vessel, will create an operational suspended sediment plume and discharge plume of fine particulate material (Oebius et al., 2001; Gillard et al., 2019; Kim et al., 2021) that could extend beyond the mineral extraction area (Baker and Beaudoin., 2013). The suspended plume of particles will disperse with distance, which means that there will be a greater impact near the mining zone (Boschen et al., 2013). The intensity and direction of the suspended plume travel depends on the discharge volume, bottom currents speed as well the particle type and composition (Sharma et al., 2001; Miller et al., 2018), where according to

Gwyther, (2008) and Boschen et al., (2013), the plume release dispersal from a sulphide test-mining point at Solwara 1 suggest that the coarsest fraction of the discarded sediments will quickly settle on the slope below the disposal site (up to 1 km) and will form over 500 mm mounds, while, lighter sediments will reach up to 10 km away as plumes near the seabed, with deposit thickness of 0.1 mm. Previous studies at CCZ area, indicate that the distance for suspended plume sediments in the water column is predicted to vary between 2.5 Km under normal flow conditions to up 8 Km during an eddy passage through the mining area (cut-off value 10 $mg.L^{-1}$). (BMWi, 2015; Gillard et al., 2019).

The sediments disposal may occur at the sea bottom, once, discharge in the surface would likely increase nutrient levels and increase turbidity, preventing the input of light to the ocean, which could lead to the decay of an entire food chain (Burns, 1980; ISBA, 2010; Miller et al., 2018) and since the velocities of horizontal currents at surface are greater than bottom currents, in which the finest sediments can reach almost 200 km (Lopes et al., 2019).

The resuspension of unconsolidated sediments and the discharge of remaining rejected mining products will be deposited onto the seafloor covering the sediment-water interface and burying the benthos ecosystems to varying depths of sediments, (Glover and Smith, 2003; Aleynik et al., 2017). A previous study on the cold water coral *Lophelia pertusa,* showed low tolerance to buried sediment conditions in which coral mortality would rapidly increase in a short period of time (Brooke et al., 2009).

The benthic fauna besides being buried, at the near field by the sediment particles, may suffer from clogging of the respiratory surfaces causing smothering of the organism, hamper food to filter feeders or even through coverage and dilution of the already scarce food supply (Boschen et al., 2013; Aleynik et al., 2017; Gillard et al., 2019; Van Dover, 2019). Sediment plumes can affect the reproduction of the benthic fauna, including changes in larval dispersal, behaviour or mortality and cause failure in settlement of larvae or juvenile organisms, which could cause delays or prevent the faunal recolonization in ecosystems affected by sediment plumes (Gollner et al., 2017) and lead to ecosystem's collapse.

Removal of ores and disposal of rejected mined materials will probably release fine sediments and complex mixtures of potentially toxic elements to water column, such as heavy metals that are contained in the deep-sea deposits (Miller et al., 2018; Lopes et al., 2019). High concentrations of bioavailable metals could impact the ecosystem in the area and surrounding communities. At present, not much is known about the potential ecotoxicological consequences on mining of FeMn nodules compared to SMS in hydrothermal sources (Mestre et al., 2014), since leaching of heavy metals related with manganese and iron oxides is quite low. Nevertheless, Mn-rich particles can be a potentially source of toxic heavy metals under reducing conditions, for example if anoxic sediment is unearthed or if the discharged plumes are released into Oxygen Minimum Zones (OMZ) regions (Koschinsky et al., 2001; Christiansen et al., 2020). On the contrary, at SMS deposits, in hydrothermal vents zones, heavy metals and other toxic compounds are present in large quantities (Ramirez-Llodra et al., 2015).

Metals must occur in a bioavailable form to be toxic to the fauna that exist in the impact zone and bioavailability depends on the chemical properties of each metal, where usually free metal ions are more bioavailable, and seawater physical and chemical properties (for example: pH, alkalinity, hardness, salinity, amount of organic matter, specific ion levels and complexing agents) (Lydersen et al., 2002; Niyogi and Wood, 2004; Ramirez-Llodra et al., 2015). The toxic effects will likely vary with the species and sensitivity of the organism, such as the stage of development and hydrodynamic conditions (Ramirez-Llodra et al., 2015). Potential heavy metals toxic effects studied previously include: increased mortality, growth inhibition and lower reproductive rates (Hook and Fisher, 2001; Fuchida et al., 2017), and the higher trophic levels can be the most affected due to the bioaccumulation in the food chain and even extend the impacts to surrounding ecosystems through vertical and horizontal migrations (Christiansen et al., 2020).

Copper is one of the metals related to polymetallic sulphide particles (PMS) and may be released to the seawater during the sediment plume discharge. A study carried out with the vent mussel *Bathymodiolus azoricus* exposed for 96 h to several concentrations of Cu (300, 800 and 1600 $\mu g.L^{-1}$) concluded that the stress caused by the presence of Cu in the seawater can affect the organism by increasing antioxidant enzyme activities and tissue-specific transcriptional up-regulation and caused suppression of genes related to antioxidants and immune-related gene suppression at the highest concentrations (Martins et al., 2017). Similar experiments were made to calculate Cu toxicity (LC50) for the octocoral *Dentomuricea* aff. *meteor*, in which coral mortality was visible and based on the percentage of tissue surface that changed from natural yellow to black, which indicates tissue necrosis and death. Results indicated that Cu LC50 for 96 h for the octocoral *D. meteor* was 137 µg/L. (Martins et al., 2018). Other experimental study using the gorgonian coral *Subergorgia suberosa* regarding the response to water polluted with heavy metals through histopathological and scanning electron microscopy showed polyp necrosis, increased mucus secretion, tissue expansion and increased mortality (Chan et al., 2012).

The toxic effects of heavy metals on fauna that inhabit near SMS deposits in active hydrothermal vents may be smaller, since they are less sensitive to large amounts of metals present in the bottom water. However, these metals can cause chronic effects on fauna adapted to inactive vents (Gwyther, 2008; Boschen et al., 2013).

In addition to the concentration and potential toxicity of the sediment plume the size and shape of the particles can also be one of the factors that affect different organisms. The grain size and shape will depend on the ore type and surrounding sediment, where, particles shape and sphericity can be highly variable (Ramirez-Llodra et al., 2015). Pinheiro et al., (2021) used different grain sizes (63-125 µm, 125-250 µm and 250-500 µm) of suspended sediment to discover potential effects on the juvenile *Mytilus galloprovincialis*. The results concluded that the filtration rate significantly decreased in all grain size ranges but with more severe effect for smaller particles. Liefmann et al., (2018) compared the effect of rough edged mine tailings and smooth-edged spherical glass beads on two species of soft cold-water corals (*Duva florida* and *Primnoa resedaeformis*). The results suggested that for the same size range, sharper particles caused more damage than smooth edged particles for both species.

The effects of sedimentation from dredging and runoff have been studied for years in tropical corals. Main results demonstrate that the presence of sediment in the seawater is a stress factor by smothering tissues and increasing turbidity that affect the photosynthetic productivity in zooxanthellate corals (Rogers, 1990; Anthony, 1999; Philipp and Fabricius, 2003; Fabricius, 2005; Weber et al., 2006; Duckworth et al., 2017). Other impacts on corals to sediments exposure are decreased skeletal and tissue growth, damage or tissue loss and polyp mortality (Anthony, 1999; Larsson et al., 2013). Increase in mucus excretion and ciliary activity as a mechanism to remove sediment particles from coral tissues can provoke increase of energy expenditure and cause significant metabolic changes (Rogers, 1990; Larsson et al., 2013). Previous experiments of suspended sediments exposure (10 $mg.L^{-1}$) for 40 days, reduced O:N ratios (higher respiratory and excretion rates) on the gorgonian cold-water coral *Primnoa resedaeformis* (Scanes et al., 2018). Larsson et al., (2013), exposed the cold-water coral *Lophelia pertusa* to concentrations of 25 $mg.L^{-1}$ and 5 $mg.L^{-1}$ of suspended particles (<63 µm) for 12 weeks, and observed that skeletal growth and polyp extension were significantly lower under the higher concentrations of suspended sediments.

In addition, several studies show that sediment exposure can impair coral reproduction in different ways, and in tropical corals, sediment plumes mainly affect larval development and survival and the sediment deposition mainly affect larval settlement, metamorphosis and recruit survival, where previous experimental data suggest that the pre-settlement stages, gamete production, egg fertilization, larval development and survival are negatively affected (Gilmour, 1999; Fabricius, 2005). An experiment using the planulae of *Lophelia pertusa* exposed to 25 mg L^{-1} of fine fraction drill cuttings result into a significant mortality (67%) after 4 days of exposure (Larsson et al., 2013).

There are also some cases of positive responses to sedimentation, since cold-water corals are heterotrophic, suspended sediments can provide sources of organic matter and yield nutritional advantages, with reports of certain levels of exposure leading to increase of skeletal and tissue growth rates (Anthony., 1999; Anthony and Fabricius., 2000).

It is difficult to access potential ecological risks of deep-sea mining due to insufficient knowledge about the structure and vulnerability of its ecosystems (Washburn et al., 2019), even so, it is known that many of the recovery processes of these ecosystems are slow, due to the lack of availability, low quality and irregular distribution of food, low temperatures, slow biological rate processes and low abundance of species (Jones et al., 2017). Therefore, it is important to develop studies and quantify the variation in biological responses to mining activities. The present thesis focuses on the impacts of deep-sea mining on the octocoral *Dentomuricea* aff. *meteor*. This octocoral was selected as a model species because is an important element of the communities that inhabits the seamounts and island slopes in the Azores region between 150 to 600 m depth (Braga-Henriques., 2013) In addition, *D. meteor* has been used for several aquaria experiments in the deep-sea lab facilities, DeepSeaLab of IMAR/OKEANOS and for which we have physiological baseline information (Martins et al., 2018; Rakka et al., 2021).

Aim, objectives and hypotheses tested:

The aim of the present study was to evaluate the impacts caused by sediment plumes generated during mining activities for the extraction of nodule in the Clarion-Clipperton Fracture Zone (CCZ), and from a hydrothermal vent field in the Mid-Atlantic Ridge (MAR) region of the Azores (NE Atlantic), on the cold-water octocoral *Dentomuricea* aff. *meteor*. This was achieved by conducting an aquaria experiment, with a duration of 28 days, in which corals were exposed to two different concentrations (~10 and ~50 $mg.L^{-1}$) of simulated suspended plumes of sediments from nodule fields and hydrothermal vent fields and a control treatment with no sediment addition. Specific objectives of the study were to evaluate the effects of sediments on the survival, tissue condition, polyp activity and respiration rates of the octocoral *D. meteor*.

The hypothesis tested in the present dissertation were:

1 - The cold-water octocoral *Dentomuricea meteor* is affected by sediment plumes generated by deep-sea mining impact with respect to tissue condition, polyp activity and respiration

2 - *Dentomuricea meteor* responds differentially to the exposure of sediment plumes generated by the extraction of seafloor massive sulphides and ferromanganese nodules

3 - *Dentomuricea meteor* responds differently to different concentrations of sediments (~10 and ~50 $mg.L^{-1}$)

This thesis was part of a large experimental study carried out within the scope of the JPIOMiningImpact2 and iAtlantic projects. The study evaluates other coral responses at the level of the organism (nutrient excretion), tissues (C:N ratios, bioaccumulation of metals) and cell (enzymes involved in the oxidative stress and detoxification) not included in this dissertation.

2. Materials and methods

2.1. Study areas

2.1.1. Clarion-Clipperton fracture zone (CCZ)

The Clarion-Clipperton Fracture Zone (CCZ) located in the Northeast Pacific (NE Pacific) is expected to be the largest zone for potential deep-sea mining and even greater than land-mining, on the scale of several hundred square kilometres of seafloor each year per operation (Fig.7) (Ardron et al., 2019). The CCZ extend from the west coast of Mexico to Hawaii and covers an area of 5.2 million km^2 (Kersken et al., 2018) with a commercial interest area of 4.2 million km^2 (Petersen et al., 2016). Previous studies indicate that there are about 21,100 million dry metric tonnes of nodules in the CCZ regions (Petersen et al., 2016) containing the highest concentration of known nodule fields and would produce nearly 6000 million tonnes of manganese, which is more than the global manganese land-based reserves. These region contains 270 million tonnes of nickel and 44 million tonnes of cobalt. This is three to five times larger than the entire land-based reserves and 226 million tonnes of copper, and could yield 30% of the land-based reserves (Petersen et al., 2016).

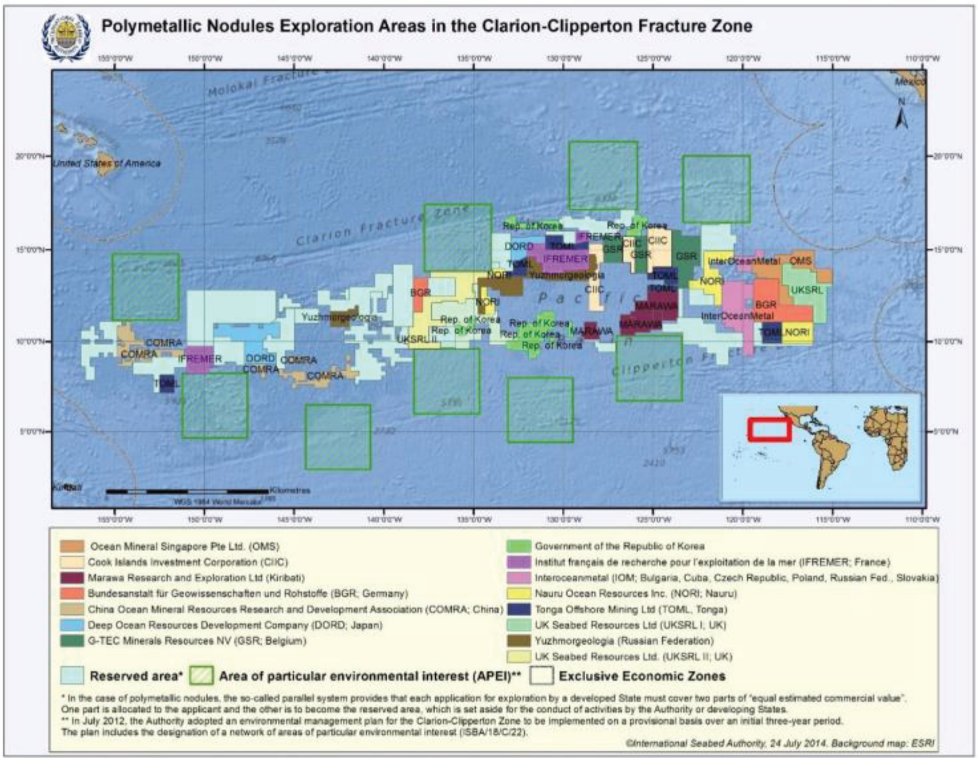

Figure 7: Polymetallic nodules exploration contract areas in the CCZ, NE Pacific Ocean (status July 2014). Source: International Seabed Authority (ISA); BMWi, 2015).

2.1.2. Azores archipelago

The Azores archipelago is located in the middle of the Northeast Atlantic (NE Atlantic). It is associated with the rifting along the triple junction of the Eurasian (EU), North American (NA) and African/ Nubia (NU) plates and its islands are spread along the Mid-Atlantic Ridge (MAR) (Fig. 8). The archipelago is characterized by narrow shelves, steep slopes and several seamounts scattering in the region (Morato et al., 2008). The Azores hosts hydrothermal vent fields (Portail et al., 2018) that are being considered for exploration of Seafloor Massive Sulphides (SMS) deposits also referred as polymetallic sulphides. The size of deposits at MAR are similar to those at the Southern Explorer Ridge, where ten of the largest sulphide deposits contain a total of 2.7-4.5 million tonnes of SMS deposits (Boschen et al., 2013).

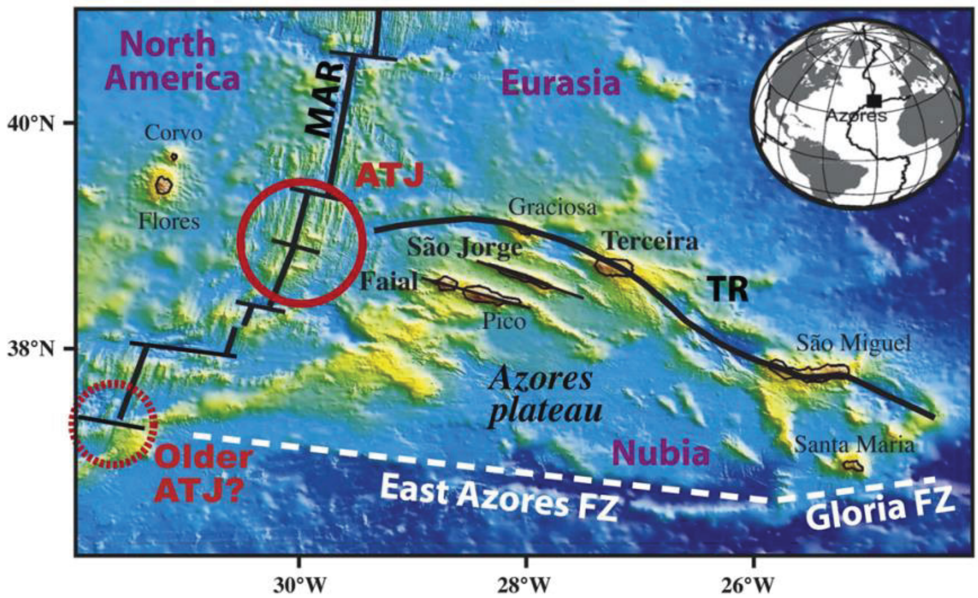

Figure 8: Azores archipelago location, near the triple junction (ATJ) between the Eurasian, North American and African (Nubia) plates; White line shows the East Azores Fracture Zone and the black lines show the Mid-Atlantic Ridge (MAR) and the Terceira Rift (TR); (Source: Hildenbrand et al., 2014; background bathymetric data from Lourenço et al., 1998).

2.2. Coral collection and maintenance

Dentomuricea aff. *meteor* colonies were collected on October 2020 from the summit of the Condor seamount, 3808’N, 2905’W, Azores Archipelago, at depths between 185-210m as by-catch from experimental long-line fisheries on R/V *Arquipelago* (CONDOR monitoring program, University of the Azores), (Fig. 9A). The colonies were transported to the DeepSeaLab facilities, IMAR/OKEANOS, in coolers and were acclimated to atmospheric pressure and maintained in two 170L aquaria supplied with seawater from a depth of 5 m from unpolluted EntreMontes bay (Cruz et al., 2010) in a flow through open system under darkness, (Fig. 9B). Seawater underwent an UV-light sterilization treatment (Vecton 600, TMC™) and was repeatedly filtered through polypropylene strainer bags (mesh size: 50 µm and 1 µm, TMC™).

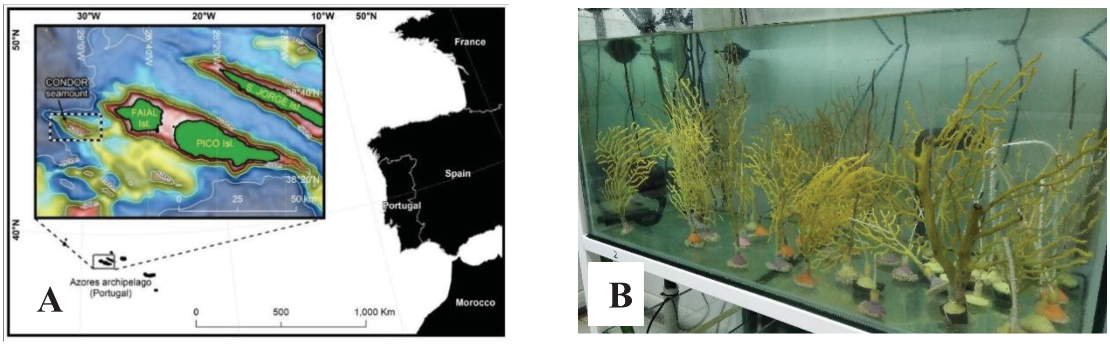

Figure 9: (A) Location of Condor Seamount in the Azores Archipelago (Portugal, NE Atlantic); (B) *Dentomuricea* aff. *meteor* acclimatization in the DeepSeaLab facilities.

The colonies were left to acclimatize for a period of 2 months under temperatures of 14.0 ± 1.0 º C, similar to the temperature recorded in their natural habitat, salinity of 36.0 ‰, and a pH of 8.08 - 8.12. The corals were fed twice a day, seven days a week, with a mixture of frozen adult *Artemia salina* and nauplii, mysids and microplakton (Ocean Nutrition™), and a food supplement composed of proteins, amino acids, lipids, vitamins, and oligoelements (Marine Active Supplement, Bentos Nutrition).

Six colonies were fragmented in December 2020 into 90 fragments containing 4-12cm in length and mounted into silicone tubing in bases made of inert epoxy putty. Coral fragments were placed into the experimental aquaria to an acclimatization that lasted 22 days.

2.3. Sediment preparation

Hydrothermal polymetallic sulphide particles (PMS) representative of mine sediment plumes produced during the extraction of massive polymetallic sulphide deposits were obtained by grinding an inactive sulphide chimney collected at 1750m of depth by the ROV VICTOR6000 on the Eiffel Tower structure at the Lucky Strike hydrothermal vent field during the research cruise MoMARSAT-2013 on the RV *Pourquoi Pas?*. Chimney fragments were grinded in a tungsten carbide ring mill pulveriser and subsequently were analysed by laser diffraction (Mastersizer 2000) at the Center of Geology, University of Lisbon - CeGUL, Portugal) for particle size analysis (MIDAS DL 5.3). The analysis revealed that 23% of the particles were less than 2 µm in size, 72% 2-63 µm (32% 2-8 um, 40% 8-63) and 5% > 63 µm. Energy Dispersive X-Ray Analysis (EDAX) revealed that PMS particles were mainly composed of barite ($BaSO_4$), pyrite (FeS_2), and chalcopyrite ($CuFeS_2$), with a minor composition of sphalerite (MIDAS DL 5.3). The PMS particle size matched the range expected by Seafloor Mining Tools excavation and by dewatering processes, according to the IHC Mining B.V. (80% 0.5-10 µm and 20% 10-70 µm). Sediment from ferromanganese nodules fields were obtained from abyssal depths of 4000 m using a multicorer in the Belgian license area for potential mining in the Clarion-Clipperton Fracture Zone (CCZ) during the research cruise Sonne 268 (JPIOceans2) in February 2019 (Linke and Haeckel., 2019). According to BMWi, (2015) reporting sediment size analysis, sediments were composed predominantly of fine-grain carbonate clays, siliceous sediment oozes and red clays and contained 20-38% clay (< 2 µm), 48-79% silt (2-63 µm) and 5-13% sand (> 63 µm). Three samples of each sediment type were burned 5h at 450 ºC to determine their organic matter content, in which was obtained an average of 4.7% for the hydrothermal vent sediment and 3.0% for the ferromanganese nodules field sediment.

2.4. Experimental design and set-up

The experiment was undertaken between 13 January and 10 February 2021, in which coral fragments were exposed for 28 days to four treatments of suspended sediments and one control with no sediment exposure. Sediments were added in 12h daily cycles (~11:30h – 23:30h), except 1 day per week when respiration measurements were performed. The sediment treatments consisted in two different types of sediment: (1) pollymetallic sulphide particles (PMS) from a hydrothermal vent and (2) sediments from ferromanganese nodules fields (NFS). Two concentrations were used for each type of sediment: 10 mg DW L^{-1} and 50 mg DW L^{-1} of suspended plumes of sediments. Sediment concentrations were selected based on plume dispersal models, as close field and far field (hundreds of meters for 50 mg DW L^{-1}) and far field (kilometres for 10 mg DW L^{-1}) plume dispersal scenarios performed within the MIDAS and JPIOceans projects (Guillard et al., 2019 for NFS and Morato et al unpublished data for PMS).

The experiment used three 13L flume aquaria per sediment treatment and six for control treatment, containing five coral nubbins per aquarium at the beginning of the experiment, (Fig.10). Flumes were designed to keep a laminar current flow (Rakka et al., 2021), with coral fragments placed in small platform 10 cm above the bottom of the aquarium to ensure a maximal exposure of the corals to the suspended sediment (Fig. 10). Corals were fed rotifers (*Brachionus* sp., Ocean Nutrition^{TM}) twice a day, seven days a week, 2 hours before the entry of sediment start and 6 hours after (time when sediment concentration in the aquarium reaches its target concentration and stabilizes). Rotifers were thawed in seawater and inserted into aquaria with a concentration of 10.7 mg/L per fragment that matched the carbon requirement needs of *D. meteor* based on previous feeding experiments (Rakka et al., 2021).

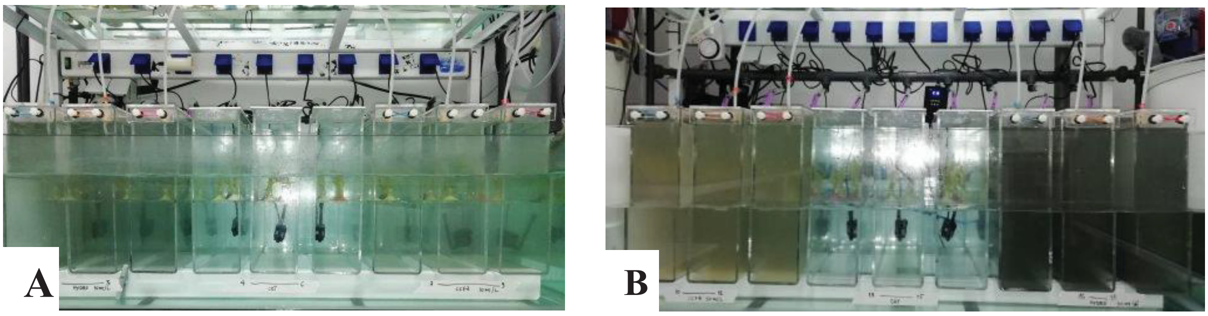

Figure 10: (A)Aquaria with *Dentomuricea aff. meteor* exposed to 10 mg L^{-1} treatments and one control treatment; (B) Aquaria with *Dentomuricea aff. meteor* exposed to 50 mg L^{-1} treatments and one control treatment.

Suspended sediment during the exposure period was manipulated using the model of Anthony (1999). For the model, sediment tests were made before the experiment started to measure the sedimentation velocities and rates to achieve the necessary adjustments in the amount of sediment input into aquaria. Sedimentation rates (SR) were calculated using the formulas provided by Anthony, (1999), (Table 1), in which three 13L aquariums including 200 $L.h^{-1}$ recirculation pumps (HL-BT200, HAILEA) were used for each treatment. Aquariums were filled with pre-filtered seawater (0,45µm) and 200ml samples were removed every 20 minutes during 1h30 min for 50 mg L^{-1} treatments while for 10 mg L^{-1} treatments, sampling were made every 10 min for 1h. Samples were filtered through 45µm GF/F pre-weighed filters, where were washed with 15mΩ water to remove salt, dried at 60ºC and weighed 48 hours later. These trials were made without new seawater input. After calculating the SR, several tests were performed to ensure there were not errors caused by the experimental set-up.

Four reservoir 114L tanks were used to prepare a homogeneous solution of each sediment concentration and filtered seawater. The tanks were built with an output at the base through which the sediment solution was continuously homogenized, circling from the bottom to the top of the tank with an external 5000 $L.h^{-1}$ pump (DC Runner 5.2, AquaMedic). Sediment "stock" solutions of each sediment type and concentration were prepared daily in 114L reservoir tanks to achieve the desired suspended sediment concentration in each aquaria (Table 1). Sediments were weighed and homogenized with filtered seawater for 30 min before being included into the reservoir tanks, to avoid particles aggregates, (Fig. 11A). After being included into the tanks, sediments were homogenized for 10 min before pumping to the aquaria, (Figs. 11B e 11C).

Table 1: Sedimentation rates calculated using the formulas provided by Anthony, (1999) and amount of sediment added to the reservoir tank for the treatments with sediments from nodule fields (NFS) and massive sulphide particles (PMS) at concentrations of 10 and 50 $mg.L^{-1}$

	NFS		**PMS**	
	10 $mg.L^{-1}$	**50 $mg.L^{-1}$**	**10 $mg.L^{-1}$**	**50 $mg.L^{-1}$**
SR ($L.min^{-1}$)	0,055	0,055	0,163	0,163
Tank Sed. (g DW)	4,0	20,4	9,4	48,9

SR = Sedimentation rate ($L.min^{-1}$) and Tank Sed. (g DW) = Dry sediment weight add to the reservoir tanks

Figure 11: (A) Homogenization of sediments before being included in the reservoir tanks (B) homogenization of the 50 mg L^{-1} NFS sediment in the reservoir tank (C) homogenization of the 50 mg L^{-1} PMS sediment in the reservoir tank.

The sediments stocks were continuously pumped using three 0,045 $L.min^{-1}$ dosing pumps (Iwaki Metering Pump, EWN – B21VCER) from reservoir tanks and were delivered to three aquaria for each treatment through silicone tubes attached to the aquarium lid. Each sediment concentration was added through short pulses from the sediment stocks reservoirs at preprogrammed time intervals. (Figs.12 e 13). Recirculation pumps 200 $L.h^{-1}$ (HL-BT200, HAILEA) were used to guarantee the water circulation and the sediment resuspension inside each aquaria.

Figure 12: (A) reservoir 114L tank used to prepared sediment “stock”; (B) Dosing pumps that delivered sediments from the reservoir tank to each aquaria; (C) silicone tubes attached to the aquarium lids.

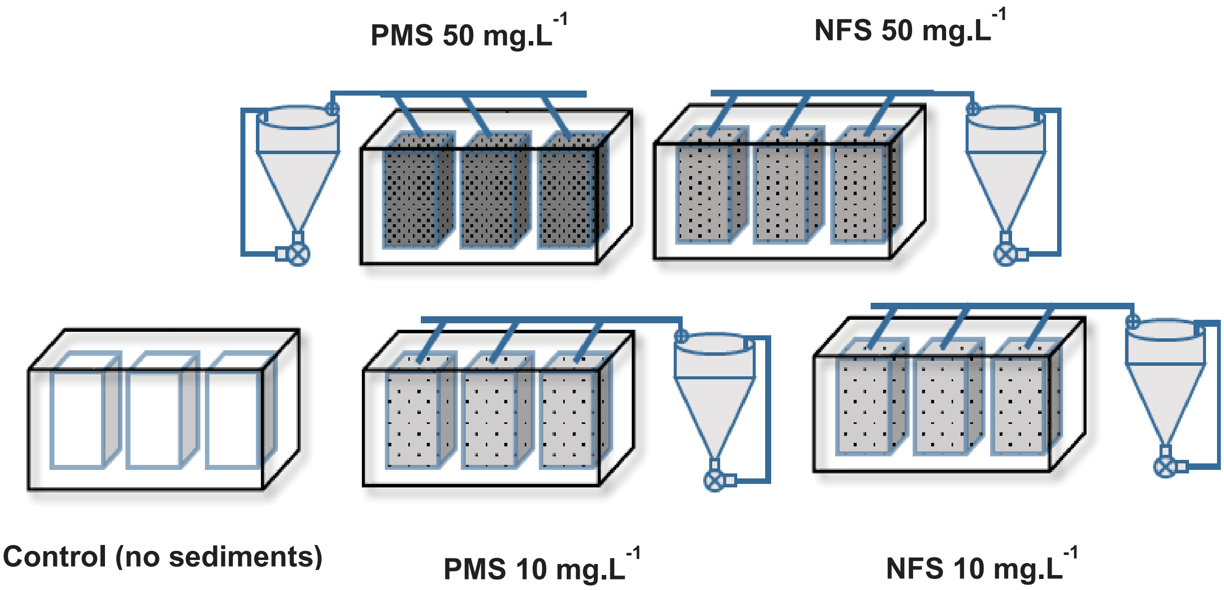

Figure 13: Experimental design used in the aquaria experiment. "PMS" indicates the polymetallic sulphide particles treatments and "NFS" indicates the nodule field sediments treatments, at concentrations of 10 and 50 mg. L^{-1}. Sediment "stock" solutions of each sediment type and concentration were prepared in 114L reservoir tanks and added to the aquaria using dosing pumps.

To achieve the required sediment concentration in the aquariums a balance between the input of seawater and sediment was needed. The flow rates of new seawater and sediment input were previously calculated using the formulas provided in Anthony, (1999). Both flow rates were measured to values of 0,045 L. min^{-1} and adjusted once a week. Excess of sediment was daily removed by siphoning each aquarium before the new cycle started.

Seawater physical-chemical parameters were measured daily in each aquarium after siphoning. Seawater salinity was measured with a S30 SevenEasy™ conductivity meter, pH and temperature with a S8 Seven2Go™ pH/Ion meter (MettlerToledo) and oxygen with a Fibox4 (PreSens) with a Oxygen Dipping Probe DP-PSt3 (Table 2). Seawater samples (12mL) were also collected (at T5, T16 and T27 days) and filtered at 0.45 µm filter (Cytiva Whatman™ GD/X Glass Micro Fiber (GMF) Syringe Filters) for inorganic nutrient analysis. The samples were frozen at -19ºC in polystyrene (PE) sampling bottles until analysis. Nutrient concentrations were determined at MARINNOVA - Marine and Environmental Innovation, Technology and Services using a colorimetric Autoanalyzer Sunplus with Segmented Flow, applying the methodologies Skalar: M461-318 (EPA 353.2), M155-008R (EPA 350.1) and M503-555R (Standard Method 450-P I). Potential metal (Mn, Cu, Ni, Cr, Cd) release in the seawater from PMS particle weathering was monitored by means of Diffusive Gradients in Thin-films for metals (DGT, DGT Research Ltd, UK), changed weekly in all aquaria and weekly collection of seawater samples (250 ml). Metal concentrations are under analysis by colleagues at the Instituto Português do Mar e a Atmosfera (IPMA) using a quadropole inductively coupled plasma mass spectrometer (Thermo Elemental, X-Series).

2.5. Monitoring of sediment levels in aquaria

During the experiment, seawater was filtered from each aquarium to control the sediment concentrations in the different treatments. Sampling was made twice a week, 6 hours after of the cycle began, except for the first week where three filtrations were performed. Samples of 1L of water were filtered using a conical filter vacuum flask and pre-weighed and pre-burned (5 hours at 450ºC) 45μm GF/F filters. The filters were washed with MilliQ water (15mΩ) and dried at 60ºC during 48 hours before being weighed again.

2.6. Monitoring of respiration and excretion rates

Dentomuricea aff. *meteor* respiration and inorganic nutrients excretion rates were measured once a week by closed-cell incubation in 250ml glass chambers with silicone stoppers with a ~10mm hole in the centre equipped with a "Planar Oxygen-Sensitive Spot" (SP-PSt3-NAU-D5-YOP). An O_2 reactive polymer was read through the glass by laser pulses using a fiber optic cable (POF-L2.5-2SMA) connected to a single channel oxygen meter Fibox4 (PreSens). Chambers were placed on top of a magnetic stirring plate in water bath maintained with temperatures of 14 ± 0.1 °C using a Profilux 4 (GHL), with digital thermometer adjusted to the tenth of a degree Cº (hysteresis of 0.1 Cº), and the water was continuously stirred using glass-coated magnetic stirrers (Fig. 14). Two coral fragments per aquarium, including the two control aquaria, were chosen to be incubated and were placed into the chamber with 0.45 µm pre-filtered seawater from the respective treatment. Dissolved O2 was measured every 30 minutes using the single channel oxygen meter Fibox4 and the Fiber Optic Cable that read the PSt3 sensors. The incubation time was 6 hours and 30 min and oxygen saturation inside the chambers never dropped below 80%. Respiration rates were calculated by the decrease of dissolved O_2 and adjustments were made using rates recorded in chambers without corals to account for microbial respiration.

Water samples (12mL) for analysis of nutrient concentration released by the coral nubbins were taken using syringes from the chambers at the beginning and the end of the incubation and were filtered using a 0.45 µm filter (Cytiva Whatman™ GD/X Glass Micro Fiber (GMF) Syringe Filters). The samples were frozen at -19ºC in polystyrene (PE) sampling bottles until analysis. Coral excretion of inorganic nutrients is not presented in this thesis due to sample contamination problems.

Initial photographs of all corals nubbins were taken 5-7 days before the experiment started with a 1 cm size scale for the purpose of calculating the surface area of coral tissue for each coral fragment. Photographs of coral fragments were analysed in the ImageJ software (Rasband, 2018) to take length and width measurements. The surface area of the coral fragments was quantified using the mathematical formula of the surface area of a cylinder based on Naumann et al (2011). Both respiration and excretion rates were standardized to the coral nubbin tissue surface area.

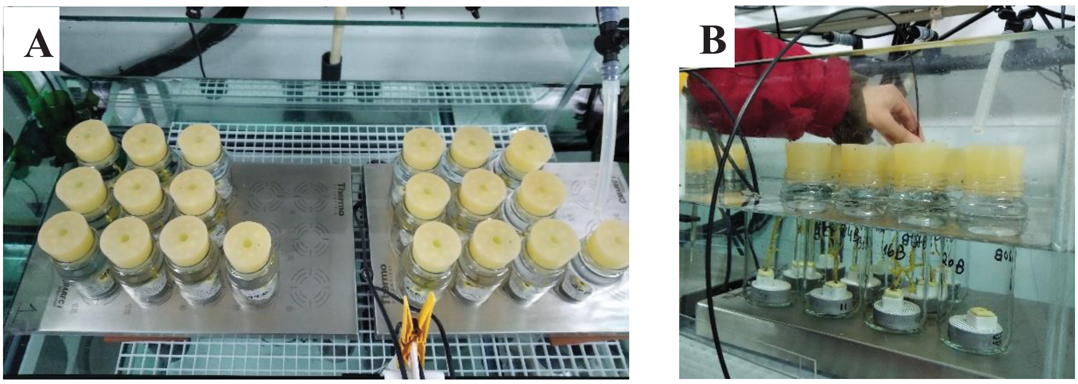

Figure 14: (A) Incubation system with magnetic stirring plate in water bath maintained with temperatures of 14 ± 0.1 °C and 250ml glass chambers with rubber lids where the oxygen PSt3 sensor are read with the oxygen meter Fibox4; (B) O_2 measurement with the oxygen meter Fibox4.

2.7. Polyp activity and tissue condition

Polyp' activity was observed and photographed twice a day, 1 hour before started the sediments entry into the aquarium and 6 hours after the 12h cycle started. To stimulate the polyps to open and assess the capacity to feed, the corals were fed with rotifers 1 hour before observations. The observation was made to all coral fragments although occasionally was difficult to count at the highest concentrations. Polyp activity was divided in three categories: (1) open, (2) partially open and (3) closed. The category of open polyps was assigned to fragments that had more than 80% of the polyps fully open (Fig. 15A), while the category of partially open polyps was assigned when the fragments had from one open polyp to 80% of the open polyps (Fig. 15B), and the category of closed polyps was attributed to the fragments that had all polyps closed (Fig. 15C).

After each incubation (once a week), each incubated fragment was photographed using a 2 cm size and color scale to quantify the coral tissue surface area lost over the experimental time (Fig. 15D). ImageJ software was used to analyse the photographs of coral fragments in order to take length measurements. The length of coral branch without tissue or polyps tissue length obtained after measured each week was subtracted from the initial length of the coral nubbins and the results were presented in percentage tissue loss.

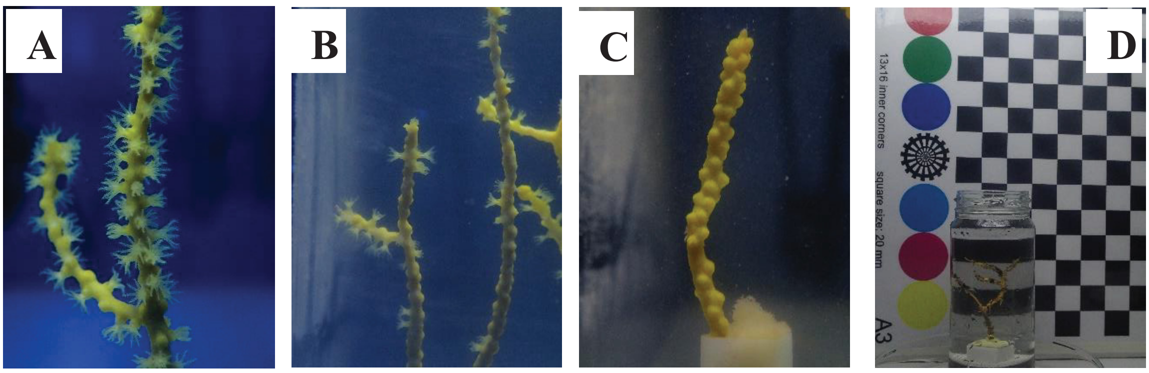

Figure 15: (A) Open polyps' category, fragment under Control treatment (28th day of the experience); (B) partially open polyps' category, fragment under NFS 50 treatment (28th day of the experience); (C) closed polyps' category, fragment under NFS 10 treatment (28th day of the experience); (D) 2 cm size and color scale to quantify the coral tissue surface area lost over the experimental time.

2.8. Histological analysis

Coral fragments were placed in polypropylene (PP) sampling bottles with 10% seawater formalin for further histological analysis, following the histological procedure described in Rakka et al (2021). Four polyps were removed from the skeletal axis of two coral fragments for each treatment. Polyps were placed in eppendorfs and rinsed repeatedly with distilled water until the next day. Posteriorly, polyps were decalcified in a solution of 10% formic acid for a period of 25-30 min. Then, dehydration and cleaning under vacuum using a conical filter vacuum flask were carried out by subsequent immersions in ethanol solutions (70%, 80% for 30 min, 90%, 95% for 15 min and 100% for 20 min x 3 times) and xylol for 20 min, followed by infiltration in paraffin (Merck Histosec) at 58 ◦C for 1 h. Lastly, polyps were embedded in paraffin blocks which were sectioned into serial 7 μm transversal and longitudinal sections using a Leica 2035 microtome. The slides were stained using a standard Hematoxylin-Eosin protocol and mounted with coverslips and DPX. Histological slides were photographed with a Leica DFC420 camera attached to a LEICA DM6000 microscope (x10).

2.9. Statistical analyses

All data are reported as mean ± standard deviation (SD). Data analysis was performed with PERMANOVA (unrestricted permutation of raw data) (Anderson et al., 2008) using the software PRIMER 6 & PERMANOVA. This statistical analysis is a powerful non-parametric approach that uses a permutational technique to enable significance tests for small sample sizes to be conducted (Walters and Coen, 2006). The analyses used a resemblance matrix based on Euclidean distance and PERMANOVA was run using 9999 permutations to produce p-values using the Monte Carlo (MC) method.

Seawater properties (temperature, pH, salinity, oxygen, nitrate, ammonia, phosphates) were analyzed with a PERMANOVA, where treatments and time were treated as fixed effects.

Suspended sediment concentrations measured during the experiment were analyzed with regression analysis for each treatment over the time at SPSS software (Statistical Package for the Social Sciences).

The effects of sediment exposure on coral oxygen consumption (respiration rates) were firstly checked graphically for linearity. Subsequently a linear regression technique was used to estimate respiration rates with regression analysis for each coral fragment using the SPSS software, were oxygen decrease over the six and half hours of incubation (μmol L^{-1} min^{-1}) is given by the slope of the straight line. The first 30 min of the incubation were considered as the time required for coral to acclimate to the incubation chambers and were thus excluded from the analysis. Subsequently, the values of the oxygen consumption were normalized with the respective chambers volume and coral nubbins tissue surface area. The effects of sediment exposure on oxygen consumption rates were analyzed with a PERMANOVA, where treatments and time were treated as fixed effects.

The effects of sediment exposure on percentage polyp activity and tissue loss were log (x+1) transformed and analyzed with a PERMANOVA where treatments and time were treated as fixed effects, whereas, for tissue loss analysis the corals nubbin within treatments were treated as random effects.

3. Results

3.1. Experimental conditions

The physical-chemical properties of seawater in the experimental treatments over the 28-day period are presented in Table 2. Seawater properties varied slightly between experimental treatments and time (supplementary material 1) but remained within the levels experienced by corals in their natural habitat. Average salinities were 35.8 ± 0.4‰, pH of 8.13 ± 0.02, temperatures of 14.1 ± 0.3 ºC and oxygen concentrations of 253,7 ± 2,8 mol. L^{-1} during the experimental time (Table 2).

Table 2: Seawater physical-chemical properties in experimental aquaria during the 28 days for the treatments with sediments from nodule fields (NFS) and massive sulphide particles (PMS) at concentrations of 10 and 50 mg L^{-1} and a control treatment with no sediment addition. Data are values average ± standard deviation.

Treatment/ Aquarium monitoring	**Control**	**NFS**		**PMS**	
		10 mg.L^{-1}	**50 mg.L^{-1}**	**10 mg.L^{-1}**	**50 mg.L^{-1}**
Salinity (ppt)	35,9 ± 0,4	35,9 ± 0,4	35,8 ± 0,3	35,2 ± 0,0	35,2 ± 0,0
T º C	14,0 ± 0,3	14,0 ± 0,2	14,2 ± 0,1	13,8 ± 0,2	14,5 ± 0,2
pH	8,14 ± 0,01	8,14 ± 0,01	8,14 ± 0,02	8,12 ± 0,01	8,10 ± 0,02
O2 (µmol.L^{-1})	254,0 ± 2,8	253,8 ± 3,0	253,4 ± 2,6	253,2 ± 2,1	251,1 ± 1,8
NO_3^- (µmol.L^{-1})	2,9 ± 1,8	1,6 ± 1,6	1,6 ± 1,3	3,6 ± 0,7	3,3 ± 0,1
NH_3+NH_4^+ (µmol.L^{-1})	9,0 ± 2,2	8,8 ± 0,9	8,7 ± 0,4	9,9 ± 0,6	15,5 ± 0,1
PO_4^{3-} (µmol.L^{-1})	0,8 ± 0,2	0,9 ± 0,1	1,2 ± 0,3	0,4 ± 0,1	0,4 ± 0,1

NO_3^- = Nitrates; NH_3+NH_4^+ = Ammonia; PO_4^{3-} =Phosphates

Sediment concentrations over the experimental period, in average, were 9,07 ± 1,69 mg.L^{-1} and 45,26 ± 4,82 mg.L^{-1} for the treatments NFS 10 mg.L^{-1} and NFS 50 mg.L^{-1} respectively and 8,25 ± 1,41 mg.L^{-1} and 35,57 ± 4,43 mg.L^{-1} for PMS 10 mg.L^{-1} and PMS 50 mg.L^{-1} treatments respectively and 6,56 ± 2,33 mg.L^{-1} for the control treatment. The variation of sediment concentrations over time for each treatment is shown in Figure 16. Suspended sediment concentrations varied slightly with time, but average concentrations were in reasonable agreement with target levels (supplementary material 2).

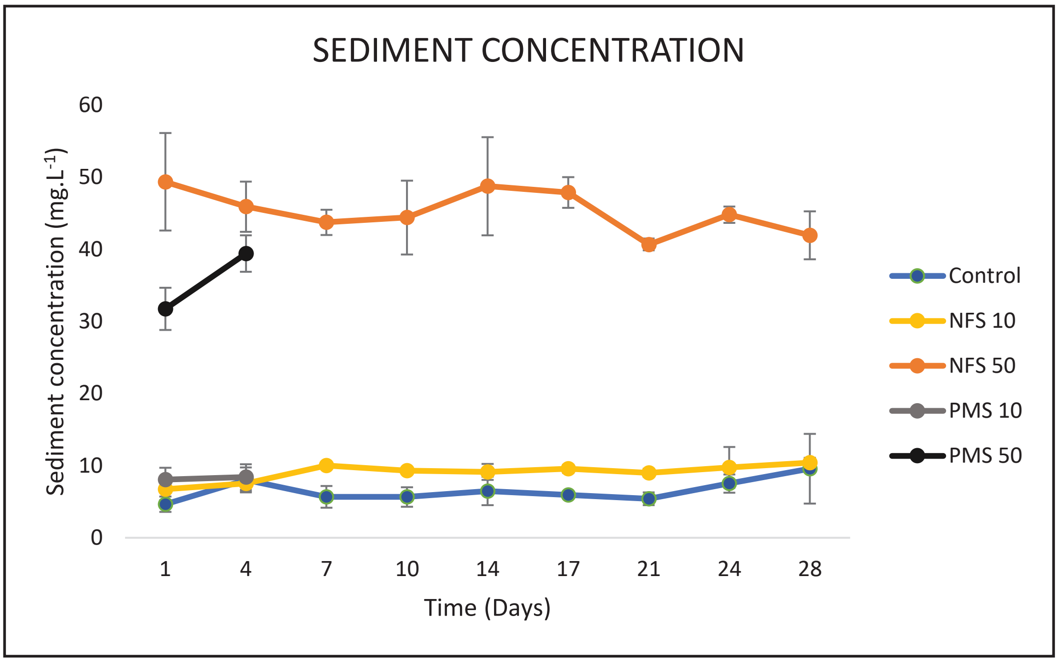

Figure 16: Sediment concentrations measured in the experimental aquaria during the 28 days for the treatments with sediments from nodule fields (NFS) and massive sulphide particles (PMS) at concentrations of 10 and 50 mg.L^{-1} and a control treatment with no sediment additions. Data are values average ± standard deviation.

3.2. General observations and survival

Coral exposure to suspended sediment treatments caused a visible deposition of sediment particles on the coral surface tissue (coenenchyme) and polyps, at both concentrations of PMS treatments. Sediment particles in the fragments exposed to PMS treatment were apparent from the first day of the experiment, and rapidly increased over the next three days of the experiment particularly in the high PMS treatment (50 mg. L^{-1}) (Fig. 17). Regarding NFS treatments, the fragments exposed to the higher concentration (50 mg. L^{-1}) showed greater sediment deposition in tissues and polyps than the corals exposed to low concentration (10 mg.L^{-1}) (Fig. 17). However, there was some variation in the amount of sediment covering the coral fragments, where some fragments were more covered than others within the same experimental aquaria.

Coral fragments died after 4 days of exposure to treatments with PMS but fully survived until the end of the experimental period (28 days) in treatments with NFS.

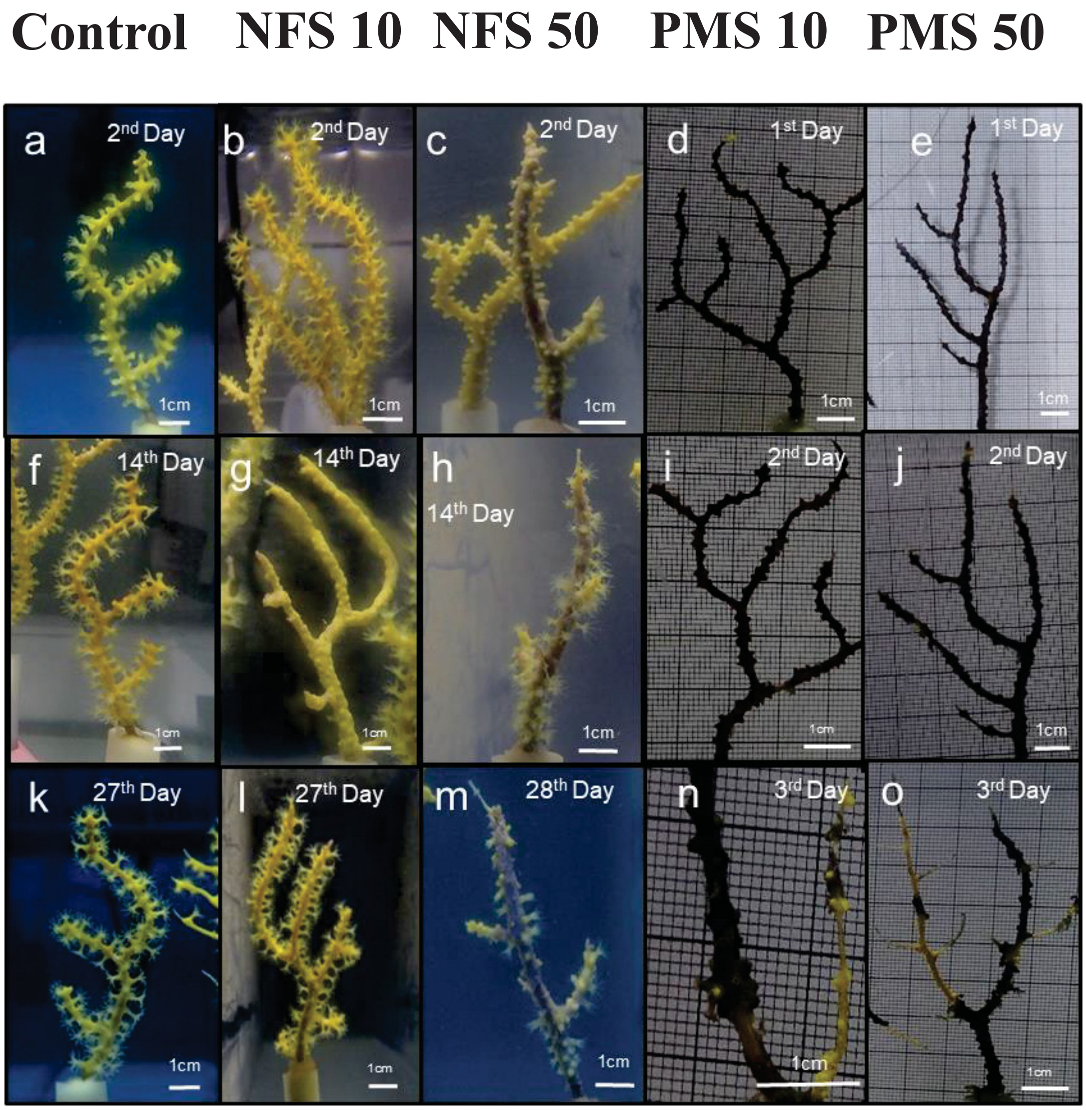

Figure 17: Images of coral fragments demonstrating the variation in the amount of accumulated sediment and the progression of tissue (coenenchyme) loss and necrosis in the different experimental treatments through time. "NFS" indicates the nodule field sediment treatments and "PMS" indicates the polymetallic sulphide particles treatments at concentrations of 10 and 50 $mg.L^{-1}$.

3.3. Tissue condition

Tissue condition of *D. meteor* also changed through time in the different treatments (Fig. 17). At both concentrations of PMS treatments, tissue necrosis and loss of polyps was evident after two days of exposure, although in many fragments exposed to the PMS 50 treatment the sediment particles on the coral surface tissue formed a compact "crust" that made it hard to evaluate tissue damage. In contrast, under the PMS 10 treatment, darkening and loss of tissue and polyps was more gradual (Fig. 17).

Corals exposed to the NFS treatment showed sediment deposition on tissues, particularly under the NFS 50 treatment, although polyps were often open and free from particles. Tissue necrosis and loss of polyps at the tips of the branches was evident for a few coral fragments exposed to the NFS 50 after 17 days of the exposure but not in NSF 10 and control treatments (Fig. 17).

Close examination of tissues and polyps under the dissecting microscope (Leica XX microscope and photographs taken with the LAS V4. 12 software) showed that polymetallic sulfide particles adhered to coral tissues and polyps, with clogging of polyps under PMS 50 treatment after only 2 days of exposure (Fig. 18c-d). Nodule field sediment particles were more difficult to observe on coral tissues due to the color similarity of particles and coral tissue. Nevertheless, close observation showed the sloughing of tissue by the end of the experiment, particularly in the NFS 50 treatment (Fig. 18h). Displacement of sclerites was also observed in some of the corals exposed to PMS 10, PMS 50 and NFS 10 treatments.

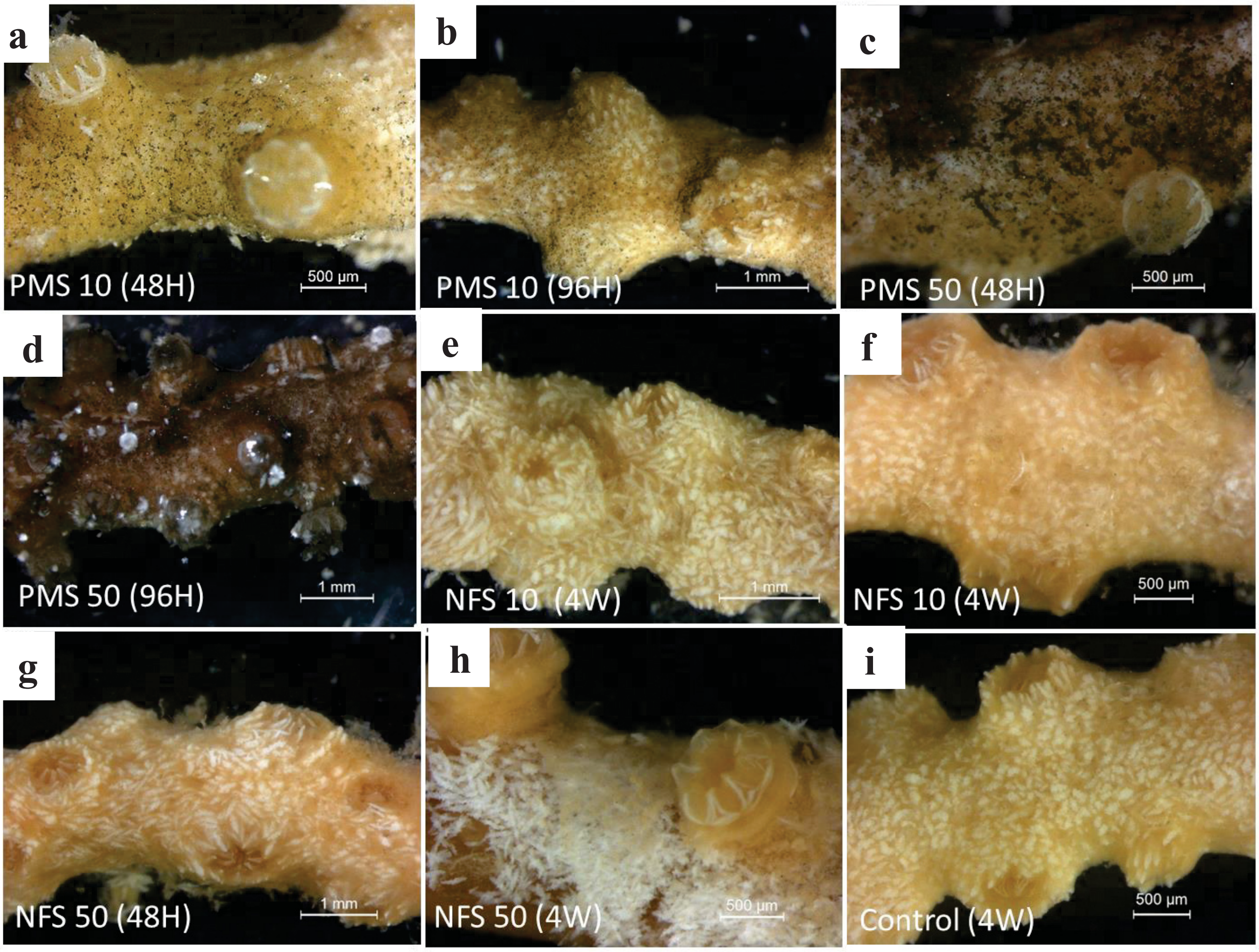

Figure 18: Photographs illustrating the tissue and polyps' condition of *Dentomuricea aff. meteor* over the experimental period of 28 days in the different treatments under the dissecting microscope: (a) coral fragments in treatments with massive sulphide particles (PMS) at concentrations of 10 $mg.L^{-1}$ after 2 days exposure and (b) 4 days exposure; (c) coral fragments in the PMS 50 $mg.L^{-1}$ treatment after 2 days and (d) 4 days (e) coral fragments in treatments with sediments from nodule fields (NFS) at concentrations of 10 $mg.L^{-1}$ after 2 days and (f) 28 days; (g) coral fragments in the NFS 50 $mg.L^{-1}$ treatment after 2 days and (h) 28 days; (i) coral fragments in the control treatment after 28 days.

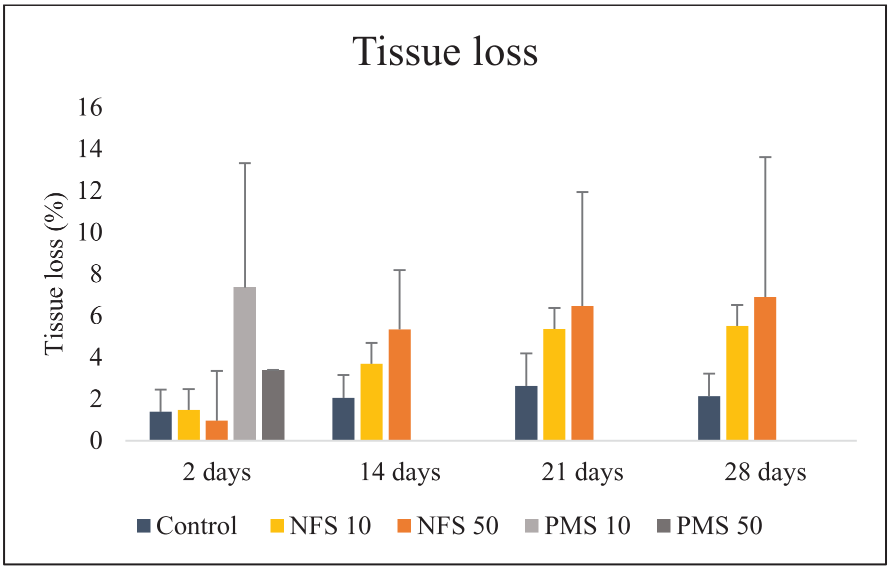

Figure 19: Loss of tissue and polyps in coral fragments of *Dentomuricea* aff. *meteor* exposed to experimental treatments with sediments from nodule fields (NFS) and massive sulphide particles (PMS) at concentrations of 10 and 50 mg.L^{-1} and a control treatment with no sediment addition during the 28 days of the experiment. Data values as average ± standard deviation.

The percentage loss of tissue and polyps of coral fragments exposed to the different treatments is presented in Figure 19. Highest tissue loss was recorded in the PMS treatments with tissue loss of 7,3% for the PMS 10 and 3,4% for PMS 50 by the second day of the experiment. Coral tissue in the PMS 50 was covered by a “crust” of particles that may have prevented the tissue from falling off and thus tissue and polyp loss could be greater than was measured. Because of the rapid death of the corals in these treatments, it was not possible to compare it statistically with the other treatments.

Results of the permutational MANOVA revealed that tissue loss changed with experimental time but not between treatments (Table 3). Coral fragments significantly loss 4,4% of their tissue in the NFS 50 between times 2 and 14 days (pairwise comparisons t= 3.9954; p= 0.0013) and although loss of tissue further increased to 6,9% by the end of the experiment, this difference was not statistically significant. Tissue loss in the NSF 10 treatments also increased from 1,5% to 5,4% between days 2 and 21 but not after other experimental times (pairwise comparisons t=2.6449; p=0.0178). Tissue loss did not significantly change in the control treatment during the experiment (pairwise comparisons t= 1.8393; p=0.0883). Although average tissue loss in treatments with NFS were 2-3 times greater than control conditions, the differences were not statistically significant due to the high variability among corals within treatments (Fig 19).

Table 3: PERMANOVA analysis of tissue loss of *Dentomuricea* aff. *meteor* fragments over the experimental time, with treatments and time treated as fixed effects and coral fragments within treatments treated as random effects.

	Effect	df	SS	MS	PseudoF	P(perm)	Unique perms	P(MC)
Tissue loss								
Treatment	Fixed	2	4.3699	2.185	0.78908	0.4562	9894	0.4743
Time	Fixed	3	5.9451	1.9817	5.585	0.0034	9949	0.0025
TreatmentxTime	Fixed	6	3.71	0.61833	1.7426	0.14	9954	0.1363
Coral ID(Treatment)	Random	14	38.766	2.769	7.8038	0.0001	9926	0.0001
Residual	Random	42	14.903	0.35483				
Total		67	67.801					

Examination of histological sections of coral polyps exposed to NFS treatments did not reveal any detectable changes in tissue integrity or the presence of sediment particles (Fig. 20). Histological analysis of corals exposed to PMS treatments was not possible due to the necrotic condition of tissues and the sediment grain interference during sectioning with the microtome.

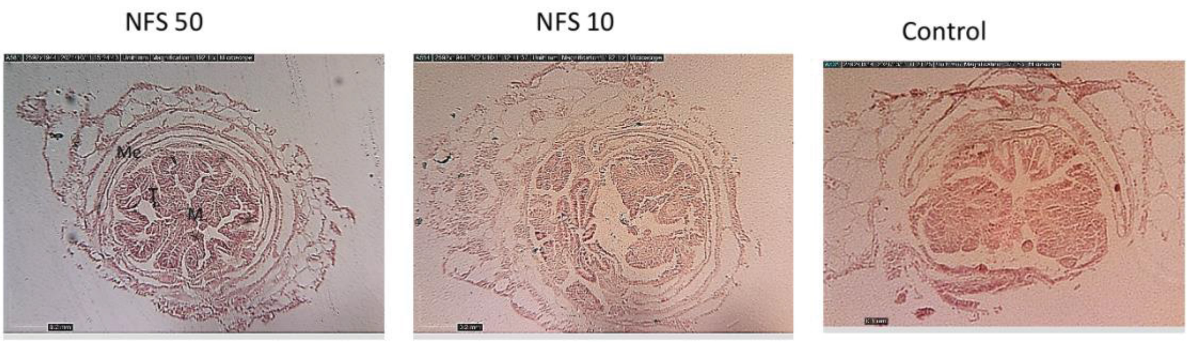

Figure 20: Microphotographs (x10) of histological sections of *Dentomuricea* aff. *meteor* polyps exposed to (a) the NFS 50 mg.L^{-1} treatment, (b) NFS 10 mg.L^{-1} treatment, and (c) control treatments after 4 weeks exposure, show tentacles (T), a mouth (M), mesenteric muscular filament (Me).

3.4. Polyp activity

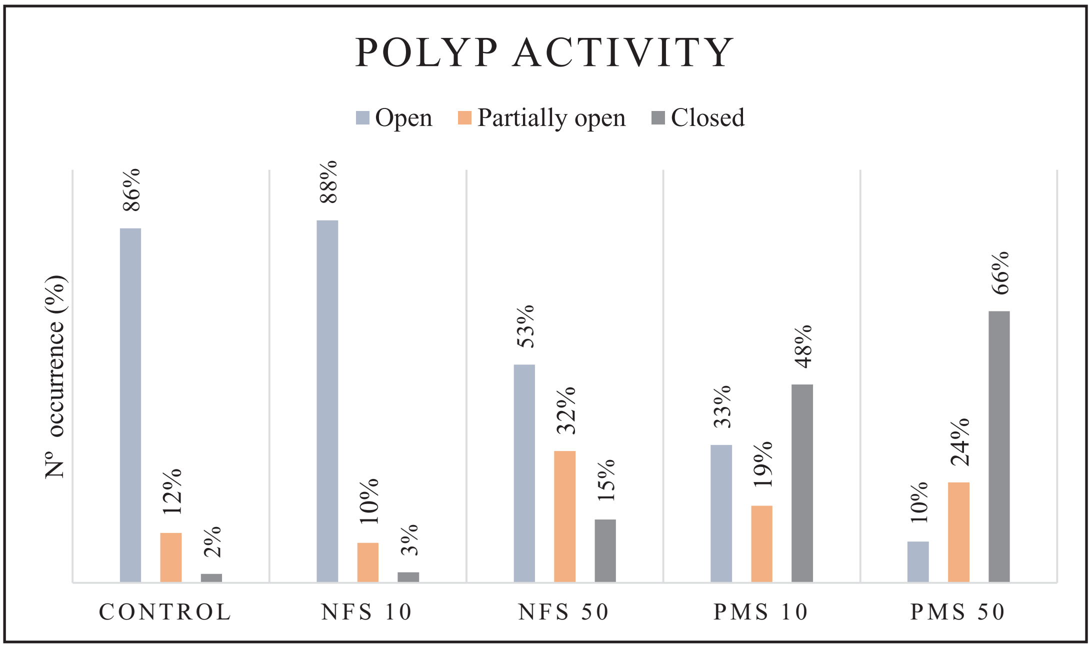

Figure 21: Polyp activity of *Dentomuricea* aff. *meteor* under different suspended sediment treatments, where, the percentage of the coral fragments that had open, partially open and closed polyps throughout the experiment was counted. Results are shown as the average percentage during the whole duration of the experiment. Nodule field sediment is designated as "NFS" and "PMS" indicates the polymetallic sulphide particles treatments at concentrations of 10 and 50 mg.L^{-1}. The results are reported as mean ± standard deviation; n=30 for control treatment and n=15 for each sediment treatment.

Polyp activity of *D.* aff. *meteor* was measured after feeding to determine their capacity to extend their polyps and feed. The permutational MANOVA showed that polyp activity varied between treatments, but not through time of the experiment (Table 4). The percentage of fragments with fully open polyps was higher in control and NFS 10 treatments when compared to other treatments, with average of 86% and 88% respectively throughout the total duration of the experiment (pairwise tests t=4.30-7.39 and p=0.0001-0.0005, Fig 21). In contrast, in the PMS treatments most fragments had closed (retracted) or partially open polyps, with 66% fragments with closed polyps in the PMS 50 followed by 48% in the PMS 10 treatment, which were statistically significantly greater than the NFS 10 and control treatments (pairwise tests t =3.37-5.14 and p=0.0001-0.0026, Fig. 21) The percentage of fragments with closed polyps was significantly greater in PMS 50 treatment but not of the NFS 50 treatment (pairwise tests t= 2.20 and p= 0.03, Fig 21) and did not differ significantly between PMS 10 and NFS 50. Polyp activity was also reduced under NFS 50 treatment when compared with control levels, with only 53% of fragments with fully open polyps (pairwise tests t=5.13 and p=0.0002).

Table 4: PERMANOVA analysis of *Dentomuricea* aff. *meteor* polyp activity over the experimental time, with treatments and time treated as fixed effects.

	Effect	df	SS	MS	PseudoF	P(perm)	Unique perms	P(MC)
Polyp activity								
Treatment	Fixed	4	149.7	37.426	12.983	0.0001	9942	0.0001
Time	Fixed	3	4.6566	1.5522	0.53845	0.8051	9932	0.8078
TreatmentxTime	Fixed	6	12.289	2.0482	0.71051	0.7592	9929	0.7526
Residual	Random	69	198.91	2.8827				
Total		82	391.57					

3.5. Metabolic activities: respiration rates

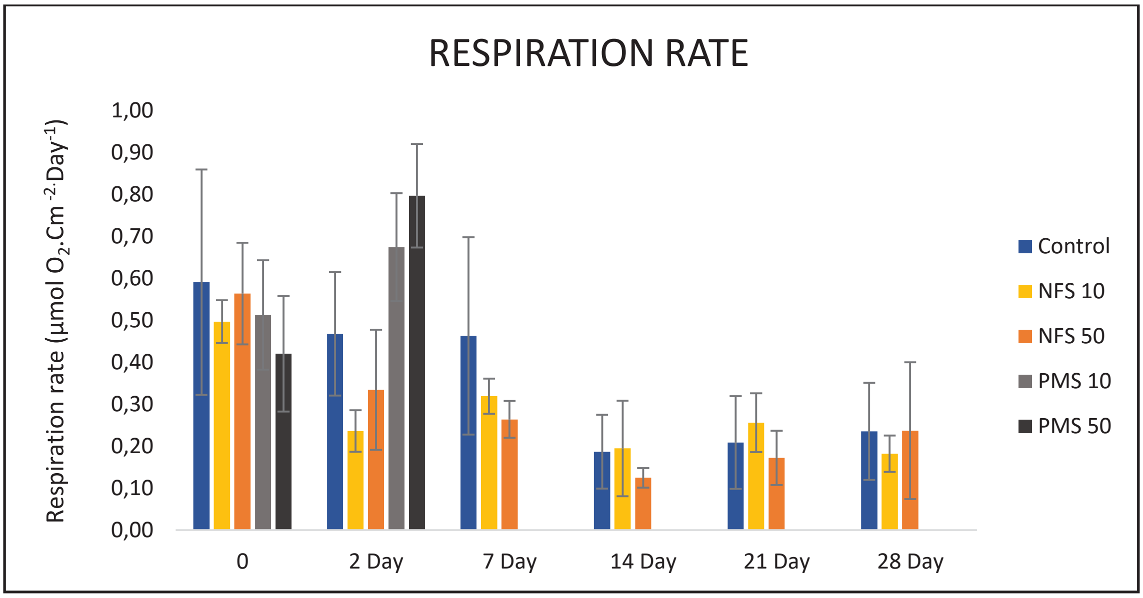

Figure 22: Respiration rates of *Dentomuricea aff. meteor* under different suspended sediment treatments. The respiration rate is normalized to the coral tissue surface area. Respiration was measured before the experiment started (time 0), two days after and once a week until the end of the experiment. "NFS" indicates the nodule field sediment treatments and "PMS" indicates the polymetallic sulphide particles treatments at concentrations of 10

and 50 mg.L^{-1}; The results are reported as mean ± standard deviation; n=12 for control treatment and n=6 for each sediment treatment except for the 2nd day was used n=6 for control treatment and n=3 for each sediment treatment.

Respiration rates of coral fragments in the different treatments were measured every week in each treatment, except for the PMS treatments in which corals died after 4 days of exposure (Fig. 22). The permutational MANOVA revealed significant differences in respiration rates between treatments that were not constant during experimental time as indicated by the significant interaction term Treatment x Time (Table 5). Respiration rates of *D. meteor* at T=0, before the beginning of the experiment, varied little between the different treatments, but after 2 days of exposure, respiration rates significantly increased under both PMS treatments, with their subsequent death on day 4 (Fig. 22, Table 5). Respiration rates in the PMS 10 treatment increased 31% from 0,51 ± 0,13 µmol O_2 cm^{-2} day^{-1} prior the experimental time to 0,67 ± 0,13 µmol O_2 cm^{-2} day^{-1}, whereas in the PMS 50 treatment increased 91% from 0,42 ± 0,14 to 0,80 ± 0,12 µmol O_2 cm^{-2} day^{-1} at the day 2 (Fig. 22). When compared to the control treatment, respiration rates under PMS 50 were 70% greater (pairwise comparisons t= 3.289 and p=0.0157). Respiration rates in both PMS treatments were also 179-142% greater than the NFS treatments for 10 and 50 mg.L^{-1} respectively (pairwise comparisons t=4.3966-4.2281 and p=0.0209-0.0135). In the NFS 10 treatment, respiration rates decreased by half after 2 days of exposure (0,50 ± 0,05 to 0,24 ± 0,05 µmol O_2 cm^{-2} day^{-1}) while for NFS 50 significantly decreased during the first two weeks (from 0,56 ± 0,12 µmol O_2 cm^{-2} day^{-1} at time = 0 to 0,12 ± 0,02 µmol O_2 cm^{-2} day^{-1} at day 14) (pairwise comparisons t= 2.58-2.97 and p=0.031-0.038). Respiration rates were significantly lower in the NFS 50 when compared to NFS 10 and control treatments on day 7 (pairwise comparisons t=2.2659, p=0.0429). Respiration rates also significantly decreased in the control treatments between times 0 and 14 (pairwise comparison t=2.52, p=0.026). During the last 15 days of the experiment, there were no significant differences between the control and NFS 10 and NFS 50 treatments (pairwise comparisons t= 0.74281-0.01155, p= 0.5037-0.9912).

Table 5: PERMANOVA analysis of *Dentomuricea* aff. *meteor* respiration rates over the experimental time, with treatments and time treated as fixed effects.

	Effect	df	SS	MS	PseudoF	P(perm)	Unique perms	P(MC)
Respiration rates								
Treatment	Fixed	5	0.29792	5.9584E-2	2.7098	0.0248	9942	0.0254
Time	Fixed	5	0.67868	0.13574	6.173	0.0001	9953	0.0001
TreatmentxTime**	Fixed	11	0.69136	6.2851E-2	2.8583	0.0047	9941	0.0036
Residual	Random	77	1.6931	2.1989E-2				
Total		98	4.6275					

4. Discussion

The present dissertation elucidates the differential impacts caused by sediment plumes potentially generated during mining activities to extract nodules in the Clarion-Clipperton Fracture Zone (NE Pacific) and seafloor massive sulphides from a hydrothermal vent field in the Mid-Atlantic Ridge region of the Azores (NE Atlantic) on the octocoral *Dentomuricea* aff. *meteor* using an aquaria-based experiment. Results of the study confirmed the hypothesis put forward in this dissertation that *D. meteor* responds differentially to sediment plumes generated by the extraction of seafloor massive sulphides and ferromanganese nodules. The results of this study showed high sensitivity of *D. meteor* to pollymetalic sulphide particles (PMS), since the survival of all coral fragments was only four days of the experiment under both concentrations tested (~10 and ~50 mg.L^{-1}). On the contrary, coral fragments exposed to sediments from nodule fields (NFS) treatments full survived until the experiment's end (28 days). The physiological responses involved in this different result are discussed below.

4.1. Polyp activity and tissue condition

One important mechanism that minimizes sedimentation damage in corals is sediment removal by the coral polyps. Previous studies with tropical corals indicate polyp retraction as an active mechanism to prevent sediment accumulation on the corals' tissue, a natural reaction that contribute to abrasion avoidance by several coral species (Erftemeijer et al., 2012). In addition to polyp retraction, specific coral responses to remove sediment from their tissues are similar, such as ciliary and tentacular activity, increase the mucus excretion, polyp inflation and others intraspecific morphological variations (Rogers, 1990; Erftemeijer et al., 2012; Larsson et al., 2013). In the present study, *D. meteor* showed different polyp activity when exposed to the different treatments (Fig. 21). During the first hours of the experiment, corals showed a reduction in polyps' activity for both PMS concentrations and since the initial moment of the experiment little expansion by the nubbins' polyps were observed. In both treatments 57% of the fragments presented retracted polyps, likely as mechanism to avoid abrasion from PMS particles. Studies about sedimentation on tropical coral species refer that polyp inflation (oral disc) with retracted tentacles allows to form a smoother surface on which sediments can slide easier, when tentacular action is not enough to remove the particles (Erftemeijer et al., 2012). Other sediment rejection mechanisms such the use of polyp expansion and mucus production may have also been used by the nubbins that maintained open or partially open polyps in the NFS treatments, although mucus release was not evident from coral observation. Coral nubbins under NFS 50 showed a reduced polyp activity (53% fragments with open polyps) compared to those in the NFS 10 and control treatments (>86% fragments with open polyps), whereby, there were no significant differences in the polyp behaviour between NFS 10 and control treatments. This points out to differential capacity of *D. meteor* nubbins to remove PMS and NFS particles, which may be related to the nature of the sediment such as due to the shape, size or toxicity.

Studies with tropical corals indicate that sediment removal rates depend on sediment properties, such as the grain size and organic and nutrient content (Fabricius and Wolansky, 2000; Webber et al., 2006; Erftmayer et al., 2012). These studies show that sandy grain size fractions were rejected more effectively than nutrient-rich fine sediments (mud and silt-sized), possibly due to the greater volume and stickiness of the latter. In the present study, the size of particles was comparable although their composition markedly differed. Nodule field sediments were composed predominantly of fine-grain carbonate clays, siliceous sediment oozes and red clays, with organic content of 3.0% while polymetallic sulphide particles were composed of barite ($BaSO_4$), pyrite (FeS_2), and chalcopyrite ($CuFeS_2$) with organic content of 4,7%. It is also likely that PMS particles were sharper than NFS although no detailed observation of the angularity of particles were made to confirm this. These different properties may have influenced on the differential responses by *D. meteor* behaviour and subsequent effects on the tissue necrosis and in their survival.

Another important difference in the two types of sediments is the metals content. Preliminary results of the metal concentration leached from the PMS particles into the seawater in the PMS 50 treatments revealed extremely high concentration of Cu (500-1100 µg/L) and also high concentrations of Mn, Co, Ni, Zn, Cd (Miguel Caetano and Joana Raimundo, unpublished data). Although the metal content in seawater from the NFS treatment has not been analysed yet, previous studies indicate that the concentration of metals in these sediments is expected to be very low (Menendez et al., 2019). Previous studies on the impact of PMS particles on *D. meteor* conducted in the framework of the MIDAS project (Carreiro-Silva et al unpublished, MIDAS DL 5.3) also reported copper concentration 3-5 times higher in treatments with PMS at 25 $mg.L^{-1}$ (18±0.5-23±10 $\mu g.L^{-1}$) in comparison to control levels. In this study a semi-open seawater system instead of an open system was used, where 8 hours of running seawater were intercalated with a 4-hour period in closed system during which the PMS particles were added in a single pulse of 25$mg.L^{-1}$. Results of the study showed coral death on the PMS treatments recorded only after the 13th day of a 27 day experiment, instead of the four days recorded in the present study. The later time of death of the corals in Carreiro-Silva et al, was probably related to the fact that the single pulse addition of PMS particles resulted in high sedimentation shortly after particle delivery, with only 2 $mg.L^{-1}$ being in suspension after two hours and the much lower concentration of dissolved Cu recorded. The preparation of the PMS stock solution in the present study resulted in a higher metal concentration leached from the PMS particles into the seawater.

In another study on the lethal and sub-lethal toxicological effects of Cu added to *D. meteor* in dissolved form, Martins et al (2018) observed that Cu concentrations twice as high as to the ones recorded in the PMS treatment in Carreiro-Silva et al (60$\mu g.\ L^{-1}$), although inflicted physiological stress, did not cause *D. meteor* death. Thus suggesting that a combination of both mechanical and toxicological effects of PMS particles in Carreiro-Silva et al resulted in the coral mortality observed, which may also be the case in the present study, but this needs further investigations.

Coral's reaction to sedimentation can also be variable between different coral species and a study made by Riegl (1995) about the sand deposition effects on different species of scleractinian and alcyonacean corals concluded that sand exposure led to increase of tentacular action in the scleractinian *Gyrosmilia interrupta* and a hydrostatic inflation of polyps in the others scleractinian and alcyonacean corals. Both coral orders developed tissue necrosis after the first week under continuous sand application but partial bleaching and death of entire colonies were only observed in alcyonacean species. In this study, the author concluded that scleractinian corals were active sediment shedders while alcyonacean corals were passive shedders, mainly depending on the water flow and gravity to expel accumulated sediment. This lower cleaning capacity of the alcyonacean compared to scleractinian corals, points to a greater vulnerability of soft corals to sediment exposition. These mechanisms may explain the differences responses between the cold-water scleractinian coral *L. pertusa* and the octocoral *D. meteor* at similar concentrations (~50 $mg.L^{-1}$), in which, results obtained by Brooke et al., (2009), demonstrate that *L. pertusa* are able to tolerate sediments conditions with high survival rates. Larsson et al., (2013) also showed that *L. pertusa* efficiently removed deposited particles under exposure concentrations of 5 $mg.L^{-1}$ and 25 $mg.L^{-1}$, which indicates an efficient cleaning reaction. On the contrary, results observed in this study demonstrate a lack of capacity or an inefficient cleaning mechanism to remove deposited sediments of *D. meteor* tissue. Despite the low mortality of *L. pertusa* to sediment exposure, the authors reported that at high sediment concentrations the polyps were less extended and therefore, might be the cause of the reduced skeletal growth (Larsson et al., 2013).

The effects of suspended particles on *D. meteor*' tissue condition was seen through time in the different treatments (Fig. 17). Tissue loss in *D. meteor* was more evident on fragments under PMS treatments, likely related to the toxicity, size or shape of PMS particles that could cause abrasive effects on the corals tissue, as referred above. Tissue degeneration for nubbins under PMS 10 was visible at the 2nd day of exposure with 7.3% of tissue loss and was evident for both concentrations of PMS treatments three days after, being possible to observe visible skeleton portions in several coral fragments. For these treatments, tissue loss was observed not only at the tips of the coral branches but also in intermediate areas of greater sediment accumulation. The photos taken with the dissecting microscope showed PMS particles aggregated on the corals tissue, with a greater accumulation on top of the contracted coral at the highest concentration suggesting high adhesion of these particles to coral tissue. This high adhesion by PMS particles may have caused smothering in a short period of time, with fragments death at the 4th day. Previous studies also suggest that sediment accumulation on corals tissue can hamper mucus production making sediment rejection difficult (Bak and Elgershuizen, 1976). Tissue necrosis and loss for nubbins under NFS 50 exposure increased over the course of the experiment and were evident for a few coral nubbins after 17 days of the exposure, where started to have a thinner appearance and tissue necrosis and loss were observed at tips of the coral branches. Photos taken with the dissecting microscope after 4 weeks exposure showed the disintegration of coral tissues with sclerite displacement as a result of high particle abrasion, also observed in the coral fragments exposed to PMS particles. Histological analysis made by Liefmann et al., (2018) show that smaller and more angular particles can cause tissue damage in corals as they can penetrate the tissue, being more harmful to corals than smooth particles, these also may explain the differences in the damages found at *D. meteor* tissue between PMS and NFS particles, although no detailed analysis of the shape of both particle types was made.

Moreover, the high adhesion of PMS particles on corals tissue and the great covering by sediments on corals surfaces may cause anoxic environment and subsequent sulphides production, creating optimal conditions for bacterial growth (Bagarinao, 1992). The perforation by sediment on the corals tissue might also add vulnerability of corals to bacterial diseases with subsequent tissue necrosis (Liefmann et al., 2018). Increased bacterial diseases correlated to sedimentation were previously reported in tropical corals (Weber et al., 2012; Pollock et al., 2014) and in the cold-water coral *L. pertusa* (Allers et al., 2013). In previous studies, the organic matter present in the sediment induce the decomposition of tissues by bacteria with the release of hydrogen sulphide, known to be harmful to corals causing tissue damage and death (Bagarinao, 1992). In this study, the sulfur present in PMS particles could have directly provoked bacterial growth which may have led to a faster tissue necrosis and induced coral death.

4.2. Respiration rates as a proxy of metabolism

The active mechanism to prevent sediment accumulation in coral tissue may increase energy expenditure and cause significant changes in metabolic rates (Rogers, 1990; Erftemeijer et al., 2012; Larsson et al., 2013). In this study, respiration rates of *D. meteor* significantly increased under both PMS concentrations after two days, with their subsequent death on day four, whereas for both NFS treatments respiration rates significantly decreased during the first week (Fig. 22). As stated in Larsson et al., (2013), previous studies on the effects of sedimentation on tropical corals report differing effects on respiration rate, as an indication of metabolic activity, such as increased respiration during sediment removal (Dallmeyer et al., 1982; Abdel-Salam et al., 1988; Telesnicki and Goldberg., 1995), decrease in respiration (Riegl and Branch., 1995) or no difference (Lirman and Manzello., 2009). In cold-water corals, increased respiration rates were reported for the octocoral *Primnoa resedaeformis* (Scanes et al., 2018) while no differences were reported for *L. pertusa* (Larsson et al., 2013). Reduced metabolism in corals have been related to a reduction in polyp activity or from tissue or polyps loss (Riegl and Branch, 1995) whereas increasing respiration have been related to increased polyp activity or increased mucus production (Riegl and Branch., 1995; Scanes et al., 2018). Studies reporting impacts on respiration rates in sponges exposed to suspended sediments have also showed contrasting results: increased respiration rates in some studies (e.g. McGrath et al., 2017) and decreased respiration rates in others (e.g. Kutti et al., 2015), and it is thought that the increase in respiratory rates is due to active mechanisms in order to remove sediment from their system, such as mucus production (McGrath et al., 2017), while respiratory rates decrease may result from a reduction in water pumping rates to prevent sediment ingestion (Cummings et al., 2020). Respiration rates in the present study, show different results under different sediments treatments and by these results, polyp activity and respiration rate did not appear to be related. Therefore, the increased respiration rates under both PMS concentrations at the 2nd day likely suggest physiological stress that may be related to the copper intoxication of corals under these treatments. Studies on the effect of copper and climate change (ocean acidficaton and warming) on a coral reef symbiont bearing foraminifer Amphistegina gibbosa (used as bioindicators of coral reefs health, Marques et al., 2020), showed that Cu exposure increased respiration, irrespective of climate scenarios, suggesting increased metabolism. This increase in respiration was interpreted by the authors as an acclimation response to Cu or increased energy allocation to cope with stress (Marques et al., 2020). A similar increase in respiration rates in response to PMS particles was found by Carreiro-Silva et al., (unpublished) where, *D. meteor* respiration rates significantly increased after 13 days of exposure to PMS particles. Increased respiration in another octocoral from the Azores, *Viminella flagellum*, in response to dissolved Cu was also recorded by Martins et al (unpublished data) suggesting a stress response to Cu on octocorals.

Despite the decrease in the respiration rate of *D. meteor* under NFS sediments until the 7th day, there was a general decreased in respiration rates in coral fragments in all treatments during the last 15 days of the experiment. This general decrease in metabolism in NFS and control treatments could be due to insufficient amount of food offered during experiment, even though the amount of carbon in the food offered per coral fragments was calculated based on the metabolic requirement on *D. meteor* in Rakka et al (2021). Another possible explanation may be general decrease in coral health after prolonged maintenance in aquaria, eventhough corals in the control treatment were generally active with vibrant color.

4.3. Limitations and way forward

To better understanding the differential responses of PMS and NFS sediment plumes in *D. meteor*, comparative analysis of particles shape and granulometry of both sediment types should be made. While there was information on particle size of the PMS particles used in the experiment from a previous study conducted within the MIDAS project, this information was based solely on the literature for the NFS sediment. Particle size tests using stainless steel sieves were tried to be done for the NFS particles before the start of the experiment, but the large flocculation and adhesion by the particles prevented the quantification of the various sizes percentage that are present in this sediment. Observation of both sediment types with scanning electron microscopy would provide more information about the general shape of the particles, the highly effect on corals under the PMS particles that are likely to be more angular than the NFS particles.

In this experiment, we used filtration to verify and control the concentrations for the different treatments, but this method of quantifying can accumulate errors throughout the process, such as sediment losses that adhere to the materials used. There are also potential errors related to the salt content of the different sediments and natural seawater which can influence the weight of the sediment filtered. This could partially explain the sediment concentration values measured in the control treatment. These errors can be resolved if turbidity measurements are made using a turbidimeter to quantify concentrations of sediment present in the aquaria.

The limited experience with histological sectioning may have prevented the identification of changes associated with coral reaction to sedimentation, such as the presence of mucocytes or other small alterations on coral tissues.

Further analysis of biochemical composition of tissues, mucocyte cell production and cellular stress proteins will help to determine the physiological condition of corals under the NFS treatments and whether they will likely be able to cope with the increased energetic costs of sediment removal under long time periods. Further metabolic analysis such as C:N ratio and nitrates and ammonia excretion rates may be complementary to respiration rates responses of corals under both sediment treatments.

To improve the assessment of the response of *D. meteor* to suspended plumes (e.g. antioxidant enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), heat-shock protein HSP70 and lipid peroxidation (LPO), it is important to evaluate the antioxidant biomarkers related to cellular stress and the detoxification pathways in *D. meteor* tissues. These will help to determine the physiological effects of *D. meteor* under both PMS and NFS treatments and whether there are distinct responses of *D. meteor* to the different sediment concentrations.

In addition, analysis of tissues and skeleton metal bioaccumulation combined to monitoring the bioavailable labile metal fraction in the seawater will clarify the causes of coral mortality under PMS treatments.

5. Conclusions

This research aimed to identify the effects of suspended sediment plumes generated by mining activities on the survival, tissue condition, polyp behaviour and respiration rates of the octocoral *Dentomuricea* aff. *meteor*, using sediments from nodules fields and massive sulphides deposits. Some of the *D. meteor* responses under sedimentation conditions in this study were already expected, such as alterations in the polyp's behaviour, tissue damage and changes in metabolic activities that may result from the redirection of energy to expel sediment of the tissue or by the explosions of bacteria populations, as the responses of corals exposed to sedimentation in previous studies are similar. As described in previous studies, these potential effects of sediment plumes can not only affect coral mortally but can also cause other sublethal effects such as reduced growth rates, reduced reproduction, increased susceptibility to diseases and increased risk of abrasion, breakage and reduced regeneration of damage to the coral tissue. The deposits of seafloor massive sulphides and ferromanganese nodules differ in mineralogy, metal composition and particle morphology, resulting in different mechanical and ecotoxicological impacts on organisms. Mining of polymetallic sulphides deposits must have a greater potential toxicity than nodules mining due metal-rich composition of PMS particles. Natural plumes of particles with high metal elements are normally present at active hydrothermal vents but are absent at inactive hydrothermal vents whereby, during mining of SMS deposits at these inactive vents, the heavy metals realized in the seawater will might be toxic to corals and other benthic organisms with potential for sublethal or lethal effects. The high mortality of corals at low and high concentrations of PMS particles, represent a great risk of entire ecosystems degradation, since, coral mortality can result in migration or death of associated fauna, suggesting low environmental tolerances of the coral reef-associated community.

In this study, the response of the *D. meteor* to PMS particles, points out to the potential impact of deep-sea mining on the cold-water octocorals and the urgent need to incorporate scientific data into International Seabed Authority (ISA).

Finally, this study constituted another contribution to the knowledge about the impacts of sediment plumes generated by deep-sea mining on the benthic fauna. Given the importance of the topic, it is considered that there is still much to be covered in the field of research in this area, which is, therefore, a work aid for other researchers.

References

Abdel-Salam, H. A., Porter, J. W., & Hatcher, B. G. (1988, August). Physiological effects of sediment rejection on photosynthesis and respiration in three Caribbean reef corals. In *Proc. 6th int. coral Reef Symp* (Vol. 2, pp. 285-292).

Aleynik, D., Inall, M. E., Dale, A., & Vink, A. (2017). Impact of remotely generated eddies on plume dispersion at abyssal mining sites in the Pacific. *Scientific reports*, *7*(1), 1-14. https://doi.org/10.1038/s41598-017-16912-2

Allers, E., Abed, R. M., Wehrmann, L. M., Wang, T., Larsson, A. I., Purser, A., & de Beer, D. (2013). Resistance of Lophelia pertusa to coverage by sediment and petroleum drill cuttings. *Marine pollution bulletin*, *74*(1), 132-140.

Amon, D. J., Ziegler, A. F., Dahlgren, T. G., Glover, A. G., Goineau, A., Gooday, A. J., Wiklund, H., & Smith, C. R. (2016). Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. *Scientific Reports*, *6*(1), 1-12. https://doi.org/10.1038/srep30492

Anderson MJ., Gorley RN. (2008). PERMANOVA for PRIMER: Guide to Software and Statistical Methods. PRIMERE.

Anthony, K. R.N. (1999). A tank system for studying benthic aquatic organisms at predictable levels of turbidity and sedimentation: case study examining coral growth. *Limnology and Oceanography*, *44*(6), 1415-1422. https://doi.org/10.4319/lo.1999.44.6.1415

Anthony, K. R.N., & Fabricius, K. E. (2000). Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of experimental marine biology and ecology*, *252*(2), 221-253. https://doi.org/10.1016/S0022-0981(00)00237-9

Ardron, J. A., Simon-Lledó, E., Jones, D. O., & Ruhl, H. A. (2019). Detecting the effects of deep-seabed nodule mining: simulations using megafaunal data from the Clarion-Clipperton Zone. *Frontiers in Marine Science*, *6(10)*, 604. https://doi.org/10.3389/fmars.2019.00604

Bagarinao, T., 1992. Sulfide as an environmental factor and toxicant: tolerance andadaptations in aquatic organisms. *Aquat. Toxicol. 24*, 21–62

Baillon, S., Hamel, J. F., Wareham, V. E., & Mercier, A. (2012). Deep cold-water corals as nurseries for fish larvae. *Frontiers in Ecology and the Environment*, *10*(7), 351-356. https://doi.org/10.1890/120022

Bak, R. P. M., & Elgershuizen, J. H. B. W. (1976). Patterns of oil-sediment rejection in corals. *Marine Biology*, *37*(2), 105-113.

Baker, E., & Beaudoin, Y. (2013). Deep Sea Minerals: A physical, biological, environmental, and technical review (Issue December)

BMWi. (2015). Federal Ministry for Economic Affairs and Energy. Regulatory Environment and Incentives for Using Electric Vehicles and Developing a Charging Infrastructure.

Boschen, R. E., Rowden, A. A., Clark, M. R., & Gardner, J. P. (2013). Mining of deep-sea seafloor massive sulfides: a review of the deposits, their benthic communities, impacts from mining, regulatory frameworks and management strategies. *Ocean & coastal management*, *84*, 54-67. https://doi.org/10.1016/j.ocecoaman.2013.07.005

Braga-Henriques, A., Porteiro, F., Sampaio, Í., Matos, V., Carreiro-Silva, M., Ocaña, O., & Santos, R. S. (2008). Cold-water corals of the Azores: preliminary assessment of distribution, diversity and associated fauna. In *Proceedings of the 4th International Deep-Sea Coral Symposium, Wellington, New Zealand, Poster* (Vol. 10, No. 2, p. 142). http://www.museodelmarceuta.com/papers/2008 poster_DeepSea_Coral_Symposium2008_AH.pdf

Braga-Henriques, A., Porteiro, F. M., Ribeiro, P. A., Matos, V. D., Sampaio, Í., Ocaña, O., & Santos, R. S. (2013). Diversity, distribution and spatial structure of the cold-water coral fauna of the Azores (NE Atlantic). *Biogeosciences*, *10*(6), 4009-4036. https://doi.org/10.5194/bg-10-4009-2013

Brooke, S. D., Holmes, M. W., & Young, C. M. (2009). Sediment tolerance of two different morphotypes of the deep-sea coral Lophelia pertusa from the Gulf of Mexico. *Marine Ecology Progress Series*, *390 (7)*, 137-144. https://doi.org/10.3354/meps08191

Buhl-Mortensen L, Vanreusel A, Gooday AJ, Levin LA, Priede IG, Buhl-Mortensen P, Gheerardyn H, King NJ, Raes M (2010) Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Mar Ecol 31(1*), 21–50.

Burns, R. E. (1980). Assessment of environmental effects of deep ocean mining of manganese nodules. *Helgoländer Meeresuntersuchungen*, *33*(1-4), 433-442. https://doi.org/10.1007/BF02414768

Cairns, S. D. (2007). Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. *Bulletin of Marine Science 81*(3), 311-322.

Cairns, S. D. (2016). New abyssal Primnoidae (Anthozoa: Octocorallia) from the clarion-clipperton fracture zone, equatorial northeastern Pacific. *Marine Biodiversity*, *46*(1), 141-150.

Carreiro-Silva, M., Andrews, A. H., Braga-Henriques, A., De Matos, V., Porteiro, F. M., & Santos, R. S. (2013). Variability in growth rates of long-lived black coral leiopathes sp. from the azores. *Marine Ecology Progress Series*, *473*(1), 189–199. https://doi.org/10.3354/meps10052

Carreiro-Silva, M., Ocana, O., Stanković, D., Sampaio, I., Porteiro, F. M., Fabri, M. C., & Stefanni, S. (2017). Zoantharians (Hexacorallia: Zoantharia) associated with cold-water corals in the Azores region: new species and associations in the deep sea. *Frontiers in Marine Science*, *4(4)*, 88. https://doi.org/10.3389/fmars.2017.00088

Chan, I., Tseng, L. C., Kâ, S., Chang, C. F., & Hwang, J. S. (2012). An experimental study of the response of the gorgonian coral Subergorgia suberosa to polluted seawater from a former coastal mining site in Taiwan. *Zoological Studies*, *51*(1), 27-37.

Christiansen, B., Denda, A., & Christiansen, S. (2020). Potential effects of deep seabed mining on pelagic and benthopelagic biota. *Marine Policy*, *114(9)*, 103442. https://doi.org/10.1016/j.marpol.2019.02.014

Costello, M. J. (2005). Cold-Water Corals and Ecosystems (Vol. 148).

Cruz, J. V., Pacheco, D., Cymbron, R., & Mendes, S. (2010). Monitoring of the groundwater chemical status in the Azores archipelago (Portugal) in the context of the EU water framework directive. *Environmental Earth Sciences, 61*(1), 173-186.

Cummings, V. J., Beaumont, J., Mobilia, V., Bell, J. J., Tracey, D., Clark, M. R., & Barr, N. (2020). Responses of a common New Zealand coastal sponge to elevated suspended sediments: Indications of resilience. *Marine environmental research*, *155*, 104886.

Cuvelier, D., Gollner, S., Jones, D. O. B., Kaiser, S., Arbizu, P. M., Menzel, L., Mestre, N. C., Morato, T., Pham, C., Pradillon, F., Purser, A., Raschka, U., Sarrazin, J., Simon-Lledó, E., Stewart, I. M., Stuckas, H., Sweetman, A. K., & Colaço, A. (2018). Potential mitigation and restoration actions in ecosystems impacted by seabed mining. *Frontiers in Marine Science, 5*(12), 1–22. https://doi.org/10.3389/fmars.2018.00467

Dallmeyer, D. G., Porter, J. W., & Smith, G. J. (1982). Effects of particulate peat on the behavior and physiology of the Jamaican reef-building coral Montastrea annularis. *Marine Biology*, *68*(3), 229-233.

D'Onghia, G., Maiorano, P., Sion, L., Giove, A., Capezzuto, F., Carlucci, R., & Tursi, A. (2010). Effects of deep-water coral banks on the abundance and size structure of the megafauna in the Mediterranean Sea. Deep-Sea Research Part II: Topical Studies in Oceanography, 57(5–6), 397–411. https://doi.org/10.1016/j.dsr2.2009.08.022

Douglas, R. H., Partridge, J. C., & Hope, A. J. (1995). Visual and lenticular pigments in the eyes of demersal deep-sea fishes. *Journal of Comparative Physiology A, 177*(1), 111–122. https://doi.org/10.1007/BF00243403

Duckworth, A., Giofre, N., & Jones, R. (2017). Coral morphology and sedimentation. *Marine Pollution Bulletin*, 125(1–2), 289–300. https://doi.org/10.1016/j.marpolbul.2017.08.036

Erftemeijer, P. L., Riegl, B., Hoeksema, B. W., & Todd, P. A. (2012). Environmental impacts of dredging and other sediment disturbances on corals: a review. *Marine pollution bulletin*, *64*(9), 1737-1765.

Erik, C., Convenor, C., Author, L., Arnaud-haond, S., Bergstad, O., Paula, A., Freiwald, A., & Roberts, J. M. (2017). Cold-Water Corals. The First Global Integrated Marine Assessment, 803–816. https://doi.org/10.1017/9781108186148.052

Fabricius, K. E., & Wolanski, E. (2000). Rapid smothering of coral reef organisms by muddy marine snow. *Estuarine, Coastal and Shelf Science*, *50*(1), 115-120

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Marine Pollution Bulletin*, 50(2), 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Foley, N. (2014). The ecological and economic value of cold-water coral ecosystems. Paper Knowledge . Toward a Media History of Documents, 1–39.

Freiwald, A., & Roberts, J. M. (Eds.). (2006). *Cold-water corals and ecosystems*. Springer Science & Business Media.

Fuchida, S., Yokoyama, A., Fukuchi, R., Ishibashi, J. I., Kawagucci, S., Kawachi, M., & Koshikawa, H. (2017). Leaching of Metals and Metalloids from Hydrothermal Ore Particulates and Their Effects on Marine Phytoplankton. *ACS Omega, 2*(7), 3175–3182. https://doi.org/10.1021/acsomega.7b00081

Gillard, B., Purkiani, K., Chatzievangelou, D., Vink, A., Iversen, M. H., Thomsen, L., & Ackley, S. F. (2019). Physical and hydrodynamic properties of deep sea mining-generated, abyssal sediment plumes in the Clarion Clipperton Fracture Zone (eastern-central Pacific). *Elementa: Science of the Anthropocene*, *7(1)*. https://doi.org/10.1525/elementa.343

Gilmour, J. (1999). Experimental investigation into the effects of suspended sediment on fertilisation, larval survival and settlement in a scleractinian coral. *Marine Biology,* 135(3), 451–462. https://doi.org/10.1007/s002270050645

Glover, A. G., & Smith, C. R. (2003). The deep-sea floor ecosystem: Current status and prospects of anthropogenic change by the year 2025. *Environmental Conservation,* 30(3), 219–241. https://doi.org/10.1017/S0376892903000225

Gollner, S., Kaiser, S., Menzel, L., Jones, D. O. B., Brown, A., Mestre, N. C., van Oevelen, D., Menot, L., Colaço, A., Canals, M., Cuvelier, D., Durden, J. M., Gebruk, A., Egho, G. A., Haeckel, M., Marcon, Y., Mevenkamp, L., Morato, T., Pham, C. K., … Martinez Arbizu, P. (2017). Resilience of benthic deep-sea fauna to mining activities. *Marine Environmental Research,* 129, 76–101. https://doi.org/10.1016/j.marenvres.2017.04.010

Grehan, A., Koslow, T., & Roberts, J. M. (2011). Cold-water Coral Reefs: out of sight - no longer out of mind. UNEP-WCMC Biodiversity Series 22. Cold-Water Coral Reefs: Out of Sight - No Longer out of Mind. *UNEP-WCMC Biodiversity Series 22*. https://doi.org/10.5962/bhl.title.45025

Gwyther. (2008). ENVIRONMENTAL IMPACT STATEMENT Solwara 1 Project. Nautilius Minerals Niugini.

Hannington, M., Jamieson, J., Monecke, T., Petersen, S., & Beaulieu, S. (2011). The abundance of seafloor massive sulfide deposits. Geology, 39(12), 1155–1158. https://doi.org/10.1130/G32468.1

Haugan, P. M., & Levin, L. A. (2020). What Role for Renewable Energy and Deep-Seabed Minerals in a Sustainable Future ? Figure 1, 0–4.

Hein, J. R., Mizell, K., Koschinsky, A., & Conrad, T. A. (2013). Deep-ocean mineral deposits as a source of critical metals for high- and green-technology applications: Comparison with land-based resources. *Ore Geology Reviews, 51*, 1–14. https://doi.org/10.1016/j.oregeorev.2012.12.001

Herring, P. J., Gaten, E., & Shelton, P. M. J. (1999). Are vent shrimps blinded by science? *Nature, 398*(6723), 116. https://doi.org/10.1038/18142

Hildenbrand, A., Weis, D., Madureira, P., & Marques, F. O. (2014). Recent plate re-organization at the Azores Triple Junction: Evidence from combined geochemical and geochronological data on Faial, S. Jorge and Terceira volcanic islands. *Lithos*, 210, 27–39. https://doi.org/10.1016/j.lithos.2014.09.009

Hook, S. E., & Fisher, N. S. (2001). Reproductive toxicity of metals in calanoid copepods. *Marine Biology, 138*(6), 1131–1140. https://doi.org/10.1007/s002270000533

Huang, C. Y. M., & Rasband, M. N. (2018). Axon initial segments: structure, function, and disease. *Annals of the New York Academy of Sciences*, *1420*(1), 46.

IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp

ISBA. (2010). ISBA/16/LTC/7 Legal and Technical Commission Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for polymetallic nodules in the Area 1 Issued by the Legal and Technical Commiss. *International Seabed Authority, 64217*(11), 1–22.

Jones, D. O. B., Kaiser, S., Sweetman, A. K., Smith, C. R., Menot, L., Vink, A., Trueblood, D., Greinert, J., Billett, D. S. M., Arbizu, P. M., Radziejewska, T., Singh, R., Ingole, B., Stratmann, T., SimonLledó, E., Durden, J. M., & Clark, M. R. (2017). Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. *PLoS ONE*, 12(2), e0171750. https://doi.org/10.1371/journal.pone.0171750

Jones, D. O. B., Ardron, J. A., Colaço, A., & Durden, J. M. (2020). Environmental considerations for impact and preservation reference zones for deep-sea polymetallic nodule mining. *Marine Policy, 118*(10), 103312. https://doi.org/10.1016/j.marpol.2018.10.025

Kaiser, S., Smith, C. R., & Arbizu, P. M. (2017). Editorial: Biodiversity of the Clarion Clipperton Fracture Zone. *Marine Biodiversity, 47*(2), 259–264. https://doi.org/10.1007/s12526-017-0733-0

Kersken, D., Janussen, D., & Arbizu, P. M. (2018). Deep-sea glass sponges (Hexactinellida) from polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCFZ), northeastern Pacific: Part I – Amphidiscophora. *Marine Biodiversity, 48*(1), 545–573. https://doi.org/10.1007/s12526- 017-0727-y

Kim, M. G., Hyeong, K., Yoo, C. M., Lee, J. Y., & Seo, I. (2021). Characterization of fines produced by degradation of polymetallic nodules from the clarion–clipperton zone. Minerals, 11(2), 205 https://doi.org/10.3390/min11020205

Koschinsky, A., Fritsche, U., & Winkler, A. (2001). Sequential leaching of Peru Basin surface sediment for the assessment of aged and fresh heavy metal associations and mobility. Deep-Sea Research Part II: *Topical Studies in Oceanography, 48*(17–18), 3683–3699. https://doi.org/10.1016/S0967- 0645(01)00062-5

Kutti, T., Bannister, R. J., Fosså, J. H., Krogness, C. M., Tjensvoll, I., & Søvik, G. (2015). Metabolic responses of the deep-water sponge Geodia barretti to suspended bottom sediment, simulated mine tailings and drill cuttings. *Journal of Experimental Marine Biology and Ecology, 473*, 64–72. https://doi.org/10.1016/j.jembe.2015.07.017

Lake, R. G., & Hinch, S. G. (1999). Acute effects of suspended sediment angularity on juvenile coho salmon (Oncorhynchus kisutch). *Canadian Journal of Fisheries and Aquatic Sciences, 56*(5), 862– 867. https://doi.org/10.1139/f99-024

Lange, E., Petersen, S., Rüpke, L., Söding, E., & Wallmann, K. (2014). Marine Resources - Opportunities and Risks. *World Ocean Review, 3*, 165.

Larsson, A. I., van Oevelen, D., Purser, A., & Thomsen, L. (2013). Tolerance to long-term exposure of suspended benthic sediments and drill cuttings in the cold-water coral Lophelia pertusa. *Marine Pollution Bulletin, 70*(1–2), 176–188. https://doi.org/10.1016/j.marpolbul.2013.02.033

Levin, L. A., & Le Bris, N. (2015). The deep ocean under climate change. *Science*, *350*(6262), 766-768.

Levin, L. A., Baco, A. R., Bowden, D. A., Colaco, A., Cordes, E. E., Cunha, M. R., Demopoulos, A. W. J., Gobin, J., Grupe, B. M., Le, J., Metaxas, A., Netburn, A. N., Rouse, G. W., Thurber, A. R., Tunnicliffe, V., Van Dover, C. L., Vanreusel, A., & Watling, L. (2016a). Hydrothermal vents and methane seeps: Rethinking the sphere of influence. *Frontiers in Marine Science, 3*(5), 72 https://doi.org/10.3389/fmars.2016.00072

Levin, L. A., Mengerink, K., Gjerde, K. M., Rowden, A. A., Van Dover, C. L., Clark, M. R., RamirezLlodra, E., Currie, B., Smith, C. R., Sato, K. N., Gallo, N., Sweetman, A. K., Lily, H., Armstrong, C. W., & Brider, J. (2016b). Defining "serious harm" to the marine environment in the context of deep-seabed mining. *Marine Policy, 74*(9), 245–259. https://doi.org/10.1016/j.marpol.2016.09.032

Levin, L. A., Amon, D. J., & Lily, H. (2020). Challenges to the sustainability of deep-seabed mining. *Nature Sustainability, 3(*10), 784–794. https://doi.org/10.1038/s41893-020-0558-x

Levin, L. A., Wei, C. L., Dunn, D. C., Amon, D. J., Ashford, O. S., Cheung, W. W. L., Colaço, A., Dominguez-Carrió, C., Escobar, E. G., Harden-Davies, H. R., Drazen, J. C., Ismail, K., Jones, D. O. B., Johnson, D. E., Le, J. T., Lejzerowicz, F., Mitarai, S., Morato, T., Mulsow, S., … Yasuhara, M. (2020). Climate change considerations are fundamental to management of deep-sea resource extraction. *Global Change Biology, 26*(9), 4664–4678. https://doi.org/10.1111/gcb.15223

Liefmann, S., Järnegren, J., Johnsen, G., & Murray, F. (2018). Eco-physiological responses of cold-water soft corals to anthropogenic sedimentation and particle shape. *Journal of Experimental Marine Biology and Ecology,* 504(2), 61–71. https://doi.org/10.1016/j.jembe.2018.02.009

Linke, P., Haeckel, M. (2019). Short Cruise Report . RV SONNE SO268/1+2. https://www.ldf.uni-hamburg.de/sonne/wochenberichte/wochenberichte-sonne/so267-2-268-3/so268-scr.pdf

Lirman, D., & Manzello, D. (2009). Patterns of resistance and resilience of the stress-tolerant coral Siderastrea radians (Pallas) to sub-optimal salinity and sediment burial. *Journal of Experimental Marine Biology and Ecology*, *369*(1), 72-77.

Lopes, C. L., Bastos, L., Caetano, M., Martins, I., Santos, M. M., & Iglesias, I. (2019). Development of physical modelling tools in support of risk scenarios: A new framework focused on deep-sea mining. *Science of the Total Environment, 650,* 2294–2306. https://doi.org/10.1016/j.scitotenv.2018.09.351

Lourenço, N. L. J. F., Miranda, J. M., Luis, J. F., Ribeiro, A., Victor, L. M., Madeira, J., & Needham, H. D. (1998). Morpho-tectonic analysis of the Azores Volcanic Plateau from a new bathymetric compilation of the area. *Marine Geophysical Researches*, *20*(3), 141-156. https://doi.org/10.1023/A:1004505401547

Lydersen, E., Löfgren, S., & Arnesen, R. T. (2002). Metals in Scandinavian surface waters: Effects of acidification, liming, and potential reacidification. *Critical Reviews in Environmental Science and Technology,* 32(2–3), 73–295. https://doi.org/10.1080/10643380290813453

Marques, J. A., Abrantes, D. P., Marangoni, L. F., & Bianchini, A. (2020). Ecotoxicological responses of a reef calcifier exposed to copper, acidification and warming: A multiple biomarker approach. *Environmental Pollution*, *257*, 113572.

Martins, A. Q., & Europeia, U. (2016). E s t a t í s t i c a A p l i c a d a c o m o S . P . S . S . June. https://doi.org/10.13140/RG.2.1.3571.9282

Martins, I., Goulart, J., Martins, E., Morales-Román, R., Marín, S., Riou, V., Colaço, A., & Bettencourt, R. (2017). Physiological impacts of acute Cu exposure on deep-sea vent mussel Bathymodiolus azoricus under a deep-sea mining activity scenario. *Aquatic Toxicology,* 193(5), 40–49. https://doi.org/10.1016/j.aquatox.2017.10.004

Martins, I., Godinho, A., Goulart, J., & Carreiro-Silva, M. (2018). Assessment of Cu sub-lethal toxicity (LC50) in the cold-water gorgonian Dentomuricea meteor under a deep-sea mining activity scenario. *Environmental Pollution, 2*40, 903–907. https://doi.org/10.1016/j.envpol.2018.05.040

McGrath, E. C., Smith, D. J., Jompa, J., & Bell, J. J. (2017). Adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges. *Journal of Experimental Marine Biology and Ecology*, *496*, 74-83.

Menendez, A., James, R.H., Lichtschlag, A., Connelly, D., Peel, K. (2019). Controls on the chemical composition of ferromanganese nodules in the Clarion-Clipperton Fracture Zone, eastern equatorial Pacific. *Marine Geology 409*, 1–14.

Mestre, N. C., Calado, R., & Soares, A. M. V. M. (2014). Exploitation of deep-sea resources: The urgent need to understand the role of high pressure in the toxicity of chemical pollutants to deep-sea organisms. *Environmental Pollution,* 185, 369–371. https://doi.org/10.1016/j.envpol.2013.10.021

Mewes, K., Mogollón, J. M., Picard, A., Rühlemann, C., Kuhn, T., Nöthen, K., & Kasten, S. (2014). Impact of depositional and biogeochemical processes on small scale variations in nodule abundance in the Clarion-Clipperton Fracture Zone. Deep-Sea Research Part I: *Oceanographic Research Papers, 91*, 125–141. https://doi.org/10.1016/j.dsr.2014.06.001

Miller, K. A., Thompson, K. F., Johnston, P., & Santillo, D. (2018). An overview of seabed mining including the current state of development, environmental impacts, and knowledge gaps. *Frontiers in Marine Science, 4*(1), 418. https://doi.org/10.3389/fmars.2017.00418

Morato, T., Machete, M., Kitchingman, A., Tempera, F., Lai, S., Menezes, G., Pitcher, T. J., & Santos, R. S. (2008). Abundance and distribution of seamounts in the Azores. *Marine Ecology Progress Series,* 357, 17–21. https://doi.org/10.3354/meps07268

Myriam Lacharite. (2012). Lasp-1 Regulates Podosome Function. *PloS One,* 7(4), 1–10. https://doi.org/10.1371/Citation

Naumann, U. (2011). *The art of differentiating computer programs: an introduction to algorithmic differentiation*. Society for Industrial and Applied Mathematics.

Niyogi, S., & Wood, C. M. (2004). Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environmental Science and Technology*, 38(23), 6177–6192. https://doi.org/10.1021/es0496524

Oebius, H. U., Becker, H. J., Rolinski, S., & Jankowski, J. A. (2001). Parametrization and evaluation of marine environmental impacts produced by deep-sea manganese nodule mining. Deep-Sea Research Part II: *Topical Studies in Oceanography, 4*8(17–18), 3453–3467. https://doi.org/10.1016/S0967-0645(01)00052-2

Paulus, E. (2021). Shedding Light on Deep-Sea Biodiversity—A Highly Vulnerable Habitat in the Face of Anthropogenic Change. *Frontiers in Marine Science*, *8*, 281.

Petersen, Sven, & Hein, J. R. (2013). The Geology of Sea-Floor Massive Sulphides. In Deep Sea Minerals; *Sea-Floor Massive Sulphides*. http://dsm.gsd.spc.int/public/files/meetings/TrainingWorkshop4/UNEP_vol1A.pdf

Petersen, S., Krätschell, A., Augustin, N., Jamieson, J., Hein, J. R., & Hannington, M. D. (2016). News from the seabed – Geological characteristics and resource potential of deep-sea mineral resources. *Marine Policy, 70*, 175–187. https://doi.org/10.1016/j.marpol.2016.03.012

Petersen, S., Lehrmann, B., & Murton, B. J. (2018). Modern seafloor hydrothermal systems: new perspectives on ancient ore-forming processes. *Elements: An International Magazine of Mineralogy, Geochemistry, and Petrology*, *14*(5), 307-312.https://doi.org/10.2138/gselements.14.5.307

Pham, C. K., Diogo, H., Menezes, G., Porteiro, F., Braga-Henriques, A., Vandeperre, F., & Morato, T. (2014). Deep-water longline fishing has reduced impact on Vulnerable Marine Ecosystems. *Scientific Reports, 4,* 1–6. https://doi.org/10.1038/srep04837

Philipp, E., & Fabricius, K. (2003). Photophysiological stress in scleractinian corals in response to short-term sedimentation. *Journal of Experimental Marine Biology and Ecology, 287*(1), 57–78. https://doi.org/10.1016/S0022-0981(02)00495-1

Pinheiro, M., Oliveira, A., Barros, S., Alves, N., Raimundo, J., Caetano, M., Coimbra, J., Neuparth, T., & Santos, M. M. (2021). Functional, biochemical and molecular impact of sediment plumes from deep-sea mining on Mytilus galloprovincialis under hyperbaric conditions. *Environmental Research, 195*, 110753. https://doi.org/10.1016/j.envres.2021.110753

Portail, M., Brandily, C., Cathalot, C., Colaço, A., Gélinas, Y., Husson, B., Sarradin, P. M., & Sarrazin, J. (2018). Food-web complexity across hydrothermal vents on the Azores triple junction. Deep-Sea Research Part I: *Oceanographic Research Papers, 131*(4), 101–120. https://doi.org/10.1016/j.dsr.2017.11.010

Ragnarsson, S. Á., Burgos, J. M., Kutti, T., van den Beld, I., Egilsdóttir, H., Arnaud-Haond, S., & Grehan, A. (2017). The impact of anthropogenic activity on cold-water corals. *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*, 989-102.

Rakka, M., Maier, S. R., Van Oevelen, D., Godinho, A., Bilan, M., Orejas, C., & Carreiro-Silva, M. (2021). Contrasting metabolic strategies of two co-occurring deep-sea octocorals. *Scientific reports*, *11*(1), 1-12.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., Mol, B. D., Escobar, E., German, C. R., & Vecchione, M. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences*, *7*(9), 2851-2899.

Ramirez-Llodra, E., Tyler, P. A., Baker, M. C., Bergstad, O. A., Clark, M. R., Escobar, E., Levin, L. A., Menot, L., Rowden, A. A., Smith, C. R., & van Dover, C. L. (2011). Man and the last great wilderness: Human impact on the deep sea. *PLoS ONE, 6*(8), e22588. https://doi.org/10.1371/journal.pone.0022588

Ramirez-Llodra, E., Trannum, H. C., Evenset, A., Levin, L. A., Andersson, M., Finne, T. E., Hilario, A., Flem, B., Christensen, G., Schaanning, M., & Vanreusel, A. (2015). Submarine and deep-sea mine tailing placements: A review of current practices, environmental issues, natural analogs and knowledge gaps in Norway and internationally. *Marine Pollution Bulletin, 97*(1–2), 13–35. https://doi.org/10.1016/j.marpolbul.2015.05.062

Ramirez-Llodra, E. (2020). "Chapter 2 Deep-Sea Ecosystems: Biodiversity and Anthropogenic Impacts". In *The Law of the Seabed*. Leiden, The Netherlands: Brill | Nijhoff. doi: https://doi.org/10.1163/9789004391567_004

Rasband, W. S. (2018). ImageJ, U. S. Bethesda, MD: National Institutes of Health.

Riegl, B. (1995). Effects of sand deposition on scleractinian and alcyonacean corals. *Marine Biology*, *121*(3), 517-526.

Riegl, B., & Branch, G. M. (1995). Effects of sediment on the energy budgets of four scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. *Journal of Experimental Marine Biology and Ecology*, *186*(2), 259-275.

Roberts, J. M., Wheeler, A. J., & Freiwald, A. (2006). Reefs of the deep: The biology and geology of cold-water coral ecosystems. *Science, 312*(5773), 543–547. https://doi.org/10.1126/science.1119861

Rogers, C. S. (1990). Responses of coral reefs and reef organisms to sedimentation. Marine Ecology Progress Series, *Oldendorf*, *62*(1), 185–202. https://doi.org/10.3354/meps062185

Rogers, A. D. (1999). The biology of Lophelia pertusa (Linnaeus 1758) and other deep-water reef-forming corals and impacts from human activities. *International Review of Hydrobiology, 84*(4), 315–406. https://doi.org/10.1002/iroh.199900032

Rossi, S., Bramanti, L., Gori, A., & Orejas, C. (2017). An overview of the animal forests of the world. *Marine Animal Forest, Rossi, S., Springer*, 1-25.

Rye, H., Reed, M., Frost, T. K., Smit, M. G. D., Durgut, I., Johansen, O., & Ditlevsen, M. K. (2008). Development of a numerical model for calculating exposure to toxic and nontoxic stressors in the water column and sediment from drilling discharges. *Integrated Environmental Assessment and Management, 4*(2), 194–203. https://doi.org/10.1897/IEAM_2007-039.1

Sampaio, I., Braga-Henriques, A., Pham, C., Ocaña, O., de Matos, V., Morato, T.,et al. (2012). Cold-water corals landed by bottom longline fishery in the Azores,J. Mar. *Biol. Assoc*. UK92, 1547–1555. doi: 10.1017/S0025315412000045

Scanes, E., Kutti, T., Fang, J. K. H., Johnston, E. L., Ross, P. M., & Bannister, R. J. (2018). Mine waste and acute warming induce energetic stress in the deep-sea sponge Geodia atlantica and coral Primnoa resedeaformis; results from a mesocosm study. *Frontiers in Marine Science, 5,* 129.https://doi.org/10.3389/fmars.2018.00129

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), 671-675.

Sharma, R., Nagender Nath, B., Parthiban, G., & Jai Sankar, S. (2001). Sediment redistribution during simulated benthic disturbance and its implications on deep seabed mining. Deep-Sea Research Part II: *Topical Studies in Oceanography*, 48(16), 3363–3380. https://doi.org/10.1016/S0967- 0645(01)00046-7

Sharma, R. (2017). Deep-sea mining: Resource potential, technical and environmental considerations. Deep-Sea Mining: *Resource Potential, Technical and Environmental Considerations, 1*–535. https://doi.org/10.1007/978-3-319-52557-0

Somayajulu, B. L. K. (2000). Growth rates of oceanic manganese nodules: implications to their genesis, palaeo-earth environment and resource potential. *Current Science (Bangalore)*, *78*(3), 300-308.

Steiner, R. (2009). independent review of the environmental impact statement for the proposed nautilus minerals solwara 1 seabed mining project, Papua New Guinea. *Report for the Bismarck-Solomon Seas Indigenous Peoples Council (Madang)*.

Stölting, M., Wiesner, C., van Vliet, V., Butt, E., Pavenstädt, H., Linder, S., & Kremerskothen, J. (2012). Lasp-1 regulates podosome function. *PLoS One*, *7*(4), e35340.

Telesnicki, G. J., & Goldberg, W. M. (1995). Effects of turbidiy on the photosynthesis and respiration of two south Florida reef coral species. *Bulletin of Marine Science*, *57*(2), 527-539.

Tempera, F., Atchoi, E., Amorim, P., Gomes-Pereira, J., & Gonçalves, J. M. S. (2013). Atlantic Area Marine Habitats: *Adding new Macaronesian habitat types from the Azores to the EUNIS Habitat Classification*. https://doi.org/10.13140/RG.2.1.3479.8808

Thiel, H. (2003). Anthropogenic impacts on the deep sea. *Ecosystems of the Deep Oceans, 427*–471. http://cmbc.ucsd.edu/Students/Current_Students/SIO277/ch13.pdf

Thurber, A. R., Sweetman, A. K., Narayanaswamy, B. E., Jones, D. O. B., Ingels, J., & Hansman, R. L. (2014). Ecosystem function and services provided by the deep sea. *Biogeosciences, 11(*14), 3941– 3963. https://doi.org/10.5194/bg-11-3941-2014

Van Dover, C. L. (2014). Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: A review. *Marine Environmental Research, 102*, 59–72. https://doi.org/10.1016/j.marenvres.2014.03.008

Van Dover, C. L. (2019). Inactive sulfide ecosystems in the deep sea: A review. *Frontiers in Marine Science, 6,* 461 https://doi.org/10.3389/fmars.2019.00461

Vanreusel, A., Hilario, A., Ribeiro, P. A., Menot, L., & Arbizu, P. M. (2016). Threatened by mining, polymetallic nodules are required to preserve abyssal epifauna. *Scientific Reports, 6,* 1–6. https://doi.org/10.1038/srep26808

Walters, K., & Coen, L. D. (2006). A comparison of statistical approaches to analyzing community convergence between natural and constructed oyster reefs. *Journal of Experimental Marine Biology and Ecology*, *330*(1), 81-95.

Wang, X., & Müller, W. E. G. (2009). Marine biominerals: perspectives and challenges for polymetallic nodules and crusts. *Trends in Biotechnology, 27*(6), 375–383. https://doi.org/10.1016/j.tibtech.2009.03.004

Washburn, T. W., Turner, P. J., Durden, J. M., Jones, D. O. B., Weaver, P., & Van Dover, C. L. (2019). *Ecological risk assessment for deep-sea mining. Ocean and Coastal Management, 176*, 24–39. https://doi.org/10.1016/j.ocecoaman.2019.04.014

Watling, L., France, S. C., Pante, E., & Simpson, A. (2011). Biology of deep-water octocorals. *Advances in marine biology*, *60*, 41-122.

Weber, M., Lott, C., & Fabricius, K. E. (2006). Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. *Journal of Experimental Marine Biology and Ecology, 336*(1), 18–32. https://doi.org/10.1016/j.jembe.2006.04.007

Williams, A., Schlacher, T. A., Rowden, A. A., Althaus, F., Clark, M. R., Bowden, D. A., Stewart, R., Bax, N. J., Consalvey, M., & Kloser, R. J. (2010). Seamount megabenthic assemblages fail to recover from trawling impacts. *Marine Ecology, 31*, 183–199. https://doi.org/10.1111/j.1439-0485.2010.00385.x

Yesson, C., Taylor, M. L., Tittensor, D. P., Davies, A. J., Guinotte, J., Baco, A., Black, J., Hall-Spencer, J. M., & Rogers, A. D. (2012). Global habitat suitability of cold-water octocorals. *Journal of Biogeography, 39*(7), 1278–1292. https://doi.org/10.1111/j.1365-2699.2011.02681.x

List of supplementary material

Supplementary material 1

Table with PERMANOVA of the seawater quality measurements over the experimental time. For data analysis was necessary to divide the times into before (T0-T5) and after (T6-T28) the end of the PMS treatment.

	Effect	df	SS	MS	PseudoF	P(perm)	Unique perms	P(MC)
Salinity								
Treatment	Fixed	3	0,25	8,33E-02	8,1081	0,0017	3172	0,0009
Time	Fixed	2	0,18056	9,03E-02	8,7838	0,0012	2905	0,0015
TreatmentxTime	Fixed	6	0,22833	3,81E-02	3,7027	0,0109	6587	0,0092
Residual	Random	24	0,24667	1,03E-02				
Total		35	0,90556					
TºC (T0-T5)								
Treatment	Fixed	5	8,5374	1,7075	215,03	0,0001	9956	0,0001
Time	Fixed	5	0,25319	5,06E-02	6,3769	0,0002	9944	0,0001
TreatmentxTime	Fixed	25	0,62215	2,49E-02	3,134	0,0001	9914	0,0001
Residual	Random	72	0,57173	7,94E-03				
Total		107	9,9845					
TºC (T6-T28)								
Treatment	Fixed	3	6,6998	2,2333	1027,3	0,0001	9780	0,0001
Time	Fixed	22	3,9241	0,17837	82,048	0,0001	9654	0,0001
TreatmentxTime	Fixed	66	2,971	4,50E-02	20,707	0,0001	9852	0,0001
Residual	Random	184	0,4	2,17E-03				
Total		275	13,995					
pH (T0-T5)								
Treatment	Fixed	5	1,25E-02	2,50E-03	28,76	0,0001	9079	0,0001
Time	Fixed	5	1,37E-02	2,74E-03	31,517	0,0001	9057	0,0001
TreatmentxTime	Fixed	25	1,37E-02	5,48E-04	6,2915	0,0001	9278	0,0001
Residual	Random	72	6,27E-03	8,70E-05				
Total		107	4,62E-02					
pH (T6-T28)								
Treatment	Fixed	3	4,84E-04	1,61E-04	11,133	0,0001	6992	0,0001
Time	Fixed	22	2,48E-02	1,13E-03	77,675	0,0001	6495	0,0001
TreatmentxTime	Fixed	66	4,80E-03	7,27E-05	5,0174	0,0001	9675	0,0001
Residual	Random	184	2,67E-03	1,45E-05				
Total		275	3,27E-02					
O2 (T0-T5)								
Treatment	Fixed	5	176,62	35,323	22,768	0,0001	9945	0,0001
Time	Fixed	4	94,242	23,56	15,186	0,0001	9945	0,0001
TreatmentxTime	Fixed	20	71,801	3,59	2,314	0,0054	9915	0,0064
Residual	Random	60	93,087	1,5514				
Total		89	435,75					
O2 (T6-T28)								
Treatment	Fixed	3	58829	19610	1,014	0,2826	9003	0,3901
Time	Fixed	22	4,40E+05	19985	1,0334	0,0002	7805	0,427
TreatmentxTime	Fixed	66	1,28E+06	19337	0,99986	0,5249	6918	0,49

Residual	Random	184	3,56E+06	19340				
Total		275	5,33E+06					
Nitrate								
Treatment	Fixed	3	1,1496	0,38319	5,97E-02	0,9823	9961	0,9792
Time	Fixed	2	80,816	40,408	6,2963	0,0055	9953	0,006
TreatmentxTime	Fixed	6	34,46	5,7433	0,8949	0,5058	9950	0,5162
Residual	Random	24	154,03	6,4178				
Total		35	270,45					
Ammonia								
Treatment	Fixed	3	36,961	12,32	2,7588	0,0489	9960	0,0637
Time	Fixed	2	6,9634	3,4817	0,77962	0,4924	9956	0,4712
TreatmentxTime	Fixed	6	53,974	8,9957	2,0143	0,0825	9950	0,098
Residual	Random	24	107,18	4,4659				
Total		35	205,08					
Phosphate								
Treatment	Fixed	3	0,75619	0,25206	23,68	0,0001	9945	0,0001
Time	Fixed	2	0,64752	0,32376	30,416	0,0001	9970	0,0001
TreatmentxTime	Fixed	6	0,30793	5,13E-02	4,8214	0,0029	9940	0,0023
Residual	Random	24	0,25547	1,06E-02				
Total		35	1,9671					

Supplementary material 2

Table with linear regression analyses of the sediment concentrations ($mg.L^{-1}$) for each treatment over the experimental time.

	Unstandardized Coefficients B	Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
Control 1					
(Constant)	5,431	0,978		5,551	0
Time	0,111	0,059	0,35	1,869	0,073
Control 2					
(Constant)	5,192	0,642		8,085	0
Time	0,068	0,039	0,33	1,748	0,093
NFS 10					
(Constant)	7,746	0,49		15,794	0
Time	0,094	0,03	0,536	3,175	0,004
NFS 50					
(Constant)	39,175	4,473		8,758	0
Time	0,167	0,271	0,123	0,618	0,542
PMS 10					
(Constant)	7,944	1,359		5,846	0,004
Time	0,122	0,466	0,13	0,262	0,806
PMS 50					
(Constant)	29,178	2,161		13,502	0
Time	2,556	0,741	0,865	3,448	0,026