Deep–Sea Research I 208 (2024) 104311

Contents lists available at ScienceDirect

Deep-Sea Research Part I

journal homepage: www.elsevier.com/locate/dsri

Adverse effects of crushed seafloor massive sulphide deposits on the boreal deep-sea sponge *Geodia barretti* Bowerbank, 1858 and its associated fauna

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ARTICLE INFO

Keywords:
Deep-sea mining
Environmental impact
Plumes
sponge grounds
Benthic-pelagic coupling
Geodia barretti

ABSTRACT

Abundant mineral resources in the deep sea are prospected for mining for the global metal market. Seafloor massive sulphide (SMS) deposits along the Mid-Atlantic Ridge are one of the potential sources for these metals. The extraction of SMS deposits will expose adjacent marine ecosystems to suspended particle plumes charged with elevated concentrations of heavy metals and other potentially toxic compounds. Up to date there is no information about the impact of mining activities on deep-sea benthic ecosystems such as abundant deep-sea sponge grounds in the North Atlantic Ocean. Sponge grounds play a major role in benthic-pelagic coupling and represent an important habitat for a diversity of vertebrates, invertebrates and microorganisms. To simulate the effects of mining plumes on benthic life in the deep sea, we exposed *Geodia barretti*, a dominant sponge species in the North Atlantic Ocean, and an associated brittle star species from the genus *Ophiura* spp. to a field-relevant concentration of 30 mg L^{-1} suspended particles of crushed SMS deposits. Three weeks of exposure to suspended particles of crushed SMS resulted in a tenfold higher rate of tissue necrosis in sponges. All brittle stars in the experiment perished within ten days of exposure. SMS particles were evidently accumulated in the sponge's mesohyl and concentrations of iron and copper were 10 times elevated in SMS exposed individuals. Oxygen consumption and clearance rates were significantly retarded after the exposure to SMS particles, hampering the physiological performance of *G. barretti*. These adverse effects of crushed SMS deposits on *G. barretti* and its associated brittle star species potentially cascade in disruptions of benthic-pelagic coupling processes in the deep sea. More elaborate studies are advisable to identify threshold levels, management concepts and mitigation measures to minimize the impact of deep-sea mining plumes on benthic life.

1. Introduction

The deep sea, earth's largest ecosystem (Ramirez-Llodra et al., 2010), is known to host a high biological diversity and the discovery of new habitats and life forms is increasingly gaining momentum with current technological advances (Cunha et al., 2017). New species and ecosystems are being described continuously in the deep sea (Danovaro et al., 2014). Simultaneously, economic interests in lucrative metal deposits are pushing extractive industries into the earth's vastest ecosystem. A spiked interest in mining the untapped oceanic metal resources found in seafloor massive sulphide (SMS) deposits (Hoagland et al., 2010) has emerged in recent years as a consequence of a growing demand and increased metal prices (Watzel et al., 2020). Often, mineral deposits of economic value are providing a habitat for highly specialized deep-sea fauna (Purser et al., 2016; Van Dover et al., 2018). Thus, the extraction of minerals from the seafloor is posing a potential threat to the abundant deep-sea fauna (Washburn et al., 2019).

SMS deposits form on and below the seabed from high-temperature (up to 400 °C), metal loaded hydrothermal fluids in various tectonic settings (e.g. Hoagland et al., 2010; Pedersen et al., 2013). At the seabed, the hydrothermal fluids mix with the cold seawater. This leads to precipitation of sulphide minerals forming chimney structures on the seabed and particle-loaded plumes of fluids emanating from the chimneys. Surrounding the hydrothermal vents, accumulated fall-out

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https://doi.org/10.1016/j.dsr.2024.104311
Received 22 February 2023; Received in revised form 29 March 2024; Accepted 24 April 2024
Available online 30 April 2024

material from the plumes and collapsed chimney structures form SMS deposits of various sizes and composition (Humphris et al., 1995; Hannington et al., 1998; Murton et al., 2019). During mining operations, these deposits will be crushed and ground before the SMS-rich slurry is pumped up into a transport vessel. After extracting minerals from the pumped up ore it is proposed to release the remaining material in the water column close to the mining site (Gwyther, 2008). Up to date limited information is available about the details of the particle size distribution of the waste effluent, at what depth waste effluent will be released and how this material would disperse as function of distance from the seafloor and local current regimes. These parameters will be dependent on the local conditions at the mining site but no references are available up to date as deep-sea mining has not yet happened. From a first attempt to mine seafloor massive sulphide deposits it is estimated that particles as small as 8–10 μm will be released relatively close to the seafloor. The natural background concentration of suspended particles in the benthic boundary layer has been shown to be as low as 0.05–0.08 mg L^{-1} (Starodymova et al., 2023) and will most likely be exceeded by a few orders of magnitude with SMS mining activities (Gwyther, 2008; Washburn et al., 2019). Indirect effects such as particle sedimentation or the presence of suspended waste material in the water column are suggested to exceed the local mining footprint by a few orders of magnitude (Mingotti and Woods, 2020; Muñoz-Royo et al., 2021; Morato et al., 2022). Thus, indirect mining impacts affect a much larger area compared to the direct effect of sole removal of hard substrate at the location of the mining process (Washburn et al., 2019; Muñoz-Royo et al., 2021; Morato et al., 2022). In addition to particle plumes, the mobilization and weathering of sulphides is expected to release heavy metals and protons into the environment (Hu et al., 2022). The environmental impacts of the particles released by mining of SMS deposits on abundant deep-sea fauna remain largely unknown (Boschen et al., 2016).

In the North Atlantic Ocean (NAO), SMS deposits are distributed in areas with an abundant biodiversity of deep-sea fauna (Dunn et al., 2018; Cowart et al., 2020). Large areas identified as biological hotspots in the deep NAO have been found to be dominated by species of one particular animal phylum: sponges (Porifera) (Klitgaard et al., 1997). These areas, where sponges can represent up to 95 % of the benthic biomass (Maldonado et al., 2017), have been termed sponge grounds (Klitgaard and Tendal, 2004). Sponge grounds alter the physicochemical properties of the deep sea (Hanz et al., 2021), provide three dimensional microhabitats (Hogg et al., 2010) and contribute to local biodiversity (Hawkes et al., 2019). Sponges are highly efficient filter feeders (Maldonado et al., 2012) and in addition to particulate organic matter (POM) they can metabolize dissolved organic matter (DOM), an organic matter source most deep-sea fauna cannot utilize (Kazanidis et al., 2018). The retention of carbon via the so called sponge loop (De Goeij et al., 2013) has recently been described to fuel complex food webs in sponge grounds in the NAO (Bart et al., 2021). Deposit-feeding on particulate excretions of sponges or predation on sponges can channel energy to higher trophic levels such as echinoderms or anthozoans (Maier et al., 2020; Bart et al., 2021; Hanz et al., 2021). This trophic interaction highlights the ecological function of sponge-driven nutrient cycling and makes sponge grounds hotspots of benthic-pelagic coupling processes (Maldonado et al., 2017).

Industrial activities such as drilling for fossil energy resources (Fang et al., 2018), bottom trawling (Wurz et al., 2021) or mine waste disposal (Scanes et al., 2018) are impacting sponge grounds throughout the NAO. Sponges also have been shown to be abundant in areas of potential interest for mining operations along the Mid-Atlantic Ridge (Gebruk et al., 2010; Keogh et al., 2022). The demosponge species *Geodia barretti* Bowerbank, 1858 is one of the dominating species in sponge grounds throughout the NAO (Klitgaard and Tendal, 2004; Cárdenas and Rapp, 2015). Further this sponge species can be kept in aquaria facilities which makes *G. barretti* a suitable candidate for *ex situ* experimentation to assess the coping potential of deep-sea sponges to anthropogenic stressors (Hoffmann et al., 2005; Tjensvoll et al., 2013; Fang et al., 2018; Leys et al., 2018; Bart et al., 2020a, 2020b). The described effects of indigestible, suspended particles on *G. barretti* include sub-lethal responses such as increased oxygen consumption (Tjensvoll et al., 2013) and reduced lysosomal membrane stability (Edge et al., 2016). Crucial in the severity of responses seems to be the composition of particles that *G. barretti* is exposed to. While natural sediment was tolerated by *G. barretti* in concentrations up to 500 mg L^{-1}, concentrations of 10–100 mg L^{-1} of the minerals barite and bentonite (representative for *in situ* drilling operations), used in oil and gas extraction (Neff, 2005), cumulated in compromised cellular viability in this abundant deep-sea sponge (Edge et al., 2016). Mining of SMS deposits along the Mid-Atlantic Ridge could generate large volumes of suspended particle plumes consisting of particles as small as 8 μm (Gwyther, 2008) that can be dispersed over a vast area. These μm sized particles can be ingested by sponges that are filter-feeding on 1–10 μm sized bacterioplankton (Maldonado et al., 2012). While some studies suggest coping mechanisms to egest indigestible particles in some sponge species (Strehlow et al., 2017), Wurz et al. (2021) showed that long-term exposure (three weeks) to suspended natural sediment can have adverse effects on sponge physiology. Given the high levels of potentially toxic compounds in SMS deposits, exposure to this specific type of particles may intensify those adverse effects. To our knowledge, the effects of SMS particle plumes on filter-feeding sponges and their benthic-pelagic coupling capacities in deep-sea ecosystems have not been studied up to date.

The interaction of an extractive mining industry with abundant deep-sea fauna is of great concern across the scientific community (Washburn et al., 2019). Recent environmental impact assessments performed by revenue driven corporations fail to deliver insights into mining impacts on benthic fauna (Jaeckel, 2020; Tunnicliffe et al., 2020), including abundant, filter-feeding sponges and their associated fauna. Mining operations licensed by the international seabed authority (ISA) are likely to last for decades in prospected areas (Willaert, 2020), exceeding any *ex situ* experimentation capacity to study the effects of mining operations on marine fauna. Therefore, sub chronic studies on the physiological responses of abundant deep-sea fauna to mining impacts are necessary (Jaeckel, 2020; Hitchin et al., 2023). Although studies including a variety of particle sources (natural sediment (Tjensvoll et al., 2013), drilling muds (Scanes et al., 2018) suggest that *G. barretti* might be increasingly susceptible to the continuous presence of indigestible particles in seawater, these studies do not include particles from SMS deposits containing metal sulphides and other potentially toxic compounds. Furthermore, it is unknown if SMS particles accumulate in sponge tissues with potential consequences for oxygen consumption, bacterioplankton clearance rates and the composition of the abundant sponge associated microbiome (Leys et al., 2018). It remains unknown how sponge associated detritus feeders such as brittle stars, key players in transferring sponge derived carbon sources to higher trophic levels (Maier et al., 2020), respond to the exposure to mining plumes. Such data will be important for informed guidance and decision-making by the International Seabed Authority to fulfill its obligations to protect and preserve the marine environment from serious harm (Lodge and Verlaan, 2018).

For the first time, we exposed a deep-water sponge and an associated brittle star genus to a field-relevant concentration of crushed SMS deposits, mimicking plumes generated by deep-sea mining activities. Initially, this study had been set up to investigate effects of crushed SMS deposits on sponges under current ocean conditions and under a predicted scenario for future (year 2100) ocean conditions in the deep NAO: a drop in pH of 0.3 units and an increase in temperature of 4 °C (Sweetman et al., 2017). Since no effects of pH and temperature on basic sponge functioning (survival, oxygen consumption rate and uptake of bacteria) could be demonstrated, neither before (Wurz et al., 2024), nor after exposure to crushed SMS deposit, this paper focuses primarily on the effects of crushed SMS deposits on sponges and an associated genus of brittle stars.

In this study an *ex situ* approach was applied to investigate the effects of three weeks of exposure to crushed SMS particles (30 mg L^{-1}) on survival, oxygen consumption and bacterioplankton clearance rate of the deep-sea sponge *G. barretti* and on the survival of an associated brittle star species from the genus *Ophiura*. In addition, bioaccumulation of metals was assessed in sponge and brittle star tissue and the associated microbiome of *G. barretti* was investigated across different treatments.

2. Materials and methods

2.1. Collection of experimental animals

Individuals of *G. barretti* used in this experiment were collected in 2017 and 2018 by the remotely operated vehicle (ROV) ÆGIR6000 deployed from the research vessel *G.O. Sars*. Collection of sponges took place in the area Tromsøflaket East (71°35′20.4″N 21°22′35.4″E) at a depth of 330 m. Usually, individuals of *G. barretti* with a diameter of up to 10 cm were found to grow on small rocks on the soft sediment. These pebbles were grabbed by the ROV's manipulator to gently transfer the sponge into a collection box fitted to the ROV. Only undamaged individuals were stored in the collection box and brought to the surface. During sampling, resuspension of sediment was kept at a minimum. At the surface, sponges stayed submerged in seawater at all times in the ROV's collection box until they were transferred to the flow-through aquaria facilities onboard *G.O. Sars*. Temperature controlled (7 °C) seawater from 6 m depth was pumped into a header tank and from here distributed via gravitational force to multiple 40 L tanks (20 L h^{-1}) in which sponges were kept in the dark throughout the cruise. After the cruise, sponges were transferred to the laboratory facilities of the University of Bergen, Norway. Here they were kept in 40 L tanks supplied with sand bed filtered seawater from a depth of 200 m from the adjacent fjord (Fig. 1) at a flow rate of 1 L min^{-1}. No additional food was added. Each tank was holding two to three sponges. During sampling with the ROV, brittle stars were observed to be associated with *G. barretti*. To mimic this potential species-species interaction we introduced brittle stars from the genus *Ophiura* to each of the tanks. All brittle stars were collected close to Bergen, Norway (60°12′07.6″N 5°02′22.7″E) at 30 m of depth.

2.2. Experimental design

The sponges collected in 2017 had been kept for 273 days under four different seawater regimes (Control: 6.5 °C, pH 8.1; low pH: 6.5 °C, pH 7.8; high temperature: 10.5 °C, pH 8.1; low pH + high temperature: 10.5 °C, pH 7.8) prior to the crushed SMS deposit exposure. During this pre-acclimatisation phase, no treatment effects on the response variables (oxygen consumption and bacterial clearance rate) were evident (Wurz et al., 2024). The 22 pre-incubated sponges were distributed over 8 tanks (6 tanks with n = 3 sponges, 2 tanks with n = 2 sponges) in which seawater conditions were kept similar to the preceding maintenance period. All 8 tanks received dosages of crushed SMS deposit as described below. Each tank from this SMS exposure group was also holding three brittle stars from the genus *Ophiura*. These brittle stars were present during the complete 273 days of pre-acclimatisation. In addition, six sponges (collected in 2018) were divided over three additional tanks, holding two sponge individuals each. These sponges were not dosed with crushed SMS deposit and did not contain brittle stars. Due to logistical limitations it was not possible to collect brittle stars to be added to the control tanks. *G. barretti* is maintained in the aquaria facility of the Bergen University for years without brittle stars, hence we

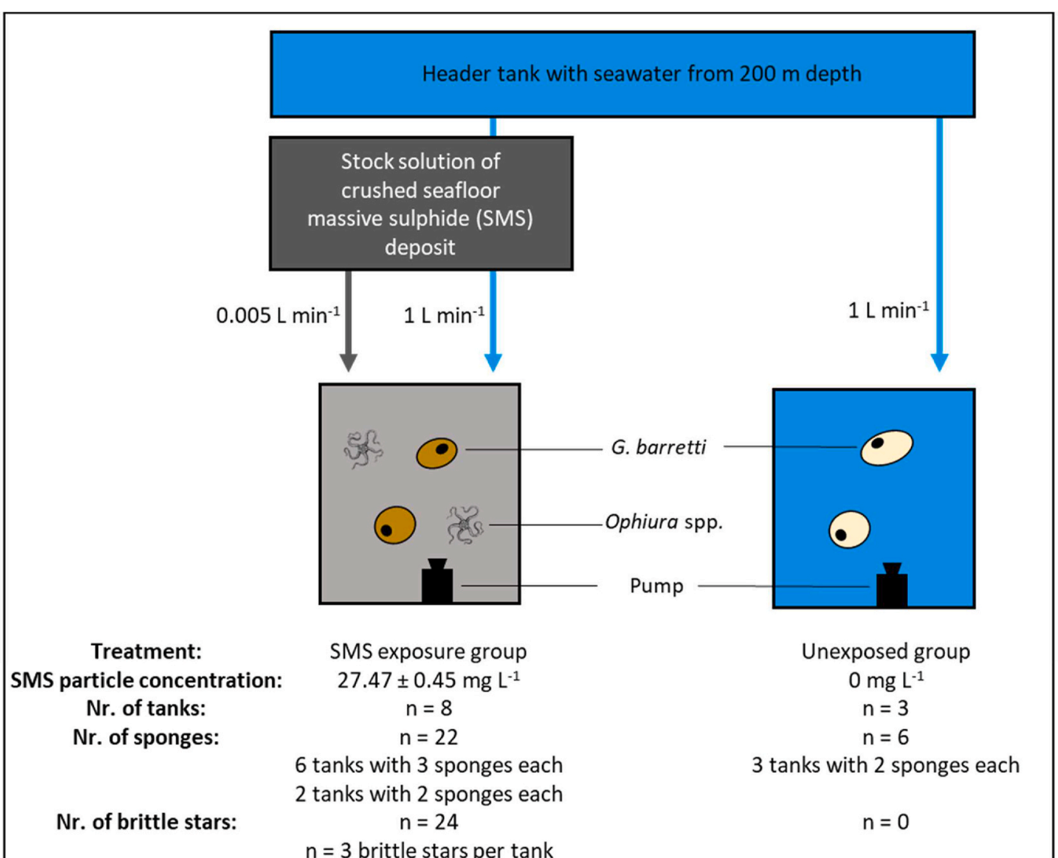


Fig. 1. Overview of the experimental design used in this study. All tanks were supplied with seawater from a header tank. Tanks assigned to the exposure group received dosages of crushed SMS deposit for 12 h per day over the course of three weeks.

consider the control tanks without brittle stars a valid control to oversee overall aquaria conditions and their suitability to support sponge health. These unexposed sponges were included in the experimental setup to safeguard that any effects observed in the exposure group are not related to changes of the conditions in the flow-through seawater facilities. However, these two groups were not compared against each other in statistical tests due to the large difference in time they were maintained in the aquaria facilities. Fig. 1 illustrates the experimental design.

2.3. Seawater manipulation

Seawater parameters of the SMS exposure study were kept similar to the preceding maintenance period in the *ex situ* aquaria facility (Control: 6.5 °C, pH 8.1; low pH: 6.5 °C, pH 7.8; high temperature: 10.5 °C, pH 8.1; low pH + high temperature: 10.5 °C, pH 7.8), each of the four treatments being applied to two tanks (n = 2). Parameters in tanks without SMS particle exposure (n = 3) were kept stable at 6.5 °C and pH 8.1. Seawater manipulation was achieved via heating elements and the addition of pressurized CO_2 (Riebesell et al., 2010). Sensors measuring seawater pH (ACQ310N-PH, Aquatronica, Italy) were calibrated and cleaned weekly following manufacturer guidelines (two-point calibration with pH = 7.00 and pH = 10.00, Certipur®, Merk, Germany).

2.4. Exposure to crushed seafloor massive sulphide deposit

To mimic the impacts of deep-sea mining, individuals of *G. barretti* and *Ophiura* spp. were exposed to ground material from a natural SMS deposit. The SMS deposit sample (SMDR7) used in this study was collected using a dredge in a transect from 73°27.62′N 7°12.85′E to 73°28.16′N 7°10.60′E at the Mohn's Treasure sulphide deposit during a research cruise with R/V Håkon Mosby in 2003. After retrieval, the sample material was dried and stored before further use and analyses. To create particles mimicking deep-sea mining activities the SMS sample was crushed to a particle size <100 μm, using a Disk Mill Pulverisette 13 classic line (Fritsch). Grain size analyses (Beckman Coulter LS 12 320) determined a particle size composition where 99 % of the sample had a grain size <50 μm and 63 % a grain size <10 μm (Fig. S1). The material was further characterized by major- and trace element analyses undertaken by Actlabs Ltd., Canada (www.actlabs.com) and the University of Bergen. The following analyses were performed at Actlabs; sample preparation (following RX1 procedure), flux fusion with lithium metaborate/tetraborate followed by ICP-AES (Thermo Jarrel-Ash ENVIRO II) analyses; four acid digestions (HClO4, HNO3, HCl and HF) followed by a dilution with *aqua regia* and ICP-AES (Thermo Jarrel-Ash ENVIRO II) analyses; Instrumental Neutron Activation Analysis (INAA) analyses and combustion/IR pyrolysis. At the University of Bergen, the sample was digested with a mix of HNO_3 and HF before ICP-AES (Thermo Scientific ICap 7600) analyses. Quantification was done by external calibration curves (multi element standard solution from Spectrapure) and Sc was used for internal standardization. For quality control and monitoring the performance during analytical runs, the synthetic water CRM SPS-SW-3 (Spectrapure standards) was analysed repeatedly. In-house seawater standards were used for additional controls. Additional investigation of the morphology and composition of crushed SMS material was performed by a scanning electron microscope (Zeiss Supra™ – 55 V Field Emission SEM) equipped with a Thermo Noran System 7 energy dispersive X-ray spectrometer (EDS) system.

Crushed SMS material (172 g dry mass) was suspended in a 30 L container to create a concentrated stock solution. Particles were kept in suspension by a pump at the bottom of the 30 L container. The stock solution was distributed to the eight exposure tanks via a peristaltic multichannel pump (Masterflex L/S EW-77919-25) with a frequency of one pulse every 3 min, resulting in a concentration of ~30 mg L^{-1}. Pumping velocity was set to a high speed, to avoid settlement of particles in the tubing delivering the stock solution to the tanks. In each tank, a pump kept the SMS particles in suspension. Exposure lasted for 21 days for 12 h day^{-1}. Settled sediment was removed with a hose daily after the 12 h exposure cycle. Particle concentration in the seawater of the exposure tanks were monitored regularly by spectrophotometry (UVmini 1240, Shimadzu, Germany), comparing the transmittance of a 10 mL water sample at 660 nm to a calibration curve based upon a dilution series of 6.25, 12.5, 25, 50 and 100 mg SMS particles L^{-1} in seawater. Natural background concentrations of suspended particles were measured in non-exposed tanks following the sampling scheme of the SMS particle exposure tanks.

2.5. Physiological responses

Oxygen consumption and clearance rates in *G. barretti* were assessed as response variables to evaluate the changes in physiological performance over time of a deep-sea sponge under the exposure to SMS particles. Chamber based incubations were performed at the beginning (Day 0), 7 and 21 days after exposure to SMS particles started. Incubations were performed in periods without SMS particle dosing. Prior to the incubations, sponges were given a 3 h recovery period after the last pulse of SMS addition to the exposure tank. Incubations were done in the respective sponge holding tank, to keep handling of experimental animals at a minimum. Dissolved oxygen concentrations were measured (HQ40D, Hach, USA) every minute over 4 h and oxygen consumption rates in μmol O_2 gDM^{-1} h^{-1} were calculated as outlined by Tjensvoll et al. (2013) using the following equation:

$$\text{Oxygen consumption}\left[\mu\text{mol } O_2 \text{ gDM}^{-1}\text{h}^{-1}\right] = \left(\left(c_2 - c_1\right) * t^{-1} * V_{net}\right) * \text{gDM}^{-1},$$

where c_1 and c_2 = the concentration of dissolved oxygen at start and end of the incubation in μmol O_2 L^{-1}, t = the time of the incubation in h, V_{net} = the volume of the incubation chamber after subtracting the volume of the respective sponge in mL and gDM = the total dry mass of the incubated animal in gram.

Water samples (5 mL) were drawn from the chamber at the beginning and the end of the 4-h incubations. Duplicate samples (2 mL) for bacterioplankton quantification were fixed with glutaraldehyde (end-concentration 0.5 %) for 10 min before flash freezing in liquid nitrogen and −80 °C storage. Bacterial abundance in the water samples was assessed by flow cytometry (Brussaard et al., 2010) and clearance rates for bacterioplankton in mL gDM^{-1} h^{-1} were calculated as outlined by Robertson et al. (2017) using the following equation:

$$\text{Clearance rate}\left[\text{mL gDM}^{-1}\text{h}^{-1}\right] = V_{net} * t^{-1} * \ln\left(n_{start} * n_{end}^{-1}\right) * \text{gDM}^{-1},$$

where V_{net} = the volume of the incubation chamber after subtracting the volume of the respective sponge in mL, t = the time of the incubation in h, n_{start} and n_{end} = the concentrations of bacterioplankton at start and end of the incubation in counts mL^{-1} and gDM = the total dry mass of the incubated animal in gram. At every time point (Day 0, 7 and 21) four empty chamber incubations were performed to control for background oxygen consumption and changes in bacterial abundance. The oxygen and bacterial concentration dynamics averaged over the empty chambers were subtracted from the sponge incubations at the respective timepoint. Oxygen consumption and clearance rate in sponges without SMS particle exposure were assessed at day 7 and 21 of the experiment. In addition to physiological parameters the presence of necrosis was observed in sponges. Tissue necrosis in *G. barretti* is recognized by areas covered in white flocculent material on the cortex of the sponge with black colored underlaying tissue. Individuals of *G. barretti* were considered dead when the necrosis covered more than 50% of the surface of the sponge. These individuals were removed from the experimental setup. Mortality rates in brittle stars were observed over time and brittle stars were considered dead when all arms were jettisoned from the central disc and brittle stars were left completely immobilized.

2.6. Histological tissue preparation and elemental analysis of tissue samples

At the end of the experiment tissue samples were extracted from experimental sponges to qualitatively describe the potential accumulation of indigestible crushed SMS particles. A small piece of cortex was prepared and embedded in epoxy resin (Agar Scientific Ltd., UK). Sections of about 1 mm were prepared with a low speed precision saw (11–1180 Isomet, Buehler, Germany). The sections were adhered to a slide, polished to a thickness of 15 μm and colored with toluidine blue under heat for several seconds. Thin sections were investigated for the presence/absence of SMS particles via light microscopy. For the elemental analysis a 1 cm^3 sample of the mesohyl from the middle of the sponge was extracted after 21 days of exposure to crushed SMS deposit. From brittle stars the central disc was sampled at the timepoint they died. Sponge and brittle star tissue samples were dried in 60 °C for 12 h and stored air tight until analysis. Concentrations of iron (Fe), copper (Cu) and sulfur (S) were analysed in a subset of samples from sponges (n = 19 for exposed group; n = 3 for control group) and brittle stars (n = 8) by ICP-AES.

2.7. Tissue and seawater sampling for microbial abundance analysis

To study potential changes in the microbial abundance in seawater and the abundance of the microbiome associated with *G. barretti,* 2 L of seawater were filtered and ~0.25 g tissue samples were extracted from sponges at the end of the experiment. In total 40 samples were processed for microbial diversity. 25 sponge samples were processed for the different experimental treatments: 6 sponges for the "SMS" treatment, 3 sponges for the "no SMS" treatment, 5 sponges for the "SMS + high temperature" treatment, 6 sponges for the "SMS + low pH" treatment, and 5 sponges for the "SMS + low pH + high temperature" treatment. In addition 15 seawater samples were processed (three replicates per experimental treatment). The DNeasy Power Soil Kit (Qiagen, Germany) was used for DNA extraction on sponge tissue and seawater filters. Sponge-derived DNA was eluted in 100 μL and afterwards diluted (1:10) with PCRgrade water. Quality of the extracts was checked using a NanoDrop spectrophotometer and gel electrophoresis after a PCR with universal 16 S primers. For sequencing, the V3 and V4 variable regions of the 16 S rRNA gene were amplified using the primer pair 341 F-806 R (5′-CCTACGGGAGGCAGCAG-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′) in a dual-barcoding approach. Verification of PCR-products was conducted with gel electrophoresis. Afterwards the samples were normalised, pooled and sequenced on a MiSeq platform (MiSeqFGx, Illumina) using v3 chemistry. Subsequently demultiplexing was performed based on 0 mismatches in the barcode sequences. The QIIME2 environment (version 2018.11) was used to process raw sequences. Based on forward reads (truncated to 270 nt), Amplicon Sequence Variants (ASVs) were generated with the DADA2 algorithm. With the FastTree2 plugin phylogenetic trees were calculated on the resulting ASVs. Classification of representative ASVs was performed using the Silva 132 99% OTUs 16 S database, with the help of a primer-specific trained Naive Bayes taxonomic classifier.

2.8. Data analysis

The oxygen consumption and clearance rates of individual sponges were averaged per tank and tank averages were used as replicates for statistical analysis (Day 0, n = 11 tanks; Day 7 and 21, n = 8 tanks). First, a pilot experiment was run to verify the absence of effects of temperature and pH on clearance and oxygen consumption rates. For this, a mixed effects (REML) model was run with tank as the random effect, and day and treatment (split into temperature and pH) as fixed effects. This model indicated no significant effect of treatment on clearance or oxygen consumption rates (Appendix Table S2). Therefore, subsequent analyses were run only comparing time points within the sediment exposure group without treatment (temperature and pH). A general linear model analysis of variance (one-way ANOVA) was applied to test the effect of exposure to crushed SMS deposits over time (n = 11 tanks for Day 0, all sponges unexposed; n = 8 tanks for Days 7 and 21, exposed sponges only). The dataset met the requirement of normality tested with a Shapiro-Wilk test. Additionally, QQ and Residual plots were assessed visually. When a significant ($\alpha = 0.05$) effect was detected, Tukey's multiple comparison analysis was performed to further investigate the effect. An unpaired *t*-test was performed to investigate physiological differences over time within the unexposed group in between the two timepoints (Day 7 and 21). Differences in end-concentrations of analysed elements (Fe, Cu and S) in tissues from SMS particle exposed (n = 19) and non-exposed group (n = 3) were investigated using an unpaired *t*-test. Survival of animals was tested for differences with a Log-rank (Mantel-Cox) test in between the pre-acclimatisation period and SMS exposure period. Values are given in mean ± standard deviation (SD) unless stated otherwise. All statistical tests were performed with GraphPad Prism 8 Version 8.2.1 (441), August 20, 2019. For analysis of the microbial community beta-diversity a Bray-Curtis distance matrix was computed and sample separation in ordination space subsequently analysed with a Non-metric Multidimensional Scaling (NMDS) approach. Bray-Curtis indices (calculated on feature level, i.e. on amplicon sequence variant level) were used as basis for computing dendrograms using Ward-clustering, and to perform Analyses of similarities (ANOSIM) for assessing statistical significance (significance level $\alpha = 0.05$). Shannon diversity indices were calculated as alpha-diversity measure and used as basis for Mann-Whitney/Kruskal-Wallis tests. With help of the Linear Discriminant Analysis Effect Size (LEfSe) algorithm, microbial phyla which differ significantly between treatments and unexposed sponges were determined and ranked according to estimated effect sizes.

3. Results

3.1. Geochemistry of SMS deposit

The whole rock analyses revealed that the SMS deposit used in this study was relatively rich in S (>20 %). The metals Fe and Cu were most abundant in the sample followed by Ba (Table 1). Relatively minor amounts of Mn, Co, Zn, Hg and Ni were present. SEM/EDS analyses (Fig. 2) revealed a relative dominance of iron- and copper-sulphides, barite (barium sulphate) and smaller particles of iron-oxyhydroxides.

3.2. SMS exposure levels and accumulation of SMS particles in sponges and brittle stars

Individuals of *G. barretti* and *Ophiura* spp. have been exposed to a concentration of 27.47 ± 0.45 mg L^{-1} of crushed SMS deposit over the course of the 21 day long experiment. Sediment concentrations in the tanks of the exposure group were elevated throughout the whole experiment compared to the unexposed tanks without crushed SMS deposit addition. Natural background concentration of indigestible particles in unexposed tanks was 0.94 ± 0.16 mg L^{-1} averaged over the 21 day experiment. Over time, particles were accumulating on sponges exposed to crushed SMS deposit. Particles were observed to settle on the cortex of the sponges as well as protruding long spicules (Fig. 3). On the mesh covering the oscula of *G. barretti* SMS particles were evident. In individuals from the unexposed group, particles were completely absent from cortex, spicules and the mesh covering the oscula.

Light microscopy of slides prepared from cortex samples revealed that SMS particles, settled on the cortex of the sponge, were intruding the sponge's mesohyl via ostia (Fig. 4A). Passing through the eurraster and sterraster spicule dominated cortex SMS particles were found to be accumulated in the walls of channels and subdermal spaces within the sponge's mesohyl (red arrows in Fig. 4B). This pathway is highlighted by red (pathway through cortex) and yellow (accumulation in channel

Table 1
Concentration of major compounds in the SMS sample material, given in percent mass fraction, and trace elements, given in mg kg^{-1} and μg kg^{-1}, used in this experiment. Here, S* represents the percentage of sulphide sulfur of the total amount of S in the sample. LOI = Loss of ignition.

Compound	Mass fraction [%]	Element	[mg kg^{-1}]	Element	[mg kg^{-1}]	Element	[mg kg^{-1}]	Element	[μg kg^{-1}]
SiO_2	0.92	**Fe**	623 909	**Y**	<1	**Se**	<3	**Au**	424
Al_2O_3	0.09	**Cu**	33 528	**Zr**	14.2	**Ta**	<1		
Fe_2O_3	62.36	**Ba**	31 531	**Ag**	2.6	**Th**	<0.5		
MnO	0.05	**Cd**	1.1	**As**	73	**U**	15.6		
MgO	0.09	**Ni**	10	**Br**	6	**W**	700		
CaO	0.4	**Zn**	815	**Co**	609	**La**	0.3		
Na_2O	0.08	**Ag**	2.6	**Cr**	<1	**Ce**	<3		
K_2O	<0.01	**Pb**	<5	**Cs**	<0.5	**Nd**	<5		
TiO_2	0.005	**Bi**	<2	**Hf**	<0.5	**Sm**	<0.1		
P_2O_5	0.02	**Cd**	1.1	**Hg**	18	**Eu**	0.5		
LOI	31.67	**Mo**	83	**Ir**	<5	**Tb**	<0.5		
Total	94.29	**Be**	<1	**Rb**	<20	**Yb**	<0.1		
S	>20.0	**Sr**	1209	**Sb**	5.9	**Lu**	<0.05		
S*	40.2	**V**	<5	**Sc**	0.2	**Li**	n.a.		

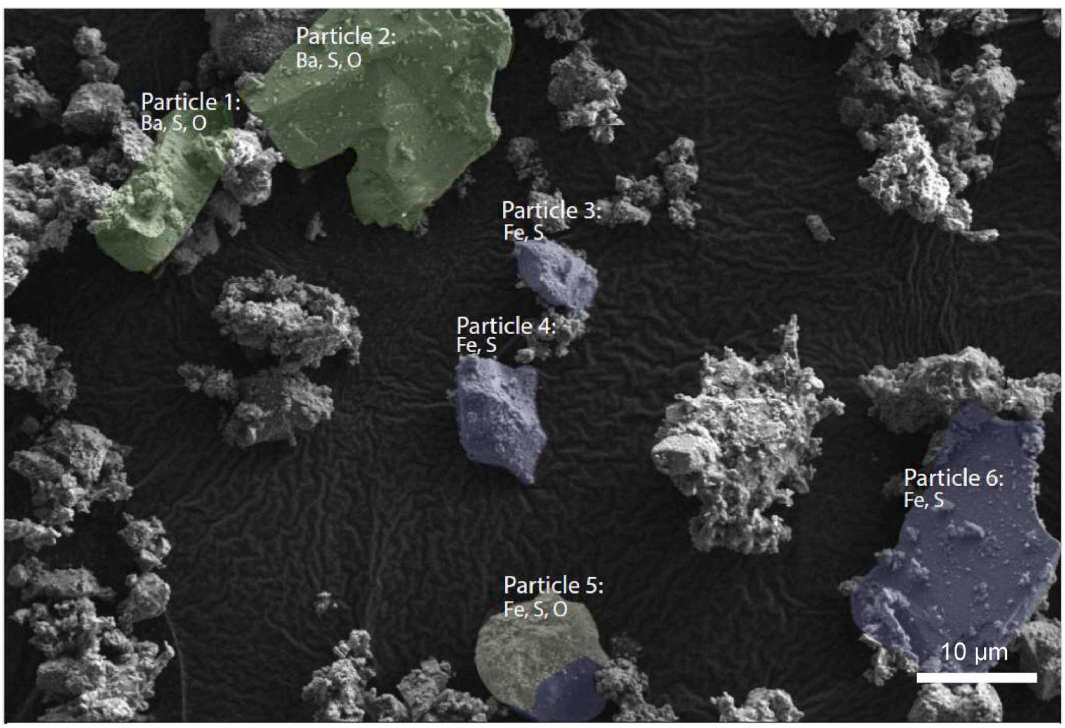


Fig. 2. Scanning electron microscopy close-up image of the SMS particles used in this experiment. Highlighted are distinct particles and their main elemental composition (Ba = Barium, S = Sulfur, O = Oxygen, Fe = Iron) is given.

walls) arrows in Fig. 5A. SMS particles were absent from the cortex and subdermal spaces of sponges from the unexposed group (Fig. 5B).

After termination of the 21 day long exposure sponges were cut in half for sample extraction. Individuals from the SMS deposit exposed group were found to be colored black throughout the whole body (Fig. 6), while individuals from the unexposed group expressed a beige, common coloration for *G. barretti* (Fig. 6). Analysis of selected elements (Fe, Cu and S) showed significant, ~ twentyfold higher concentrations of iron and copper in tissue samples from SMS exposed sponges ($mean_{copper} = 201.2 \pm 29.1$ μg gDW^{-1}; $mean_{iron} = 2283.86 \pm 492.17$ μg gDW^{-1}) compared to individuals from the unexposed group ($mean_{copper} = 12.5 \pm 1.5$ μg gDW^{-1}; $mean_{iron} = 119 \pm 25$ μg gDW^{-1}) ($p < 0.01$, respectively, $df_{iron, copper} = 20$) (Table 2). Concentrations of sulfur were comparable in the exposed and unexposed group ($mean_{exposed} = 11.38 \pm 0.7$ μg gDW^{-1}; $mean_{control} = 9.4 \pm 0.7$ μg gDW^{-1}) ($p = 0.12$ df = 20). Concentrations of iron, copper and sulfur in SMS exposed brittle stars are stated in Table 2.

3.3. *Brittle star mortality and necrosis rates in sponges*

Brittle stars that were included in the SMS particle exposure tanks were thriving throughout the 10 months long maintenance in the aquaria system preceding the described experiment (Table 3). With the start of the exposure to crushed SMS deposit brittle stars started to shed spines from their arms, cumulating in the detachment of complete arms followed by total immobility. Ultimately, ten days after the onset of exposure, all brittle stars were dead (Table 3) indicating a significant increase of mortality rate ($p < 0.01$) following SMS particle exposure. Sponges in the SMS exposure experiment expressed a significant ($p < 0.01$), tenfold higher incidence rate of necrosis when compared to the period of the preceded 10-months long maintenance in the aquaria facility (Table 3). Sponges in the three unexposed tanks did not show any signs of tissue necrosis over the 21 day long experiment.

Fig. 3. Images of the same sponge before (left) and after (right) 3 days of exposure to crushed SMS deposit for 12 h per day. Particles are settling on the spicules and the cortex of the sponge. The individual has a diameter of 8 cm.

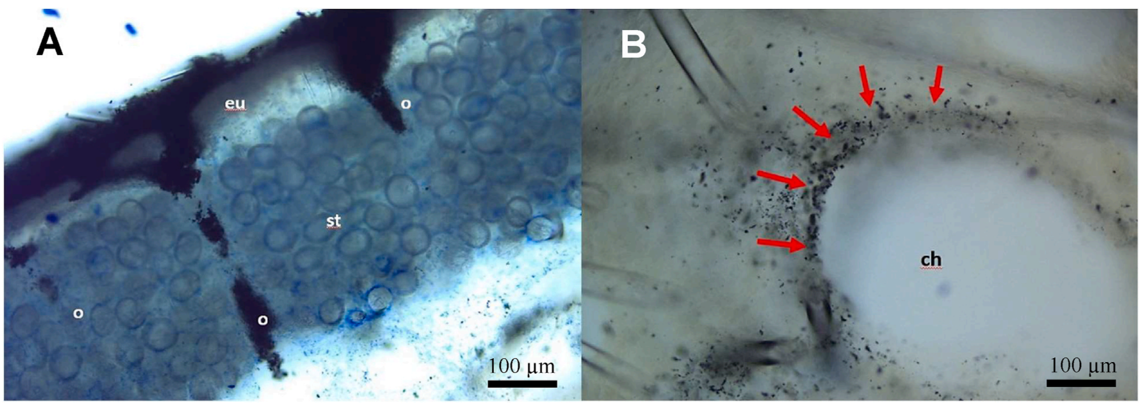


Fig. 4. Sections of the cortex and subdermal area of *G. barretti* exposed to crushed SMS deposits for 21 days, 12 h per day. A: SMS particles can be observed to intrude the sponge via the oscula (o) passing the eurraster (eu) and sterraster (st) spicules. B: Particles are accumulating along the walls of the channels (ch and red arrows).

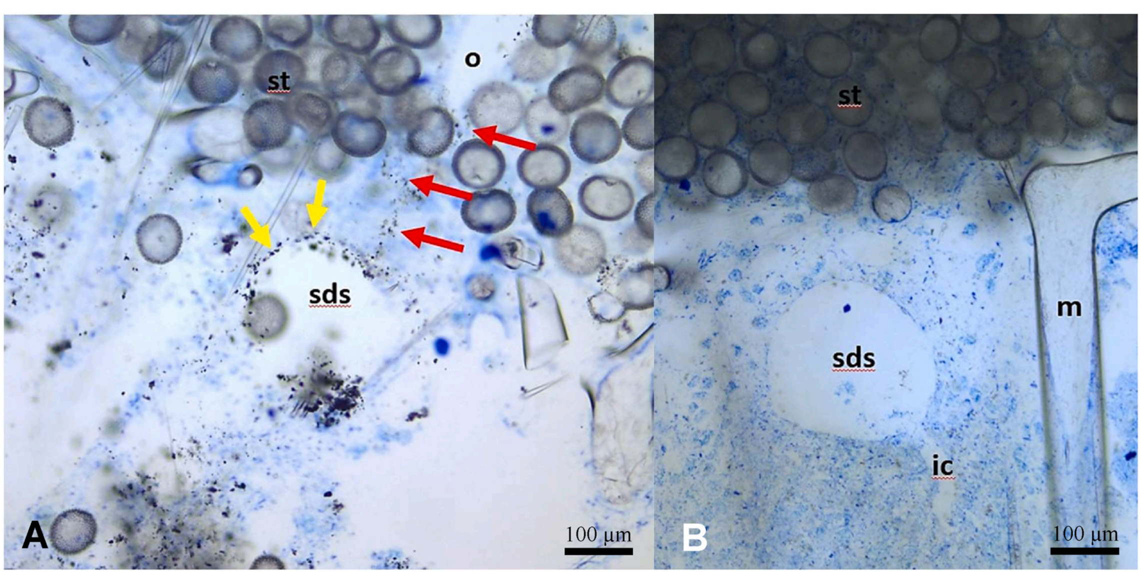


Fig. 5. Sections of the cortex and subdermal area of *G. barretti*. A: A section of a sponge exposed to crushed SMS deposits for 21 days, 12 h per day. Black particulate matter can be observed intruding the sponge via the oscula (o) being transported to subdermal spaces (sds and red arrows) and becomes accumulated in the walls of these spaces (yellow arrows). B: The cortex section of a sponge from the unexposed group is free of SMS particles in the sds, incurrent channels (ic), spaces between the megascleres (m) and sterraster spicules (st).

Fig. 6. Cut through *Geodia barretti*. Left: Unexposed individual. Right: Individual from the experimental group exposed to crushed SMS deposits for 21 days, 12 h a day. Accumulated SMS particles have colored the mesohyl black throughout the sponge.

Table 2
Overview of elemental concentrations in SMS exposed sponges, SMS exposed brittle stars and unexposed sponges.

Element	Concentration in SMS exposed sponges [μg gDW^{-1}]	Concentration in unexposed sponges [μg gDW^{-1}]	Concentration in SMS exposed brittle stars [μg gDW^{-1}]
Fe	2283.86 ± 492.17	119 ± 25	1534.5 ± 1816.6
Cu	201.2 ± 29.1	12.5 ± 1.5	229.7 ± 112.8
S	11.38 ± 0.7	9.4 ± 0.7	10.0 ± 2.4

3.4. Physiological rates

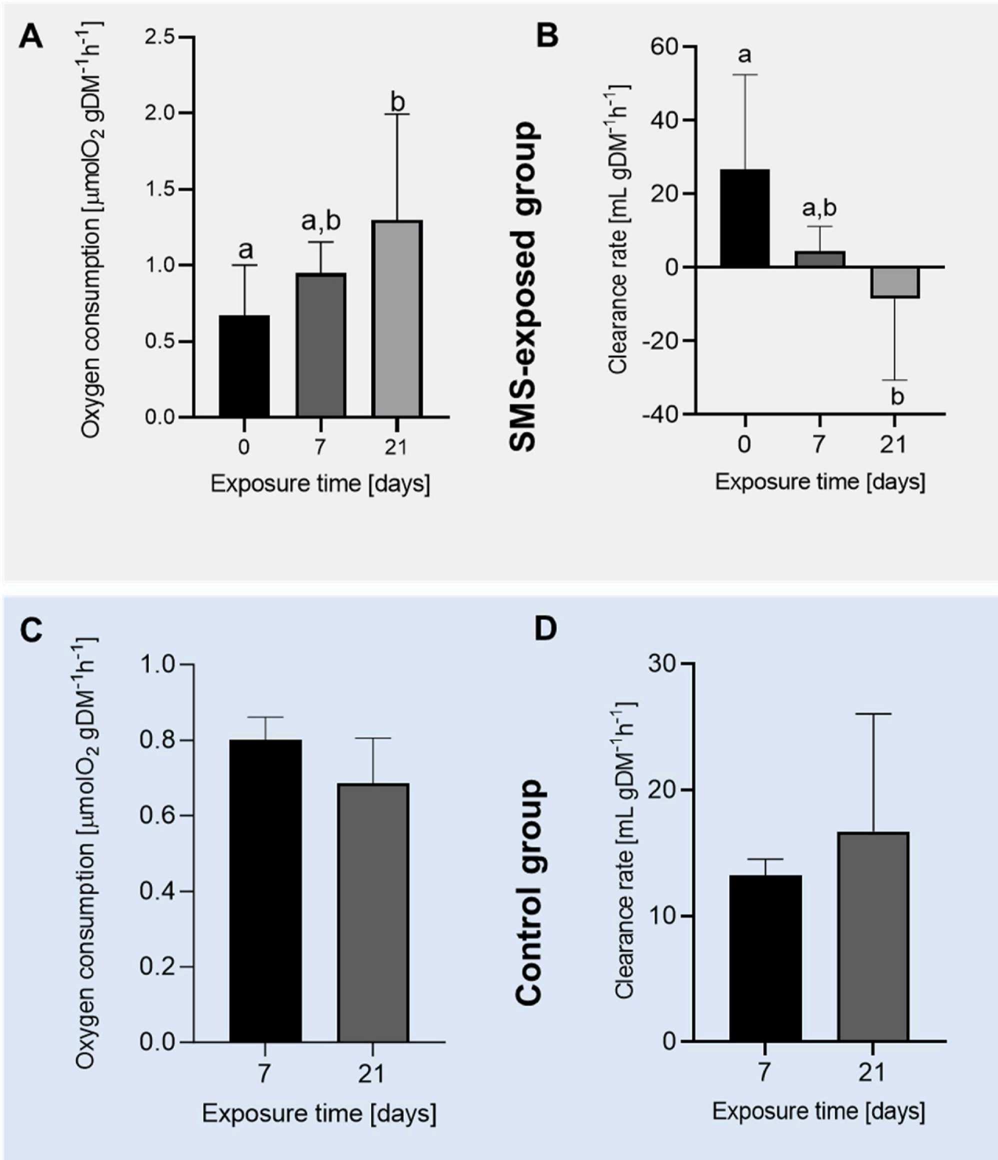
Oxygen consumption and clearance rate were assessed as response variables in *G. barretti* under the exposure to crushed SMS deposit and in individuals from the unexposed group. No effect of manipulated seawater parameters (pH and temperature) was evident (Supplementary Material Tables S1 and S2) throughout the 21-day long experiment (Supplementary material: Figs. S2 and S3). SMS deposit exposure had a significant impact on sponge oxygen consumption ($p = 0.02$ ($F_{(2, 24)} = 4.57$) (Fig. 7A and E). From Day 0 to Day 21 oxygen consumption rates in SMS exposed sponges expressed a significant ($p = 0.01$), almost 100 % elevation. At the same time oxygen consumption rates in unexposed sponges remained stable and no difference between the two time points was detected ($p = 0.21$) (Fig. 7C and E). Similar to oxygen consumption, clearance rates in sponges from the unexposed group were stable over time ($p = 0.56$) (Fig. 7D and E). However, sponges exposed to SMS deposits expressed a significant ($p < 0.01$ $F_{(2, 24)} = 6.99$) (Fig. 7B and E), 130 % decrease in clearance rates after 21 days compared to rates before SMS deposit exposure commenced.

3.5. Microbial abundances in seawater and sponge tissue

Clear shifts in the seawater microbial community composition were observed across the treatments (Fig. 8A). Although less distinct, effects of the treatments were also observed in the sponge-associated microbial communities (Fig. 8A). Microbiomes of those sponges that were exposed to SMS deposit clustered apart from unexposed samples (Fig. 8, B1) and were significantly different (Table 4). *Entotheonellaota* were significantly depleted in sponges from SMS particle exposure treatment, concomitantly *Actinobacteria* were significantly enriched. Microbiomes of sponges from the high temperature treatment clustered apart from only SMS treated samples (Fig. 8A, B2) and were significantly different in their composition (Table 4). This was not only the case in terms of beta diversity, but also in terms of alpha diversity, as the lowest microbial richness was observed in the high temperature treatments (Table 5). *Chloroflexi* were significantly depleted in the high temperature treatment, concomitantly *Firmicutes* were significantly enriched. While strong shifts occurred in the seawater microbial community composition, sponge microbiomes were not significantly different in the low pH treatments compared to the SMS only treatment (Fig. 8A) and B) 3–4; Table 5).

Table 3
Overview of *G. barretti* with signs of necrosis and mortality in brittle stars during the 273 day long maintenance period and the 21 day long exposure to crushed SMS deposit.

Experiment	Species	Animals at start [n]	Duration of experiment [days]	Individuals with necrosis [n]	Dead animals [n]	Necrosis and mortality rates [$\%_{Animals}$ day^{-1}]
SMS exposure experiment	*Geodia barretti*	21	21	5	Not applicable	1.13
Long-term maintenance	(Sponge)	31	273	10	Not applicable	0.12
SMS exposure experiment	*Ophiura* spp.	24	21	Not applicable	24	10.00
Long-term maintenance	(Brittle star)	24	273	Not applicable	0	0.00



E

Exposure Time [Days]	Group	Oxygen consumption rate [µmol O_2 gDM^{-1} h^{-1}]	Clearance rate [mL gDM^{-1} h^{-1}]
0	SMS-exposed	0.67 ± 0.32	26.6 ± 25.7
7	SMS-exposed	0.95 ± 0.19	4.6 ± 6.5
21	SMS-exposed	1.29 ± 0.65	-8.56 ± 22.0
7	Unexposed	0.80 ± 0.05	13.2 ± 1.3
21	Unexposed	0.69 ± 0.09	16.7 ± 9.3

20

Fig. 7. Oxygen consumption rate (A, C) and clearance rate (B, D) of *G. barretti* under crushed SMS exposure (A, B) and under control conditions (C,D) over the course of 21 days. Data represents averages of tanks (Exposure group: Day 0, n = 11; Day 7, 21, n = 8; Control: n = 3). Corresponding letter pairs (a,b) indicate significant differences ($p < 0.05$). Table (E) provides the detailed data from the graphs A-D. Error bars in graphs and errors stated in table represent standard deviation.

4. Discussion

This is the first study to describe the impact of SMS mining plumes on the physiology of a deep-sea sponge species and a sponge-associated brittle star. The average exposure to 27 mg L^{-1} of crushed SMS (up to 100 mg L^{-1} is field-relevant) caused a tenfold higher incidence of tissue necrosis in *G. barretti* and mortality of all *Ophiura* spp. individuals after 10 days of exposure. At the time of their death, concentrations of copper, iron and sulphate in *Ophiura* spp. were one magnitude higher than reported for a starfish species from a fjord polluted by discharged mine tailings (Coteur et al., 2003). Sponges accumulated SMS particles in their tissue and the elements copper and iron were concentrated ten times higher in tissues of exposed sponges compared to non-exposed individuals. While oxygen consumption in exposed sponges doubled over 21 days, clearance rates completely ceased over the course of the experiment. Below, the effects of SMS on *G. barretti* and *Ophiura* spp. and

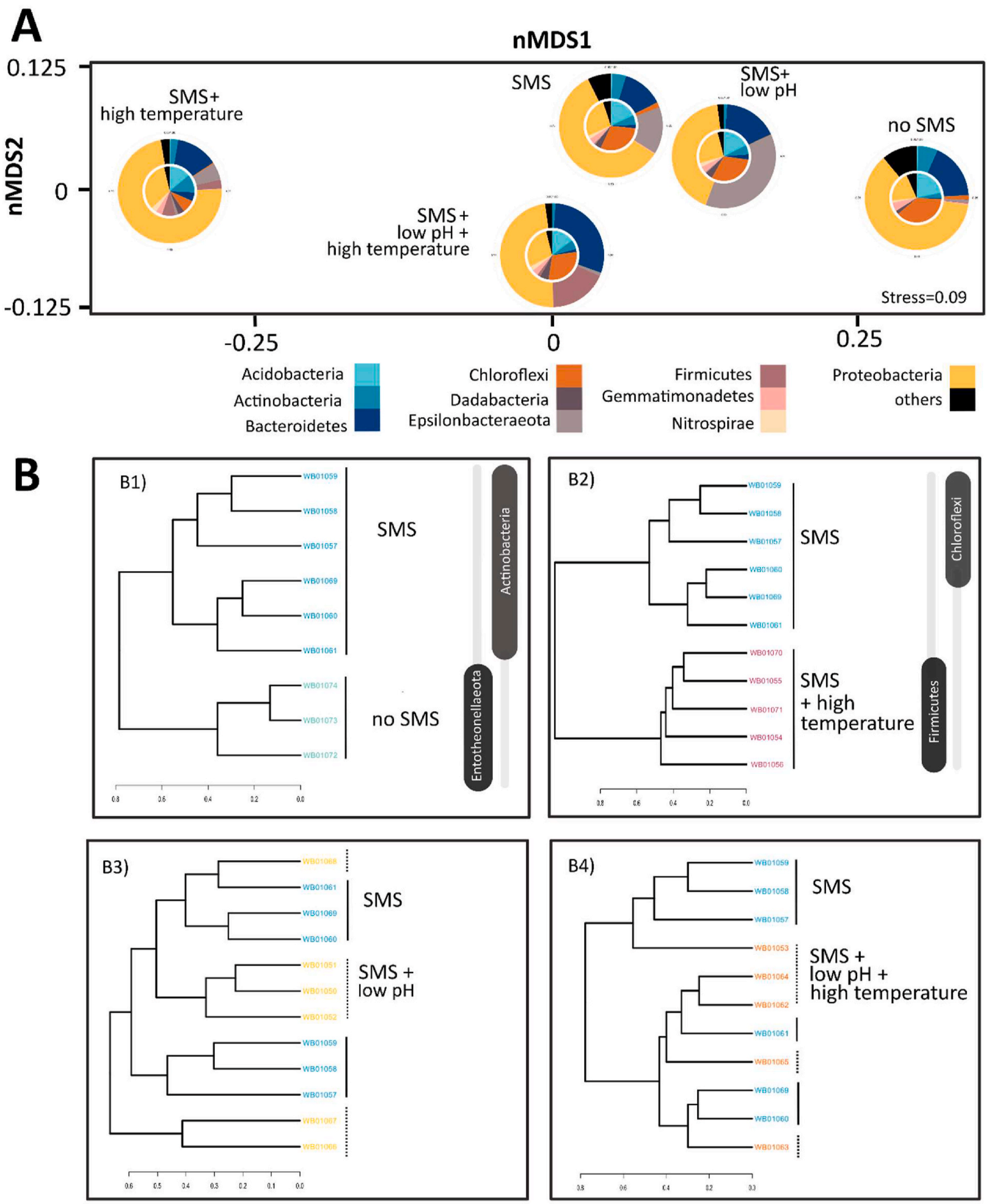


Fig. 8. A) 10 most abundant microbial phyla for sponges (inner rings) and seawater (outer rings) in SMS particle exposed and unexposed sponges. Donut diagrams show average microbial communities and are plotted at the positions of centroids for each treatment cluster, which were determined in an ordination of Bray-Curtis distances based on sponge microbial communities (feature level). B) Dendrograms illustrating similarities of microbial communities between different sponge individuals. Ward-clustering was done based on Bray-Curtis indices calculated on microbial feature level (i.e. on amplicon sequence variant level). Sponge individuals are colored according to their respective experimental treatment, including unexposed animals: blue = sediment addition; green = no sediment addition, red = sediment + high temperature, yellow = low pH, orange = high temperature + low pH treatment (Fig. 8 B1 – B4). Next to the dendrograms, the microbial phylum which is most significantly enriched and the phylum which is most significantly depleted in the respective experimental treatment are indicated (as determined in a Linear Discriminative Analysis).

Table 4
Diversity statistics for sponge associated microbial communities. For alpha diversity analyses Mann-Whitney/Kruskal-Wallis tests were performed on Shannon diversity indices (feature-level). For Beta diversity, Analyses of similarities (ANOSIM) were conducted on Bray-Curtis indices (feature-level).

comparison	α - diversity		β - diversity	
SMS vs. no SMS	M = 13; p: 0.381		R = 0.568; p: <0.023	*
SMS vs. SMS + high temperature	M = 25; p: 0.008	*	R = 0.824; p: <0.008	*
SMS vs. SMS + low pH	M = 18; p: 1.000		R = 0.059; p: <0.269	
SMS vs. SMS + high temperature + low pH	M = 21; p: 0.329		R = 0.157; p: <0.093	

Table 5
Sponge-associated microbial community richness across different treatments, calculated on microbial feature level.

Treatment	Shannon Index (mean ± standard deviation)
SMS	7.44 ± 0.15
No SMS	7.57 ± 0.05
SMS + high temperature	6.74 ± 0.22
SMS + low pH	7.44 ± 0.08
SMS + high temperature + low pH	7.21 ± 0.27

the ecological implications of these findings are discussed.

4.1. Practical considerations and limitations

Working with deep-sea animals is a logistically challenging and resource intensive endeavour. In this study we used 21 sponges that had been collected in the wild and maintained in the laboratory for a period of 273 days. The animals remained healthy and were not negatively influenced by manipulated seawater parameters to resemble future ocean conditions (T = 10.5 °C, pH = 7.8). An additional number of six new animals were added to the experimental setup and given two weeks to acclimatize before the SMS experiment commenced. A direct comparison of physiological rates between the SMS exposed and unexposed sponges was obviated because of the different periods at which these two groups of sponges had been kept under *ex situ* conditions. The physiological functioning of the unexposed sponges did not change over the course of the *ex situ* maintenance and was similar to rates reported for this species (Kutti et al., 2015; Leys et al., 2018; Bart et al., 2020a) demonstrating that effects observed in the SMS exposed group of sponges were not induced by changes in the experimental conditions.

4.1.1. Body burdens of SMS particles and associated elements in brittle stars and sponges

The elements that are associated with hydrothermally derived minerals (Cu, Cd, Ni, Zn, Co, As and Hg) are relatively available in nature and their potentially toxic effects is well-studied in aquatic life (Clearwater et al., 2002; Depew et al., 2012; Deforest and Meyer, 2015). Exposure of organisms to potentially toxic elements occur via the water phase as well as via accumulated particles. Over time, the body burden may rise up to toxic levels (De Jonge et al., 2013). Brittle stars (Deheyn and Latz, 2006) and sponges (Perez et al., 2004; Genta-Jouve et al., 2012) can accumulate biologically available heavy metals from the water phase and from accumulated particles. In this study, brittle stars were thriving well under ex situ conditions for 273 days however started to express signs of stress a few days after the SMS exposure commenced. Within ten days all individuals in SMS exposure tanks perished after complete immobilization due to the shedding of arms. Elemental analysis revealed that body burdens of copper were a magnitude higher than reported for a star fish species from a contaminated fjord in Norway (Coteur et al., 2003). Macleod et al. (2013) described sub-lethal responses such as lethargy and surfacing in the temperate brittle star species *Amphiura elandiformis* when exposed to copper concentrations similar to levels evident in tissues of brittle stars from this SMS exposure study. Thus, the high concentrations of copper in tissues of *Ophiura* spp. might have resulted in the mortality observed in response to SMS exposure.

Accumulation of SMS particles was evident in *G. barretti*. After 21 days of exposure to SMS particles, *G. barretti* individuals were colored black throughout their entire tissue. Measurements of particle size distribution revealed that the majority of SMS particles added to the exposure tanks was within the size class (1–10 μm) of bacterioplankton that *G. barretti* is able to retain from seawater (Bart et al., 2020a). The particle size used in this study (<1 μm–100 μm; mean size 11 μm) is comparable to the particles that are expected to be distributed above the seafloor during the initial cutting process and the return of dewatered unwanted materials and sediment during mining operations (Gwyther, 2008). Histological examination of the sponge cortex area revealed that the black coloration resulted from accumulated SMS particles. SMS particles, small enough to be ingested by *G. barretti*, had entered the sponges via ostia, traversing the eurraster and sterraster dominated cortex and entering the sponge's aquiferous system. Not excreted, indigestible particles can accumulate with adverse implications for sponge health (Grant et al., 2019). Although it has been suggested that *G. barretti* can arrest pumping for short (4 h) time intervals (Tjensvoll et al., 2013) to avoid ingestion of indigestible particles, the 12 h exposure cycle applied in this study apparently caused exceedance of the capacity of this protective mechanism. In addition to the accumulated particles, the concentrations of copper and iron were significantly elevated inside the tissue of the SMS exposed sponges. This could be due to direct uptake from the water or the metals may have been released from the particles accumulated inside the sponges. Their predominantly unselective filter-feeding (Maldonado et al., 2012) makes sponges prone to the ingestion of biologically available, particle-bound pollutants and elements (Illuminati et al., 2016). Hence, concentrations of such compounds found inside sponges may reflect levels of exposure. Concentrations of copper in the unexposed group were similar to concentrations in polar demosponge species from an unpolluted site (e.g. *Sphaerotylus antarcticus*, Cu = 53 ± 6 μg gDM^{-1} (Illuminati et al., 2016)). Individuals of *G. barretti* exposed to SMS particles had accumulated copper and iron up to concentrations comparable to temperate demosponges from urban wastewater outlet sites (Perez et al., 2004). In a *Geodia* species from the Mediterranean, exposure to copper was found to alter cell functions and compromise the immune system (Saby et al., 2009). The observed increase in necrosis in exposed sponges may be an early indication for adverse effects of SMS deposit plumes.

4.2. Physiological responses of G. barretti to crushed SMS exposure

Oxygen consumption of *G. barretti* in the unexposed group and in sponges from the exposed group at Day 0 (no SMS particles dosed yet) were within the range reported for this species (Bart et al., 2020a). With the exposure to SMS particles, oxygen consumption of *G. barretti* increased over time and had doubled (Fig. 7) after 21 days. Rapid respiratory responses to suspended particles have been reported for *G. barretti* (Tjensvoll et al., 2013; Kutti et al., 2015). However, several studies focusing on the effect of suspended natural sediment on deep-sea sponge species have reported drops in oxygen consumption as a response to concentrations of 10–100 mg L^{-1} (Tompkins-Macdonald and Leys, 2008; Tjensvoll et al., 2013; Mobilia et al., 2021).These studies, focusing on the effects of natural sediment starkly contrasted results from this SMS exposure experiment. Here, oxygen consumption rates increased over the course of 21 days as a response to frequent exposure to crushed SMS deposit. The increased respiration rates found in this experiment indicate a different response of *G. barretti* to SMS particles than suspended natural sediment and could point towards the activation of different coping mechanisms in *G. barretti* when exposed to deep-sea mining-borne particles. The decline in oxygen consumption as an immediate response to natural sediment exposure is thought to indicate a temporarily (4 h) cessation of pumping activity, hypothesized as a mechanism to reduce particle accumulation in the sponges body. In addition to ceasing pumping activity, mucus production has been identified as a coping mechanism towards suspended particles in demosponges (Gerrodette and Flechsig, 1979; Turon et al., 1999; Kowalke, 2000; Bannister et al., 2012; Strehlow et al., 2017). Mucus production may affect oxygen consumption and Bannister et al. (2012) observed a 40 % increase in oxygen consumption in the common reef sponge *Rhopaloeides odorabile* following a 24 h-long exposure to sediment and linked this to the enhanced production of mucus. The metabolic requirements associated with mucus production in sponges are not yet known, but high energetic cost of mucus production under sediment influence have been reported in corals (Brown and Bythell, 2005). The observed increased oxygen consumption rates in the SMS exposed group over time could represent energy expenditures of increased mucus production as an effort to expel indigestible particles that evidently accumulated in tissues of *G. barretti* (Fig. 6). Pumping in sponges has been hypothesized to be a function of oxygen consumption (Reiswig, 1971; Gerrodette and Flechsig, 1979) and in *G. barretti*, pumping has been shown to be associated with filter-feeding (Leys et al., 2018). However, in this experiment, increased consumption of oxygen coincided with reduced retention efficiency for bacterioplankton, suggesting that oxygen consumption and pumping are not strongly correlated in *G. barretti*. Furthermore, Leys et al. (2018) showed that only 5 % of the volume of *G. barretti* are comprised of sponge cells and that separating the sponge's tissue activity and oxygen needs are hard to disentangle from those of the other components within the holobiont. Thus, in addition to the potential production of mucus, the observed increased respiration rates could be driven by microbiome mediated activities supplied with oxygen via diffusive processes (Hoffmann et al., 2008; Leys et al., 2018).

Clearance rates of sponges before the SMS exposure and in the unexposed group were similar to rates reported for this species (Leys et al., 2018; Bart et al., 2020a). The frequent exposure to particles of crushed SMS deposits over the course of 21 days significantly hampered the retention efficiency for bacterioplankton and cumulated in a net expulsion of bacteria. *G. barretti* does not possess bypasses that channel water towards the osculum without being filtered (Leys et al., 2018). Therefore, indigestible particles could easily clog choanocyte chambers that generate movement of water through the sponge's body (Reiswig, 1971). The absence of clearance of bacterioplankton in SMS-exposed individuals of *G. barretti* could result from a smothering of choanocyte chambers culminating in the disability to maintain filter-feeding (Lohrer et al., 2006). It remains unknown if *G. barretti* is able to meet its increased energy demands under SMS particle exposure by phagocytosis of symbiotic microbes (Leys et al., 2018). The observed increase in concentrations of bacterioplankton in the incubation chambers might point towards a comprised energy acquisition pathway if associated symbionts, that usually are phagocytosed, are expelled under SMS particle exposure. The observed increased oxygen consumption rates in combination with reduced feeding abilities ultimately would further impair sponge health. The net-release of bacteria could also be linked to an effort of expelling accumulated, indigestible particles. This coping mechanism is linked to increased mucus production (Strehlow et al., 2017) and might be corroborated by the increased oxygen consumption rates of *G. barretti* under SMS particle exposure. Clearly, under our experimental conditions this pathway of particle evacuation was not efficient enough to prevent the observed accumulation of indigestible particles (Fig. 6).

4.3. Effects of mining plumes on the sponge associated microbiome

Geodia barretti hosts a complex microbial community (Leys et al., 2018) that plays a role in carbon and nutrient cycling within the sponge's holobiont (Radax et al., 2012). The present study shows that *G. barretti* maintains a seawater distinctive microbiome across long time spans (273 days) under *ex situ* conditions. However, the addition of SMS particles related with shifts in both seawater and sponge microbial community compositions. Increased seawater temperature and the presence of SMS particles had the largest effect on the sponge associated microbiome. Although no treatments effects of manipulated seawater parameters on physiological rates were evident (Supplementary Tables S1 and S2), the temperature increase of 4 °C resulted in an altered microbial community composition in *G. barretti* individuals after 21 days of SMS exposure. For example, a significant depletion of *Chloroflexi* bacteria was evident. This phylum is known to be an integral part of healthy *G. barretti* individuals (Radax et al., 2012). At the same time bacteria belonging to the phylum *Firmicutes*, associated with necrosis events in *G. barretti* (Luter et al., 2017), were significantly enriched under high temperature and SMS exposure. Along these lines, our results underline a link between changes in the microbiome of *G. barretti* and the high necrosis described in this study, which might be a result of the changes in oxygen consumption and clearance rate under the impact of a mimicked deep sea-mining plume. In addition to *Chloroflexi* bacteria, *Entotheonellaota* were significantly depleted under SMS exposure. This phylum is known for its production of antibiotic substances in sponges (Bhushan et al., 2017) and a reduced abundance of these microbes could potentially compromise *G. barretti's* defense against contagious bacteria strains. In combination with the increased abundance of contagious bacteria phyla this has the potential to adversely affect the fitness of *G. barretti.* A stable microbiome has been identified as an important indicator of sponge health (Pita et al., 2018; Slaby et al., 2019) As a habitat structuring key species, the microbial responses to SMS particle exposure in this study have the potential to cascade into larger scale impacts on sponge-driven deep-sea ecosystems.

4.4. Ecological implications

Deep-sea sponge grounds are hotspots of biodiversity and nutrient cycling (Hogg et al., 2010). Recent discoveries prove that sponges fuel food webs in the vast plains of the deep sea (Rix et al., 2016; Bart et al., 2020b) and play a pivotal role in the transfer of organic carbon from food sources inaccessible to a majority of deep-sea fauna to higher trophic levels (Bart et al., 2021). In addition, sponges have been shown to eliminate contagious viruses and bacteria (Welsh et al., 2020), but in this study SMS particle exposed *G. barretti* shifted from clearing to releasing bacteria when exposed to crushed SMS deposits. Hence, deep-sea mining plumes could compromise *G. barretti*'s ecological function of bacterioplankton- and virus-removal with potential adverse implication for the health of sponge-driven deep-sea ecosystems. Increased respiratory demands in combination with ceased uptake of food particles might cause shifts in energy allocation in individuals of *G. barretti.* These shifts can lead to compromised metabolic activities such as growth or tissue maintenance. Cell growth and cell maintenance in sponges have been shown to be of fundamental importance to fuel complex marine food webs in the deep sea (Bart et al., 2021). If these biological functions are downregulated in order to cope with indigestible SMS particles, this benthic pelagic coupling and food provision functioning of sponges might be compromised by active and passive mining impacts. Detritivorous brittle stars utilize excreted organic material of filter feeders in deep-sea environments and thus represent a pathway of energy transfer to higher trophic levels (Maier et al., 2020). In addition to the potential reduction in organic matter excretion by sponges the deleterious effects of SMS particles on the survival of *Ophiura* spp. described here would completely eliminate an important pathway channelling energy into higher trophic levels. Lastly the increased rates of necrosis and mortality of *G. barretti* under exposure to SMS particles would compromise its important ecological role as habitat structuring ecosystem-engineer (Murillo et al., 2012; Howell et al., 2016).

4.5. Knowledge gaps and considerations for future studies

The environmental consequences of industrial scale deep-sea mining operations can only be evaluated appropriately when assessments are based upon decent knowledge of ecosystem functioning in the deep-sea habitats where mining is about to happen (Christiansen et al., 2020). Here, we presented the first experimental data on potential effects of deep-sea mining on two common benthic species from the NAO. Our data indicate that effects of crushed SMS deposits on deep-sea benthic fauna are more severe than effects of comparable particle loads of natural suspended sediments. Follow up experiments should be extended by the inclusion of a broader spectrum of SMS concentrations and particle size classes. This will yield information about the impact of plumes as a function of distance from the active mining site. However, responses are most likely species specific and will depend on the environmental settings mining will occur in (e.g. background concentrations of suspended particles), the feeding ecology and mobility of abundant fauna (van der Grient and Drazen, 2022). Thus, it is highly recommended to perform response studies with location specific fauna under conditions resembling the environmental conditions at the prospected mining site.

4.6. Conclusions

This study shows that deep-sea mining plumes are likely to have ecotoxicological effects on deep-sea benthic fauna. A 21-day exposure to SMS particles compromised the metabolism of the abundant, habitat-forming deep-sea sponge *Geodia barretti* (higher expenditure of metabolic energy, but lower uptake of food particles) and caused rapid mortality in individuals of the sponge-associated brittle star *Ophiura* spp. We strongly advise to follow a precautionary approach when prospecting deep-sea habitats for metal extraction to secure that sponge-mediated, benthic-pelagic coupling mechanisms and other ecosystem services are not affected by indirect effects of mining operations.

Funding

This work was funded through the SponGES—Deep-sea Sponge Grounds Ecosystems of the North Atlantic: an integrated approach towards their preservation and sustainable exploitation, under H2020- the EU Framework Programme for Research and Innovation (Grant Agreement no. 679849).

CRediT authorship contribution statement

Erik Wurz: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Linn M.Brekke Olsen:** Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Kathrin Busch:** Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Tone Ulvatn:** Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Hans T. Rapp:** Funding acquisition, Methodology, Resources, Supervision. **Ronald Osinga:** Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. **Albertinka J. Murk:** Conceptualization, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work is dedicated to our friend Hans Tore Rapp who sadly passed away at a far too young age, on March 7, 2020. We thank the captain, crew and scientific participants of the cruise on the Norwegian research vessel *GO Sars* for successful operations and maintenance of sampled sponges onboard. Thanks to the ÆGIR6000 team for efficient and delicate sampling of sponges by remotely operated vehicle. We thank Jasper de Goeij (University of Amsterdam, UvA) and Martijn Bart (UvA) for frequent content meetings and their assistance on the research cruises and laboratory work in Bergen. Finally, we thank Detmer Sipkema and Rob Joosten for generously sharing the flow cytometer and help with analyzing samples and Rolf B. Pedersen (Centre for Deep Sea Research at University of Bergen) for providing the SMS sample material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2024.104311.

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