Deep-sea sponges in an Anthropocene ocean

Erik Wurz
Propositions

1. Deep-sea sponges cope with climate change without intervention of other anthropogenic activities. (this thesis)

2. Hidden from the public consciousness the deep sea is being industrialized. (this thesis)

3. Individual psychology is an integral part of modelling in decompression theory.

4. Quantifying economic value of ecosystem services prevents the decline of ecosystems providing them.

5. Traditions of the few limit the progress of the collective.


Propositions belonging to the thesis, entitled:

Deep-sea sponges in an Anthropocene ocean.

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Deep-sea sponges in an Anthropocene ocean

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Summary

Sponges (Porifera) are among the oldest multicellular animals on planet earth and are abundant throughout all oceans. From shallow, warm waters to the dark, cold deep sea. Sponges move large quantities of seawater through their body while efficiently removing dissolved and particulate nutrients. Their large filter capacity makes them important links in marine food webs as they are able to access nutrient sources that are unavailable to the majority of marine fauna and channel this energy to higher trophic levels. In the North Atlantic Ocean (NAO) sponges form dense aggregations, so called sponge grounds, that provide ecosystem services such as habitat provision, nutrient cycling and provision of novel, bioactive compounds. It remains unclear whether these deep-sea sponge grounds can continue to provide these services in a changing ocean that is increasingly industrialized. The physicochemical properties of the North Atlantic Ocean will be altered by human induced climate change. The aim of this thesis is to address this knowledge gaps by quantifying the basic eco-physiological processes such as oxygen consumption, clearance rate and uptake/release of inorganic nutrients of two habitat forming deep-sea sponges under the cumulative impacts of warmer, acidified seawater and the exposure to different types of re-suspended particles. The research underlying this thesis was part of the EU-funded research project Deep-sea Sponge Grounds Ecosystems of the North Atlantic: an integrated approach towards their preservation and sustainable exploitation (SponGES). Two model deep sea sponge species were used, *Geodia barretti* and *Vazella pourtalesi*. *G. barretti* collected from 300 m water depth in the Barents Sea, were exposed to four treatments resembling future ocean conditions (no treatment, 4 °C increase in seawater temperature, decrease of seawater pH by 0.3, and a combination of the high temperature, low pH). Over the course of 39 weeks, oxygen consumption, dissolved inorganic nutrient fluxes, and bacterioplankton clearance rates were measured as indicators of metabolic activity. All indicators within each sponge individual and per treatment were highly variable over time, and no effect of manipulated seawater treatments on these parameters could be demonstrated. Oxygen consumption rates in all groups closely followed a seasonal pattern, potentially caused by (a)biotic cues in the natural seawater flowing through the experimental aquaria. While similar metabolic rates across all treatments suggest that *G. barretti* physiologically coped with simulated future ocean conditions, tissue necrosis that developed in experimental animals might indicate that the response of the complex, high microbial *G. barretti* sponge (i.e., sponge host and microbial symbionts) to future ocean conditions may not be reflected in basic physiological processes. In addition to large scale changes of ocean conditions, also bottom trawling activities
interact with the dense sponge aggregations. Bottom trawling has been identified as the most severe direct industrial threat to abundant sponge grounds by removing sponge biomass and indirect by re-suspending bottom sediments. Plumes of re-suspended sediment potentially smother and clog the aquiferous system of filter-feeding sponges with unknown implications for their health. The physiological responses of repeated exposure to natural sediment were studied in the glass sponge *Vazella pourtalesii*, which forms dense sponge grounds in Emerald Basin off Nova Scotia, Canada. *Ex situ* chamber-based measurements of bacterial clearance and oxygen consumption (respiration) rates indicated that the animals were able to cope with elevated concentrations of suspended sediment, as they expressed normal clearance and respiration rates over 7 days of sediment exposure. However, clearance rates significantly declined after 14 days of sediment exposure and the animals visibly accumulated sediment in their tissue. Therefore, long-term exposure to elevated concentrations of suspended sediment should be avoided in order to minimize adverse effects on the abundant *Vazella* sponge grounds.

While sponges seem to cope with environmental changes and limited exposure to suspended particles as occurs in their natural environment, the response to cumulative stressors indicated impaired health. Exposure to a field relevant concentration of suspended sediment (50 mg L⁻¹) and future ocean conditions (pH decrease of 0.2 units, temperature increase of 3 °C) on the physiological performance of *Geodia barretti* resulted in a cessation of pumping. Oxygen consumption rates remained unchanged under low pH and high temperature treatments and indicate mechanisms of pumping-independent mass transfer of oxygen. A small, but statistically significant shift in the microbiome associated with *G. barretti* was observed and possibly related to coping with cumulative stressors in this deep-sea sponge species. The synergistic nature of the treatment-specific effects has the potential to adversely affect the physiological fitness of this dominating sponge species in the North Atlantic Ocean. In addition to deep-sea fisheries the nascent industry of subsea mining is prospecting abundant mineral resources present in the deep sea. The extraction of subsea minerals, such as seafloor massive sulphide (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. To simulate the effects of mining plumes on benthic life in the deep-sea, *Geodia barretti* and an associated brittle star genus were exposed 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 already 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. Sponges were shown to express high coping capacities towards fluctuations of environmental parameters within their habitat. However, additional stressors or persistence of sub-optimal conditions over extended time scales can challenge sponge’s ability to endure cumulative effects. Given the ecosystem services sponge grounds in the North Atlantic Ocean provide, industrial operations should ascertain refuges for deep-sea sponges faced with global ocean changes.
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Abstract

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Exposure to crushed seafloor massive sulphide deposit

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Tissue and seawater sampling for microbial abundance analysis

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General discussion

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Chapter 1

General introduction
The deep sea represents the largest ecosystem on planet earth (Eakins and Sharman, 2010). This biosphere is hypothesized to be the origin of all life (Jordan et al., 2019), yet is the most hostile environment known to humankind as several kilometres of water column exert immense physical forces on the ocean floor. These extreme conditions impose technological and logistical challenges to researchers, resulting in a poor understanding of a significant proportion of the world’s oceans (Webb et al., 2010; Clark et al., 2016). Only since a few decades, scientists have access to technologies such as remotely operated vehicles (ROVs) and pressure resistant high definition cameras enabling extensive exploration of deep-sea habitats (Clark et al., 2016). Recent exploration efforts are shedding light on a highly diverse deep-sea fauna that can occur in dense aggregations forming so called animal forests (Rossi and Rizzo, 2020). One example of these hotspots of biodiversity in the deep North Atlantic Ocean (NAO) are dense aggregations of sponges (Maldonado et al., 2017) (Figure 1). Despite the knowledge of the presence of these large aggregations throughout the NAO, their ecological functioning and value for healthy seas and humankind remain largely unknown.

Figure 1. *Pheronema carpenteri* dominated deep-sea sponge ground at circa 400 m of depth around the Azores. Photo courtesy: Rebikoff Foundation.

To fill the knowledge gaps around these sponge dominated ecosystems in the deep NAO the research project *Deep-sea Sponge Grounds Ecosystems of the North Atlantic: an integrated approach towards their preservation and sustainable exploitation* (SponGES) was funded by the European Union. This multidisciplinary project aimed at answering pressing questions such as: When did these ecosystems form and how are such high numbers of animals sustained by
the scarce food resources in the deep sea? Which goods and services are they providing to humankind and how do they influence their surroundings? How are they responding to anthropogenic drivers and can they maintain their ecological function under future ocean conditions? Within the framework of the SponGES project, this thesis is aiming at addressing the knowledge gap on the physiological responses of deep-sea sponges to anthropogenic drivers.

**Deep-sea sponge grounds in the North Atlantic**

Dense aggregations of sponges that form under specific oceanographic conditions are termed sponge grounds (Hogg et al., 2010). Here, sponges represent the dominating proportion of benthic biomass and can account for up to 90% of the local standing carbon stock (Maldonado et al., 2017). With their diverse and three-dimensional growth forms, sponges function as ecosystem engineers and provide a structural habitat for other organisms in the deep sea (Hogg et al., 2010). Dense aggregations of sponges are present throughout the whole NAO (Figure 2, middle) at depths ranging from 70 m to over 4000 m (Maldonado et al., 2017).

**Figure 2.** Middle: Occurrences of deep-sea sponge grounds across the North Atlantic Ocean. Different colours represent grounds with different species compositions driven by sea water temperature. **Blue frame:** Sponge aggregation characteristic for cold-water sponge grounds. Large specimen of *Schaudinnia rosea* and *Asconema foliata* growing on a thick spicule matt and living tetractinellid sponges. **Green frame:** Boreal sponge ground dominated by the Demosponge *Geodia barretti*. **Red frames:** Examples of temperate sponge grounds dominated by *Vazella pourtalesii* (upper red frame) and *Pheronema*
Depending on the local environmental conditions, there are regional differences in the species composition of sponge grounds across different latitudes (Howell et al., 2016). On the shelves off Greenland and seamounts along the mid-Atlantic ridge under the influence of cold, arctic bottom water, dense communities of *Schaudinnia rosea* and *Asconema foliata* are growing on a thick spicule matt and living tetractinellid sponges (*Geodia parva, Geodia hentscheli* and *Stelletta rhaphidiophora*) (Figure 2, upper left corner) (Roberts et al., 2018). Spanning from the Barents Sea and Norwegian shelf to the slopes off Newfoundland and Labrador on the Canadian coast, boreal grounds are the predominant type of sponge grounds. These grounds are dominated by species from the genus *Geodia* (Demospongiae). Sponges of this genus mostly exhibit massive growth forms (Figure 2 lower left corner) and some species grow as tall as 1 m in diameter (Klitgaard and Tendal, 2004). Further south on the Scotian shelf off the Canadian East Coast, the glass sponge (Hexactinellidae) *Vazella pourtalesii*, a representative species of temperate sponge grounds, is highly abundant in depths from 70 to 200 m (Figure 2, right upper corner) (Beazley et al., 2018). At temperate latitudes in the Western part of the NAO from the Porcupine Seabight to the Azores the glass sponge *Pheronema carpenteri* can be found in dense aggregations (Figure 2, lower right corner) to a depth of 4000 m (Rice et al., 1990).

**The ecological role of sponges in the ocean**

Sponges are sessile, filter-feeding animals that move large quantities of seawater through their bodies (Maldonado et al., 2010). While passing through the sponge’s body, seawater is channelled along a large number of choanocyte chambers (Reiswig, 1971). These chambers clear suspended matter such as marine bacteria from the seawater with a retention efficiency of up to 99% for particles as small as 0.1 µm (Maldonado et al., 2010). In addition to particulate matter sponges can utilize dissolved organic matter (DOM), a food source that is largely unavailable to other marine fauna (De Goeij et al., 2013). The enormous filtration capacity of sponges for particulate and dissolved matter makes them key-players in benthic-pelagic coupling processes and drivers of nutrient recycling in oligotrophic systems such as tropical coral reefs and deep-sea habitats (Rix et al., 2016; Bart et al., 2021). When present in high densities, filter-feeding sponges have the potential to greatly influence nitrogen, silica and carbon cycling processes in deep-sea environments (Maldonado et al., 2017). Furthermore, sponge grounds are thought to provide valuable ecosystem services to humankind: they clear seawater from viruses (Welsh et al., 2020), might hold novel medical compounds to fight future
diseases (Mehbub et al., 2014) and provide a habitat and breeding ground for industrially exploited fish species (Koen-Alonso, 2018). Despite their high abundance and potential significance in ecosystem service provision, the importance of sponge grounds for deep-sea ecosystems still remains poorly investigated and receive little public attention. While marine scientists just start to unravel the ecological complexity of sponge dominated deep-sea habitats, these unique ecosystems are currently already posed with potential threats from industrial activities.

**The North Atlantic Ocean in the Anthropocene**

Sponge-dominated habitats are facing various anthropogenic drivers of stress, such as exposure to different kinds of re-suspended sediment and climate change induced stress. Since the industrialisation of the fishing fleet, fisherman are deploying bottom trawling gear as deep as 2000 m, targeting economically valuable demersal fish species. Guided by up to 5000 kg heavy doors on both sides of the opening, these nets are dragged over the seafloor. Often, the up to 200 m wide net opening is equipped with a chain of metal balls to crush any obstacles in the trajectory of the net, preventing it from snagging and damage. Frequently, these obstacles are century-old deep-sea coral reefs or sponge grounds. Direct impacts of these nets with dense sponge aggregations are removal, damaging or burrowing of the animals (Pham et al., 2019).

The potential for recovery of the slow-growing cold-water sponge community is almost non-existent with trawl marks still being evident ten years after trawling in a sponge ground 1300 m deep (Freese, 2001; Ospar Commision, 2010; Morrison et al., 2020; Colaço et al., 2022). In addition to direct effects bottom trawling is re-suspending large amounts of sediment that have the potential to impact sponge communities kilometres away from the initial trawling site (Durrieu De Madron et al., 2005). Given the large scale extent of bottom trawling in the NAO, this impact was identified as the most severe threat to deep-sea sponge grounds (Smith and Hughes, 2008; Jørgensen et al., 2016). Re-suspended, small grained sediment particles can stay suspended in the water column for several days and be distributed over vast distances by ocean currents (Puig et al., 2012). Suspended in the water column, these particles can be ingested by mainly unselectively filter-feeding sponges (Grant et al., 2018). Ingested particles could clog the delicate, fine-branching aquiferous system of sponges with adverse implications for overall animal health. While some deep-sea demosponge species might possess the ability to reduce intake by arresting pumping when exposed to short-term elevated concentrations of suspended sediment (Tjensvoll et al., 2013), hexactinellid cold-water sponges are highly susceptible to low concentrations of suspended particles and arrest pumping for extended periods of time (Leys, 2013). The arrest of pumping can compromise the sponge’s intake of food, excretion of
metabolic waste products and tissue oxygenation (Grant et al., 2019). When sponges function as habitat-providing ecosystem engineers and drivers of regional nutrient cycles, the decrease in individual fitness under elevated sediment concentrations has the potential to cascade in ecosystem-wide consequences that may persist over a long time-span. Ocean warming (OW) and ocean acidification (OA) are altering the physicochemical conditions in the deep sea and have been identified as the second severe impact on sponge grounds in the NAO after bottom trawling. By the year 2100 the water masses in the deep NAO are expected to experience a decrease of seawater pH of up to 0.3 units and a temperature increase of up to 4 °C in areas with abundant sponge grounds (Sweetman et al., 2017). While recent research has highlighted that *V. pourtalesii* might benefit from increased seawater temperatures by the potential extension of its geographical distributional range (Beazley et al., 2021), *G. barretti* was shown to express increased mortality under heat wave events (temperatures of 4 °C above average), which might occur with an increased frequency under future conditions in the NAO (Guihen et al., 2014). Furthermore, the combination of OA and OW has been shown to cause adverse physiological effects such as compromised pumping capacity and skeleton stability in deep-sea hexactinellid sponges (Stevenson et al., 2020). Sponges are often referred to as holobionts as they harbour a vast diversity of associated microorganisms within their tissues (Webster and Taylor, 2012). These symbionts are involved in various processes such as chemical defence (Pita et al., 2018), aerobic and anaerobic nitrogen cycling (Schläppy et al., 2010; Fiore et al., 2013) or fixing of inorganic carbon (van Duyl et al., 2020). Given the diverse, specialized abilities of their associated microbes, sponges with an abundant microbiome are hypothesized to be more resilient to lowered seawater pH conditions (Goodwin et al., 2014) than taxa with a low abundance of associated microbes. While Bell et al. (2018) evaluated the impact of ocean acidification on shallow water sponges as moderate, the above mentioned studies highlight the complex and species-specific sensitivity of cold-water adapted sponge species to changes in environmental conditions (Strand et al., 2017). Up to date knowledge about the interactive effects of OA, OW, pollution and suspended sediment on deep-sea sponge holobionts remains limited (Carballo and Bell 2017).

In the deep sea, areas with an abundant, highly specialized marine fauna coincide with geological formations that contain metals of interest to the nascent industry of deep-sea mining. Geological formations such as ferromanganese crusts, polymetallic nodules and seafloor massive sulphide (SMS) deposits form over millions of years under specific oceanographic conditions and contain high grades of e.g. cobalt, nickel, copper and gold (Levin et al., 2020). Many metals found in deep-sea deposits are thought to be crucial for the renewable energy
transition and for developments in the information and communication technology. The extraction of metals from the ocean floor will have direct impacts on the deep-sea seafloor environment such as habitat loss, compaction of sediments and the release of potentially toxic chemical components (Washburn et al., 2019). Indirectly, deep-sea habitats will be affected by the return of dewatered residues (Washburn et al., 2019). Dewatered residues contain particles from sediments and crushed geological formations as small as 8 µm (Coffey Natural Systems, 2008). These particles have the potential to be dispersed over vast distances by ocean currents and can affect sessile suspension- and filter-feeding organisms kilometres away from the initial mining site (Rolinski et al., 2001; Aleynik et al., 2017; Gillard et al., 2019). The discharge of dewatered residues could lead to increased sedimentation rates or smothering of feeding structures of key-players in benthic pelagic coupling processes, such as sponges. While currently, the International Seabed Agency is working towards a code of conduct for deep-sea mining operations to avoid significant harm for pelagic and benthic deep-sea fauna, knowledge is too scarce to define the term “harm” to deep-sea fauna and ecosystems in the first place (Levin et al., 2016). To enable efficient monitoring programs that govern the environmental impacts of any future deep-sea mining operation, it is crucial to understand the physiological responses of sponges to indirect effects of mining activities and the cascading effects of different stressors that potentially decrease individual fitness for sponge dominated deep-sea habitats.

**Knowledge gaps and aim of thesis**

Up to date, knowledge about sponge dominated deep-sea ecosystems is limited. At the same time, a broad knowledge base is required to support evidence-based management decisions that warrant the consistency of deep-sea ecosystem services under the influence of large-scale environmental changes in interaction with ongoing and future industrial activities. No knowledge exists how the habitat forming glass sponge *V. pourtalesii* responds to sediment that is re-suspended by bottom trawling activities adjacent to dense aggregations of this sponge species. Experimental work has been done on the response to different types of suspended particles in the demosponge *G. barretti*, but it remains unknown how cumulative exposure to warmer, more acidic seawater is affecting the coping capacity towards suspended natural sediment in this sponge species. Some studies have addressed the physiological responses of deep-sea sponge species to short term increases in seawater temperature (Strand et al., 2017). However, their acclimatisation potential to these conditions in combination with more acidic seawater over an extended amount of time remains unknown. A completely new frontier to marine sciences is studying the ecological consequences from the proposed mining of
geological formations in deep-sea ecosystems. While technological developments are making fast progress, it remains unknown what environmental impact the mineral-rich particles, released during mining operations, will have on filter-feeding deep-sea sponges. The aim of this thesis is to address this knowledge gaps by quantifying the basic eco-physiological processes such as oxygen consumption, clearance rate and uptake/release of inorganic nutrients of two habitat forming deep-sea sponges under the cumulative impacts of warmer, acidified seawater and the exposure to different types of re-suspended particles in a series of aquaria-based *ex situ* experiments. To reach this aim the following key research questions are addressed in this thesis:

1. How does the glass sponge *V. pourtalesii* physiologically respond to a field relevant concentration of suspended, natural sediment?
2. How do explants of *G. barretti* physiologically respond to warmer, more acidic seawater and the simultaneous exposure to suspended, natural sediment?
3. How do small, intact individuals of *G. barretti* physiologically cope with warmer, more acidic seawater over an extended time scale?
4. How do *G. barretti* and an associated brittle star species physiologically respond to exposure to mining-borne sediments of crushed geological formations over the cause of three weeks?

Figure 3 is providing an overview of which model species is used in which chapter and states the investigated interaction of the model species used with an anthropogenic driver.
**Model species**

In this thesis two model deep sea sponge species are used. Firstly, the Demosponge *Geodia barretti* (Bowerbank, 1857) (Figure 4A) which is one of the main habitat builders in boreal and arctic sponge grounds (Figure 4B) and highly abundant on the Norwegian continental shelf and throughout the NAO (Klitgaard and Tendal, 2004). In distinct areas of the Norwegian shelf the average biomass of *G. barretti* is 1,4 kg m$^{-2}$, but it can reach peaks of up to 45 kg m$^{-2}$ wet weight (Maldonado et al., 2017).

![Figure 4.](image)

**Figure 4.** (A) Individual of *Geodia barretti* (Bowerbank, 1858) (Cárdenas and Rapp, 2015); (B) Sponge ground dominated by *G. barretti* in 1581 m water depth at the Flemish Cap. Each sponge individual measures 10-20 cm (Cárdenas et al., 2013).

The sponge grounds on the Norwegian shelf, dominated by *G. barretti*, filter up to 250 000 000 m$^3$ of seawater per day and are estimated to consume up to 60 t of carbon daily (Kutti et al., 2013). Thus, this species plays a key-role in benthic deep-sea habitats and is therefore a relevant model organism to assess the acclimatization potential of boreal and arctic deep-sea sponge grounds to changing environmental conditions and industrial interactions. *G. barretti* is considered a high microbial abundant sponge as it harbors up to $3.1 \times 10^{11}$ associated microbes per ml sponge tissue (Leys et al., 2018). The complex microbiome of *G. barretti* is thought to enable this sponge species to produce biologically active antifouling compounds (Hedner et al., 2008) and to possess efficient aerobic and anaerobic energy acquisition pathways (Hoffmann et al., 2005, 2008). Its high abundance of associated microbes renders *G. barretti* a suitable model species for HMA deep-sea sponges in the NAO. As *G. barretti* in the North-East Atlantic, the hexactinellid sponge species *Vazella pourtalesi* (Schmidt, 1870) (Figure 5) is a
highly abundant key species in temperate sponge grounds of the North-West Atlantic (NWA). With densities up to 16 individuals per square meter, *V. pourtalesi* represents a habitat-forming species at depths greater than 75 m contributing to a three-dimensional structure and heterogeneity of benthic deep-sea habitats in the NWA (Fuller, 2011). *V. pourtalesi* has a vase-shaped body with a distinct osculum (Figure 5). Large, fragile spicules cover the external surface in which flocculent material can accumulate. Individuals can reach a height of up to 110 cm with an average size of 21,71 cm in height and 14,36 cm in width (Fuller, 2011). Its high abundance and ecosystem engineering function make *V. pourtalesi* a relevant model species to assess the effects of industrial interactions on temperate sponge grounds.

**Figure 5.** Several individuals of *Vazella pourtalesi* (Schmidt, 1870) in their natural habitat. Copyright: DFO
Thesis outline

This thesis comprises of four research chapters (Chapters 2-5) with an introduction (Chapter 1) and a discussion synthesizing the acquired insights (Chapter 6). The links between the chapters are shown in Figure 1.3. Each chapter will contribute the following to answer the research questions:

Chapter 2 is focusing on the response of *Vazella pourtalesii* to suspended natural sediment. This sponge species is highly abundant in areas on the Scotian shelf in the North-West Atlantic Ocean. Here, the distribution area of *V. pourtalesii* is overlapping with bottom trawling dominated fishing activities. This type of fisheries is generating plumes of sediment potentially impacting the fitness of sponges adjacent to the impacted areas. To assess the response of *V. pourtalesii* to suspended particles, ten sponges were exposed to a field relevant concentration of suspended sediment. Over the course of 21 days, oxygen consumption and bacterioplankton retention rates were recorded in an exposed and unexposed group of sponges. Scanning electron microscopy was applied to follow a potential accumulation of particles in the sponge’s tissues.

Chapter 3 is focusing on the deep-sea sponge *G. barretti* and its response to the simultaneous exposure to changing environmental conditions and the presence of bottom-trawling-borne re-suspended sediment particles. 80 Sponge explants were exposed to seawater parameters resembling seawater pH and temperature conditions of the deep NAO in the year 2100 (pH decrease by 0.3, temperature increase by 4 °C) for six months before exposure to a field relevant concentration of suspended sediment for 12 h per day over the course of 19 days. In addition to the physiological parameters, oxygen consumption and bacterial clearance rate, the treatment-induced changes in the specific microbiome associated with *G. barretti* were investigated.

Chapter 4 is following up on the results from Chapter 3, exposing larger individuals of *G. barretti* for a longer time period to manipulated seawater conditions to get a better picture of the long-term response of this species to future conditions in the NAO. Long-term monitoring of the physiological parameters oxygen consumption and bacterial clearance rate is critical to understand the acclimatisation potential of *G. barretti* to future ocean conditions. Long-term responses may differ from initial short-term responses triggered by rapidly changing environmental conditions such as heat waves. In an experimental aquarium facility supplied with seawater from the deep sea, individuals of *G. barretti* were exposed to manipulated seawater conditions in a multifactorial design over the course of 10 months. Chamber based incubations were performed six times over the course of the experiment to describe the potential
of *G. barretti* to cope with the anticipated future physicochemical seawater conditions of the NAO.

**Chapter 5** is focusing on the environmental impact of the nascent subsea mining industry pushing into deep-sea habitats. During the mining process, small particles will be released which can be distributed over vast distances by ocean currents, potentially affecting filter-feeding sponges. In an *ex situ* experiment, we exposed the model sponge *G. barretti* and an associated brittle star species to field relevant concentrations and size fractions of SMS particles. Over the course of 21 days we assessed the survival of brittle stars and the physiological parameters oxygen consumption and bacterial clearance rate of *G. barretti*. After terminating the exposure experiment, tissues were sampled to investigate the effect of SMS particle exposure on the microbiome of *G. barretti* and the potential accumulation of chemical compounds originating from SMS deposits in both sponges and brittle stars.

Finally **Chapter 6** summarizes the findings from Chapter 2-5 and discusses those results in the larger context of knowledge gained over the course of the SponGES project.
Chapter 2

The hexactinellid deep-water sponge *Vazella pourtalesii* (Schmidt, 1870) copes with temporarily elevated concentrations of suspended natural sediment

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Abstract

Plumes of re-suspended sediment potentially smother and clog the aquiferous system of filter-feeding sponges with unknown implications for their health. For the first time we examined the physiological responses of repeated exposure to natural sediment in the glass sponge *Vazella pourtalesii*, which forms dense sponge grounds in Emerald Basin off Nova Scotia, Canada. *Ex situ* chamber-based measurements of bacterial clearance and oxygen consumption (respiration) rates indicated that individuals subjected to elevated concentrations of suspended sediment expressed normal clearance and respiration rates over 7 days of sediment exposure, indicating an ability to cope with elevated concentrations of indigestible sediment particles. However, clearance rates significantly declined after 14 days of sediment exposure, suggesting an inability to cope with long-term exposure to increased sediment load. Therefore, long-term exposure to elevated concentrations of suspended sediment should be avoided in order to minimize adverse effects on the abundant *Vazella* sponge grounds.

Keywords: Sponge grounds, *Vazella pourtalesii*, re-suspended sediment, bottom-trawling, marine conservation areas
Introduction

Sponges (phylum Porifera) play a pivotal role within the marine ecosystems they inhabit from tropical shallow waters to abyssal depths. Providing three dimensional structures and secondary substrates through a diversity of growth forms, they function as microhabitats themselves, increasing local faunal biodiversity (Beazley et al., 2013, 2015; Hawkes et al., 2019). Sponges are also key players in benthic-pelagic energy transfer and biochemical processing by filtering large volumes of seawater (Maldonado et al., 2012; De Goeij et al., 2013; Maldonado et al., 2017; Pham et al., 2019). The use of advanced technologies such as remotely operated vehicles (ROVs) has provided evidence of the vast diversity and abundance of sponges in the majority of deep-sea ecosystems (Maldonado et al., 2017). In specific oceanographic settings they can represent up to 94 % of the benthic biomass (Murillo et al., 2012). Over the past decade, such sponge-dominated deep-sea habitats have been characterized as sponge grounds (Hogg et al., 2010).

With the increasing discovery of these important sponge communities in the deep-sea also comes evidence of their destruction, caused mainly by the use of bottom-tending fishing gears (Hogg et al., 2010). Bottom trawling has direct and indirect effects on sessile, filter-feeding sponges. In addition to direct removal, damage, or burying, fishing gear re-suspends bottom sediments (Hogg et al., 2010). Depending on the gear used, seabed structure and distance from the impact site, particle concentrations of up to 500 mg L$^{-1}$ can be reached behind the path of a trawl (Durrieu De Madron et al., 2005). In a disturbance experiment in the Mediterranean Sea, the average concentration behind a trawl was measured at 50 mg L$^{-1}$, with the predominant proportion of re-suspended particles made up of silty clay (grain size 1 to 63 µm) (Durrieu De Madron et al., 2005). Plumes of particles within the micron scale may stay suspended for days and be transported by oceanic currents over vast distances (Puig et al., 2012). Thus, generated plumes have the potential to indirectly impact habitats kilometres away from the impact site over an extended period of time (Hogg et al., 2010; 2012; Mengual et al., 2016; Grant et al., 2019).

Previous studies have found that sponges are negatively impacted by low concentrations of suspended particles. Exposure to suspended particle concentrations between 10 to 95 mg L$^{-1}$ has resulted in decreased pumping efficiency and filtering capacity in some species (Bell et al., 2015; Grant et al., 2018). Strehlow et al. (2017) showed that the demosponge Ianthella basta (Pallas, 1766) (Family Ianthellidae) incorporated suspended sediment particles as small as 4.2 µm from the surrounding sea water into its mesohyl. The authors postulated that this particle size is well within the range of bacterioplankton that filter-feeding sponges predominantly utilize as a food source. Its uptake therefore could cause serious deleterious
effects on sponge health. At the same time, some sponge species have developed mechanisms to exclude or excrete ingested sediment. These mechanisms include mucus production, exclusion of particles by incurrent pores, closure of oscula and pumping cessation, expulsion of particles from the aquiferous system, and tissue (sensu Leys and Hill, 2012) regression to reduce the volume of the aquiferous system and limit pumping and clearance capacity (Strehlow et al., 2017). These active mechanisms require additional energetic expenditures, and it is unknown if sponges can sustain those over long time periods (Bell et al., 2015; Schönberg, 2016a; Schönberg, 2016b; Strehlow et al., 2016).

While the effects of sediment exposure on glass sponges are less understood compared to Demosponges, Tompkins et al., 2008 demonstrated that *Rhabdocalyptus dawsoni* (Lambe, 1893) (Family Rossellidae) arrests pumping when subjected to suspended particle concentrations of 36 mg L⁻¹, confirming a response of hexactinellids to suspended particles. An *in situ* study (Grant et al., 2018) on the reef-forming glass sponge *Aphrocallistes vastus* Schulze, 1886 (Family Aphrocallistidae) from the northeast Pacific showed that suspended particles in concentrations as low as 4.4 mg L⁻¹ caused sponges to arrest their pumping with prolonged phases of “coughing” patterns in pumping activities. The arrests and “coughing” patterns are thought to protect the sponge’s aquiferous system from clogging and help remove ingested particles. At the same time, reduced pumping and thus filtering activity limits feeding and waste product excretion and can result in adverse effects on overall metabolism and health of individual sponges and consequently the biogenic habitats they form (Grant et al., 2018).

The hexactinellid sponge *Vazella pourtalesii* (Schmidt, 1870) (Family Rossellidae) is distributed along the continental margin of eastern North America, from the Florida Keys in the southeaster US to the Scotian Shelf off Nova Scotia, Canada, where it forms extensive sponge grounds between 160 and 200 m depth (Figure 1 and Beazley et al., 2018). The densest known aggregations of *Vazella pourtalesii* occur in Emerald Basin, a deep-water basin on the central Scotian Shelf, where this species reaches heights (up to 40 cm) and densities (up to 4 individuals per m²) (Maldonado et al., 2020) not observed elsewhere across its distribution. The presence of *Vazella pourtalesii* sponge grounds in Emerald Basin was first noted by fisherman when fishing for pollock and redfish using otter trawl gear (Fuller, 2011; Beazley et al., 2018).
Figure 3. Sponge grounds formed by *Vazella pourtalesii* in the Sambro Bank Sponge Conservation Area, Emerald Basin, Nova Scotia, Canada. Image is a frame grab from video collected using the ROV ROPOS in 2017. Depth is 155 m, location is 43°53.6513 N, -63°4.6513 W. Scale bar in the lower left corner: 10 cm.

The highest biomass/densities of *Vazella pourtalesii* from commercial bycatch (where catch biomass up to 5900 kg in a single tow has been reported), research vessel trawl catch data, and *in situ* camera surveys (described in Beazley *et al.*, 2018) are reported to occur in two localized areas of Emerald Basin: the eastern flank of Sambro Bank, and on a shallower saddle between Emerald Basin’s Main and Northern Basins, to the west of the area known to fisherman as ‘The Patch’, (see Figure 2 Fuller, 2011; Beazley *et al.*, 2018). Conservation of sponge grounds has gained international attention since they were listed as vulnerable species to bottom contact fishing gears by the Food and Agriculture Organization of the United Nations (FAO, 2009) in the international guidelines written in response to the United Nations General Assembly resolutions aimed at protecting vulnerable marine ecosystems. Deep-sea sponge aggregations are included in the OSPAR List of threatened and/or declining species and habitats (OSPAR agreement 2008-6), and regional fisheries management organizations such as the North Atlantic Fisheries Organization have introduced management measures to protect sponge habitats (NAFO, 2021). In Canada, sponge grounds may qualify as sensitive benthic areas, the Canadian equivalent of vulnerable marine ecosystems (DFO, 2017a). In order to protect these dense aggregations from adverse impacts of bottom trawling, in 2013 Fisheries and Oceans Canada (DFO) implemented two sponge conservation areas (the Sambro Bank and Emerald Basin...
Sponge Conservation Areas, referred to herein as SCAs) prohibiting the use of bottom-tending gears. However, groundfish fishing activities continue to occur almost immediately adjacent to the borders of these two conservation areas (DFO, 2017b), potentially affecting the sponge grounds and abundant megafaunal communities within the SCAs (Hawkes *et al.*, 2019) via re-suspension of sediment.

Here, in a laboratory setting, we report for the first time the physiological response of *Vazella pourtalesii* to long-term exposure of an ecologically relevant concentration (50 mg L$^{-1}$) (Tjensvoll *et al.*, 2013; Kutti *et al.*, 2015) of suspended natural bottom sediment, thereby evaluating an indirect effect of bottom trawling activity. Animal performance was quantified by assessing oxygen removal rates and clearance rates for bacterioplankton, thus providing the first insight into the physiological responses of this species to sediment exposure. Subsequent recovery after sediment exposure was evaluated using scanning electron microscopy (SEM) techniques and qualitative observations. Knowledge on the responses of *Vazella pourtalesii* to repeated sediment exposure may help provide insight into the biological considerations needed for effective closure area design and management of glass sponge grounds in the northwest Atlantic.
Materials and Methods

Collection of animals

During an oceanographic mission to the *Vazella* sponge grounds of Emerald Basin in 2017 in support of the EU-funded Horizon 2020 project SponGES (Figure 2; and see Beazley et al., 2017 for mission details), the remotely operated vehicle (ROV) ROPOS (https://www.ropos.com/), a 40 hp Science/Work Class ROV owned and operated by the non-profit Canadian Scientific Submersible Facility (CSSF), was deployed from the Canadian Coast Guard Ship *Martha L. Black* to collect specimens of *Vazella pourtalesii* for *ex situ* experimentation. A total of 23 sponges were collected over four separate deployments of ROPOS, 3 of which occurred in the centre of the Sambro Bank SCA at approximately 160 m depth (Collection Location 1 in Figure 2), and one that spanned across the southern border of the closure at approximately 217 m depth (Collection Location 2, Figure 2). During collection, the manipulator arm of ROPOS was carefully used to collect sponges by their substrate attachment point (pebbles, cobbles) and placed them in the ROPOS ‘biobox’ where they remained submerged upon recovery. Once onboard, the sponges were transferred in a submerged state to an insulated 500 L polyethylene holding tank inside a refrigerated shipping container. The sponges were placed in individual compartments (10 cm by 10 cm) of a grid at the bottom of the holding tank to help keep them stationary. Subsurface water (< 5 m) was pumped into a tank on deck using a portable pump, which was then distributed to a tank inside the refrigerated container, chilled to 9 ºC, and then slowly pumped via a peristaltic pump (1.2 L min⁻¹) to the holding tank containing the sponge specimens. The chilled water tank was refilled twice a day, resulting in 4 water exchanges per day.

Sampling deep-water fauna for experimentation is a logistical and technological challenge. Sampling via the ROV’s manipulator arm required delicate and precise handling of sponges within the target size range. Due to this time-consuming process, along with impediments to sampling from inclement weather, only a limited number of individuals could be collected. At the same time, *Vazella pourtalesii* has been recognized as a vulnerable species (Beazley et al., 2018) and as a result, removal of animals for experimentation should be kept to a minimum. Due to these limitations and challenges, the study was performed with a minimum number of individuals to account for independent replication.
Figure 2. Location of collection sites for *ex situ* experiments in the Sambro Bank Sponge Conservation Area, Emerald Basin, Nova Scotia. Also shown is the location of sediment collection north of Emerald Basin, and the presence-absence of *Vazella pourtalesii* from commercial bycatch records, and DFO optical (camera) and multispecies research vessel trawl surveys.

**Acclimatisation to ex situ experimental conditions**

The sponges were transferred to facilities at the Bedford Institute of Oceanography (BIO), Nova Scotia, Canada, where they were maintained in a 500 L holding tank supplied with coarsely filtered saltwater from the adjacent Bedford Basin, the intake of which is 200 m from shore and at a depth of 17 m at low tide (~3 m off bottom). Salinity was 30.5 ± 0.2 PSU over the course of ten months. To ensure full recovery from sampling and transportation, sponges were acclimatized under the above conditions for seven months. A non-axenic phytoplankton culture was routinely added to the sponge holding system to stimulate bacterial growth in the header tank during this time period. Two to three times per week, 3 – 7 L of cultured phytoplankton at 1 – 5 \(10^6\) cells mL\(^{-1}\) was added to the header tank that supplied the sponge holding tank.
Collection of sediments

In order to emulate the natural sediment regime of the *Vazella* sponge grounds, sediment for use in the exposure trials was collected 22 nautical miles north of the sponge collection locations in Emerald Basin (Figure 2). There, a Van Veen grab sampler was deployed at 170 m depth until 100 L of sediment was collected. The surficial sediments here are described as LaHave Clay, a loosely compacted silty clay that is mainly confined to the basins and depressions of the Scotian Shelf (King, 1970). LaHave Clay overlays Emerald Silt and Scotian Shelf Drift (i.e., glacial till), the latter of which the densest aggregations of *Vazella pourtalesii* are most associated with (Beazley *et al.*, 2018). This location was chosen due to its proximity to several presence records of *Vazella pourtalesii*. Prior to exposure to the sponges, the collected sediment was sieved to a fraction < 63 µm in the laboratory and kept cool (4 °C).

Suspended sediment exposure

Of the 23 sponges collected, three perished during the 7-month acclimatisation period, leaving 20 specimens for experimentation. After acclimatisation, ten 40 L tanks with a flow-through sea water supply were set up as experimental units for replication between two treatment groups. Sea water (9°C) was supplied to each individual tank with a flow rate of ~0.5 L min⁻¹. Water temperature in the experimental tanks was stable over the course of the experiment (mean = 8.96 ± 0.05 °C). Equally-sized sponges were distributed randomly between two treatments, with each of the control and treatment (i.e., sediment-exposed) groups consisting of 10 individuals each, with two individuals per tank (Figure 3).

Prior to the start of the experiment, sponges in the control were unintentionally exposed to air bubbles that potentially formed due to temperature-induced off gasing of oxygen-saturated seawater (temperature difference of 0.5 from header tank to experimental unit). Upon notice of micro bubbles, sponges were returned to the 500 L maintenance tank until the experimental setup was improved. Sponges assigned to the sediment-exposed group remained in the 500 L holding tank at all times during this time period. Once the experimental setup was operational, sponges were placed back in their respective tanks the experiment commenced. No deleterious effects of micro-bubble exposure were evident in the control sponges upon the start of the experiment. We subsequently acquired control data on the respiration and clearance rate from colleagues working on this same species in the same aquaria facilities at another time (Bart *et al.* 2020) in order to compare these rates with our potentially compromised control sponges.

The sediment-exposed group received a concentration of suspended natural bottom sediments of 50 mg L⁻¹ for 12 h per day for 21 days, while the control group remained
untouched. The 12 h exposure cycle was chosen to emulate the natural tidal oscillation experienced off Nova Scotia. Each of the five sediment treatment tanks received pulses of high concentration sediment stock solution (Figure 3). Pulses of stock solution were delivered by five membrane pumps (EW-F31VC-20EPF5, IWAKI). Average frequency was one pulse of one-minute length per five minutes, resulting in 25 mL stock solution being added per pulse to each of the tanks in the exposed group. Aquarium pumps (CompactON 300, Eheim) kept dosed sediment volumes in suspension. The end-concentrations of suspended sediment and particle size distribution in each of the five tanks were monitored with a particle analyser (LISST-100X, Sequoia) at Days 0, 7, 14 and 21. Settled sediment was removed from the bottom of each tank by careful siphoning after each exposure cycle of 12 h.

Figure 3. Experimental design used in this study. Ten individual tanks, each holding two sponges (yellow shapes) were supplied with sea water. Each tank was equipped with a pump (black shapes). Five tanks received dosages of a sediment stock solution for 12 h per day. Particle concentrations in the sediment-exposed tanks were 44.95 ± 2.2 mg L\(^{-1}\) throughout the 21 day experiment.

Physiological measurements
The physiological performance (respiration rate, bacterial clearance rate) of *Vazella poutalesii* assigned to the control and sediment-exposed groups was assessed with chamber-based incubations after 7, 14 and 21 days. Before each incubation sediment-exposed sponges
were given a three-hour recovery period after the last pulse of sediment. During this period, the
tank volume was turned over by fresh seawater approximately four times, so that sponges were
exposed to clear water prior to the start of each incubation. The bottom plate of the incubation
chamber was put in its respective tank and a sponge was placed on the plate. Sponges were left
to recover for one hour before an acrylic chamber that sealed to the bottom plate was placed
over each sponge. The lid of the chamber was equipped with a stirrer for circulation and a
dissolved oxygen sensor (HQ30D equipped with LDO sensors, Hach) calibrated to 100% saturation
according to the manufacturer. Dissolved oxygen concentrations were measured every minute
over four hours and respiration rates (R) in µmol O₂ h⁻¹ gDM⁻¹ were calculated as outlined by Tjensvoll
et al. (2013) using the following equation:

\[ R[µmol O₂ h⁻¹ gDM⁻¹] = ((c₂ - c₁) t⁻¹ * V_{net}) gDM⁻¹, \]

where \(c₁\) and \(c₂\) = the concentration of dissolved oxygen at start and end of the incubation in
µmol O₂ 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 g. Wet mass was measured on a scale after taking the sponge out of
the water and allowing the water to drain for 10 s. Volume of individual sponges was measured by
water replacement in a graded beaker prior to sampling for microscopy approaches (see section
on recovery and electron microscopy). To make the rates assessed in this study comparable to
published data sets we used the conversion factor (mL:gDM = 5.2) from Bart et al. 2020 to
calculate sponge volume from dry mass.

Water samples were drawn from the chamber at the beginning and the end of the four-
hour incubations via tubes inserted through the lid. Samples 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 concentration in the water samples were assessed
by flow cytometry (Brussaard et al., 2010) and clearance rates for bacterioplankton in
mL mL⁻¹ min⁻¹ were calculated as outlined by Robertson et al. (2017) using the following
equation:

\[ \text{Clearance rate [mL mL}^{-1} \text{ min}^{-1}] = V_{net} t^{-1} \ln(n_{start} n_{end}^{-1}) V_{sponge}^{-1}, \]

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 min, \(n_{start}\) and \(n_{end}\) = the concentrations of
bacterioplankton at the start and end of the incubation in counts mL⁻¹ and \(V_{sponge}\) = the total volume
of the incubated animal in g. Empty chamber incubations (n = 2 per treatment group) without a
sponge were performed twice throughout the experiment to account for background respiration
and non-sponge related dynamics in bacterioplankton. The average of the four empty chamber
incubations was subtracted from the sponge incubations of the respective treatment.
**Data analysis**

Statistical tests were performed with GraphPad Prism 8 Version 8.2.1(441), August 20, 2019 using a general linear model analysis of variance (Sediment; two levels nominal 0 or 50 mg L\(^{-1}\), \(n = 5\)) with multiple comparisons. For analysis, measurements of individual sponges were averaged per tank resulting in five independent replicates per treatment. We tested for tank effects prior to the analysis (Kutti *et al.* 2015) and influence of tank was identified as low (Control group: clearance rate: \(p = 0.12\); respiration rate: \(p = 0.14\) Sediment-exposed group: clearance rate: \(p = 0.78\) respiration rate: \(p = 0.20\)). Values are given in mean ± standard deviation (SD) if not stated otherwise.

**Recovery potential and electron microscopy**

After three weeks of exposure to suspended sediment we followed the potential recovery of exposed individuals of *Vazella pourtalesii* for up to 38 days after the last exposure, with unexposed sponges as the control group. In order to determine the level of clogging by sediment in the aquiferous system, tissue from sediment-exposed sponges was collected for examination using scanning electron microscopy (SEM) techniques. Due to financial limitations analysis of tissues of all sponges was unfeasible and only a minimum number of replicates was analysed to account for individual differences between sponges. Three hours after the last exposure to sediment (considered Day 0 of the recovery phase), one sediment-exposed sponge was removed from its tank and subsampled by extracting a ~1 cm\(^3\) tissue sample through the body wall of the lower and upper halves of the sponge (specimens were halved at their mid-point based on total height). Two control sponges were sampled after 3 and 12 days of the recovery phase. On the final day of the recovery phase (Day 38), two sediment-exposed sponges (Sponges 14, 19) and one control (Sponge 8) were subsampled for examination using SEM to determine if sediment was still present in their tissues.

Subsamples were preserved in a 1:4:5 ratio of 25% glutaraldehyde, 0.34 M sodium chloride (NaCl), and 0.4 M phosphate-buffered saline (PBS) (i.e., “Fixative 1”). The Fixative 1 solution was changed after 0.5 hours, and the samples were placed in a fridge (4°C) where they remained for ~2 months. The final step for SEM fixation involved rinsing the samples of Fixative 1 using a 1:1 ratio of 0.4 M PBS and 0.6 M NaCl and fixing the samples in a 1:1 ratio of 4% osmium tetroxide and 0.6 M PBS (i.e., “Fixative 2”) for approximately 2 hours. Samples were then rinsed of Fixative 2 three times in distilled water for 10 minutes each and dehydrated through a series of ascending alcohol concentrations diluted from 100% anhydrous ethyl alcohol: 50% and 70% alcohol (10 min each), 80, 90, 95, and 100% alcohol (3 x 10 min each). In order to create even surfaces from which the intact aquiferous system could be observed, the
1 cm³ subsamples were freeze fractured in liquid nitrogen. Fractured pieces were then dried using liquid carbon dioxide in a Leica EM CPD300 (Critical Point Dryer) at the Scientific Imaging Suite at Dalhousie University, Halifax, Nova Scotia.

After critical point drying, samples were mounted on SEM stubs using a carbon-based paste, and sputter coated using a Leica EM ACE200 vacuum coater at a speed of 30 mA for 325 seconds, which equated to a 25 nm-thick coating of gold/palladium. To determine the degree of clogging, the coated samples were initially viewed and imaged using a Zeiss 1455VP Scanning Electron Microscope operating with an acceleration voltage of 20 kV, and SmartSEM V05.05 SEM operating software.

Initial SEM examination of the sediment-exposed sponge sampled on recovery Day 1 (Sponge 16) revealed poor results from freeze fracturing, likely due to the high density of spicules. Instead of freeze fracturing with liquid nitrogen, the 1 cm³ subsamples collected on all subsequent days were sectioned into smaller pieces using a sharp razor to create both cross sections across the atrial surface, and longitudinal sections through the body wall from the dermal to atrial surfaces. Both the longitudinal and cross sections were fixed for SEM according to the techniques described above.

To identify the potential presence of accumulated particles in sediment-exposed sponge tissue, the tissue’s elemental composition was examined using Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) techniques. Samples were examined and imaged using a Hitachi S-4700 Cold-Field Emission SEM operating at 10 kV (also at Dalhousie University). The atomic weight as a percentage of total weight was calculated for each of four or five randomly chosen point locations over the unknown matter. Samples of the matter were taken using an attached 80 mm² X-Max EDS detector (Oxford Instruments, Concord, MA, USA) and associated Inca software. All elements were analysed (normalized). Given that the mounted tissue of Vazella pourtalesii was not flat, polished, or homogeneous, which are requirements for standardized quantitative analysis using EDS, we chose to convert the atomic weight information into elemental presence/absence. Due to the use of carbon-based mounting media, the presence of carbon in the samples must be discounted, as well as the presence of osmium, which is likely a remnant of the fixation process. The elemental composition of the sediment used in the experiment was also examined using SEM/EDS techniques to serve as a comparison to the matter found in the sponge tissue. Sediment (unfixed) was mounted on an SEM stub using carbon-coated double-sided tape, and gold/palladium coated to a thickness of 15 nm.
Results

Survival

All ten sponges in the treatment group survived repeated exposure to suspended sediment over the course of the 21-day experiment (indicated by observations of active pumping and by respiration and clearance rate assessments) and no tissue necrosis or other signs of decreased health status were observed in the treatment group over the course of the experiment. Initially, sediments accumulated only on the long spicules of the exposed individuals, but with repeated exposure, the atrial surfaces eventually became covered in sediment particles and turned brown in colour (Figure 4). In the control group, three sponges showed signs of necrosis (grey/black coloured tissue) after 10 days in the experimental tanks and eventually perished (Supplementary Figure 1). These sponges deteriorated relatively quickly (within 3 days after the initial signs of discoloration) and no signs of necrosis were evident in the remaining sponges throughout the 21-day experiment. It cannot be ruled out that the mortality in the control group was induced by a short exposure to micro-bubbles at the beginning of the experiment. Thus, all sponges from the control group may have been compromised in their physiological performance. For this reason, data from the control group evaluated in this study were augmented by a published dataset (Bart et al., 2020) on the physiological rates of *Vazella pourtalesii*. Respiration and clearance rates of *Vazella pourtalesii* described by Bart et al. (n = 7) were assessed in a similar manner as in this study (chamber-based incubations) and were performed in the same aquaria facilities.
Figure 4. Individual of *Vazella pourtalesii* from the control (left) and the exposure (right) group after 7 days in the experiment. The long spicules and atrial surface of the exposed sponges eventually became densely covered with sediment particles.

**Suspended sediment exposure**

The average suspended particle concentration in the treatment tanks during the 12 h exposure phase over the course of the 21-day experiment was 44.95 ± 2.2 mg L⁻¹. Particle concentrations at the beginning of the experiment (33.6 ± 2.5 mg L⁻¹) were below the targeted threshold of 50 mg L⁻¹, but steadily increased up to 56.9 ± 2.1 mg L⁻¹ at Day 21 (Figure 5). The control treatment had an average suspended particle concentration of 1.98 ± 0.87 mg L⁻¹. Particles < 6 µm were prevalent in the suspended sediment representing a fraction of 73% of the total concentration (mg L⁻¹). A fraction of 10% was represented by particles as large as ~184 µm (Figure 6).
Figure 5. Average of end-concentrations ± SD of suspended sediment in exposure (n = 5) and control tanks (n = 5) throughout the experiment. Overall average exposure level was 44.95 ± 2.2 mg L⁻¹.

Figure 6. Percentage of concentration per size class of the suspended sediment in end-concentration. 73 % of suspended particles is represented in the size class from 1 to 6 µm.
Physiological performance

The respiration and clearance rates of *Vazella pourtalesii* in the two treatment groups measured during the chamber incubations on days 7, 14, and 21 are summarized in Table 1. Decrease in oxygen concentration in the closed chambers was linear over time. Respiration rates of individuals from the control and sediment-exposed groups were comparable at each of the three measured time points (Day 7, 14 and 21; Figure 7). However, respiration rates increased slightly in the sediment exposed group over the 21-day experiment, with rates ranging from 1.89 ± 0.15 µmol O\textsubscript{2} h\textsuperscript{-1} gDM\textsuperscript{-1} at Day 7 to 2.66 ± 0.51 µmol O\textsubscript{2} h\textsuperscript{-1} gDM\textsuperscript{-1} at Day 21. No effect of sediment treatment or time of exposure was detected (F (3, 18) = 1.96, p = 0.156).

Clearance rates (Figure 8) significantly differed between the sediment-exposed and the control group from Bart *et al.* (2020) on Day 14 and 21 (p = 0.028 and p = 0.006 respectively; and see Table 1). Clearance rates in the sediment-exposed group declined over time with rates ranging from 0.97 ± 0.17 mL mL\textsuperscript{-1} min\textsuperscript{-1} at Day 7 to 0.53 ± 0.15 mL mL\textsuperscript{-1} min\textsuperscript{-1} at Day 21. From 14 days of sediment exposure clearance rates differed significantly from the control group and continued to decrease until the last assessment after 21 days (Day 14: F (3, 18) = 6.40, p = 0.028: Day 21: F (3, 18) = 6.40, p = 0.006).

**Table 1:** Overview of the assessed physiological parameters in *Vazella pourtalesii* under elevated concentrations of suspended particles (Sediment, n = 5) and the literature control group (n = 7) (Bart *et al.*, 2020). Mean values ± SD. Significant differences are indicated by corresponding letter pairs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration rate</strong></td>
<td>Sediment, this study</td>
<td>1.89 ± 0.15</td>
<td>2.28 ± 0.53</td>
<td>2.66 ± 0.51</td>
</tr>
<tr>
<td>[µmolO\textsubscript{2} gDM\textsuperscript{-1} h\textsuperscript{-1}]</td>
<td>Bart <em>et al.</em> 2020</td>
<td>3.39 ± 1.76</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Clearance rate</strong></td>
<td>Sediment, this study</td>
<td>0.97 ± 0.17</td>
<td>0.83 ± 0.20 \textsuperscript{b}</td>
<td>0.53 ± 0.15 \textsuperscript{b}</td>
</tr>
<tr>
<td>[mL mL\textsuperscript{-1} min\textsuperscript{-1}]</td>
<td>Bart <em>et al.</em> 2020</td>
<td>2.22 ± 1.26 \textsuperscript{a}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 7. Respiration rate ± SD of *Vazella pourtalesii* in the treatment (n = 5 per timepoint) and literature control group (n = 7) over time (Bart et al., 2020). Pairs of letters (ab, cd, e.g.) indicate significant differences ($p < 0.05$) among means as indicated by tests for contrasts after 7, 14 and 21 days in the treatment respectively.
Figure 8. Clearance rate ± SD of *Vazella pourtalesii* in the sediment treatment group over time compared to a literature control (Bart *et al.*, 2020). Differing letters indicate significant differences ($p < 0.05$) among means ($n = 5$ per sediment treatment, $n = 7$ for literature control) common letters indicate no significant difference was detected.
**Observations of accumulated material after sediment exposure**

After each 12-hour cycle of sediment exposure, settled sediment was removed from the bottom of each tank. Within 30 minutes after sediment removal, flocculate matter, possibly representing sloughed mucous-coated sediment, was observed accumulating next to the sponges (Figure 9). Particles had a flocculated appearance; small pellets of sediment clumped together by a translucent material and settled on the bottom of the aquaria. This matter was not present at the bottom of the tanks of the control group. Video observations of outflow of sediment-exposed individuals showed that the large, accumulated particles were expelled with high velocity from the osculum (see video in supplementary materials).

![Image](image_url)

**Figure 9.** Left: *Vazella pourtalesii* in top view with flocculated matter (black arrows) accumulated in the tank after initial removal of settled sediment particles. Right: *Vazella pourtalesii* in experimental tank with accumulated sediment particles on the tank bottom after 12 h of exposure.
Recovery potential and scanning electron microscopy examination

The atrial surfaces of most individuals in the sediment-exposed group returned to their pre-exposure state approximately 14 days after last exposure. Of the three sponges left to recover to Day 38, all appeared to clear their dermal surface of sediment completely. Two sediment-exposed sponges appeared to be in the process of dying, with dark sections of tissue indicating necrosis. A sediment-exposed sponge (Figure 10), while appearing to nearly recover, self-dislodged from its basal attachment and floated to the top of the tank on Day 38 and was dissected. Some tissue necrosis was also evident in this individual.

Figure 10. Succession of recovery of a sediment-exposed Sponge at 6, 11, and 21 days after sediment exposure stopped. This sponge was sampled for SEM/EDS analysis 38 days after sediment exposure.

SEM/EDS analysis (Supplementary Table 1) revealed that the sediment used in the exposure experiment consisted of a wide variety of grains and elemental composition, with textural differences between grains observed. The presence of aluminium, silicate, potassium, iron, magnesium and chloride were evident. The presence of both calcium and silicate suggests the sediment grains are both calcareous and siliceous in origin. These elements were largely absent from the three control sponges sampled, where the observed matter was comprised mostly of carbon (although note contamination from mounting media), oxygen, and silicate.

Due to the poor results of freeze fracturing and manual razor sectioning of the tissue, observations of the exact location of sediments within the aquiferous system of the sponges were not possible. Nonetheless, observations on the composition/origin of matter observed amongst the spicules of the mesohyl were made to assess recovery. The elemental composition of the matter observed within the mesohyl of a sediment-exposed sponge (sampled on Day 1 of
the recovery phase) was most similar to that of the two analysed sediment samples. Here, iron, aluminium, magnesium and potassium were all constituents of the sample, suggesting the presence of sediments inside the sponge. Examination of the sediment-exposed sponges (Sponges 14 and 19) sampled on Day 38 revealed an elemental composition more similar to control sponge 8 (sampled on Day 38) than sediment-exposed Sponge 16 and the two controls. For sediment-exposed Sponge 19, the observed matter was comprised of carbon, nitrogen, oxygen, sodium, silica, aluminium and sulphur. The sulphur was possibly related to the decaying state of the sponge prior to sampling.

The similar elemental composition found in the sediment-exposed and the control sponges (longitudinal sections), and the lack of iron, potassium, aluminium, and calcium, which were indicative of the sediments, indicates that sediment-exposed individuals had mostly cleared the sediment from their tissues over the course of the 38-day recovery period. However, observations of the cross-section sample from sediment-exposed Sponge 14 revealed the presence of matter with a similar elemental composition as that of the sediment, suggesting high variability in the location of sediment within the tissues of the same individual.
Discussion

Physiological response of Vazella pourtalesii to natural suspended sediment

Under the laboratory conditions of this study, Vazella pourtalesii survived repeated exposure to seawater with particle concentrations up to 57 mg L\(^{-1}\) over the course of 21 days. There was no difference in respiration rates between the sediment-exposed and literature control group throughout the experiment. These findings are in line with experimental work on deep-water demosponges exposed to a similar sediment concentration as used in this study. For example, Kutti et al. (2015) found that oxygen consumption rates in Geodia barretti Bowerbank, 1858 remained stable over time when exposed to natural suspended sediment (50 mg L\(^{-1}\)) for up to 50 days, indicating that this species is adapted to cope with periods of higher natural water turbidity.

However, in this study Vazella pourtalesii individuals exposed to sediment expressed reduced bacterial clearance rates after 14 days of exposure. In situ and ex situ studies showed that some species of glass sponges arrest pumping when exposed to different concentrations of natural sediment, whereas others do not. For instance, the glass sponge Aphrocallistes vastus maintained pumping during exposure to sediment concentrations up to 36 mg L\(^{-1}\) in an aquaria-based study (Tompkins–Macdonald and Leys, 2008), while Grant et al. (2019) found that concentrations as low as 2.8 – 6.4 mg L\(^{-1}\) caused arrests in the glass sponge Rhabdocalypthus dawsoni from the Hecate Strait reef complex, but no arrests were observed in Farrea occa Bowerbank, 1862 when exposed to similar concentrations as reached in our study (57 mg L\(^{-1}\)). Grant et al. (2018) reported short-term arrests of pumping activities in glass sponges as a response to elevated particle concentrations. These reported arrests never exceeded intervals of ~80 min, likely to avoid oxygen depletion in the sponge’s tissue. While we have not directly measured or observed whether short-term cessation in pumping occurred in our experiment, the increasing brown coloration of the sponges over the course of the experiment, as well as the presence of sediments in the tissues of exposed Vazella pourtalesii individuals as indicated by SEM, suggests that the animals were actively pumping during the 12 h exposure cycles.

Sponges respond to sediment stress via closure of incurrent pores (the ostia) and osculae and the reduction of the aquiferous system via tissue regression. These morphological responses result in the cessation of pumping. In addition, expulsion of particles from the aquiferous system, and mucus production (Strehlow et al., 2017) have been observed after sediment exposure. Particles up to 1 mm in diameter were observed in the excurrent flow of the sponges after sediment exposure (video in supplemental material). Particles had a flocculated appearance, and small pellets of sediment, clumped together by a translucent material and
settled on the bottom of the aquaria. No accumulation of such particles was observed at any time throughout the experiment in the control sponges. Excretion of pellets of indigestible particulate matter is linked to an increase in mucus production, a known stress response exhibited by both demosponges (Strehlow et al., 2017; Grant et al., 2019) and hexactinellids (Kahn et al., 2018). However, mucus production is an energy-demanding process (McGrath et al., 2017), and prolonged exposure to suspended natural sediment likely has an adverse effect on a sponge’s ability to maintain this coping mechanism. A decrease in bacterial clearance rates with sediment exposure, as observed in our study, is indicative for sediment induced cessation of pumping. If the sponges reduce their pumping activity, energy input will decrease and hence, continued coping with sediment will start to consume energy at the expense of other processes. Thus, processes such as growth, tissue maintenance (Shore, 1971; De Goeij et al., 2009), reproduction and cell renewal (Bell, 2002; Alexander et al., 2014) might be compromised under prolonged events of elevated suspended particle concentrations. This could result in an energy deficiency and adverse effects on overall sponge health (Grant et al., 2019).

Recently, Vazella pourtalesii has been shown to predominantly utilize dissolved organic carbon to fuel its metabolic requirements (Bart et al. 2020). It remains to be investigated whether this species can increase the uptake of dissolved organic matter to counteract the reduced uptake of bacterioplankton resulting from repeated sediment exposure. Also unknown is to what extent pathways for dissolved organic matter uptake in Vazella pourtalesii are dependent on pumping and as such affected by the presence of elevated levels of natural suspended sediment. Additionally, a decrease in pumping could result in a reduced capacity to evacuate indigestible sediment particles and accumulation within the sponge’s tissues.

**Recovery after sediment exposure**

Individuals left to recover for up to 38 days after last sediment exposure appeared to clear their dermal surfaces of sediment and were similar in appearance to sponges in the control group. However, SEM/EDS techniques indicated that after 38 days, sediment particles were still present in certain tissue sections of one individual, suggesting high variability in the location of sediment within the tissues and possibly high variability between individuals in the ability to expel accumulated, indigestible particles. Visual observations made during the experiment indicated that sediments tended to accumulate inside and at the base of the atrium of this barrel-shaped species. It is likely that tissues closer to the base will take longer to clear indigestible particles. In our study, all samples for SEM were extracted from the bottom portion of the sponge (based on total height) suggesting that tissues located further away from the base of the sponge might have been cleaned more efficiently from accumulated sediment particles.
than the sampled regions of sponge tissue. Despite the visual recovery of some individuals, a number of *Vazella pourtalesii* perished over the course of the 38-day recovery phase. As some individuals in the control group also showed signs of tissue necrosis and perished, the cause of death cannot evidently be linked solely to exposure to suspended particles. To assess the physiological recovery potential of *Vazella pourtalesii* after long-term sediment exposure, parameters such as oxygen uptake and bacterial clearance rates should be monitored throughout the recovery period in future studies.

**Potential adaptations to a dynamic environment**

It is hypothesised that glass sponges in deep-sea environments may utilize sediment-borne bacteria as an additional food source. Stable carbon and nitrogen isotope examinations of glass sponges collected off the Pacific coast of Canada showed ingestion of sediment-borne bacteria re-suspended by tidal oscillations (Kahn et al., 2018). Sediments in deep-sea environments have been found to be 100-1000 times more rich in heterotrophic bacteria than the overlying water column (Kuwae and Hosokawa, 1999). Furthermore, Grant and Roberts-Regan (1987) showed that the fine silty sediments on the Scotian Shelf and particularly in Emerald Basin contain a high proportion of easily re-suspended organic particles and postulated that lateral transport of organic material makes the Scotian Shelf a sink for organic carbon. The potential trophic interaction of *Vazella pourtalesii* and sediment borne re-suspended organic matter has not been investigated up to date. However, our incubation-based assessments of physiological parameters suggest that *Vazella pourtalesii* tolerates re-suspended particles, rather than utilizing and benefiting from the potentially bacteria-and particle-enriched water layers. The assessed respiration rates suggest that there is no metabolic benefit of the presence of suspended sediment enriched seawater for these sponges.

Emerald Basin is subjected to higher tidal variability and mixing compared to other areas of the Scotian Shelf (Petrie and Smith, 1977). Tidal oscillations and storm events have the potential to re-suspend small grain particles that may remain suspended in the water column for several days (Bogucki et al., 1997; Boegman and Stastna, 2019). In addition to seasonal short term resuspension events, persistent nepheloid (sediment enriched) layers with particle concentrations of up to 8 mg L$^{-1}$ have been described to occur on the Scotian Shelf (Spinrad et al., 1983; Pilskaln et al., 1998). Similar resuspension events with a duration of several days were observed during a 10-month deployment of an autonomous benthic lander in the Sambro Bank Sponge Conservation Area (Hanz et al., 2020 and see Beazley et al. (2017) for deployment details). Dynamic oceanographic characteristics and sponge occurrences are often linked in the North Atlantic. For instance, the distribution of the glass sponge species
Pheronema carpenteri (Thomson, 1869) (Family Pheronematidae) at the Porcupine Seabight (northeast Atlantic) is thought to be linked to areas where bottom currents are intensified due to internal waves (Rice et al., 1990). The causal link was suggested to be an increase in the supply of food related to the occurrence of the internal waves. The same oceanographic settings might be a possible explanation for the occurrence of dense demosponge grounds in the Faroe-Shetland (Davison et al., 2019). Our findings suggest that Vazella pourtalesii might be adapted to tolerate natural suspended sediment up to a certain concentration and exposure time. This is corroborated by the proliferation of this species in this highly dynamic environment subjected to periodic re-suspension events. However, the observed reduction in clearance efficiency with sediment exposure over time indicates that potential coping mechanisms and adaptations are compromised when sediment exposure occurs over a longer time scale.

**Implications for conservation management**

While the results of this study indicate no adverse physiological effects (respiration and clearance rates) of short-term sediment exposure (7 days), prolonged (14 to 21 days) exposure to re-suspended sediment from bottom trawling may impair physiological rates in Vazella pourtalesii as a response mechanism. Over time, this may result in an energy deficit and reduction in overall sponge health. With a concentration well below the described re-suspension potential of a trawl (up to 500 mg L\(^{-1}\), see Introduction), these likely negative impacts on the physiology of Vazella pourtalesii could be amplified in its natural setting if exposed to re-suspended sediments from trawling. Future studies should include different concentrations of suspended sediment in combination with longer exposure times to detect potential thresholds for adverse effects of suspended sediments on Vazella pourtalesii.

The Sambro Bank and Emerald Basin Sponge Conservation Areas were designed to protect two of the more significant concentrations of Vazella pourtalesii on the Scotian Shelf identified from biomass/density observations from commercial bycatch records, research vessel trawl catch data, and in situ camera observations. However, kernel density analyses applied to research vessel trawl survey catch data to identify hotspots in sponge biomass (all Porifera, including Vazella pourtalesii; Kenchington et al., 2016), indicated that less than 2% of the significant concentrations formed primarily by Vazella pourtalesii in the area known as the Scotian Gulf, which includes Emerald Basin, are encompassed by the closed areas. Similar results were found when examining the proportion of protected suitable habitat of Vazella pourtalesii generated from Random Forest species distribution modelling; see Beazley et al. (2018). The percentage overlap between the significant concentrations of all Porifera, including Vazella pourtalesii, and groundfish fishing effort using mobile-gears revealed that 29.4% of
the area occupied by these significant concentrations was subjected to some level of fishing (DFO, 2017; Koen-Alonso et al., 2018), with most effort occurring in the lowest percentile (low effort). Nonetheless, this lower effort does not indicate that significant adverse impacts of fishing to *Vazella pourtalesii* are not occurring on these sensitive benthic areas (Koen-Alonso et al., 2018). Depending on the type of gear and composition of the benthic community, few interactions can cause serious irreversible damage (NAFO, 2011), and the greatest impacts to these ecosystems, such as irreversible damage or complete removal, are often caused by the first fishing events (DFO, 2006; Pham et al., 2019).

Grant et al. (2018) modelled the indirect footprint of a trawl adjacent to an existing bottom fishing closure and estimated that the re-suspended sediment plumes spread as far as 6 km from the initial trawling site. They concluded that fishing effort along the boundaries of sponge conservation area rendered the dense sponge grounds inside it vulnerable to suspended sediments. Although the trajectory and retention rates of sediment plumes from bottom fishing in Emerald Basin were not measured in this study, the close proximity of existing bottom fishing to the SCAs on the Scotian Shelf (Koen-Alonso et al., 2018) may have the potential to impact the dense *Vazella* grounds that reside within them. Here, buffer zones of a sufficient dimension could be established around the SCAs to minimize the indirect impact of downstream sediment re-suspension by bottom gear to the sponge ground community inside the closed areas. Although there was no adverse effect evident the first 7 days of sediment exposure, an additional mitigation measure could be a temporal staggering of fishing activities within the same area to allow the sponges to recover following trawling events. In this area, the groundfish fishery operates year-round, from April 1st – March 31st but has a 3-month period of high fishing activity from July to September with individual trips occurring on the scale of 5 days and with multiple sets spread out over multiple areas (D. Fenton, Fisheries & Oceans Canada, pers. comm.). Thus, the 21-day period chosen for this experiment likely represents a worst-case scenario of sustained fishing activity from different vessels in the vicinity of the sponge grounds within the peak fishing period. The Scotian Shelf is subjected to ongoing structural developments such as offshore oil and gas extraction (Breeze et al., 2005). The impacts of sediments contaminated with drilling muds or potential spills of crude oil on glass sponges are at present poorly understood. The present study on the physiological response of *Vazella pourtalesii* to a concentration of natural suspended sediment may act as a blueprint for the design of these elaborate experiments.
Conclusion

In this aquaria-based ex situ study *Vazella pourtalesii* was found to cope with increased levels of natural suspended sediment for a period of 7 days. As this glass sponge species thrives in a dynamic shelf environment, *Vazella pourtalesii* is likely adapted to periodic, current-induced re-suspension events. However, our observations of decreasing clearance rates over time could indicate impaired filtration capacities cumulating in negative impacts on biological functions such as growth, tissue repair mechanisms and reproduction, and a metabolic deficit with adverse implications for overall animal health. Following the precautionary approach, the application of buffer zones to the current management measures for this species on the Scotian Shelf, and temporal coordination of sediment re-suspending activities may help mitigate the potentially adverse long-term effects on the unique ecosystem the *Vazella* grounds represent on the Scotian Shelf.

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Chapter 3

Over the edge: Synergistic effects of suspended natural sediment and future climate conditions decrease metabolic fitness in the boreal deep-water sponge *Geodia barretti* Bowerbank, 1858

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Abstract

The depths of the North Atlantic Ocean comprise vast ecosystems called sponge grounds. These sponge-dominated habitats are hotspots of biodiversity and nutrient recycling. Bottom trawling has been identified as the most severe industrial threat to these abundant sponge grounds by removing sponge biomass and resuspending bottom sediments. At the same time the physicochemical properties of the North Atlantic Ocean will be irreversibly altered by human induced climate change. This study explored the interactive effects of a field relevant concentration of suspended sediment (50 mg L⁻¹) and future ocean conditions (pH decrease of 0.2 units, temperature increase of 3 °C) on the physiological performance of Geodia barretti Bowerbank, 1858, a dominating sponge species in boreal sponge grounds. Physiological performance was defined as oxygen consumption, bacterial clearance rate and the stability of the microbial community associated with G. barretti. We found that cumulative stressors had synergistic effects on clearance rates resulting in a cessation of pumping. At the same time indications are evident that bacterial clearance might be a sub-optimal proxy for sponge performance for the high microbial abundant G. barretti. Oxygen consumption rates remained unchanged under low pH and high temperature treatments and indicate mechanisms of pumping-independent mass transfer of oxygen. A small, but statistically significant shift in the microbiome associated with G. barretti could be interpreted as an early warning sign of a limited coping capacity towards cumulative stressors in this deep-sea sponge species.

Keywords: sponge grounds, benthic-pelagic coupling, bottom trawling, climate change, holobiont
**Introduction**

From abyssal, polar depths to shallow, tropical waters sponges (Porifera) have been identified as an integral part of benthic fauna communities (reviewed in Bell, 2008). Sponges are filter-feeding organisms that process large volumes of seawater. While pumping seawater through their bodies sponges retain a wide range of energy sources including particulate and dissolved organic matter (DOM) (Maldonado *et al.*, 2012). Retention efficiency for small particulate resources is usually high and sponges can retain up to 95% (Yahel *et al.*, 2006) of ultraplankton particles smaller than 10 μm (Witte *et al.*, 1997). Their efficient filtering apparatus and high water processing capacities make sponges key-players in terms of benthic-pelagic coupling by transferring energy from lower to higher trophic levels (Ribes *et al.*, 2005) in tropical shallow-water (De Goeij *et al.*, 2013) and deep-sea ecosystems (Rix *et al.*, 2016).

In the North Atlantic Ocean (NAO) the demosponge *Geodia barretti* Bowerbank, 1858 (Geodiidae) is highly abundant and can locally represent up to 90% of the benthic biomass (Murillo *et al.*, 2012) with up to 45 kg m\(^{-2}\) wet mass (Maldonado *et al.*, 2017). These sponge dominated habitats have been termed sponge grounds (Klitgaard *et al.*, 1997). Kutti *et al.* (2013) calculated that the standing stock of *G. barretti* in an area of 300 km\(^2\) on the Norwegian Shelf could filter approximately 250 million m\(^3\) of water and consume 60 t of organic carbon daily. This capacity to sequester carbon is in the same order of magnitude as of other marine habitats (Alongi, 2012) that are considered to play a significant role in the regulation of both local and global climate (Nellemann *et al.*, 2009; Pendleton *et al.*, 2012).

Industrial fishing activities and global environmental changes are simultaneously affecting *G. barretti* dominated sponge grounds in the NAO. Given the vast dimensions of fishing activities and the heavy contact with the seabed, bottom trawling has been identified as the most severe threat to sponge grounds in the NAO (Ospar Commission 2010; Smith and Hughes, 2008). The seabed of the Barents Sea is subjected to ~ 27 000 hours of fishing activities per year (Tjensvoll *et al.*, 2013). These fishing activities are dominated by the use of bottom trawling gear (Bradshaw *et al.*, 2012), which deliberately disturbs the seabed in order for demersal target species to swim up in the net (Bradshaw *et al.*, 2012). This deliberate disturbance can mobilize and resuspend the top 20 cm of the bottom sediment layer (Hiddink *et al.*, 2006). Particle concentrations up to 500 mg L\(^{-1}\) have been reported to be resuspended behind a bottom trawl (Durrieu De Madron *et al.*, 2005). Mobilized, resuspended particles < 10 μm can be dispersed over large distances by oceanic currents impacting ecosystems kilometres away from the initial trawling site (Lepland and Mortensen, 2008; Puig *et al.*, 2012). Concentrations of re-suspended sediments have the potential to impede the physiology of filter-feeding sponges by entering their aquiferous system and choanoecyte chambers (Tompkins-
Macdonald and Leys, 2008; Strehlow et al., 2017). *G. barretti* has been shown to respond to short term (four hours) sediment exposure with a rapid decrease in its rate of respiration followed by a normalization of respiration rates once the sediment exposure ceased (Tjensvoll et al. 2013). On longer time scales (up to 29 days) exposure to suspended natural sediment did not affect respiration in *G. barretti* (Kutti et al., 2015). However, it remains unknown how potentially accumulated, indigestible sediment particles impact the water processing capacities that make *G. barretti* a key-player in benthic-pelagic coupling. In addition, information on combined effects of local sediment resuspension events and predicted global ocean changes in the next centuries on deep-sea sponges is still lacking.

The Barents Sea and the Norwegian Shelf with their *G. barretti* dominated sponge grounds have been identified as areas to be highly impacted by ocean acidification and warming in the near future (Sweetman et al., 2017). Under the business as usual scenario (IPCC, 2014) these regions are predicted to experience a decrease in seawater pH of up to 0.4 units and a temperature increase of 3.5 °C down to 300 m water depth until the year 2100 (Sweetman et al., 2017). The effects of changing environmental conditions on sponge physiology have been studied in a variety of shallow water, tropical sponge species (Carballo and Bell, 2017; Bell et al., 2018). However, effects of changing environmental conditions on species that are structuring deep-sea habitats are poorly understood. Heat wave-induced short term increases in seawater temperatures have been reported to have caused mass mortalities in *G. barretti* in different locations across its distributional range (Howell et al., 2016; Strand et al., 2017). Although the rapid seawater temperature increase could not be identified as the sole cause of mass mortality events (Strand et al., 2017), these occurrences showcase the potentially limited coping capacities of deep-sea sponges to climate change. Stress responses have also been evident in *Geodia atlantica* (Stephens, 1915) (Geodiidae) when exposed to cumulative stressors of acute warming and mine waste (Scanes et al., 2018). Ocean warming (OW) in sponge dominated habitats in the NAO will be accompanied by a decrease in seawater pH (Sweetman et al., 2017), so called ocean acidification (OA). Studies have identified a number of sponge species to tolerate low pH seawater conditions (Duckworth et al., 2012; Morrow et al., 2015). This resilience is hypothesised to be especially prominent in high microbial abundant sponge species (Goodwin et al., 2014). The broad spectrum of associated microbes might enable sponges to restructure their symbiotic microbial community with bacteria strains that are coping with changes in seawater pH (Ribes et al., 2016). While Bell et al. (2018) evaluate the impact of ocean acidification on shallow water sponges as moderate, they stress that responses are most likely species-specific and that sponge associated microorganisms play a key role in the capacity of sponges to cope with changes in their ambient environments. Furthermore Carballo
and Bell (2017) stress, that up to date the knowledge about the interactive effects of OA, OW and pollution or suspended sediment on the sponge holobiont is limited.

Currently, the understanding of the responses of deep-sea sponges to near future ocean conditions and their interaction with fishing activities in the NAO is limited. Filter capacity of ultraplankton, an important metric for filter-feeding sponges, has not been included in recent studies on the impact of suspended sediment on deep-sea sponges. Clearance rate has been shown to be a proxy for active pumping of water in *G. barretti* (Leys *et al.*, 2018) as there are no bypasses evident in this species that would channel water through the sponge’s body without being filtered. Thus, clearance of ultraplankton (assessed in this study) can be used as a proxy for pumping. When exposed to suspended sediment, the unselective filter-feeding ultimately results in uptake of sediment particles in the sponge holobiont (Leys *et al.*, 2018). It remains unknown how this potential accumulation of indigestible particles is affecting active pumping and how the abundant microbiome associated with *G. barretti* responds to potentially compromised physiological rates of the sponge host. The microbial symbionts of *G. barretti* are one important component in the microbe-sponge assemblage (sponge holobiont), contributing to a variety of processes including food uptake (Leys *et al.*, 2018), inorganic nitrogen cycling (Hoffmann *et al.*, 2009) and production of secondary metabolites (Hedner *et al.*, 2006; Lind *et al.*, 2013). A stable microbiome in sponges has been identified as a proxy for sponge health (Slaby *et al.*, 2019) and monitoring the microbial community was proposed as a diagnostic tool for early warning indicators for stress at the holobiont level (Glasl *et al.*, 2017). While the microbiome associated with *G. barretti* explants remained stable under laboratory conditions and a temperature increase of 5 °C (Strand *et al.*, 2017), some species express major shifts in the microbial community in response to warming seawater (Ramsby *et al.*, 2018). Up to date it is not described how increasing seawater temperature, accompanied by a decrease in seawater pH and suspended sediment exposure will impact the high microbial abundant (HMA) deep-water sponge holobiont *G. barretti*. Multifactorial experiments, which encompass a variety of potential future ocean characteristics and maintain these conditions for an extended range of time (months), are needed to investigate the response of *G. barretti* to cumulative stressors (Riebesell *et al.*, 2010).

In this study, the habitat structuring sponge species *G. barretti* was exposed to cumulative stressors under laboratory conditions for eight months. In a multifactorial experiment sponges were pre-exposed for seven months to near future ocean conditions (pH decrease of 0.2 units, temperature increase of 3 °C) followed by an exposure to a concentration of suspended natural sediment (50 mg L⁻¹) for 19 days. Sponge oxygen consumption, bacterial clearance rates and diversity of the associated microbiome were assessed as response variables.
This is the first study in which a boreal deep-water sponge species has simultaneously been subjected to a scenario of climate change and suspended natural sediment in an ecologically relevant concentration (Tjensvoll et al., 2013; Kutti et al., 2015). Quantifying the physiological rates and microbial stability of a habitat structuring, highly abundant deep-sea sponge species under cumulative stressors is critical to evaluate the impact of global changes and local interactions on sponge driven benthic-pelagic coupling mechanisms (Rix et al., 2016; Maier et al., 2020) and ecosystem services (Johnson et al., 2018).
Materials and methods

Experimental animals and manipulated seawater acclimatisation

Explants of *Geodia barretti* were prepared via fragmentation according to Kutti *et al.*, (2015) and were kept for seven months at 700 m depth in a sheltered fjord near Bergen (Norway). This allowed for recovery of the explants and development of an operational aquiferous system, often with visible oscules. Regenerated explants (*n* = 60; volume = $35.1 \pm 11.4$ ml; dry mass = $9.1 \pm 3.4$ g; wet mass = $35.8 \pm 13.0$ g) were exposed to four treatments of seawater pH and temperature: 1. Control (pH 8.0, 8.5 °C); 2. Increased temperature (termed: High T, pH 8.0, 10.5 °C); 3. Decreased pH (termed: Low pH, pH 7.7, 8.5 °C); 4. Increased temperature and decreased pH (termed: Low pH high T pH 7.7, 10.5 °C). Treatment thresholds are based on climate predictions for the year 2100 according to the scenario RCP6.0-RCP8.5 (IPCC, 2014; Sweetman *et al.*, 2017). Per treatment five tanks contained three sponge explants each, resulting in 15 sponge replicates per treatment (Figure 1). Seawater (35 PSU), drawn from 160 m depth from the Langenuen Fjord (60° 5’ 16.91”N, 5° 16’ 9.84”E), was pre-treated in 150 L basins with CO$_2$ before supplied to the header tank of the respective pH treatment. In the temperature-controlled header tanks the pH manipulated seawater was mixed before it was feed into the replicate tanks via gravitational force (flow rate 1 L h$^{-1}$). No additional food was added to the tanks. Previous studies utilizing these facilities have shown that the sand filtered seawater contains sufficient particles smaller than 10 μm as food supply (Kutti *et al.*, 2015; Strand *et al.*, 2017). Exposure to the seawater treatments started seven months before the sediment exposure experiments commenced.
Figure 1. Schematic overview of the experimental design used in this study. Four treatments with five tanks each were supplied with preconditioned seawater from an individual header tank. Header tanks assigned to low pH treatments received dosages of low pH preconditioned seawater (yellow arrows). Each of the tanks was holding three enclosures with sponges (grey shapes) of which two were individually dosed with a sediment stock solution (brown arrows) 12 h day⁻¹. Every enclosure was supplied with preconditioned seawater with a flow rate of 0.5 L h⁻¹ (blue arrows).

Sediment collection and analysis

Natural bottom sediment was collected by a van Veen grab at 130 m depth in Raunefjord, Norway. The sediment was 63 μm wet sieved and kept at a temperature below 10 °C, while settling for 5 days. After the supernatant water was removed, sediment was frozen (-20 °C) until use in the experiment. Homogenized samples (n = 5) were collected, weighed with an analytical balance, dried in a drying oven at 60 °C for 24 h to determine the wet/dry mass ratio. Organic carbon content was measured by combusting 72 h dried samples for 4 hours at 450 °C. Particle size distribution of the natural sediment was determined with a Portable Particle Counter (PAMAS S4031GO; Germany).
**Sediment exposure**

For the natural sediment exposure three sponge replicates residing in each of the 20 tanks were maintained individually in enclosures (1225 mL chambers) that were placed inside the tanks (Figure 1). In this way a series of sponges could be exposed to two different sediment regimes: exposure for 12 hours per day (termed: Sediment; n = 10 per seawater treatment) and no sediment exposure (termed: Control; n = 5 per seawater treatments). Sediment exposure lasted for 19 days. The small enclosures had an individual water supply of 4 L h\(^{-1}\) and were equipped with a circulation pump to keep the seawater mixed and dosed sediment in suspension. Pulses of a sediment stock solution were delivered by four membrane pumps (EW-F31VC-20EPF5, IWAKI) dependent of seawater flow-through rates in the respective aquaria and the concentration of the stock solution. Average frequency was one pulse of one-minute length per five minutes delivering approximately 20 mL of stock solution. Sediment concentrations in the exposure enclosures were monitored regularly by spectrophotometry (Shimadzu UV-160), hereby 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 dry sediment L\(^{-1}\) in seawater. Settled sediment was siphoned off the bottom of the enclosures weekly.

**Physiological measurements**

The physiological rates (respiration rate, bacterial clearance rate) of *G. barretti* were assessed by chamber-based incubations after 7, 14 and 19 days in the two groups (Control and sediment) within their respective seawater treatment. Sponges subjected to sediment exposure were given a three-hour recovery period after the last pulse of sediment before incubations started. Dissolved oxygen concentrations were measured (Oxy 10-mini, Presens, Germany) every 15 seconds over four hours and respiration rates (R) in µmol O\(_2\) h\(^{-1}\) gDM\(^{-1}\) were calculated as outlined by Tjensvoll *et al.* (2013) using the following equation:

\[
R[\mu\text{mol } O_2 \text{ h}^{-1} \text{ gDM}^{-1}] = ((c_2 - c_1) \ t^{-1} \times V_{\text{net}}) \ g\text{DM}^{-1},
\]

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, \(V_{\text{net}}\) = 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 g. Sponge mass was assessed at the end of the experiment by taking the respective sponge out of the water and let water drip from the sponge for 10 s before placement on the scale.

Water samples (5 mL) were drawn from the chamber at the beginning and the end of the four-hour incubations. Samples (2 mL) for bacterioplankton quantification were fixed with glutaraldehyde (final 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 h⁻¹ gDM⁻¹ were calculated as outlined by Robertson et al. (2017) using the following equation:

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

where \( V_{\text{net}} \) = the volume of the incubation chamber after subtracting the volume of the respective sponge in mL, \( t \) = the time of the incubation in h, \( n_{\text{start}} \) and \( n_{\text{end}} \) = 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 g. Per treatment and time point five empty enclosure incubations were performed to account for background respiration and non-sponge related dynamics in bacterioplankton. The five replicate blank incubations were averaged and subtracted from the respective rates of sponges in the corresponding treatment.

**Microbiome**

Sponge samples were taken according to Table 1 before and after the sediment exposure. Sponges were removed from the aquaria after sample extraction to not introduce compromised individuals to the experiment. Structural components were extracted by cutting through the sponge with a sterile single use scalpel. Three cubes of approximately 5 mm length were extracted from each sponge, placed in a 2 mL vial and flash frozen in liquid nitrogen prior to storage in -80 °C until analysis. The DNeasy Power Soil Kit (Qiagen) was used for DNA extraction on sponge components (~0.25g). Sponge-derived DNA was eluted in 100 µL and afterwards diluted (1:10) with PCR grade water. Quality of the extracts was checked using a NanoDrop spectrophotometer and gel electrophoresis after a PCR with universal 16S primers. For sequencing, the V3 and V4 variable regions of the 16S rRNA gene were amplified using the primer pair 341F- 806R (5’-CCTACGGGAGGCAGCAG-3’ and 5’-GACTACHVGGGTWTCTAAT-3’) in a dual-barcoding approach. Verification of PCR-products was conducted with gel electrophoresis after a PCR with universal 16S primers. Based on forward reads (truncated to 270nt), 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 16S database, with the help of a primer-specific trained Naive Bayes taxonomic classifier.
Table 1.  Overview of component samples of *G. barretti* used for microbiome analysis.

<table>
<thead>
<tr>
<th>Before/after exposure</th>
<th>Climate treatment</th>
<th>Sediment</th>
<th>Nr. of samples [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>Control</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>After</td>
<td>Control</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>Before</td>
<td>High T</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>After</td>
<td>High T</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>After</td>
<td>High T</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>Before</td>
<td>Low pH</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>After</td>
<td>Low pH</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>After</td>
<td>Low pH</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>Before</td>
<td>Low pH high T</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>After</td>
<td>Low pH high T</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>After</td>
<td>Low pH high T</td>
<td>Yes</td>
<td>3</td>
</tr>
</tbody>
</table>

**Data analysis**

Statistical tests were performed with GraphPad Prism 8 Version 8.2.1(441), August 20, 2019. A one-way ANOVA was performed to compare sediment concentrations between treatments. The endpoint measurements of the two sediment exposed sponges per tank were averaged to avoid pseudoreplication. Clearance and respiration rates were analysed using a general linear model analysis of variance (Sediment; two nominal levels 0 (n = 5 tanks per seawater treatment) or 50 mg L⁻¹ (n = 5 tanks per seawater treatment)). 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. Values are given in mean ± standard deviation (SD) if not stated otherwise. To statistically assess the treatment effects on the *G. barretti* associated microbiome weighted UniFrac distances (calculated on feature level) were used as basis to perform pairwise PERMANOVAs (significance level $\alpha=0.05$).
Results

Seawater characteristics

Manipulated seawater parameters in the long-term acclimatisation period met the thresholds specified in the climate scenario RCP6.0-RCP8.5 (IPCC, 2014). The treatments low pH and low pH high T had pH values of 7.72 ± 0.04 and 7.68 ± 0.05, respectively (Table 2). The treatments control and high T without active pH manipulation had pH values of 8.09 ± 0.05 and 8.08 ± 0.03, respectively (Table 2). In the high temperature treatments seawater had temperatures of 10.51 ± 0.06 °C (High T) and 10.54 ± 0.08 °C (Low pH high T). In the treatment low pH and control without active temperature manipulation temperatures were 8.15 ± 0.14 °C and 8.15 ± 0.19 °C, respectively.

Table 2. Seawater parameters in the experimental treatments. Values are means over time and given with standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature [°C]</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.15 ± 0.19</td>
<td>8.09 ± 0.05</td>
</tr>
<tr>
<td>Low pH</td>
<td>8.15 ± 0.14</td>
<td>7.72 ± 0.04</td>
</tr>
<tr>
<td>High T</td>
<td>10.51 ± 0.06</td>
<td>8.08 ± 0.03</td>
</tr>
<tr>
<td>Low pH high T</td>
<td>10.54 ± 0.08</td>
<td>7.68 ± 0.05</td>
</tr>
</tbody>
</table>

Sediment characteristics and concentration over time

The sediment had a median grain size of 1.22 μm. More than 99 % of the total particle counts consisted of particles < 10 μm (Figure 2). Throughout the experiment, the average suspended sediment concentration was 59 ± 22 mg L⁻¹ and no differences in particle concentration between sediment treatments were detected ($p = 0.093$) (Figure 3). The organic matter content, measured as ash-free dry weight, was 0.6 %. At the end of the exposure period structural component samples have been extracted from the sponges. While individuals from the control treatment resembled a natural colouration for *Geodia barretti*, sediment exposed individuals expressed a grey coloration (Figure 4).
Figure 2. Percentage of total counts of particles per size class. 99% of particles of the sediment used in this study had a grain size smaller than 10 μm.

Figure 3. Concentrations of natural suspended sediment in the four respective treatments over time. The control refers to the treatment that received sediment dosages but no manipulation of seawater parameters. The dotted line represents the overall average of particle concentration over time (59 ± 22 mg L⁻¹).
Physiological responses

Respiration and bacterial clearance rates were highly variable among replicate sponges, as is reflected in large standard deviations (Figure 5). Moreover, repeated measurements on the same sponge individual over time showed high variability. For bacterial clearance rates, response patterns were different for the three timepoints at which the measurements were done (1, 12 and 19 days after the start of the sediment exposure) and were not consistent. This probably relates to the large variability within and among individuals. Hence, the three measurements per sponge over time were averaged. To further account for the large variation in individual rates, we only compared future ocean conditions (Low pH high T – herein after also referred to as “climate change”) to current ocean conditions under sediment exposure and without sediment exposure. Analysis of these averaged data (represented in Figure 5) showed no statistical effects of climate change and sediment on respiration (2-way ANOVA, Table 3A). However, the treatments “control sediment” and “low pH high T” expressed a slight increase in oxygen uptake rates (1.32 ± 0.38 and 1.51 ± 0.35 µmol O$_2$ h$^{-1}$ gDM$^{-1}$, respectively). Despite the non-significance of the differences between means in the four groups, a trend of increasing respiration rates can be observed (0.1>p>0.05, Table 3A). In the cumulative stressor treatment (low pH high T sediment) respiration rates were comparable to rates in the control group (1.12 ± 0.37 and 1.16 ± 0.23 µmol O$_2$ h$^{-1}$ gDM$^{-1}$, respectively). Bacterial clearance was
significantly reduced by sediment exposure (2-way ANOVA, Table 3B). This main effect was primarily caused by explants exposed to combined sediment stress and climate change conditions (Tukey post hoc comparison, Table 4). While sponges in the climate change treatment expressed clearance rates comparable to the control group (9.76 ± 6.26 and 10.94 ± 5.54 mL h⁻¹ gDM⁻¹, respectively), sponges in the cumulative stressor treatment expressed significantly lower (-4.74 ± 9.65 mL h⁻¹ gDM⁻¹) bacterial clearance rates than sponges from the control group (p = 0.01) and the climate change group (p = 0.02). Clearance and respiration rates expressed a limited correlation in sponges from this study. In all treatments the correlations between the two physiological parameters is described by R² values < 0.45 (Figure 6).

Figure 5. Average respiration rates (left) and bacterial clearance rates (right) over time for explants of *G. barretti* under current ocean conditions (control) and future ocean conditions (low pH high temp) with and without exposure for 12 hours per day to 60 mg L⁻¹ suspended natural sediment. Dashed lines in left figure indicate trends ((0.1>p>0.05)) in changes in respiration rates in response to single (red dotted line) and combined treatments (suspended sediment and climate change, blue dashed line). In the right graph (bacterial clearance), significant differences between treatments are indicated with characters (a,b), treatments sharing the same character not being significantly different from each other (Tukey post hoc analysis following 2-way ANOVA). Error bars indicate standard deviations (n = 5 per treatment group).
Table 3. Results of 2-way ANOVA on the data presented in Figure 5.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>(F(1,16))</th>
<th>(p)-value</th>
<th>Factor</th>
<th>DF</th>
<th>(F(1,16))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>1</td>
<td>3.196</td>
<td>0.478</td>
<td>Sediment</td>
<td>1</td>
<td>2.120</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Low pH high T</td>
<td>1</td>
<td>0.5277</td>
<td>0.620</td>
<td>Low pH high T</td>
<td>1</td>
<td>10.45</td>
<td>0.084</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.2551</td>
<td>0.093</td>
<td>Interaction</td>
<td>1</td>
<td>3.387</td>
<td>0.165</td>
</tr>
</tbody>
</table>

Table 4. Tukey post hoc comparison test on the data for bacterial clearance presented in Figure 5.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. control sediment</td>
<td>0.602</td>
</tr>
<tr>
<td>Control vs. low pH high T</td>
<td>0.993</td>
</tr>
<tr>
<td>Control vs. low pH high T sediment</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>Low pH high T vs. control sediment</td>
<td>0.760</td>
</tr>
<tr>
<td>Low pH high T vs. low pH high T sediment</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>Control sediment vs. low pH high T sediment</td>
<td>0.132</td>
</tr>
</tbody>
</table>
Figure 6. Clearance rate as a function of respiration rate in *Geodia barretti* under different sediment (n = 10 per sediment treatment) and seawater treatments (n = 5 per seawater treatment). Trendlines and associated $R^2$ values indicate the level of correlation between clearance and respiration rate per treatment group.
**Microbiome**

The combination of exposure to natural suspended sediment and climate change conditions did not have large effects on the overall microbiome structure (microbial phylum-level) of *G. barretti* (Figure 7). However, significant differences in the microbial community composition were observed on a finer-resolution (feature-level) between groups that were exposed to cumulative sediment and climate treatments (Table 6).

**Figure 7.** Effects of exposure to suspended natural sediment on the relative abundance in the microbiome (microbial phylum-level) of *Geodia barretti* under different seawater conditions. From top to bottom: 1. Low pH high T treatment, no sediment exposure (n = 5) 2. Low pH high T treatment, 12 h sediment exposure (n = 3) 3. Low pH treatment, no sediment exposure (n = 5) 4. Low pH treatment, 12 h sediment exposure (n = 4) 5. High T treatment, no sediment exposure (n = 3) 6. High T treatment, 12 h sediment exposure (n = 4) 7. Control treatment, no sediment exposure (n = 5) 8. Control treatment, 12 h sediment exposure (n = 4)
Table 5. Results of pairwise Permanova analyses on sponge-associated microbial communities within each seawater (Control, low pH, high T, low pH high T) treatment. Permutations for each comparison were 999.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Sample size</th>
<th>pseudo-F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Before</td>
<td>Sediment</td>
<td>13</td>
<td>2</td>
<td>0.11</td>
</tr>
<tr>
<td>Control</td>
<td>Before</td>
<td>No sediment</td>
<td>10</td>
<td>0.8</td>
<td>0.54</td>
</tr>
<tr>
<td>Control</td>
<td>Sediment</td>
<td>No sediment</td>
<td>13</td>
<td>0.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Low pH</td>
<td>Before</td>
<td>Sediment</td>
<td>13</td>
<td>2.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Low pH</td>
<td>Before</td>
<td>No sediment</td>
<td>9</td>
<td>2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Low pH</td>
<td>Sediment</td>
<td>No sediment</td>
<td>14</td>
<td>1.3</td>
<td>0.32</td>
</tr>
<tr>
<td>High T</td>
<td>Before</td>
<td>Sediment</td>
<td>11</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>High T</td>
<td>Before</td>
<td>No sediment</td>
<td>6</td>
<td>1.5</td>
<td>0.36</td>
</tr>
<tr>
<td>High T</td>
<td>Sediment</td>
<td>No sediment</td>
<td>11</td>
<td>1.1</td>
<td>0.42</td>
</tr>
<tr>
<td>Low pH high T</td>
<td>Before</td>
<td>Sediment</td>
<td>13</td>
<td>1.3</td>
<td>0.34</td>
</tr>
<tr>
<td>Low pH high T</td>
<td>Before</td>
<td>No sediment</td>
<td>10</td>
<td>1.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Low pH high T</td>
<td>Sediment</td>
<td>No sediment</td>
<td>13</td>
<td>0.6</td>
<td>0.64</td>
</tr>
</tbody>
</table>
Table 6: Results of pairwise Permanova analyses on sponge-associated microbial communities between seawater (Control, low pH, high T, low pH high T) and sediment treatments. Permutations for each comparison were 999.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Sample size</th>
<th>pseudo-F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control before</td>
<td>Low pH before</td>
<td>9</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>Control before</td>
<td>High T before</td>
<td>8</td>
<td>0.6</td>
<td>0.68</td>
</tr>
<tr>
<td>Control before</td>
<td>Low pH high T before</td>
<td>10</td>
<td>1.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Low pH before</td>
<td>High T before</td>
<td>7</td>
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<tr>
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</tr>
<tr>
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<td>0.13</td>
</tr>
<tr>
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<td>16</td>
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<td>0.02</td>
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</tbody>
</table>
**Discussion**

This study assessed the physiological performance and the stability of the associated microbiome of *Geodia barretti* explants under the impact of future ocean conditions and suspended natural sediment exposure simulating bottom trawling activities. The exposure to cumulative stressors resulted in a trend of antagonistic responses in respiration rates. The single factors caused a moderate increase in respiration, whereas in the combined treatment rates went back to control levels. Treatment effects on clearance rates were synergistic. While the exposure to sediment resulted in a ~50% reduced clearance rate, the exposure to sediment under future ocean conditions caused a complete cessation of clearance. The microbiome associated with *G. barretti* expressed first signs of a shift in relative abundances under the combined treatment of manipulated seawater parameters and sediment exposure.

Multiple stressor experiments are important to understand the implications of global and local changes for marine organisms (Gunderson et al., 2016). In the present study, water manipulation was applied over an extended range of time (seven months) under otherwise stable conditions. The described variation of physiological rates in *G. barretti* and the multifactorial design limited the statistical power to differentiate between all treatment groups. Bacterial clearance rates in particular proved to be a very unpredictable response variable. To explain variability in clearance and pumping, Osinga et al. (2001) hypothesized that some sponges exhibit temporal or spatial shutdown of pumping activity to accommodate their associated anaerobic microorganisms. This hypothesis may particularly apply to HMA sponges such as *G. barretti*. Working with deep-sea species remains challenging. Yet, this study provides important knowledge on the synergistic nature environmental changes can pose on *G. barretti*.

**Synergistic effects of future ocean conditions and sediment**

Sponges express a variety of responses to unfavourable environmental conditions (Tjensvoll et al., 2013; Bell et al., 2015; Grant et al., 2018). Tjensvoll et al. (2013) described a rapid decline in respiration rates in response to suspended natural sediments (>50 mg L⁻¹) in larger (1-2 L of volume) *G. barretti* individuals and it was hypothesized that the rapid respiratory response was linked to an active cessation of sponge pumping to prevent the accumulation of indigestible particles in the sponge’s aquiferous system. The interior body components of sponges from the sediment exposed groups in this study expressed a brown coloration after 19 days of sediment exposure. This indicates that sponges were actively pumping during the 12 h of sediment exposure cycles. In this study, small explants of *G. barretti* expressed respiration rates within the range reported for this species (Kutti et al., 2013; Leys et al., 2018; Bart et al., 2020) throughout all treatments with a trend of increasing
respiration rates in the treatments control sediment and low pH high T. When exposed to these conditions simultaneously, *G. barretti* expressed antagonistic responses and oxygen uptake rates were comparable to individuals in the control group. Coinciding with the respiration rates returning to control levels was a cessation of pumping. The complete absence of clearing activity under cumulative stressors of sediment exposure and future ocean conditions would be expected to result in a decline in oxygen uptake as sponge’s respiration has been described as a function of pumping (Reiswig, 1971; Gerrodette and Flechsig, 1979). However, this study indicated that in *G. barretti* respiration and pumping are not strongly correlated and respiration rates seem to be unaffected by the cessation of pumping. Hoffmann *et al.* (2005) suggested that small choanosomal explants of *G. barretti* can cover their oxygen demands via diffusion of oxygen via the cortex, resulting in an aerobic area with a width of 1 mm, deeming the majority of biomass of *G. barretti* anoxic. These anoxic conditions are thought to promote anaerobic microbial energy pathways (Hoffmann *et al.*, 2005). The respiration rates described under cumulative stressors in this study corroborate findings from Kutti *et al.*, (2015) that described stable respiration rates in small *G. barretti* explants under sediment exposure. Although oxygen supply might be possible by diffusion along concentration gradients in smaller individuals as used in this study, active pumping of water through the sponge’s body removes waste products, indigestible particles and supplies the associated microbiome with energy sources (Morganti *et al.*, 2019). Thus a limited movement of water through the sponge holobiont may compromise overall animal health.

The results in this study indicate a trend towards reduced clearance rates with sediment exposure. This reduced clearance could be a result of the accumulation of sediment particles indicated by a brown coloration of structural components in sediment exposed sponges in this study. Suspended sediment particles can clog ostia, be accumulated in the choanocyte chambers and aquiferous system of sponges (Strehlow *et al.*, 2017). The accumulation of indigestible particles has been shown to result in reduced pumping in a variety of sponge species (Tompkins-Macdonald and Leys, 2008; Grant *et al.*, 2018) and is thought to have adverse effects on sponge health. Recently, filter-feeding on bacterioplankton has been identified to cover only a small proportion (less than 1 %) of the energy budget of *G. barretti* (Bart *et al.*, 2020). Although a lack of pumping can compromise feeding activities in sponges (Bell *et al.*, 2015), food sources other than bacterioplankton have been described to be accessible to deep-sea sponges (Rix *et al.*, 2016; Leys *et al.*, 2018). If these food sources are accessible to *G. barretti* via passive diffusion, as hypothesized for the uptake of oxygen in small explants of *G. barretti*, the access to a diversity of energy sources might enable small individuals of *G. barretti*
to maintain their metabolic requirements under unfavourable conditions. To what temporal extent these unfavourable conditions can be tolerated remains unknown.

Contrasting a proposed cessation of pumping (this study), the negative clearance rates under combined stressors described in this experiment could also be interpreted as an active expelling of bacteria by *G. barretti* explants. Thus, a negative clearance rate could indicate that *G. barretti* was actively expelling bacteria. An increased waterflow through sponge’s mesohyl has been identified as potential mechanism to evacuate indigestible sediment particles (Tompkins-Macdonald and Leys, 2008). The negative clearance rates under the cumulative stressor treatment could be linked to an increased effort of evacuating indigestible particles that accumulated in channels and chambers of the aquiferous system (Grant *et al.*, 2018). If the efforts of removing indigestible particles are accompanied by the expelling of potentially symbiotic bacteria, this might have adverse consequences for the sponge holobiont (Slaby *et al.*, 2019). The microbial symbionts of *G. barretti* are known to be important partners in the sponge holobiont (microbe-sponge assemblage). They contribute to a variety of processes including food uptake (Leys *et al.*, 2018), inorganic nitrogen cycling (Hoffmann *et al.*, 2009), production of secondary metabolites (Hedner *et al.*, 2006; Lind *et al.*, 2013). A stable microbiome in sponges has been identified as a proxy for sponge health (Slaby *et al.*, 2019). Therefore, slight deviations of the microbiome as observed in the present study, could be an early indication of compromised sponge health (Pita *et al.*, 2018; Slaby *et al.*, 2019), and a sign for limitations to the potential of *G. barretti* to cope with combined stressors in a future ocean (Lesser *et al.*, 2016; Slaby *et al.*, 2019). Observed microbial shifts under the exposure to combined stressors might have been initiated by ceased pumping activities, or the active expelling of bacteria. Alternatively, a shift in the abundances of symbiotic microbiome communities may also be a potential coping mechanism in holobionts facing global oceanic changes (Pita *et al.*, 2018). Whether the slight, but significant shifts in microbial community compositions observed in the presented study between sponges exposed to cumulative stressors are of temporary or permanent nature, and beneficial, harmful or irrelevant for sponge health remains open for further investigations. Overall, the basic microbial community structure remained unaffected (on phylum level), which suggests that sponge-associated microbiomes may possess a buffering capacity against climatic changes, which in turn may enhance physiological robustness of *G. barretti*.

**Ecological implications in a future ocean**

Sponges and their associated fauna play a major role in habitat structuring, nitrogen cycling and carbon fixation in the deep-sea (Danovaro *et al.*, 2008; Ribes *et al.*, 2012; Danovaro
et al., 2014; Cathalot et al., 2015). The treatment-specific physiological responses described in this study could result in a metabolic deficit in G. barretti. With increasing energetic costs for potential sediment coping mechanisms (Strehlow et al., 2017) this energy deficiency could be exacerbated and compromise sponge driven benthic-pelagic coupling mechanisms (Rix et al., 2016; Maier et al., 2020). Field observations of mass mortalities of G. barretti in association with changing environmental conditions highlight the vulnerability of habitat forming deep-sea sponges (Guihen et al., 2014). Yet, no causal effect of increased temperatures on mortality of G. barretti could be identified in ex situ experiments (Strand et al., 2017). The implication, that increased seawater temperature was not the sole cause of sponge mass mortality urges the implementation of verification of findings resulting from experimental studies into species’ natural habitats. Complex species-species and species-environment relations in ecosystem settings could have implications for sponge resilience that go undetected in controlled, yet ecologically simplified aquaria experiments (Scanes et al., 2018). Furthermore, the metabolic parameters assessed in this study should be complimented by other biological proxies such as reproduction activity and the effect of stressors on larvae when evaluating the resilience of deep-sea sponges to global changes and interactions with industries (Pita et al., 2018).

Implications for extractive industries

Bottom trawling has been identified as the biggest threat for sponge grounds in the North Atlantic Ocean (Ospar Commission, 2010) and sediment resuspension as an indirect effect of bottom trawling (Hogg et al., 2010) has been shown to inhibit sponge fitness in a future ocean (this study). Throughout the North Atlantic Ocean 35.3 % of soft bottom sponge grounds overlap with intermediate to very high fishing intensity (Buhl-Mortensen et al., 2019). In these areas, G. barretti is highly abundant (Klitgaard et al., 1997; Howell et al., 2016; Davison et al., 2019) and can be the dominating sponge species (Murillo et al., 2012). This study underlined the species-specific responses of a habitat forming deep-sea sponge to global environmental changes in combination with local industrial interactions. Given the physiological constraints induced by experimental treatments the authors suggest to follow the precautionary approach and refrain from bottom trawling in areas with high sponge abundances that have been identified as vulnerable marine ecosystems (Buhl-Mortensen et al., 2019; Vieira et al., 2019). Global climate change is thought to limit the effectiveness of area based management tools for deep-sea ecosystems (Johnson et al., 2018) and areas with high abundances of sponge biomass could be set aside as refugia with strictly limited human interactions.
**Conclusion**

In this study we found *G. barretti* to be physiologically inhibited by the combined stressors of future ocean conditions and elevated concentrations of suspended natural sediment. A complete cessation of bacterial clearance was coinciding with a slight change of relative abundances in the microbiome associated with *G. barretti*. A treatment-induced shift in microbial community can be interpreted as a first warning sign of compromised physiological fitness in this habitat forming deep-water sponge. The synergistic nature of the treatment-specific effects has the potential to adversely affect the physiological fitness of this dominating sponge species in the North Atlantic Ocean. Extractive industries should refrain from bottom trawling activities in areas with high sponge abundances to ascertain refuges for marine species faced with global ocean changes.

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Chapter 4

The deep-sea ecosystem engineer Geodia barretti (Porifera, Demospongiae) maintains basic physiological functions under simulated future ocean pH and temperature conditions

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Abstract

Global ocean warming and acidification will alter the physicochemical conditions in the deep North-Atlantic Ocean. Here, extensive sponge grounds, often dominated by the demosponge species *Geodia barretti*, provide three-dimensional structure, habitat and significantly contribute to benthic-pelagic coupling and nutrient cycling processes in the deep sea. It is unknown if *G. barretti* remains physiologically functional under the future physicochemical properties of an Anthropocene ocean. In this study, individuals of *G. barretti* collected from 300 m water depth in the Barents Sea, were exposed to four treatments resembling future ocean conditions (no treatment, 4 °C increase in seawater temperature, decrease of seawater pH by 0.3, and a combination of the high temperature, low pH). Over the course of 39 weeks, oxygen consumption, dissolved inorganic nutrient fluxes, and bacterioplankton clearance rates were measured as indicators of metabolic activity. We found that all indicators within each sponge individual and per treatment were highly variable over time and no effect of manipulated seawater treatments on these parameters could be demonstrated. Oxygen consumption rates in all groups closely followed a seasonal pattern, potentially caused by (a)biotic cues in the seawater flowing through the experimental aquaria. While similar metabolic rates across all treatments suggest that *G. barretti* physiologically coped with simulated future ocean conditions, observed tissue necrosis in experimental animals might indicate that the response of the complex, high microbial *G. barretti* sponge (i.e., sponge host and microbial symbionts) to future ocean conditions may not be reflected in basic physiological processes.

Keywords: sponge grounds, deep sea, climate change effects, *Geodia barretti*, ecophysiology
**Introduction**

The North Atlantic Ocean (NAO) harbours vast assemblages of sponges (Porifera) (Cárdenas and Rapp, 2015; Hawkes et al., 2019). In these so called deep-sea sponge grounds, sponges can represent up to 98 % of the benthic biomass (Hogg et al., 2010) under specific oceanographic settings (Beazley et al., 2018; Dijkstra et al., 2021; Hanz et al., 2021b; Roberts et al., 2021). These dense assemblages of sponges form complex three-dimensional structures, otherwise scarce in the deep sea, that provide habitat and increase local biodiversity (Beazley et al., 2013; Hawkes et al., 2019). Often, economically important fish species are associated with sponge grounds (Kenchington et al., 2013; Kazanidis et al., 2018), highlighting their ecological and economic importance. In addition to their role as ecosystem engineers, sponges are key-players in nutrient cycling by filtering large volumes of seawater, removing particulate and dissolved organic matter and regenerate inorganic nutrients (Maldonado et al., 2012; de Goeij et al., 2017). In a confined area (300 km²) on the Norwegian shelf the standing stock of the deep-sea sponge species Geodia barretti has been estimated to consume 60 t of particulate organic carbon daily (Kutti et al., 2013). These magnitudes of organic carbon removal indicate that G. barretti dominated sponge grounds recycle organic carbon in the same order of magnitude as other marine invertebrate dominated habitats (Coppari et al., 2019). Moreover, in tropical coral-reef ecosystems sponges have been identified as crucial recyclers of dissolved organic matter (DOM) through the so-called sponge loop (de Goeij et al., 2013). Recently, also dominant deep-sea sponges, including G. barretti, were shown to be able to feed on DOM (Bart et al., 2020b) and a deep-sea sponge loop has been identified (Rix et al., 2016; Bart et al., 2021). Given that the deep sea represents the largest habitat on the planet (approximated at 360 million km², Danovaro et al., 2014) and contains a high abundance of sponges, sponge-driven processes have the potential to play a crucial role in providing global ecosystem services (Armstrong et al., 2019) like habitat provision and carbon and nutrient cycling (Ottaviani, 2020; Rossi and Rizzo, 2020). However, to date it is unknown if deep-sea sponges can maintain their ecological function under future changing ocean conditions.

Human-induced climate change is altering the physicochemical seawater properties of the deep NAO (Sweetman et al., 2017) and Geodia-dominated sponge grounds in the South-Western Barents Sea have been identified as areas impacted by ocean acidification (OA) and ocean warming (OW) (Kazanidis et al., 2019). By the year 2100, the water masses in the deep NAO are expected to experience a decrease of seawater pH of up to 0.3 units and a temperature increase of up to 4 °C (Sweetman et al., 2017). While tropical shallow water reefs and their sponge fauna have received extensive scientific attention over the past decades (Carballo et al., 2017), knowledge about the physiological responses of deep-sea sponges to future ocean conditions is scarce and limited to a few species.
*Geodia barretti*, one of the most abundant and well-studied *Geodia* species in sponge grounds in the NAO has been shown to express an increased mortality under heat wave events (temperatures of 4 °C above average) (Guihen *et al.*, 2014), highlighting the sensitivity of cold-water adapted sponge species to changes in environmental conditions (Strand *et al.*, 2017). The interaction of OW and OA has been found to have adverse effects on the pumping capacity and tissue integrity in a deep-sea glass sponge from the North East Pacific (Stevenson *et al.*, 2020). However, information is lacking about the physiological response of *Geodia* to (combined) near-future ocean conditions and the implications for sponge-driven ecosystem services. Given the large impact climate change will have on all vulnerable deep-sea habitats in the NAO (Johnson *et al.*, 2018), and the significance of sponge-driven ecosystem services in the deep sea, it is crucial to understand how basic physiological functions of highly abundant sponge species like *G. barretti* will respond to near-future ocean conditions.

Oxygen consumption, the clearance of bacterioplankton, and fluxes of inorganic nutrients (e.g., ammonium, nitrate, phosphate) have been used extensively in assessing the metabolic or physiological function of deep-sea sponges, including *G. barretti* (Leys *et al.*, 2018; Bart *et al.*, 2020, 2021b; de Kluijver *et al.* 2021). In *G. barretti*, the pumping of water is directly linked to the retention of bacterioplankton due to the lack of bypasses that would shunt water through the sponge holobiont without being filtered (Leys *et al.*, 2018). Thus, the clearance rate for bacterioplankton can be used as a proxy for the volume of water processed. To cope with the constraints of a warmer, more acidic future ocean, it is of pivotal importance that *G. barretti* can maintain its basic metabolic functions under the predicted future physicochemical seawater conditions. This study is the first to assess the long-term acclimatisation potential of *G. barretti* to near-future sea water pH (decrease by 0.3 units) and temperature conditions (increase of 4 °C). In an aquaria-based, multifactorial experiment, the basic physiological functions of oxygen consumption and bacterioplankton clearance will be assessed over the course of ten months. The assessment of oxygen consumption rates and bacterioplankton clearance rates under manipulated seawater parameters will provide baseline information about the resilience of sponge-driven benthic-pelagic coupling mechanisms in a future ocean. Additionally, the multifactorial design applied in this study will allow for the evaluation of single and combined stressor effects to better understand the effect of (a combination of) future climate effects on our model sponge.
Materials and methods

Sponge collection

Individuals of *G. barretti* with a maximum diameter of ~10 cm were collected by the remotely operated vehicle (ROV) ÆGIR6000 deployed from the Norwegian research vessel GO Sars in the area Tromsøfjorden East (71°35´20.4”N 21°22´35.4”E) at a water depth of 330 m. Only undamaged individuals were stored in the collection box and brought to the surface. During sampling at the seafloor, re-suspension 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 GO Sars. Temperature controlled (7 °C, 20 L min⁻¹) seawater from 6 m depth was pumped into 40-L tanks on board in which the sponges were kept in the dark throughout the expedition, after which the sponges were transferred to the running seawater aquarium facilities of the University of Bergen, Norway.

pH and temperature treatments

Sponges were kept in twelve 40-L tanks (Figure 1; 2-3 sponges per tank, 31 sponge individuals in total), supplied with sand-bed filtered seawater obtained from 200 m water depth from the adjacent fjord at a flow rate of 1 L min⁻¹. No additional food was added. Three replicate tanks were assigned to one of the following experimental treatment groups: Control (7 sponges), low pH (9 sponges), high temperature (T) (7 sponges) and low pH combined with high T (8 sponges). Seawater manipulation (temperature increase by 4 °C, pH decrease by 0.3 units) was achieved via computer-controlled (Evolution Controller, Aquatronica, Italy) heating elements and the dosing of pressurized CO₂ (Riebesell *et al.*, 2010). pH electrodes (acq310n-ph, Aquatronica, Italy) in the experimental units were triggering low-pressure solenoid valves that opened and closed, when respectively, the upper (pH 8.1) or lower (pH 7.8) setpoint was reached. Sensors measuring seawater pH were cleaned and calibrated weekly following manufacturer guidelines (two-point calibration with pH = 7.00 and pH = 10.00, Certipur®, Merk, Germany). Temperature was increased from ambient to treatment levels by 0.6 °C 24 h⁻¹, whereas seawater pH was lowered by 0.2 units 24 h⁻¹ until reaching treatment levels. An overview of the treatment-specific seawater parameters over the course of the 10-month long experiment can be found in Table 1. Throughout the experiment sponges were visually monitored for signs of tissue necrosis. Tissue necrosis in *G. barretti* was characterized by black spots on the sponge’s cortex that were eventually covered in white, flocculent material (Luter
et al., 2017). Sponges with signs of necrosis were removed from the experiment to not impact other individuals within the same tank.

Metabolic rate measurements

Oxygen consumption, bacterioplankton clearance, and inorganic nutrient uptake or release rates of *G. barretti* in the four treatment groups were assessed by chamber-based incubations after 3, 11, 25, 28, 31, and 39 weeks (note: inorganic nutrient concentrations were only assessed in week 11). Therefore, within its respective tank, a sponge individual was carefully manoeuvred onto the bottom plate of a respiration chamber (volume = 6.2 L). Sponges were given a 3 h recovery time before a transparent cylinder and a lid equipped with an optical oxygen probe, and magnetic stirrer for constant water mixing, were fitted onto the bottom plate complementing the air-tight incubation chamber. Individuals were incubated for 4 h.
Figure 1. Experimental design used in this study. Individuals of *G. barretti* were exposed to four different sea water treatments. Each treatment had three replicate tanks holding up to three sponges. The tanks in the treatments were individually supplied with seawater from the nearby fjord from a depth of 200 m. Each tank was equipped with a pH electrode and temperature sensor connected to a computer. The treatments high T, low pH and low pH high T were equipped with a heater, CO₂ dosing, and combination of those, respectively. The heater and CO₂ dosing were controlled by a computer to maintain treatment parameters. An aquarium pump in each tank provided circulation and mixing to avoid temperature and pH gradients within the tank.
Table 1. Overview of the sea water parameters in the experimental tanks. Values represent averages over time (39 weeks) and the three tanks per treatment. Values given ± Standard deviation.

<table>
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<th>high T</th>
<th>low pH high T</th>
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<tbody>
<tr>
<td>Seawater temperature [°C]</td>
<td>6.91 ± 0.19</td>
<td>7.40 ± 0.17</td>
<td>10.63 ± 0.14</td>
<td>10.61 ± 0.33</td>
</tr>
<tr>
<td>Seawater pH</td>
<td>8.10 ± 0.12</td>
<td>7.77 ± 0.15</td>
<td>8.03 ± 0.12</td>
<td>7.79 ± 0.16</td>
</tr>
</tbody>
</table>

Oxygen consumption rates

Concentrations of dissolved oxygen (O$_2$) were assessed min$^{-1}$ with an optical sensor (HQ30D53315000, Hach, Colorado, USA) and oxygen removal rates in µmol O$_2$ g DM$^{-1}$ h$^{-1}$ were calculated following Tjensvoll et al. (2013) using the following equation:

$$O_2 \text{ removal} [\mu \text{mol } O_2 \text{ g } DM_{Sponge}^{-1}h^{-1}] = (c_2 - c_1) t^{-1} \cdot V_{net} \cdot g DM_{Sponge}^{-1},$$

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 g DM = the total dry mass of the incubated animal in g. Sponge mass was assessed at the end of the experiment by taking the respective sponge out of the water and let water drip from the sponge for 10 s before placement on the scale. To determine the dry mass for each individual, per sponge three 1 cm$^3$ tissue samples containing cortex and mesohyl were extracted randomly, weighted, dried for 12 h at 60 °C and weighted again. Sponge volumes were assessed at the end of the experiment by measuring the volume of replaced seawater by each individual.

Bacterioplankton clearance rates

Duplicate water samples (2 mL) were drawn from the chamber at the beginning and the end of the 4-h incubations. Samples for bacterioplankton quantification were fixed with glutaraldehyde (final concentration 0.5 %) for 10 min before flash freezing in liquid nitrogen and -80 °C storage until further analysis. Bacterioplankton abundances were assessed by flow cytometry (Attune Nxt – Acoustic focusing cytometer, Invitrogen, Thermo Fisher, USA). Therefore, 500 µL TE buffer were stained with 5 µL of a 1 × working solution of SYBR® green I (Invitrogen, Thermo Fisher, USA) and kept in the dark for 10 min at room temperature. 40 µL of the stained TE buffer were analysed with a flow speed of 25 µL min$^{-1}$. Then, seawater samples (50 µL) were diluted in 450 µL TE buffer and stained with 5 µL of a 1 × working solution of SYBR® green I. After 10 min in the dark at room temperature, 40 µL of stained
seawater sample were analysed with a flow speed of 25 µL min\(^{-1}\) while keeping the rate of events s\(^{-1}\) below 500 to avoid clogging of the machine. Gates were manually set around stained cells that were detected when compared against the stained TE buffer. Clearance rates for bacterioplankton in mL mL\(_{\text{sponge}}\)\(^{-1}\) h\(^{-1}\) were calculated as outlined by Robertson et al. (2017) using the following equation:

\[
\text{Bacterioplankton clearance rate } \left[ \text{mL mL}^{\text{\text{-1}}} \text{mL}_{\text{sponge}}^{\text{\text{-1}}} \text{h}^{\text{-1}} \right] = \frac{V_{\text{net}} \cdot t^{-1} \ln (n_{\text{start}} n_{\text{end}}^{-1})}{mL^{\text{\text{-1}}} \text{mL}_{\text{sponge}}},
\]

where \(V_{\text{net}}\) = the volume of the incubation chamber after subtracting the volume of the respective sponge in mL, \(t\) = the time of the incubation in h, \(n_{\text{start}}\) and \(n_{\text{end}}\) = the concentrations of bacterioplankton at start and end of the incubation in counts mL\(^{-1}\) and mL\(_{\text{sponge}}\) = the volume of the incubated sponge individual in mL. Seawater-only incubations were performed (\(n = 4\) in weeks 11 and 39, \(n = 3\) in weeks 3, 25, 28 and 31) to account for background respiration and non-sponge related dynamics in bacterioplankton. Sponge incubations were corrected for seawater-only chamber dynamics in oxygen consumption and bacterioplankton concentration. For the correction the average of the seawater-only chamber incubations per timepoint was used.

\[\text{Dissolved inorganic nutrient fluxes}\]

Duplicate water samples (10 mL) were taken at the beginning (\(t_0\)) and end (\(t_2\)) of the 4-h incubation to assess the removal and release rates of dissolved inorganic nutrients (ammonium: NH\(_4^+\), nitrate: NO\(_3^-\), nitrite: NO\(_2^-\), and phosphate: PO\(_4^{3-}\)) by \(G. \text{barretti}\) (only in week 11). Water samples were filtered over a sterile 0.2 µm polyether sulfone filter (Puradisc, Whatman, UK), collected in high-density polyethylene vial (Pony vial, Perkin Elmer, USA) and stored at -20 °C until further analysis. Concentrations of inorganic nutrients were detected with an automated Wet Chemistry Analyzer (SAN++, Skalar Analytical, Breda, The Netherlands). Nitrate concentrations were derived as: [NO\(_3^-\)] = [NO\(_x^+-\)] – [NO\(_2^-\)]. Exchange rates, normalized to sponge dry mass and given in µmol g DM\(^{-1}\) h\(^{-1}\), were calculated from the difference in concentration over time and the chamber volume, corrected by changes in a control chamber incubation without a sponge.

\[\text{Data analysis}\]

Physiological rates of individual sponges were averaged per experimental unit (tank), resulting in \(n = 3\) per treatment. The datasets of respiration and clearance rates passed the Shapiro-Wilk test for normality. To investigate the differences between the treatments, we tested for significance at the 5 % level by using a repeated measure, mixed-model analysis of
variance with the factors time, temperature and pH. To investigate the relations between individual timepoints, Tukey’s multiple comparisons were performed. When time was not found to have a significant effect, timepoints were averaged and a two-way ANOVA with the factors temperature and pH with Tukey’s multiple comparisons was performed. Treatment effects on nutrient uptake and excretion rates were assessed using dissolved inorganic nutrient specific two-way ANOVAs. Rates of tissue necrosis in *G. barretti* individuals from the four different treatment groups were analysed by a log-rank (Mantel-Cox) test. All tests were performed with GraphPad Prism 8 Version 8.2.1(441), August 20, 2019. Values are given in mean ± standard deviation (SD) if not stated otherwise.
Results

Metabolic rates under different pH and T treatments

Oxygen consumption rates

Sponges in the control group expressed a trend \( (p = 0.09; \text{PROVIDE STATS}) \) of increased oxygen consumption over time (Figure 2A) ranging from \( 0.65 \pm 0.21 \mu\text{mol O}_2 \text{ gDM}^{-1} \text{ h}^{-1} \) in Week 3 (time of the year: November; mean ± SD unless stated otherwise) to \( 1.36 \pm 0.11 \mu\text{mol O}_2 \text{ g DM}^{-1} \text{ h}^{-1} \) in Week 28 (time of the year: July) before returning to levels observed at the start of the experiment in Week 39 (time of the year: September). Oxygen consumption rates were not affected by manipulated seawater conditions (Temperature: \( p = 0.07, \text{pH} = 0.92, \) Table 3) in the respective treatment over time (Figure 2A-C). The low pH and combined low pH + high T treatments generally followed the trend of increasing oxygen consumption rates over time, with generally higher (low pH) or lower average rates (low pH + high T) compared to the control group (although not significant), whereas the high-T treatment fluctuated around average rates observed in the control group. Respiration rates across the four treatments were significantly impacted by the factor time \( (p = 0.008, \text{Table 3, Figure 3}) \). A significant elevation of oxygen consumption rates averaged over the four treatments (Figure 3) was evident from Week 3 \( (0.8 \mu\text{mol O}_2 \text{ gDM}^{-1} \text{ h}^{-1}) \) to Week 28 \( (1.3 \mu\text{mol O}_2 \text{ gDM}^{-1} \text{ h}^{-1}) \) \( (p = 0.014; \text{Table 3}) \). This increase over the course of 25 weeks was followed by a significant decline to \( 0.68 \mu\text{mol O}_2 \text{ gDM}^{-1} \text{ h}^{-1} \) over the course of eight weeks from Week 31 to 39 \( (p = 0.007) \) (Figure 3).

Table 3. Overview of the test results for respiration rate over time (Repeated measure mixed effect analysis). For the multiple comparisons, only comparisons with a test result of \( p < 0.05 \) are stated.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>( p )-value</th>
<th>( F (\text{DFn, DFd}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>\textbf{0.008}</td>
<td>( F (2.81, 20.84) = 5.293 )</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.07</td>
<td>( F (1, 8) = 4.11 )</td>
</tr>
<tr>
<td>pH</td>
<td>0.92</td>
<td>( F (1, 8) = 0.009 )</td>
</tr>
<tr>
<td>Time × Temperature</td>
<td>0.64</td>
<td>( F (5, 37) = 0.68 )</td>
</tr>
<tr>
<td>Time × pH</td>
<td>0.76</td>
<td>( F (5, 37) = 0.51 )</td>
</tr>
<tr>
<td>Temperature × pH</td>
<td>0.12</td>
<td>( F (1,8) = 3.01 )</td>
</tr>
<tr>
<td>Time × Temperature × pH</td>
<td>0.18</td>
<td>( F (5, 37) = 1.62 )</td>
</tr>
</tbody>
</table>

Multiple comparisons (Post-hoc test)

<table>
<thead>
<tr>
<th></th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 3 vs. week 28</td>
<td>\textbf{0.014}</td>
</tr>
<tr>
<td>Week 31 vs. week 39</td>
<td>\textbf{0.007}</td>
</tr>
</tbody>
</table>

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Figure 2. Oxygen consumption (A, B, C) and clearance rates (D, E, F) of *G. barretti* in the control group displayed with rates from the low pH treatment group, high temperature treatment group and low pH + high temperature treatment group over the course of 39 weeks. Values represent the mean of three tanks per treatment and timepoint.
**Figure 3.** Average of oxygen consumption rates of *G. barretti* across all four treatments over time. Values represent average means of the four treatments per timepoint (*n* = 12 per time-point). Corresponding letter pairs (ab, cd) indicate a significant difference.

**Bacterioplankton clearance rates**

Bacterioplankton clearance rates of *G. barretti* were stable throughout time in the control group (*p* = 0.99; Figure 2D). The pH and T treatments followed the same trend over time, with no effect of exposure time on the clearance rate (*p* = 0.98, see Table 4). While the average bacterioplankton clearance rates of the low pH and high T treatment were lower compared to the control treatment, this difference was not significant (*p* = 0.66 for low pH; *p* = 0.21 for high T, Table 4). A significant interaction (*p* = 0.03; Table 4) of high seawater temperature and low pH was detected. This interaction was further explored with a 2-Way ANOVA without the factor “time” but the performed Post-hoc tests did not yield more information about the specifics of the detected interaction (Table 5). Noteworthy, however, is the trend (*p* = 0.09; Table 5) of increased seawater temperature affecting clearance rates in *G. barretti*. This trend is absent in the combined treatments (*p* = 0.64; Table 5) and might point towards an alleviating effect of decreased seawater pH on temperature induced responses.
Table 4. Overview of the test results for clearance rate over time (repeated measure mixed-effect analysis)

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>p-value</th>
<th>F (DFn, DFd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0.98</td>
<td>F (5, 37) = 0.13</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.21</td>
<td>F (1, 8) = 1.86</td>
</tr>
<tr>
<td>pH</td>
<td>0.66</td>
<td>F (1, 8) = 0.20</td>
</tr>
<tr>
<td>Time × Temperature</td>
<td>0.78</td>
<td>F (5, 37) = 0.48</td>
</tr>
<tr>
<td>Time × pH</td>
<td>0.82</td>
<td>F (5, 37) = 0.43</td>
</tr>
<tr>
<td>Temperature × pH</td>
<td>0.03</td>
<td>F (1.8) = 6.29</td>
</tr>
<tr>
<td>Time × Temperature × pH</td>
<td>0.77</td>
<td>F (5, 37) = 0.49</td>
</tr>
</tbody>
</table>

Multiple comparisons (Post-hoc test)

No significance detected

Table 5. Overview of the two-way ANOVA results for clearance rate with the factors temperature and pH.

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>p-value</th>
<th>F (DFn, DFd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>0.24</td>
<td>F (1, 8) = 1.86</td>
</tr>
<tr>
<td>pH</td>
<td>0.66</td>
<td>F (1, 8) = 0.20</td>
</tr>
<tr>
<td>Temperature × pH</td>
<td>0.03</td>
<td>F (1.8) = 6.29</td>
</tr>
</tbody>
</table>

Multiple comparisons (Post-hoc test)

| Control × high T              | 0.09    |
| control × low pH              | 0.21    |
| control × low pH + high T     | 0.64    |
| high T × low pH + high T      | 0.93    |
| low pH × low pH + high T      | 0.47    |
| high T × low pH               | 0.78    |

Dissolved inorganic nutrient fluxes

Geodia barretti individuals in the control treatment showed a net release of ammonium (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻) and phosphate (PO₄³⁻) (Fig. 5; positive value depicts release rates). Fluxes of inorganic nutrients in the pH and T treatments were not significantly affected compared to the control group (p > 0.3 in all dissolved inorganic nutrient specific two-way ANOVAs, see details of the tests in the Supplemental Table S1). However, sponges switched from net average release to a net average removal of NO₂⁻ in the pH and T treatments, compared to the control.
Figure 5. Dissolved inorganic nutrient (A: NH₄⁺ and PO₄⁻; B: NO₃⁻; C: NO₂⁻) exchange rates of *G. barretti* in four different treatments at Week 11. Here, positive values represent release, negative values uptake of the respective dissolved inorganic nutrient.

**Tissue necrosis**

Throughout the experiment, several individuals from the four treatment groups expressed signs of tissue necrosis. The lowest number of animals showing signs of necrosis was observed in the control group (1 out of 7 over 39 weeks) and the highest number (4 out of 8 over 39 weeks) was observed in the combined treatment group (low pH + high T) (Table 7). However, under the experimental approach, no statistical effect of the treatment on tissue necrosis was evident ($p = 0.19$). Animals found to express signs of necrosis were removed from the experiment.
Table 7. Overview of numbers of individuals at the start ($n_{\text{start}}$) and the end ($n_{\text{end}}$) of the long-term acclimatisation period after 39 weeks. Sponges were removed from the experiment when expressing signs of tissue necrosis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n_{\text{start}}$</th>
<th>$n_{\text{end}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>low pH</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>high T</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>low pH high T</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
Discussion

Throughout a ten-month *ex situ* study in a flow-through aquarium with *in situ* seawater supply, the metabolic rates (i.e. oxygen consumption, bacterioplankton clearance and dissolved inorganic nutrient removal/release rates) of the deep-sea sponge *G. barretti* were, in general, not significantly affected by simulated future ocean conditions (lower pH, higher temperature). Instead, oxygen consumption rates across all treatments were significantly affected by time of the year with highest rates expressed in late summer. Here, the ecological implications of these findings are discussed, while drawing attention to the complex eco-physiology of the holobiont *G. barretti* and the urgency for holistic approaches to quantify the effects of global changes on this highly abundant deep-sea ecosystem engineer.

**Metabolic rates of *G. barretti* under present and future pH and T conditions**

The measured oxygen consumption rates of individuals in the control treatment of this long-term experiment (0.55-1.36 O$_2$ g DM$^{-1}$ h$^{-1}$) were within the range reported for *G. barretti* (1.13–1.38 O$_2$ gDM$^{-1}$ h$^{-1}$) under *ex situ* conditions similar to those in the presented experiment (Strand et al., 2017; Leys et al., 2018; Bart et al., 2020b). Biological processes, such as oxygen consumption rates (i.e., as measure for respiration rate) are often driven by the thermodynamics of chemical reactions (Clark and Fraser 2004). This temperature dependency of biological processes is often described by a Q$_{10}$ factor, which represents the magnitude by which the process rate changes with a temperature increase of 10 $^\circ$C. A Q$_{10}$ value of 2.3 has been reported for respiration in temperate sponges (Coma et al., 2002). When assuming a similar Q$_{10}$ value for *G. barretti*, the oxygen consumption rates expressed by *G. barretti* exposed to a temperature increase of 4 $^\circ$C were expected to be elevated by approximately 40 %. However, this was not corroborated by the results from the present experiments, in which significant effects of temperature on oxygen consumption could not be demonstrated. Correspondingly, comparison of average oxygen consumption rates in high temperature treated sponges with rates in control sponges resulted in a Q$_{10}$ value of only 1.18. In contrast, twice higher oxygen consumption rates, corresponding to a Q$_{10}$ value of 6.2, were found in explants of *G. barretti* when exposed to acute heat stress (i.e., a 4–5 $^\circ$C elevation in temperature within 24 h) (Strand et al., 2017). Acute exposure to a “heat wave” may trigger acute physiological responses that do not necessarily reflect the long-term acclimatization potential of sponges to elevated temperatures. Despite the more gradual acclimation to higher temperature that was applied in the present study (0.3 $^\circ$C elevation per 12 h in the present experiment versus 2–3 $^\circ$C elevation per 12 h in the experiment by Strand et al., 2017), an initial response was also apparent here, the Q$_{10}$ in Week 3 of the present experiment being 4.8. These findings stress the importance of executing
long-term experiments to study acclimation of sponges to future ocean conditions. This is reflected by observations made in two other treatments: The lower pH treatment showed a generally lower average oxygen consumption compared to the control group, but the combined treatment of low pH and high T showed generally higher average oxygen consumption rates. While there is a trend that temperature has an effect \((p = 0.07)\) on oxygen consumption rates, the data does not allow for a conclusion if long-term elevated seawater temperatures are causing an increase or decreased of oxygen consumption rates in \(G.\ barretti\) under ex situ conditions. Changes in oxygen consumption rates can be caused by both favourable (e.g., higher respiration due to elevated nutrition Ribes et al., 1999) and unfavourable (e.g., lower respiration due to seizing of pumping Hoffmann et al., 2008) or higher respiration due to higher maintenance costs under suboptimal environmental conditions (Pörtner et al., 2005). Although the manipulated physicochemical parameters were not affecting respiration rates, time of the year was found to have an effect on the oxygen consumption rate in \(G.\ barretti\). Respiration rates were found to significantly increase in late summer. It has been shown that reproduction in fjord populations of \(G.\ barretti\) coincides with phytoplankton blooms, gametogenesis/spawning taking place in late summer (Spetland et al., 2007). Both reproductive activities and seasonal patterns of environmental conditions have been described to cause fluctuations in physiological rates in sponges (Koopmans and Wijffels, 2008; Koopmans et al., 2011; Morley et al., 2016). Thus the changes in respiration rates over time in this long-term study could be an indication for reproductive activities in the experimental animals, triggered for example by the influx of biological cues from the wild \(G.\ barretti\) fjord population or by changes in abiotic parameters other than temperature and seawater pH occurring outside the location of the experimental facilities.

While ex situ experiments have the sole purpose to minimize the biological complex environment to controlled conditions, the flow-through design with unfiltered in situ deep-sea water was a compromise between keeping sensitive deep-sea sponges alive and the controlled manipulation of physicochemical seawater parameters. One of the environmental variables that was found to vary over time was the abundance of bacterioplankton in the seawater flowing into the experimental facilities. This fluctuation of bacterioplankton indicates that biological and/or chemical in situ cues may have affected the sponges in the experimental ex situ setup.

Clearance rates for bacterioplankton are another important measure to assess the physiological fitness of filter-feeding sponges (Maldonado et al., 2012). The clearance rates obtained in this experiment \((\text{control} = 144 \pm 58 \ \text{mL mL}^{-1} \ \text{h}^{-1})\) are higher than reported for this species \((22 – 68 \ \text{mL mL}^{-1} \ \text{h}^{-1})\) (Leys et al., 2018). These higher clearance rates could be explained by the size of experimental animals. De Kluijver et al. (2021) have described that
smaller individuals of *G. barretti* assimilate more bacterioplankton than larger individuals. While Leys *et al.* (2018) have used sponges with an average volume of 1 L (maximum 3 L), in this study sponges had an average volume of 0.14 L. Similar to the respiration, we found no significant difference of clearance rates between the treatments and the control group. However, clearance rates in the two single stressor treatments were showing a trend of being lower compared to the controls, implying that filter-feeding may be negatively affected by (the combined effects of) pH and T. While this effect was not tested significant, the interaction of pH and high T showed a significant effect. This might imply, that potential effects of single stressors might be alleviated by low pH as clearance rates in the combined treatment largely overlapped with rates observed in the control group. Future studies with higher numbers of replicates are, however, needed to investigate this relation in further details, as the interaction could not be explored to full details with the dataset generated in this experiment.

A third indicator of metabolic rates investigated in this experiment were uptake/release rates of dissolved inorganic nutrients by sponges. *Geodia barretti* is known to exhibit many metabolic nitrogen pathways, mediated by both the host and its microbial symbionts (Leys *et al.*, 2018; Rooks *et al.*, 2020; de Kluijver *et al.*, 2021). No significant effects could be detected between the control group and the pH and T treatments over time in terms of inorganic nutrient fluxes, indicating no major metabolic changes in the sponges. But changes in average fluxes throughout treatment show interesting trends. In terms of N-cycling, the average release rates of nitrate are increased (i.e. predominantly in the combined pH and T treatment), whereas the fluxes of ammonium and nitrite are decreased, to even a net average uptake of nitrite (Fig. 4). This can imply an increase of aerobic nitrification (i.e., the oxidation of ammonium into nitrite and subsequently nitrate) and/or, but less likely, increased anaerobic processes, such as ammonium oxidation (i.e., loss of nitrogen through transformation of ammonium into nitrite and nitrogen gas). Both processes occur in *G. barretti* and are mediated by the sponge’s microbiome (de Kluijver *et al.*, 2021). Additionally, an increase in the average phosphate (P) release was observed in the combined pH and T treatment compared to the control. Although not conclusive, the increased release of both N and P indicate an increased assimilation of food under these future scenarios. Unfortunately, we were not able to quantify inorganic nutrient fluxes through time and further studies are needed to relate the fluxes of inorganic nutrients to seasons (e.g., temperature and food availability).

We conclude that the most consistent pattern observed during our 39-week long experiment, namely a summer peak in oxygen consumption, was likely a seasonal response to
environmental conditions. Sponge metabolic rates, such as oxygen consumption, but also growth and reproduction, are found to be closely linked with ambient food conditions (Ribes et al., 1999; Spetland et al., 2007; Koopmans and Wijffels, 2008; Morley et al., 2016). For example, it has been shown that reproduction in fjord populations of G. barretti coincides with phytoplankton blooms, gametogenesis/spawning taking place in late summer (Spetland et al., 2007). Thus, the recorded physiological changes of the sponges over time in our long-term study may have been caused predominantly by (a)biotic cues in the in situ water flowing through the experimental setup.

**Eco-physiology of G. barretti in an Anthropocene ocean**

In the North Atlantic Ocean, G. barretti has been described to be abundant from depths of 2000 m with temperatures ranging from 3–5 °C until 30 m of water depth in Norwegian fjords ecosystems where individuals can be exposed to seawater temperatures as high as 15 °C (Cárdenas et al., 2013). Geodia barretti’s distributional range points towards a broad ecological potential. This could explain the continuity of basic physiological parameters across a variety of climate treatments as evidenced in this study and might point towards a resilience of G. barretti towards environmental parameters of future NAO conditions. Sponges that contain high abundances of microbial symbionts, such as G. barretti (Leys et al., 2018), have been hypothesized to be more resilient to low seawater pH conditions given the diverse, specialized abilities of their microbiome (Goodwin et al., 2014). Tissue necrosis is an early warning sign that can result in sponge death, even to in situ mass mortality events of G. barretti (Guihen et al., 2014). Tissue necrosis has been described as response of sponges to high CO2 concentration and increased temperature (Bennett et al., 2017; Strand et al., 2017) and can be an indication for a disruption of the stable state, or changes in the community composition, of the microbial symbionts associated with the holobiont G. barretti (Vacelet and Donadey, 1977; Hentschel et al., 2003; Luter et al., 2017). At the same time, G. barretti heavily relies on the phagocytosis of symbiotic eukaryotes to meet its metabolic carbon requirements (Leys et al., 2018), and the high processing rates of dissolved organic matter (DOM) in G. barretti (Bart et al., 2021b) are thought to be partly driven by microbial symbiont activities (Bart et al., 2020). A shift in the microbial community could therefore affect two energy acquisition pathways in G. barretti, limiting its coping potential in a future ocean. While the results of this study show metabolic resilience of G. barretti to changing environmental settings, in situ observations of mass mortalities following acute temperature increases stress the necessity to acquire in situ physiological and metabolic data on deep-sea sponges. Furthermore, first evidence shows that
the resilience of *G. barretti* may be overruled when sponges are exposed to multiple stressors, such as re-suspended sediment caused by bottom trawling (Wurz *et al.*, 2021, submitted).

**Limitations of the study**

Deep-sea research is a logistically challenging and expensive endeavour (Ruth, 2006), limited by the access to deep-sea organisms and the availability and operation of facilities suitable to maintain them. Despite these limitations, in this experiment, basic physiological parameters in more than 30 deep-sea sponges were assessed. Large fluctuations of physiological rates were evident in the experimental sponges over time. Given this dynamic nature of physiological rates in *G. barretti*, at least five (based on power analysis of here presented data with R `wp.anova` function) replicates of experimental (tanks) and measurement (sponges) units are advised for future studies, to provide better detection limits for treatment effects. Given the technical challenges to maintain *G. barretti* under _ex situ_ conditions, life history traits (age, size, reproductive status, etc.) of the experimental animals most likely differed from natural communities in *G. barretti* dominated sponge grounds (de Kluijver *et al.* 2021). Here, we used individuals with a diameter of maximum 10 cm and up to 500 g of wet weight. While these smaller-sized sponges are well manageable in the context of aquaria experiments and chamber-based incubations, they deviate from the ambient community structure of *G. barretti* dominated habitats. *Geodia barretti* can reach up to a meter in diameter with a wet weight of up to 24 kg (Klitgaard and Tendal, 2004) and size has recently been found to be a major factor in determining the metabolic rates of sponges (Morganti *et al.*, 2019; de Kluijver *et al.* 2021). Furthermore, a recent study suggested that male and female individuals of *Geodia* species spend different amounts of energy on maintenance of reproductive tissues and reproduction (Koutsouveli *et al.*, 2020), potentially affecting the gender-specific coping potential towards changing environmental conditions.

**Conclusions**

In this _ex situ_ study, the deep-sea sponge species *G. barretti* was found to cope with seawater temperature and pH conditions representing future physicochemical parameters in the deep North-Atlantic Ocean. The basic metabolic metrics of oxygen consumption, bacterioplankton clearance and inorganic nutrient exchange rates were not significantly affected by the manipulated seawater temperature and pH treatments. However, the trend towards an effect of elevated seawater temperatures on decreased clearance rates, the observations of potential changes in microbially-mediated nitrogen cycling of *G. barretti*, and the highest level of tissue necrosis in the combined treatment stress the need for further
investigation of climate change effects on this deep-sea sponge species. A more holistic approach is needed to further evaluate the eco-physiological resilience of *G. barretti* to future ocean conditions, which should include *in situ* measurements and the broad spectrum of (microbially-mediated) energy acquisition (e.g., the processing of phytoplankton and DOM) processes in *G. barretti*. Care must be taken to include potential interactions between climate change effects and other anthropogenic stressors (e.g., Wurz *et al.*, submitted 2021) when assessing the coping capacity of *G. barretti* towards future ocean conditions.

**Acknowledgements**
The Authors thank Heikki and Tone from the University of Bergen for support in maintaining the aquaria facility and technical support when needed. We also thank Detmer Sipkema (WUR) and Rob Joosten (WUR) for generously sharing the flow cytometer and help with analyzing samples.

**Funding**
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Chapter 5

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

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Abstract

Abundant mineral resources present 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. These 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 its associated brittle star species Ophiura sp. 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.

Keywords: deep-sea mining, environmental impact, plumes, sponge grounds, benthic-pelagic coupling, Geodia barretti
Introduction

The deep-sea, earth’s largest ecosystem (Robison, 2004), 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). In this habitat, new species and ecosystems are being described continuously (Danovaro et al., 2014), while simultaneously economic interests for lucrative metal deposits are pushing extractive industries into the deep-sea. 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) hydrothermal fluids in various tectonic settings (Hoagland et al., 2010; Pedersen et al., 2013). At the seabed, the hydrothermal fluids mix with the cold seawater and precipitate sulphide minerals forming chimney structures. Plumes of black particle-loaded fluids emanate from these chimneys. Surrounding the hydrothermal vents, accumulated fall-out material from the plumes and collapsed chimney structures form SMS deposits of various sizes and composition (Cuyvers et al., 2018). During mining operations, these deposits will be crushed and grinded before the SMS-rich slurry is pumped up into a transport vessel. Onboard the vessel, the material is dewatered, and waste effluent containing grains < 8 μm will be pumped back into the ocean 25-50 m above the seafloor and evenly spread out over a large area (Gwyther, 2008). The indirect impact of mining operations on the area surrounding the active mining site through the generated plumes is suggested to exceed the local mining footprint by a few orders of magnitude (Mingotti and Woods, 2020). Thus, indirect mining impacts affect a much larger area compared to the sole removal of hard substrate at the location of the mining process (Washburn et al., 2019). 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 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., 2021b), 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 untap (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 anthozoa (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. 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), which makes it a suitable model 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., 2020). The described effects of 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\(^{-1}\) (representative for *in situ* drilling operations) of the minerals barite and bentonite, 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 (Coffey Natural Systems, 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 in some sponge species to egest indigestible particles (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.

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 are likely to last for years in prospected areas, 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). 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). A decrease in metabolic activity in abundant deep-sea sponges can have cascading effects on sponge-driven benthic-pelagic coupling processes. 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 environmental harm (Lodge and Verlaan, 2018). Adverse effects of SMS mining related particles on sponges as key players in benthic-pelagic coupling and deposit-feeding brittle stars, might cascade in effects on the integrity of energy pathways in sponge dominated habitats in deep-sea ecosystems.

This study

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 ocean conditions in the 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 in our study, neither before, 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 short, we used an *ex situ* approach to study how three weeks of exposure to crushed SMS particles (30 mg L⁻¹) affects survival, oxygen consumption and bacterioplankton clearance rate of the deep-sea sponge *G. barretti* and survival of an associated brittle stars from the genus *Ophiura*. In addition, bioaccumulation of metals was assessed in sponge and brittle star tissue.
Materials and methods

Collection of experimental animals

Individuals of *G. barretti* used in this experiment were collected in 2017 and 2018 by the remotely operated vehicle (ROV) ÁEGIR6000 deployed from the Norwegian research vessel GO 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 pebbles 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 GO Sars. Temperature controlled (7 °C, 20 L min⁻¹) seawater from 6 m depth was pumped into the 40 L tanks 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 (Figure 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 three 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.

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.*, in preparation). The in total 21 pre-incubated sponges were distributed over 8 tanks 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 6 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. These unexposed sponges were included
in the experimental setup to show that the effects observed in the exposure group are not related to changes of the conditions in the flow-through seawater facilities. Figure 1 illustrates the experimental design.

**Figure 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 12h per day over the course of three weeks.

**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; Wurz et al., in preparation), 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 CO₂ (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).
Exposure to crushed seafloor massive sulphide deposit

To mimic the activities of deep-sea mining, individuals of *G. barretti* and *Ophiura* sp. were exposed to ground material from a natural SMS deposit. The SMS deposit sample (SM-03-DR7) 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 that were small enough to stay suspended in seawater 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 (Figure 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 manually grinded using agata mortar, digested with a mix of HNO3 and HF before ICP-AES (Thermo Scientific ICap 7600) analyses. Quantification was done by external calibration curves and (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 analyses was performed to investigate the mineralogy using a scanning electron microscope ((SEM) Zeiss Supra™ – 55V Field Emission SEM (Gemini)) 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 three minutes, 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 terminated. 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.

**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 and 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 four hours and oxygen consumption rates in µmol O₂ gDM⁻¹ h⁻¹ were calculated as outlined by Tjensvoll *et al.* (2013) using the following equation:

\[
\text{Oxygen consumption} [\mu\text{mol O}_2 \ \text{gDM}^{-1} \ \text{h}^{-1}] = ((c_2 - c_1) \ t^{-1} \ V_{\text{net}}) \ \text{gDM}^{-1},
\]

where \(c_1\) and \(c_2\) = the concentration of dissolved oxygen at start and end of the incubation in µmol O₂ L⁻¹, \(t\) = the time of the incubation in h, \(V_{\text{net}}\) = 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 four-hour 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:

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

where \(V_{\text{net}}\) = the volume of the incubation chamber after subtracting the volume of the respective sponge in mL, \(t\) = the time of the incubation in h, \(n_{\text{start}}\) and \(n_{\text{end}}\) = 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.

**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 following Boury-Esnault
(2002). Sections of about 1 mm were prepared with a low speed precision saw (11-1180 Isomet,
Buehler, Germany). The sections then were adhered to a slide, polished to a thickness of 15 µm
and coloured 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 cm³ 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) and brittle stars (n = 8) by ICP-AES.

**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, clearance and oxygen consumption rates were analysed using a
mixed effects model to describe possible effects of manipulated seawater treatments. Since
manipulated seawater treatment effects were not evident 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
(α = 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) and end-
concentrations of analysed elements (Fe, Cu and S) in tissues from SMS particle exposed
(n = 19) and non-exposed group (n = 6). 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. For
analysis of the microbial community 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) 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. All statistical tests were performed with GraphPad Prism 8 Version 8.2.1(441), August 20, 2019.

**Results**

**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 the mineral Ba (Table 1). Relatively minor amounts of Mn, Co, Zn, Hg and Ni were present. SEM/EDS analyses (Figure 2) revealed a relative dominance of iron- and copper-sulfides, covered with a layer of FeHO$_2$ and the presence of BaSO$_4$. 

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Figure 2. Scanning electron microscopy close-up 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.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass fraction [\%]</th>
<th>Fe [mg kg(^{-1})]</th>
<th>Y [mg kg(^{-1})]</th>
<th>Zr [mg kg(^{-1})]</th>
<th>Ta [mg kg(^{-1})]</th>
<th>Se [µg kg(^{-1})]</th>
<th>Au [µg kg(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO(_2)</td>
<td>0.92</td>
<td>623 909</td>
<td>&lt; 1</td>
<td>14.2</td>
<td>&lt; 1</td>
<td>&lt; 3</td>
<td>424</td>
</tr>
<tr>
<td>Al(_2)O(_3)</td>
<td>0.09</td>
<td>Cu 33 528</td>
<td>2.6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeO(_3)</td>
<td>62.36</td>
<td>Ba 31 531</td>
<td>1.1</td>
<td>2.6</td>
<td>1.1</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>MnO</td>
<td>0.05</td>
<td>Cd 1.1</td>
<td>As 73</td>
<td>&lt; 1</td>
<td>6</td>
<td>1.1</td>
<td>15.6</td>
</tr>
<tr>
<td>MgO</td>
<td>0.09</td>
<td>Ni 10</td>
<td>Br 6</td>
<td>1.1</td>
<td>6</td>
<td>1.1</td>
<td>15.6</td>
</tr>
<tr>
<td>CaO</td>
<td>0.4</td>
<td>Zn 815</td>
<td>Co 609</td>
<td>La 0.3</td>
<td>1.1</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>Na(_2)O</td>
<td>0.08</td>
<td>Ag 2.6</td>
<td>Cr 1</td>
<td>Ce 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(_2)O</td>
<td>&lt; 0.01</td>
<td>Pb &lt; 5</td>
<td>Cs &lt; 0.5</td>
<td>Nd 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TiO(_2)</td>
<td>0.005</td>
<td>Bi &lt; 2</td>
<td>Hf &lt; 0.5</td>
<td>Sm 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(_2)O(_5)</td>
<td>0.02</td>
<td>Cd 1.1</td>
<td>Hg 10</td>
<td>Eu 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOI</td>
<td>94.29</td>
<td>Be &lt; 1</td>
<td>Rb &lt; 20</td>
<td>Yb 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94.29</td>
<td>Mo 83</td>
<td>Ir 5</td>
<td>Tb 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|       | S > 20.0             | Sr 1 209            | Sb 5.9           | Lu 0.05          |
|       | S* 40.2              | V < 5               | Se 0.2           | Li n.a.          |
SMS exposure levels and accumulation of SMS particles in sponges and brittle stars

Individuals of *G. barretti* and *Ophiura* sp. 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 (Figure 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.

![Figure 3.](image) 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.

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 (Figure 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 Figure 4B). This pathway is highlighted by red (pathway through cortex) and yellow (accumulation in channel walls) arrows in Figure 5A. SMS particles were absent from the cortex and subdermal spaces of sponges from the unexposed group (Figure 5B).
Figure 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).

Figure 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).

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 (Figure 6), while individuals from the unexposed group expressed a beige, common coloration for *G. barretti* (Figure 6). Elemental analysis of selected compounds (Fe, Cu and S) showed significant, ~ twentyfold higher concentrations of iron and copper in tissue samples from SMS exposed sponges compared to individuals from the unexposed group (*p* < 0.01, respectively) (Table 3). Concentrations of sulfur were comparable in the exposed and unexposed group (*p* = 0.12). Concentrations of iron, copper and sulfur in SMS exposed brittle stars are stated in Table 3.

**Table 3.** Overview of elemental concentrations in SMS exposed sponges, SMS exposed brittle stars and unexposed sponges.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration in SMS exposed sponges [µg gDW⁻¹]</th>
<th>Concentration in unexposed sponges [µg gDW⁻¹]</th>
<th>Concentration in SMS exposed brittle stars [µg gDW⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>2283.86 ± 492.17</td>
<td>119 ± 25</td>
<td>1534.5 ± 1816.6</td>
</tr>
<tr>
<td>Cu</td>
<td>201.2 ± 29.1</td>
<td>12.5 ± 1.5</td>
<td>229.7 ± 112.8</td>
</tr>
<tr>
<td>S</td>
<td>11.38 ± 0.7</td>
<td>9.4 ± 0.7</td>
<td>10.0 ± 2.4</td>
</tr>
</tbody>
</table>

**Figure 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.

_Brittle star mortality and necrosis rates in sponges_
Brittle stars that were introduced to the SMS particle exposure tanks were thriving throughout the 10 months long maintenance in the aquaria system preceding the described experiment (Table 4). With the start of the exposure to crushed SMS deposit brittle stars started to shed movable 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 4) 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 4). Sponges in the three unexposed tanks did not show any signs of tissue necrosis over the 21 day long experiment.

**Table 4.** 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.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Species</th>
<th>Animals at start [n]</th>
<th>Duration of experiment [days]</th>
<th>Individuals with necrosis [n]</th>
<th>Dead animals [n]</th>
<th>Necrosis and mortality rates [%Animals.day$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMS exposure experiment</td>
<td><em>Geodia barretti</em></td>
<td>21</td>
<td>21</td>
<td>5</td>
<td>Not applicable</td>
<td>1.13</td>
</tr>
<tr>
<td>Long-term maintenance</td>
<td>(Sponge)</td>
<td>31</td>
<td>273</td>
<td>10</td>
<td>Not applicable</td>
<td>0.12</td>
</tr>
<tr>
<td>SMS exposure experiment</td>
<td><em>Ophiocomina</em> spp.</td>
<td>24</td>
<td>21</td>
<td>Not applicable</td>
<td>24</td>
<td>10.00</td>
</tr>
<tr>
<td>Long-term maintenance</td>
<td>(Brittle star)</td>
<td>24</td>
<td>273</td>
<td>Not applicable</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**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 Table S1 and S2) throughout the 21-day long experiment (Supplementary material: Figure S2 and S3). SMS deposit exposure had a significant impact on sponge oxygen consumption ($p = 0.02$ ($F_{(2, 24)} = 4.57$) (Figure 7A, Table 5). 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 ($p = 0.21$) (Figure 8A, Table 5). Similar to oxygen consumption, clearance rates in sponges from the unexposed group were stable over time ($p = 0.56$) (Figure 8B, Table 5). However, sponges exposed to SMS deposits expressed a significant ($p < 0.01$ $F_{(2, 24)} = 6.99$) (Figure 7B, Table 5), 130 % decrease in clearance rates after 21 days compared to rates before SMS deposit exposure commenced.
Figure 7. Oxygen consumption rate (A) and clearance rate (B) and of *G. barretti* under crushed SMS exposure over the course of 21 days. Data represents averages of tanks (Day 0, n = 11; Day 7, 21, n = 8). Corresponding letter pairs (a,b) indicate significant differences (p < 0.05).

Table 5. Overview of oxygen consumption and clearance rates of *G. barretti* in the SMS exposed and unexposed group over time.

<table>
<thead>
<tr>
<th>Exposure Time [Days]</th>
<th>Group</th>
<th>Oxygen consumption rate [µmol O₂ gDM⁻¹ h⁻¹]</th>
<th>Clearance rate [mL gDM⁻¹ h⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>SMS-exposed</td>
<td>0.6 ± 0.3</td>
<td>26.6 ± 25.7</td>
</tr>
<tr>
<td>7</td>
<td>SMS-exposed</td>
<td>1.0 ± 0.2</td>
<td>4.6 ± 6.5</td>
</tr>
<tr>
<td>21</td>
<td>SMS-exposed</td>
<td>1.2 ± 0.6</td>
<td>-8.56 ± 22.0</td>
</tr>
<tr>
<td>7</td>
<td>Unexposed</td>
<td>0.8 ± 0.06</td>
<td>13.2 ± 1.3</td>
</tr>
<tr>
<td>21</td>
<td>Unexposed</td>
<td>0.6 ± 0.1</td>
<td>16.7 ± 9.3</td>
</tr>
</tbody>
</table>
Figure 8. Oxygen consumption rate (A) and clearance rate (B) and of *G. barretti* from the unexposed group over the course of 14 days. Data represents averages of tanks (n = 3).
**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 of 27 mg L^{-1} 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* sp. individuals after 10 days of exposure. Sponges strongly accumulated the 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. At the time of their death, concentrations of copper, iron and sulphate in *Ophiura* sp. were one magnitude higher than reported for a starfish species from a fjord polluted by discharged mine tailings (Coteur *et al.*, 2003). While oxygen consumption in exposed sponges doubled over 21 days, clearance rates in these sponges completely ceased over the course of the experiment. Below, we discuss these effects of SMS on *G. barretti* and *Ophiura* sp. in detail and elaborate on ecological implications of these findings.

**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 his species (Kutti *et al.*, 2015; Leys *et al.*, 2018; Bart *et al.*, 2020) demonstrating that effects observed in the SMS exposed group of sponges were not induced by changes in the experimental conditions.

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

The elements Cu, Cd, Ni, Zn, Co, As and Hg that are associated with hydrothermally derived minerals 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 occur via the water phase as well as via accumulated particles and 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, individuals of the brittle star genus Ophiura 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, Eriksen and Meyer (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 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 coloured 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 filtering from the seawater (Bart et al., 2020). The particle size used in this study (< 1 µm to 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 the dewatered ores (Coffey Natural Systems, 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 further 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. Alternatively, 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 (e.g. Cliona viridis, Fe = 1806.1 ± 432.2 µg gDM⁻¹; Cacospongia scalaris, Cu = 151.6 ± 2.7 µg gDM⁻¹, 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.

**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., 2020). With the exposure to SMS particles, oxygen consumption of G. barretti increased over time and had doubled (Table 5 and Figure 7) after 21 days. Rapid respiratory responses to suspended particles have been reported for G. barretti (Tjensvoll et al., 2013). However, the reported rapid drop in oxygen consumption as a response to concentrations of 10 - 100 mg L⁻¹ (Tjensvoll et al., 2013) 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 decline in oxygen consumption as an immediate response to natural sediment exposure is thought to indicate a temporarily (4h) cessation of pumping activity, hypothesized as a mechanism to reduce particle accumulation in the sponge’s 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 (Figure 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., 2020). 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 sponges body (Reiswig, 1971). The absence of clearance of bacterioplankton in SMS-exposed individuals of G. barretti could result from a smothering of choanocyte chambers cumulating 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 (Figure 6).

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., 2020) 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 clearance 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 deep-sea ecosystems. Also, the transfer of energy to higher trophic levels might be compromised due to higher metabolic rates of G. barretti under exposure to indigestible SMS particles (this study). The detritivorous brittle stars utilize excreted organic material of filter feeders in deep-sea environments and thus represent a pathway of energy transfer (Maier et al., 2020, Bart et al., 2021). Also, this link can be weakened by changes in the metabolism of G. barretti, but especially the direct deleterious effects of SMS particles on the survival of Ophiura sp. described here would completely eliminate this pathway. In addition, increased rates of necrosis and mortality of G. barretti would compromise its important ecological contributions such as benthic pelagic-coupling and the habitat structuring characteristics (Murillo et al., 2012; Howell et al., 2016).

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. This study examined the effects of deep-sea mining on two model species. However, responses are most likely species specific and will depend on the environmental settings mining will occur in. Thus, it is highly recommended to perform response studies with location specific fauna under conditions resembling the environmental conditions at the prospected mining site.
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) and caused rapid mortality in individuals of the sponge-associated brittle star *Ophiura* sp. 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.

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 ÅGIR6000 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.

Funding

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Supplementary material

Figure S1. Grain size distribution of the crushed SMS deposit used in the experiment.

Figure S2. Oxygen consumption rates of *G. barretti* across four seawater treatments combined with SMS exposure over time.

Table S1. Overview of the test results for oxygen consumption over time (Repeated measure mixed-effects analysis).

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>p-value</th>
<th>F (DFn, DFd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0.02</td>
<td>F (2, 8) = 5.81</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.85</td>
<td>F (1, 7) = 0.03</td>
</tr>
<tr>
<td>pH</td>
<td>0.92</td>
<td>F (1, 7) = 3.35</td>
</tr>
</tbody>
</table>
Table S2. Overview of the test results for clearance rate over time (Repeated measure mixed-effects analysis).

<table>
<thead>
<tr>
<th>Fixed factor</th>
<th>p-value</th>
<th>F (DFn, DFd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0.03</td>
<td>F (1.42, 5.68) = 7.25</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.86</td>
<td>F (1, 7) = 0.03</td>
</tr>
<tr>
<td>pH</td>
<td>0.97</td>
<td>F (1, 7) = 0.001</td>
</tr>
<tr>
<td>Time × Temperature</td>
<td>0.43</td>
<td>F (2, 8) = 0.92</td>
</tr>
<tr>
<td>Time × pH</td>
<td>0.96</td>
<td>F (2, 8) = 0.04</td>
</tr>
<tr>
<td>Temperature × pH</td>
<td>0.11</td>
<td>F (1, 7) = 3.35</td>
</tr>
<tr>
<td>Time × Temperature × pH</td>
<td>0.70</td>
<td>F (2, 8) = 0.37</td>
</tr>
</tbody>
</table>

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 ~0.25g tissue samples were extracted from sponges at the end of the experiment. 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 16S primers. For sequencing, the V3 and V4 variable regions of the 16S rRNA gene were amplified using the primer pair 341F-806R (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 270nt), 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 16S database, with the help of a primer‐specific trained Naive Bayes taxonomic classifier. For seawater reference samples 2 L of seawater from the exposed and unexposed tank were filtered over a 200 µm filter. Three replicates per tank were filtered and frozen in -80 °C until analysis as described above.

**Microbial abundances in seawater and sponge tissue**

Clear shifts in the seawater microbial community composition were observed across the treatments (Figure S4). Although less distinct, effects of the treatments were also observed in the sponge‐associated microbial communities. Microbiomes of those sponges that were exposed to SMS deposit clustered apart from unexposed samples (Figure S4, B1) and were significantly different (Table S3). *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 all other SMS treated samples (Figure S4, B2) and were significantly different in their composition (Table S3). 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 temperature treatments (Table S4). *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 (Figure S4, A and S4, B3 - 4; Table S3).
Figure S4.  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) Ward-clustering of Bray-Curtis indices calculated on feature level showing each treatment together with respective unexposed animals (Figure 9 B1 - B4). Next
to the dendrograms, the microbial phylum which is most significantly enriched and the phylum which is most significantly depleted determined in a Linear Discriminative Analysis are indicated.

Table S3. Microbial diversity statistics. 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).

<table>
<thead>
<tr>
<th>comparison</th>
<th>α - diversity</th>
<th>β - diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMS vs. no SMS</td>
<td>M= 13; p: 0.381</td>
<td>R= 0.568; p: &lt;0.023  *</td>
</tr>
<tr>
<td>SMS vs. high temperature</td>
<td>M= 25; p: 0.008 *</td>
<td>R= 0.824; p: &lt; 0.008  *</td>
</tr>
<tr>
<td>SMS vs. low pH</td>
<td>M= 18; p: 1.000</td>
<td>R= 0.059; p: &lt; 0.269</td>
</tr>
<tr>
<td>SMS vs. high temperature + low pH</td>
<td>M= 21; p: 0.329</td>
<td>R= 0.157; p: &lt; 0.093</td>
</tr>
</tbody>
</table>

Table S4. Microbial community richness across different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shannon Index (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMS</td>
<td>5.07 ± 0.1</td>
</tr>
<tr>
<td>control</td>
<td>5.18 ± 0.03</td>
</tr>
<tr>
<td>high temperature</td>
<td>4.58 ± 0.17</td>
</tr>
<tr>
<td>low pH</td>
<td>5.07 ± 0.07</td>
</tr>
<tr>
<td>high temperature + low pH</td>
<td>4.92 ± 0.19</td>
</tr>
</tbody>
</table>

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 table 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*. As a habitat structuring key species, these species-specific responses might cascade into larger scale impacts on sponge driven deep-sea ecosystems.
Chapter 6

General discussion
The EU-funded project *SponGES* was a dedicated, large-scale effort to enhance our knowledge about abundant deep-sea sponge grounds throughout the North Atlantic Ocean (NAO) and to bring this knowledge to the attention of the general public and policy makers. This thesis aimed to contribute to our understanding of the acclimatisation potential of deep-sea sponges towards large-scale environmental changes in combination with local interactions of various industries operating in the deep NAO. To this end, the basic eco-physiological response variables in habitat forming deep-sea sponges were investigated under the cumulative impacts of future ocean condition with warmer and acidified seawater plus the simultaneous exposure to different types of re-suspended particles (Figure 1) using aquaria based experimental approaches.

**Figure 1.** Graphical summary of the main findings from this thesis. Picture copyright: DFO

Below the main findings from this thesis are summarized per chapter.

In **Chapter 2**, it was demonstrated that the glass sponge species *Vazella pourtalesii* was able to cope for up to seven days with elevated suspended particle concentrations (50 mg L⁻¹), simulating bottom-trawling generated plumes of natural sediment. This coping potential might be related to the dynamic nature of the habitat this sponge species is thriving in. Internal waves and seasonal weather constellations on the Scotian shelf can re-suspend sediment and expose filter-feeding sponges on a regular basis. Expulsion of sediment particles was observed that were potentially clustered by mucus. While this observed evacuation of mucus-bound particles probably represents a strategy to remove accumulated indigestible matter, this mechanism was not effective enough to prevent accumulation of all sediment it was exposed to on the long run.
Continuous exposure to 50 mg L\(^{-1}\) sediment longer than seven days notably affected sponge physiology and resulted in reduced retention efficiency for bacterioplankton. This potentially compromises sponge health as well as sponge-mediated nutrient and energy cycles.

**Chapter 3** reveals that exposure of explants of the demosponge species *Geodia barretti* to acidified (minus pH 0.2), warmer (plus 3 °C) seawater and suspended natural sediment (50 mg L\(^{-1}\)) triggered an arrest pumping and increased respiration rates after 19 days. As the individual stressors did not yield a noticeable decrease in metabolic fitness, it was concluded that synergistic effects of multiple stressors in *G. barretti* can push this habitat forming species over the edge of its ecological potential to deal with unfavourable environmental conditions. In line with metabolic changes, we observed a shift in the composition of microbes associated with *G. barretti* underpinning a limited coping capacity of this deep-sea sponge species towards cumulative stressors under future environmental conditions in the deep NAO.

**Chapter 4** reports the study of physiological response variables oxygen consumption and bacterial clearance rate in small, intact individuals of *G. barretti* over a ten months period to evaluate long-term acclimatisation potential of this deep-sea sponge species to future environmental conditions. *G. barretti* expressed a remarkable resilience to acidified (minus pH 0.2) and warmer (plus 4 °C) seawater over ten months. Chemical or biological cues other than the ones controlled in this *ex situ* experiment likely caused some fluctuating patterns of oxygen consumption that were observed over time, but did not result in changes in basic physiological functioning under the stable experimental conditions. It would be interesting to further study the holobiont (i.e., sponge host and microbial symbionts) when evaluating the coping potential of this high microbial abundant sponge species towards future ocean conditions.

The way how *G. barretti* and an associated brittle star species cope with exposure to particles released by deep-sea mining operations under future physicochemical conditions of the NAO is described in **Chapter 5**. Over three weeks individuals of *G. barretti* and brittle stars were exposed to a field relevant concentration (30 mg L\(^{-1}\)) of crushed seafloor massive sulphide (SMS) deposit. Brittle stars experienced high mortality rates and after ten days all experimental animals had perished, indicating a high toxicity of crushed SMS deposit suspended in seawater. Sponges had accumulated µm-sized particles and were coloured black throughout their body after three weeks of exposure and oxygen consumption and clearance rate for bacterioplankton were significantly affected. The microbial composition was changed and could have induced or be a consequence of increased necrosis rates of sponge tissues. It was concluded that suspended
crushed SMS deposit has a detrimental, sub-lethal effect on *G. barretti* and an associated brittle star species. In contrast to experiments in chapter three climate conditions had no additional effect on the response of sponges to crushed SMS suspensions.

**Suspended particulate matter exposure as indirect effect of bottom trawling on deep-sea sponges**

On the East Canadian Shelf, the hexactinellid deep-sea sponge *Vazella pourtalesii* forms dense aggregations in depths from 70-200 m. In 2013, two sponge conservation areas (SCA), the Sambro Bank SCA (area: 62 km²) and Emerald Basin SCA (area:195 km²), have been implemented to protect these aggregations from removal or damage by bottom-tending fishing gear frequently deployed in the area (DFO, 2017). However, within the direct vicinity of the SCAs, bottom-tending bottom trawling continues to re-suspend bottom sediments. Particle plumes can disperse over large distances, directly exposing sponge assemblages within the SCA. In aquaria based experiments described in this thesis, *V. pourtalesii* (Chapter 2) was found to maintain oxygen removal and clearance rates for bacterioplankton similar to literature values for over seven days when exposed to suspended natural sediment, mimicking the indirect effects of bottom trawling activities. This indicates a tolerance of *V. pourtalesii* towards concentrations of suspended sediment of up to 50 mg L⁻¹ over short time scales. This could be related to the dynamic nature of the Scotian shelf where the sponges are present. It is a dynamic habitat were natural phenomena such as internal waves (Boegman and Stastna, 2019) and seasonal weather constellations (Hanz *et al.*, 2021) potentially increase particle concentrations close to the soft-sediment dominated seafloor. Thus, as *V. pourtalesii* is exposed to turbid waters on a regular basis, it must have developed mechanisms to prevent accumulation of indigestible particles within its tissues as described in other sponge species (Strehlow *et al.*, 2017). However, these mechanisms seemed to be insufficient when the particle concentrations remained elevated for longer than seven days as would be the case with extensive bottom trawling activities. After one week of sediment exposure, basic physiological parameters in *V. pourtalesii* started to show significant changes, indicating adverse impacts of prolonged exposure to suspended sediment. While the established SCA protect a small population of *V. pourtalesii* from the direct effects of removal or damage, the results from this thesis highlight the necessity to improve the protection of *V. pourtalesii* from extended exposure to the indirect effects of bottom trawling. The protection from indirect bottom trawling effects becomes increasingly pressing in the face of predicted climate change that is predicted to increase the frequency of storm induced re-suspension events on the Scotian Shelf (Hanz *et al.*, 2021). Ongoing fishing activities together
with a higher frequency of natural re-suspension events have the potential to exceed the coping potential of *V. pourtalesii* towards suspended sediment exposure. Compromised fitness under the influence of suspended sediment and climate change effects such as ocean warming and acidification has been shown to compromise skeletal integrity and ecological functioning in a cold-water hexactinellid sponge species from the North Pacific Ocean (Stevenson *et al.*, 2020). With up to 6 ± 5 million individuals of *V. pourtalesii* within an area of 2104 km² potential decreases in sponge health and functioning would have large scale implications for ecosystem service provision by this unique assemblage of *V. pourtalesii* in the North West Atlantic Ocean.

**Deep-sea sponges under future physicochemical seawater properties**

Deep-sea sponge species that are forming vast grounds on the bottom of the NAO will have to cope with large-scale changes of physicochemical seawater properties over the next decades (Sweetman *et al.*, 2017). Under *ex situ* conditions as described in this thesis, the model sponge species *G. barretti* tolerated the seawater pH and temperature that are predicted for the deep NAO for the next century (Chapter 4). *G. barretti* has a broad distributional range and is abundant from sub-arctic depths of up to 2000 m with temperatures around 3 °C to depths as shallow as 30 m in fjord ecosystems on the Norwegian south coast where seawater temperatures can reach up to 12 °C (Guihen *et al.*, 2014). This large distributional range suggests a broad ecological potential of *G. barretti* to cope with a large range of seawater temperatures which might enable this species to adapt to the projected temperature increase in the deep NAO. This assumption is corroborated by recent hindcast models that describe abrupt permanent shifts in water masses and related changes of seawater temperature, salinity, oxygen- and particulate organic matter-concentration across areas with high abundances of *G. barretti* (Samuelsen *et al.*, 2022). The described presence of large individuals of slow-growing deep-sea sponges that are at least a few decades old (Prado *et al.*, 2021) within these habitats suggest that *G. barretti* can withstand fluctuations of environmental parameters and persists to form dense aggregations that resemble hotspots of biodiversity, provide stepping stones of connectivity, and greatly determine nutrient cycling (Hogg *et al.*, 2010). The observed tolerance of *G. barretti* towards decreased seawater pH might be related to its vast numbers of associated microbes (3.1 × 10¹¹ microbial symbionts per ml sponge tissue (Leys *et al.*, 2018). Past studies have explored the possibility that sponge species with a high abundance of associated microbes, such as *G. barretti*, might be able to cope with changing environmental conditions by shifts in microbiome resulting in functional adaptation to a different seawater pH by microbially-driven processes (Ribes *et al.*, 2016; Pita *et al.*, 2018). At the same time, the observed tolerance of *G. barretti* towards ocean acidification and warming has to be evaluated with caution, as the *ex situ*
conditions of the performed experiments only represents a part of its life cycle and does not include reproduction, larval survival, settlement and growth. Also, experimental conditions can only represent the complexity of deep-sea ecosystems to a limited degree and do not include potential effects on associated species. In areas where this deep-sea sponge is abundant in vast numbers, global climate change is predicted to also cause e.g. changes in current regimes and organic fluxes in the deep NAO (Levin and Bris, 2015). The cumulating effects of multiple climate-related changes across the distributional range of this habitat-forming deep-sea sponge species might move the environmental conditions beyond this species’ full life cycle ecophysiological tolerance. This is not known yet, so it cannot yet be excluded that exposure to future ocean conditions could have farther reaching consequences for sponge-driven ecosystem services such as habitat provision for commercially important fish species (Ottaviani, 2020).

**Deep-sea sponges under cumulative environmental stress**

Anthropogenically induced climate-related changes in the NAO are coinciding with increasing industrial activities such as deep-sea bottom trawling fisheries or exploitation of metal-rich, geological formations on the seafloor. This thesis investigated the cumulative effects of two types of suspended particles in combination with decreased seawater pH and increased seawater temperature on the model sponge species *G. barretti*. While climate-related changes were found to be coped with under experimental conditions (Chapter 4) the combination with an extended period of exposure to a field relevant concentration of natural suspended sediment, mimicking bottom trawling activity, significantly hampered basic physiological parameters in this sponge species (Chapter 3). While earlier work has described a tolerance towards suspended natural sediment in *G. barretti* (Tjensvoll et al., 2013), findings from this thesis highlight the synergistic potential of cumulative stressors to push this species over the edge of its ecophysiological tolerance. The ceased retention of bacteria under cumulative stressors (Chapter 3) might not directly lead to an energy deficiency, as *G. barretti* is able to cover its metabolic demand via phagocytosis of microbial symbionts (Leys et al., 2018). This did, however, cause a shift in the relative abundance of symbiotic microbes which might point towards a disruption of the stable state of the sponge holobiont with implications for the energy acquisition via phagocytosis of associated microbes. The expulsion of accumulated sediments is an energy demanding process (Biggerstaff et al., 2017). Under adverse environmental conditions, other marine invertebrates have been shown to allocate energy towards stress protection and damage repair at the expense of reproduction, growth or investing in energy storages (Sokolova et al., 2012). Prolonged or repeated reduced reproductive fitness due to shifts in energy allocation as a response to adverse environmental conditions can affect the population structure of deep-sea...
sponge grounds (Colaço et al., 2022). A recent publication (Abdul Wahab et al., 2019) has shown that under concentrations of suspended sediment similar to those used in experiments from this thesis (Chapter 3), sponge larvae had reduced dispersal ranges and decreased survival rates throughout the pelagic and settlement phase. The potential shifts in energy allocation and decreased larvae survival as consequence of the indirect effects of bottom trawling activities can reduce sponge recruitment under future ocean conditions (Taboada et al., 2022). Shifts in abundance, size and functional diversity of habitat forming sponge species are direct effects of bottom trawling activities (Colaço et al., 2022). In combination with reduced recovery potential (Morrison et al., 2020) and reduced coping potential towards cumulative stressors under future ocean conditions (Chapter 3) can have implications for sponge-driven ecosystem services such as benthic-pelagic coupling, habitat provision (Ottaviani, 2020) and virus predation (Welsh et al., 2020).

Particles that are released into the marine environment as a consequence of industrial mining activities have been shown to cause more severe responses in deep-sea sponges than particles with a natural origin (sediment suspended by e.g. internal waves) (Edge et al., 2016). Mining of seafloor massive sulphide (SMS) deposits is known to have significant impact on marine benthic habitats (Boschen et al., 2013). Sponges and echinoderms are the most abundant animal phyla within potential areas of mining activities along the Mid-Atlantic ridge (Boschen et al., 2016). This thesis represented the first experimental effort to assess the response to crushed SMS deposit of the model sponge *G. barretti* and an associated genus of brittle star (Chapter 5). Within ten days of exposure to a field relevant concentration of suspended crushed SMS deposits, all brittle stars in the experiment perished. This suggests that brittle stars will likely vanish on a large scale when plumes of SMS particles would disperse over hundreds of square kilometers (Jankowski and Zielke, 2001; Gillard et al., 2019). Deposit-feeding brittle stars can consume particulate excretions of sponges and also represent an important link in the benthic food web of the deep NAO (Maier et al., 2020). Therefore, a die-off induced by a mining-plume can disrupt bottom up mechanisms of regional food webs in the deep sea (Drazen and Sutton, 2017). Similar to the brittle stars, sponges showed a strong response to the suspended SMS particle exposures. Oxygen consumption was significantly increased, while bacterioplankton clearance completely ceased after three weeks of exposure. SMS exposure also induced significant higher tissue necrosis rates and changed the relative abundance of their associated microbes (Chapter 5). These results are in line with earlier studies (Edge et al., 2016) and point towards a strong response of sponges to particles borne from industrial mining activities. While sponge’s response to natural sediment was more severe under future seawater
conditions, these synergistic effects were absent in sponges exposed to crushed SMS deposit. The strong responses in individuals exposed to crushed SMS exposure could have masked more subtle effects of changes in physicochemical seawater parameters on basic physiological parameters in *G. barretti*. Operators of deep-sea mining are required to avoid so-called significant harm for the marine environment. Results from this thesis suggest that the indirect effects of deep-sea mining have severe adverse effects on the most abundant animal phyla present in the vicinity to proposed mining areas along the Mid-Atlantic ridge. Thus deep-sea mining can have cascading effects on bottom-up mechanisms of regional marine food webs as it has the potential to completely erase two major links of benthic pelagic coupling mechanisms.

**Challenges and limitations of methods applied**

A constraint in biological deep-sea research is the limited access to experimental animals. The extensive logistical efforts to collect individuals from the deep sea has limited the replication of measurement units in this thesis. A further challenge for research on deep-sea sponges is that the experimental animals express a high variability in physiological rates. Not only among specimen but also among repeated measurements taken on the same individual. With a larger number of replicate sponges, the individual fluctuations of the assessed response variables could have been counterbalanced by a larger dataset and allowed to better disentangle the specific treatment effects from the natural variability of physiological rates observed throughout datasets from this thesis. Additionally, limitations to maintain large animals (*G. barretti* can reach up to 1 m in diameter, *V. pourtalesii* up to >70 cm in height) in aquaria have led to a selection of comparably small sponges to be included in the experiments. Size has been described to affect growth (Leys and Lauzon, 1998), pumping (Morganti *et al.*, 2019), assimilation of captured bacterioplankton and oxygen consumption in sponges (de Kluijver *et al.*, 2021). So the magnitude of sponge-mediated energy and nutrient fluxes as established in this thesis will be different at the community level with a more heterogeneous age- and size population structure. While *ex situ* experimental approaches allow for the control of specific environmental conditions, they are inevitably limited in their capacity to mimic important physical properties and ecological contexts of deep-sea species’ habitats. For example the hydrostatic pressure was shown to affect cellular processes such as lysosomal membrane stability (Yan *et al.*, 2022). In *G. barretti* this biomarker was found to express adverse changes under exposure to suspended particles released by drilling activities (Edge *et al.*, 2016). While these experiments were performed under atmospheric pressure, it cannot be excluded that the adverse effects on cellular processes will be greater under *in situ* conditions where animal
physiologies are adapted to real deep-sea conditions (deeper than 1000 m). Pressure has also been described to increase the toxicity of bioavailable metals for marine invertebrates (Brown et al., 2017). These findings imply that aquaria based observations on the effects of deep-sea mining borne particles on the two most abundant animal phyla underestimating the risk for indirect effects of this nascent deep-sea industry.

**What is needed more?**

To better evaluate if deep-sea sponges will remain abundant and ecologically viable a holistic approach is needed at the sponge holobiont level, taking into account the full life cycle of the organisms (including reproduction and larval stage), considering the role of the biotic environment (indirect effects via effects on facilitators) and paying attention to the abyssal adapted physiology of animals living under real deep-sea conditions with great ambient pressure (>1000m deep). The energy budget of the sponge holobiont should be disentangled to be able to determine how energy allocation for storage, activity, maintenance, growth, reproduction/development might shift under environmental conditions of the future NAO. Once these parameters are better understood under controlled conditions, these findings need to be integrated on an ecosystem wide scale to allow for evidence-based policy making that warrants the continuity of sponge-driven ecosystem services under global climate change effects and local industrial activities. In all cases it is eminent that direct and indirect consequences of deep-sea mining activities need to be monitored for the fate of compounds as well as the ecotoxicological effects. To quantify and qualify the effects, the application of environmental omics could help to develop methodologies to assess the health status and ecological viability of sponge-dominated ecosystems on a large scale under in situ conditions (Vad et al., 2021). Furthermore, the shared sponge exometabolome represents a promising biomarker to monitor ecosystem functioning and health (Bojko et al., 2019). These biomarkers can support evaluation of the effectiveness of imposed measures to avoid significant ecological harm in the context of industrial interactions with habitat forming, abundant sponge grounds. Large scale information about the health status of dense sponge populations also represent an outstanding contribution to the recent efforts of developing a digital twin of the ocean. This model will allow predictions on how changing environmental conditions and anthropogenic activities will affect marine ecosystems throughout the ocean’s depths. In the future the digital twin of the ocean can help to evaluate the effectiveness of measures to reduce adverse effects of environmental changes and interactions with industrial sectors on sponge-driven ecosystems in the deep sea. Models need reliable and, most importantly, large sets of data. The experiments performed in this thesis
provide a basis to be further developed and replicated on a larger scale and including a variety of geological formations targeted by mining operations. This would support policy making processes towards future deep-sea mining operations that respect the deep-sea species that are of more importance for the oceans than society appreciates.

**Concluding remarks**

Sponges were among the first multicellular animals to thrive on planet earth and they have endured and adapted to ever changing conditions of the ocean. The research of this thesis highlights the resilience of two deep-sea sponges from the North Atlantic Ocean towards large scale environmental changes and local, short-term events of sediment resuspension. Likely, this resilience originates in the broad ecological potential to thrive in sediment rich, dynamic shelf environments and a large distributional range across a broad spectrum of oceanographic conditions. However, this thesis also found that deep-sea sponges’ coping capacities can be challenged by cumulative anthropogenic activities that are changing natural systems on an unprecedented spatial and temporal scale. The frequency and duration of local disturbances outside natural thresholds can overstrain mechanisms to cope with these environmental drivers. Indications of physiological effects on a systemic level as identified throughout this thesis could have implications for sponge-driven, benthic-pelagic coupling mechanisms at ecosystem scales. To avoid significant harm to crucial ecosystem services provided by sponge grounds in the NAO, precaution should guide industrial scale deep-sea activities in the face of changing ocean conditions.
Acknowledgements

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I am very thankful for getting to know Hans-Tore Rapp who was coordinating the SponGES consortium and sadly passed away early 2020. He always had an open door and ear for the people around him and he was a great inspiration. You are missed as a friend, mentor and excellent sponge biologist.

Thanks to my thesis supervisors Tinka, Ronald and Jasper for their valuable input throughout the project and helpful suggestions when I needed a push in the right direction.

Many thanks to all PhDs of Marine Animal Ecology for a relaxed and fun working atmosphere in the office. Special thanks to my good friends Diede, Mischa and Chris (colleaguefriend) who joined me in the PhD quest for mental support and many good laughs.

Last but not least, thanks to my family and especially Veronika for supporting me during the long process of finishing this thesis.
References


VMEs in Arctic and sub-Arctic waters – Distribution and threats. 146.

Carballo, J. L., & Bell, J. J. (2017). Climate change, ocean acidification and sponges. C. S.
(2017). Climate Change, Ocean Acidification and Sponges. doi:10.1007/978-3-319-
59008-0.

Taxonomy, biogeography and DNA barcodes of Geodia species (Porifera, Demospongiae,

shed more light on the diversity and biogeography of North Atlantic deep-sea sponges. J.

(2015). Cold-water coral reefs and adjacent sponge grounds: Hotspots of benthic

mining on pelagic and benthopelagic biota. Mar. Policy.


in three temperate benthic suspension feeders. Limnol. Oceanogr.

Coppari, M., Zanella, C., and Rossi, S. (2019). The importance of coastal gorgonians in the

contamination of the starfish Asterias rubens by metals. Part 1: Short- and long-term

Environmental DNA (eDNA) to Assess Biodiversity of Hard Substratum Faunal
Communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and Investigate


(2008). Exponential Decline of Deep-Sea Ecosystem Functioning Linked to Benthic

Danovaro, R., Snelgrove, P. V. R., and Tyler, P. (2014). Challenging the paradigms of deep-


DFO (2017a). Delineation of Significant Areas of Coldwater Corals and Sponge-Dominated Communities in Canada’s Atlantic and Eastern Arctic Marine Waters and their Overlap with Fishing Activity. DFO Canadi.


**Training and Supervision Plan (TSP)**

**GENERAL INFORMATION**
- **Name PhD candidate**: Erik Wurz
- **Project title**: Effects of multiple environmental drivers on sponges in the deep-sea
- **Group**: Marine Animal Ecology (MAE)
- **Promotor**: Dr AJ (Tinka) Murk
- **Intended (co)promotor**: Dr R (Ronald) Osinga, Dr JM (Jasper) de Goeij
- **Project term**: From 04-07-2016 Until 31-12-2020

**EDUCATION AND TRAINING** (minimum 30 credits)

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**Education and Training Total** (minimum 30 credits)*

*One ECTS credit equals a studyload of approximately 28 hours

*36.1
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Deep-sea sponges in an Anthropocene ocean

E. Wurz 2022