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Meiofauna assemblages from cold seeps in the Arctic



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Meiofauna assemblages from cold seeps in the Arctic

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Comunidades de meiofauna em fontes frias no Ártico

Resumo

Fontes frias são um fenómeno presente em todos os oceanos, caracterizado pelo fluxo de fluídos e gases ricos em metano, sulfureto ou até petróleo bruto emanados através da interface entre sedimento e água no fundo do mar, e formados através de diversos processos como biogénicos e termogénicos, mas também abiogénicos. Apesar da libertação de fluídos e gases ricos em compostos químicos, muitas vezes tóxicos à vida animal, estes habitats são considerados *hotspots* de biomassa no fundo oceânico, dependentes fontes de energia alternativa à produção heterotrófica, nomeadamente através da quimiossíntese geralmente mediada por bactérias. Estas bactérias formam frequentemente agregados no solo marinho, denominados como “tapetes bacterianos”, constituindo a base da teia alimentar destes habitats. Ao afastar das zonas de maior concentração de fluídos ricos em hidrocarbonetos onde frequentemente se concentram os tapetes bacterianos, tipicamente é possível observar uma zonação de diferentes comunidades faunísticas presentes, frequentemente descritos como micro-habitats. Esta heterogeneidade espacial de pequena escala é não só impulsionada por diversos fatores físicos e químicos, como também por processos geológicos e atividade biológica. A zonação é frequentemente refletida em diferenças nas densidades populacionais dos diferentes grupos na estrutura das comunidades, e na diversidade.

Apesar de ser considerado um fenómeno comum, inclusive nas regiões mais a norte do nosso planeta, os estudos realizados até hoje em fontes frias no Ártico focam-se sobretudo na análise dos processos geológicos subjacentes a este fenómeno, tais como a composição geoquímica dos fluídos e sedimentos destes habitats, enquanto que estudos dedicados à biologia são limitados ao estudo de comunidade microbiana ou de organismos bentónicos da macro- e megafauna, tipicamente de dimensões superiores a 500 μm , deixando uma grande falta de conhecimento nos organismos de menor dimensões, a meiofauna. A meiofauna ($\geq 32\mu\text{m}$ a 1mm) é conhecida como o grupo faunístico mais abundante e diverso em sedimentos marinhos, dos quais o filo Nematoda por norma constitui a fração dominante (>90%). Este grupo é também um componente fundamental nas cadeias tróficas marinhas, bem como no suporte de diversas funções dos ecossistemas como ciclo biogeoquímico dos sedimentos.

Até à presente data, estudos realizados em fontes frias no Ártico são muito reduzidos, limitando-se às zonas mais a norte no mar de Barents, como no vulcão de lava Hakon Mosby, e em duas fontes-frias junto da margem norueguesa, Storegga e Nyegga. Os resultados obtidos nesses estudos demonstraram importantes observações, tais como uma clara distinção na composição da meiofauna e nematodes entre os diferentes micro-habitats, bem como de zonas de referência adjacentes às zonas de influência da fonte fria (e.x. tapetes bacterianos, campos de poliquetas do grupo Frenulata, etc.). Adicionalmente, observou-se uma relação negativa entre as densidades de meiofauna e macrofauna, suportando a hipótese de uma competição direta pelos recursos existentes, bem como evidência de alterações de comportamento e fisiologia de modo a sobreviver as condições extremas e muitas vezes tóxicas dos sedimentos em fontes frias, tais como ovoviviparidade na *Halomonhsytera disjuncta*, que permite o desenvolvimento interno de nematodes juvenis no interior do útero das fêmeas até estes terem um crescimento adequado e finalmente serem libertados já com maior resistência a xenobióticos e maior motilidade.

No âmbito do projeto AKMA – *Advancing knowledge of methane in the Arctic*, foram amostradas pela primeira vez duas fontes frias ao largo da costa de Svalbard, nomeadamente junto da região de Prins Karl Forland a 112m de profundidade, bem como na região sul da crista de Vestnesa, a cerca de 870m. Com o auxílio do ROV ÆGIR 6000, foram recolhidas três réplicas de sedimentos em zonas onde existia fluxo ativo e cobertura por tapetes bacterianos, bem como três réplicas em áreas a onde não foi observado fluxo de fluídos ou gases, como referência. Os sedimentos recolhidos tiveram por fim o estudo integrado da meiofauna, bem como características físico-químicas dos sedimentos. Em laboratório, a meiofauna foi extraída dos sedimentos através do método de centrifugação, seguido da contagem e identificação dos grandes grupos da meiofauna. Após contagem, foram recolhidos aproximadamente 150 nemátodes de modo aleatório e montadas lâminas para identificação ao microscópio até ao nível do género quando possível, bem como descrição de atributos funcionais chave de modo a relacionar com potenciais funções no ecossistema.

As composições das comunidades de meiofauna, assim como a diversidade estrutural e funcional de nemátodes, foram investigadas em relação ao tipo de micro-habitat, nomeadamente zonas de sedimento cobertas por tapetes bacterianos e referência, em ambas as zonas de estudo localizadas a profundidades distintas. Observações biológicas foram

interpretadas em relação a vários dados ambientais recolhidos, como o tipo de sedimento, a temperatura da água e a concentração de metano, entre outros.

Neste estudo foram observadas ligeiras diferenças na abundância total de meiofauna entre as zonas de fluxo ativo em comparação à referência da superestação menos profunda (Prins Karl Forland). Por outro lado, a composição de comunidade de nemátodes foi expressamente distinta, sendo que existe uma menor diversidade no geral devido à dominância por parte de dois géneros da família Monhysteridae (*Halomonhystera* sp.) e Chromadoridae (Chromadoridae msp1). Na superestação mais profunda (sul da crista de Vestnesa), a densidade geral das comunidades foi expressivamente maior nas áreas de referência sendo que, ao contrário, do habitualmente observado, os copépodes foram o grupo observado mais abundante. As características funcionais dos nemátodes apresentaram diferenças consoante os micro-habitats estudados, indicando que atributos biológicos associados a aspetos funcionais são distintos dependendo das condições do habitat. Nemátodes de grupos tróficos, tais como os *epistrate feeders*, foram consistentemente dominantes em sedimentos redutores com presença de cobertura de tapetes bacterianos, dos quais possivelmente se alimentam. A reprodução ovovivípara presente nos tapetes bacterianos, associados à emissão de fluídos e óleo na região de Prins Karl Forland, são expressão de uma importante adaptação dos progenitores para garantir a sobrevivência da descendência em ambientes altamente tóxicos como os sentidos neste local. Esta adaptação ao contrário do que observado anteriormente foi não só identificada na *Halomonhystera* sp., tal como na região do vulcão de lama Håkon Mosby, como também em mais dois géneros, *Halanonchus* sp. e um género por identificar pertencente à família Cyatholaimidae (Cyatholaimidae msp1).

Os resultados obtidos neste estudo permitiram, pela primeira vez, caracterizar comunidades de meiofauna em fontes frias na região do Ártico, sublinhando o impacto que os ambientes reduzidos podem ter nas comunidades de infauna em áreas de risco de destabilização associado às alterações climáticas.

Palavras-chave: mar profundo, ambientes quimiosintéticos, Nematoda, estrutura de comunidades, diversidade estrutural e funcional

Meiofauna assemblages from cold seeps in the Arctic

Abstract

Cold seeps are unique habitats characterized by the flow of reduced chemical compounds, often rich in methane, sulfide, and even crude oil, exuding at the seafloor. These habitats are considered energy hotspots, establishing singular conditions, for endemic fauna to thrive. Studies conducted in cold seeps in the Arctic Ocean have mainly investigated their geological and geochemical setting, while biological studies have been focused on the large-sized organisms, such as macro- and megafauna, with an unsettling lack of research on smaller-sized organisms belonging to meiofauna. Under the scope of the AKMA project, two depth-differing sites were sampled, with replicates in both active venting sites with fluids and oil covered by bacterial mats, and reference areas where no active venting was observed. Meiofaunal communities and nematode structural and functional diversity were investigated between the two micro-habitats at each shallow and deep-water site. Meiofaunal and nematode abundance were slightly higher in the bacterial mats at the shallow-water seep site and composed of a less diverse community, with the dominance of two genera from the family Monhysteridae (*Halomonhystera* sp.) and Chromadoridae (Chromadoridae msp1). At the deeper site overall communities' densities at the reduced sediments were lower compared to the reference, and meiofauna was predominantly composed of copepods in the bacterial mats, while nematodes were predominant in the reference sediments, where they also recorded a higher genus diversity. Functional traits investigated differed between micro-habitats, indicating that the nematode's functional role might vary depending on the local environmental conditions. The presence of ovoviviparous reproduction, an adaptation of which parents secure their brood survival in conditions of high toxicity, was identified in three different nematode genera, *Halomonhystera* sp., *Halanonchus* sp., and one not yet identified from the family Cyatholaimidae (Cyatholaimidae msp1), proving clues to strategies which nematodes develop to survive and even thrive in reduced environments such as cold seeps.

Keywords: deep-sea, chemosynthetic environments, Nematoda, community structure, structural and functional diversity

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General introduction

Topic relevance

Current knowledge regarding marine fauna inhabiting the seafloor, also designated as benthic communities, and their roles in the ecosystem functioning in high-latitude areas is limited, particularly when considering certain habitats, such as cold seeps. This lack of knowledge is even more expressive in studies regarding marine meiofauna, a faunal group extremely understudied, despite its importance in the ecosystem functioning. The present study aims to characterize for the first time, the metazoan meiofaunal from two cold seeps in the Arctic Ocean, based on the analysis of community structure and composition, contributing to better knowledge about faunal communities inhabiting sediments in the Arctic Ocean, more specifically in extreme environments such as cold seeps.

Cold seeps

Cold seeps were firstly discovered in 1983 in the Gulf of Mexico (Paull *et al*, 1984). These are unique, but not entirely understood, habitats characterised by the flow of reduced chemical compounds exuding the sediment-water interface, often rich in methane and other hydrocarbons, and even crude oil (asphalt) (Fig.1). Hydrocarbon-rich fluids and gas are formed under high pressures and temperatures, through various activities from biogenic, thermogenic, or other abiogenic processes (Ferré *et al*, 2012; Suess, 2014). Cold seeps are a widespread phenomenon, known to all world oceans. Since their discovery, cold seeps have been found in shallow water areas such as estuaries, continental shelves, slopes, and even in hadal zones (Suess, 2014). There are different known cold-seep structures, evidencing the distinct geological and geophysical settings. Among others are mud volcanoes, pockmarks, brine pools, carbonate mounds, etc. (Suess, 2014).

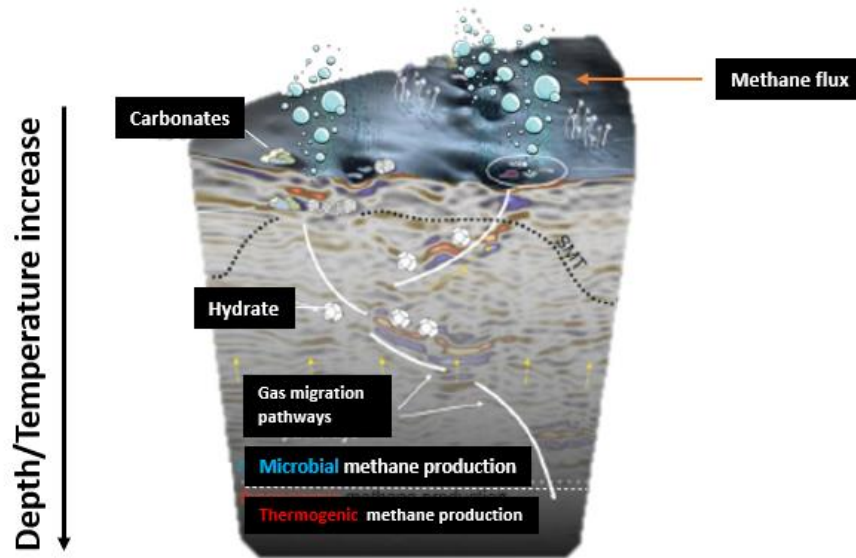


Figure 1: Schematic representation of the characteristics and processes that can occur in a methane cold seep. SMT – Sulfate-methane transition zone. **From:** Panieri *et al*, 2017.

Derived by the liberation of fluids and gas-rich compounds such as methane, hydrogen sulfide, ammonia, etc, cold seeps are characterised by holding reduced conditions. These reduced conditions represent alternately energy sources for organisms fuelled by chemoautotrophic production, especially in the deep sea, allowing specific life forms to thrive, which otherwise depend on photosynthetically derived energy from surface waters (Ramirez-Llodra *et al*, 2010). Chemosynthesis also helps limit methane emissions to the atmosphere by 20 to 80% (Boetius and Wenzhöfer, 2013) helping maintain the equilibrium of this gas concentration in the atmosphere and the ocean. Methane regulation by the ocean processes such as chemosynthesis is however threatened by climate-induced changes, namely associated with warming, acidification, and de-oxygenation of the oceans, particularly in polar regions (Hunter *et al*, 2013).

Life in cold seeps

Cold seeps provide energy hotspots on the seafloor, establishing unique conditions that can promote high local biological productivity (biomass) on the seafloor (Levin & Michener, 2002; Boetius & Suess, 2004; Boetius, 2005), particularly in the deep-sea areas (>200m depth) where food supply is limited and largely dependent on surface heterotrophic

productivity regimes (Smith *et al*, 2008; Glover *et al*, 2010). These oasis-type ecosystems are usually mentioned as the hydrocarbon-metazoan-microbe-carbonate association, giving rise to benthic seep communities that are well noticeable persistent, and good indicators of past and present seep activity (Boetius & Suess, 2004). Reduced chemical compounds in sediments become a major source of energy to the seafloor biota, sustaining chemoautotrophic production that maintains generally high productivity and the trophic web in these areas (Levin & Michener, 2002; Boetius & Suess, 2004). Nevertheless, fauna in these habitats still need to cope with adverse environmental conditions, i.e., elevated concentrations of chemical compounds, that are often toxic for most organisms, low oxygen levels at and below the sediment-water interface, etc (Zeppilli *et al*, 2018). Chemosynthesis in cold seeps (Fig.2), is usually mediated by hydrocarbon-dependent, and CO₂-fixing symbiotic bacteria, exhibiting diverse metabolic pathways to produce organic matter (Levin, 2005; Levin *et al*, 2016).

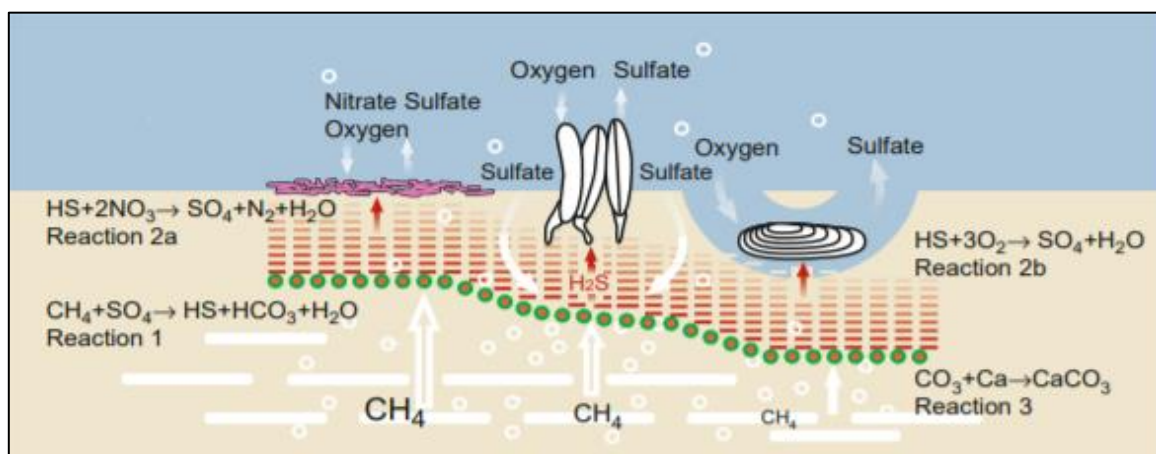


Figure 2: Schematics on the diverse metabolic pathways linked with anaerobic oxidation of methane (CH₄). Arrows point out different rates of methane flux; **Red-green circles:** AOM (anaerobic oxidation of methane)-consortia concentrated at varying depths below the seafloor. **Reaction 1:** production of hydrogen sulfide (H₂S) and bicarbonate (HCO₃⁻). **Reaction 2a:** oxidation of hydrogen in sulfide microbial mats. **Reaction 2b:** oxidation of hydrogen in sulfide by macrofauna symbionts. **Reaction 3:** precipitation of calcium carbonate (CaCO₃). **From:** Suess, 2014.

The bacterial communities present in seeps can set aggregates at the seafloor, forming mats that in some cases can cover hundreds of square meters of the seafloor surface (Niemann *et al*, 2006b; Grünke *et al*, 2011). Usually, “Bacterial mats” are formed concentrically around sulfide-rich pore water sources in the seabed and associated with the distance to the main

fluid sources, varying invertebrate fauna assemblages, creating a gradient of different communities, with bacterial assemblages at the basis of the food web (Niemann *et al*, 2006; Fischer, 2010). Symbiotic relationships at seeps are common, and often involve an invertebrate taxon as a host, associated with one or several species of methane-oxidizing bacteria that will synthesize organic carbon for direct or indirect use to the host, while benefiting from a protected environment (Van Dover *et al*, 2002; Fischer, 2010).

While most seeps seem to differ in composition, particularly at a regional level, symbiotic taxa may include for example the widespread presence of frenulate tubeworms, vesicomid clam, or bathymodiolid mussels' beds, although not necessarily at the same time or associated (Fig.3; Sahling *et al*, 2002; Boetius & Suess, 2004; Levin, 2005). Other frequently observed taxa in seep worldwide may include carnivore sponges, anemones, various crustaceans, and zoarcid fishes attracted by the elevated number of preys, showing that not only the foundation species can benefit from these habitats (Sahling *et al*, 2003; Levin, 2005; Cordes *et al*, 2010b; Åström *et al*, 2016, 2018). Identically to non-seep habitats, within the sediment, nematodes, and other polychaetes taxa besides the frenulate tubeworms can also prevail but is still unclear if these groups also display seep-associated endemism, particularly for meiofauna groups such as the nematodes, where identifications are often limited to genus level (Montagna *et al*, 1987; Olu *et al*, 1997; Van Gaever *et al*, 2006).

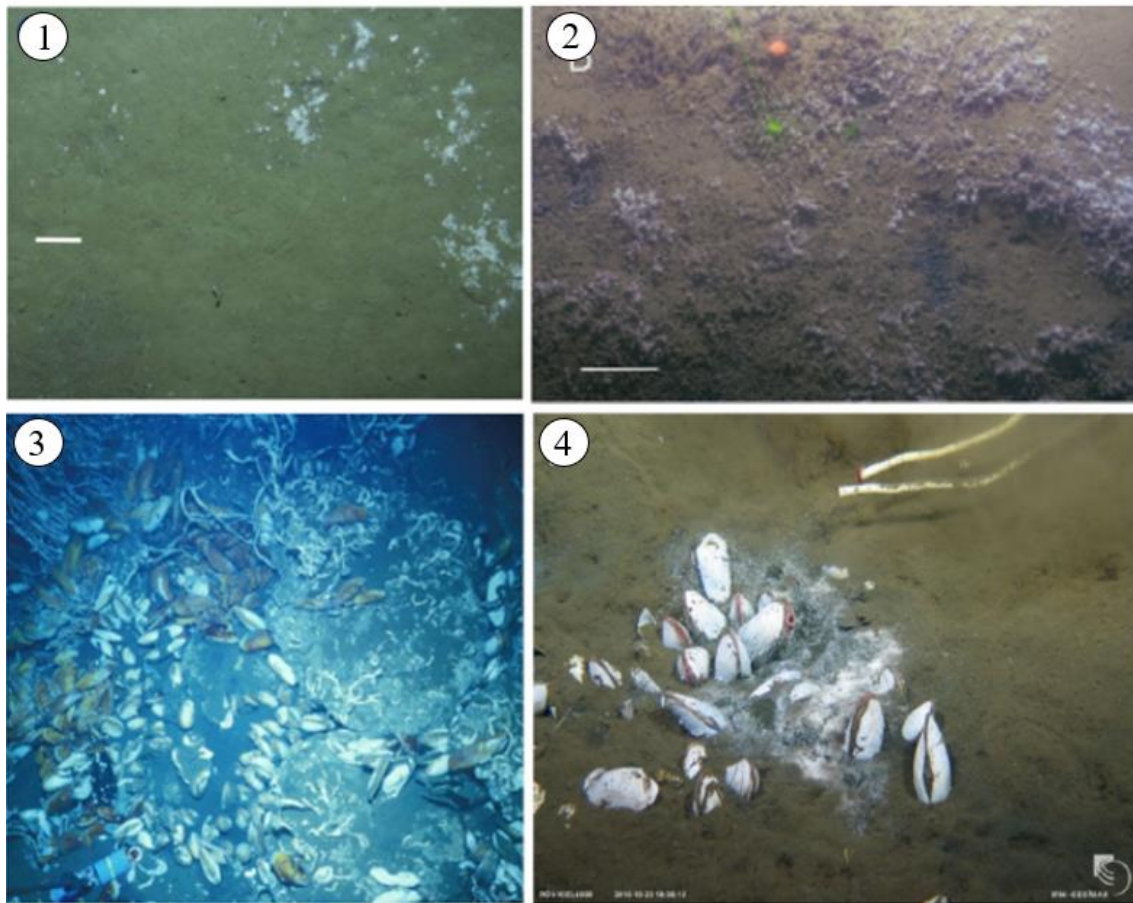


Figure 3: Different faunal communities in cold seeps from various geographical locations. **(1)** Bacterial mats on soft sediments at the seafloor in Hikurangi margin, New Zealand (**From** Bowden *et al.*,2013). **(2)** Bacterial mat and frenulate tubeworms colonized by filamentous bacteria in the Barents Sea (**From** Åström *et al.*,2019). **(3)** *Mytilid* colonies on carbonate mounds off Costa Rica (**From** Suess, 2014). **(4)** Vesicomys colony on the seabed off Concepcion, Chile (**From** Suess, 2014).

Seep communities generally present low species richness but high faunal standing stocks (density and biomass) compared with the surrounding areas, mainly explained by enhanced availability of organic matter and habitat heterogeneity (Fig.4). It is, however, important to highlight that every seep region studied to date exhibits differences from one another and is not possible to make inferences to other unstudied seep sites around the world. Physico-chemical conditions (e.g., temperature, salinity, PH, oxygen, carbon-dioxide, hydrocarbon concentration, ammonia, and metal contents), geological processes (e.g., carbonate precipitation and hard structures creation), or even biological activity (e.g., bacterial mats and tubeworm patches) are not stagnant in cold seeps, changing over space

and time as, the result of altered fluid flow regimes. This heterogeneity in biogeochemical conditions as distance increases from the main fluid seepage source, often promotes the presence of micro-habitats (zonation), increasing by itself habitat complexity at a local scale (Fisher *et al*, 2007; Levin & Sibuet, 2012), creating conditions (both shelter and food) for the establishment of infauna and epibenthic organisms (Levin & Mendonza, 2007; Vanreusel *et al*, 2009; Åström, 2018).

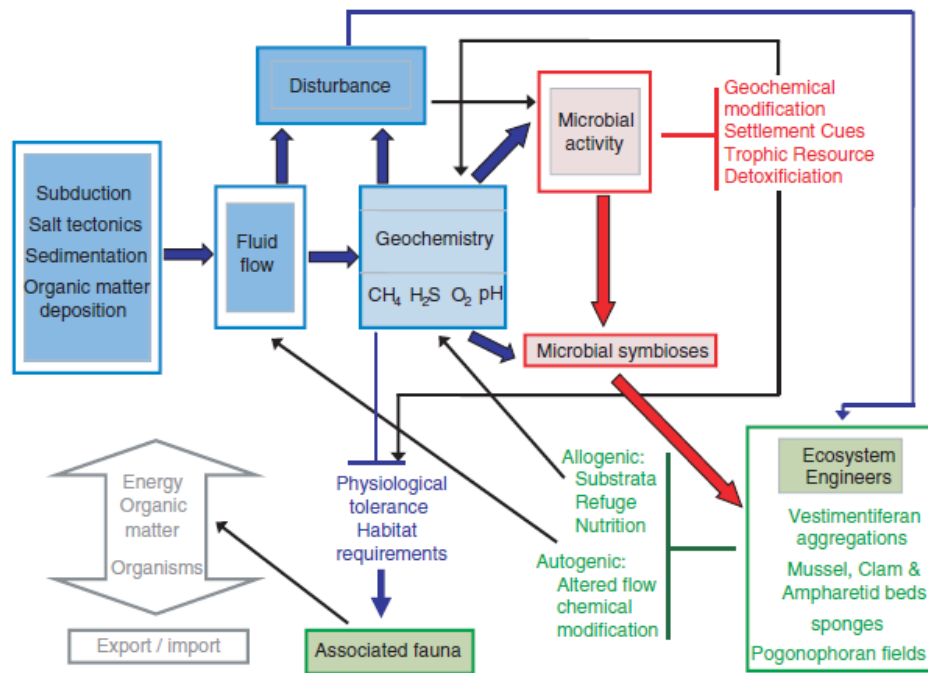


Figure 4: Biological, biogeochemical, and geological processes, creating habitat heterogeneity at cold seeps. **From:** Cordes *et al*,2010a.

Zonation in habitat complexity is particularly key in structuring faunal and trophic composition and complexity (Fig.5; Dando and Hovland, 1992; Cordes *et al*, 2010a; Portail *et al*, 2016). As distance increases from the main fluid seepage source, trophic diversity generally increases, a direct result of the less toxic environment (low fluid-flux settings) and more importantly diminished need for specific adaptations to the available food sources (Portail *et al*, 2016). In high fluid-flux settings, food-web complexity is by comparison lower, mostly due to the lower number of species able to tolerate the reduced conditions (Portail *et al*, 2016).

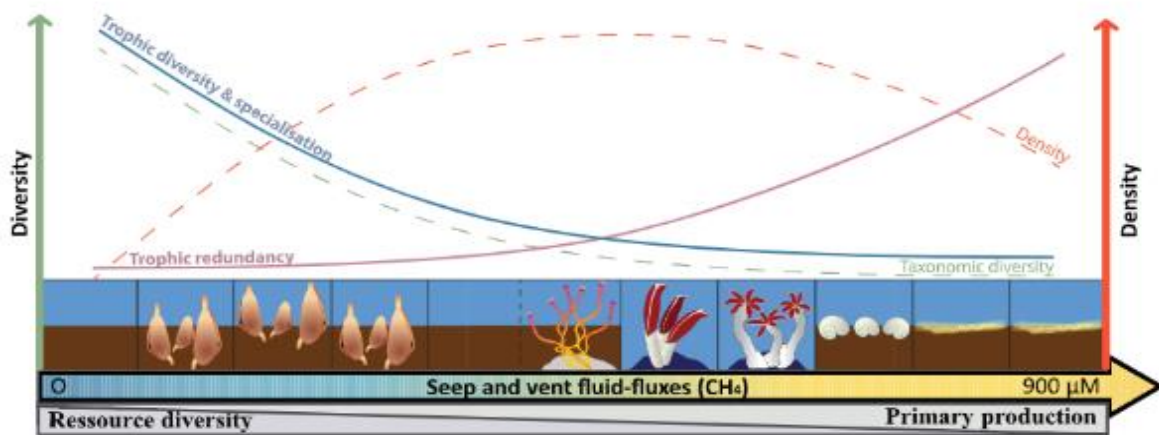


Figure 5: Conceptual diagram of faunal community and food-web patterns along fluid-flux gradients, showing the different micro-habitats at cold seeps (Example taken from Guaymas seep and vent ecosystems). **From:** Portail *et al*, 2016.

Discrepancies in seep faunal composition, at different cold seeps, can also relate to varying the water depth, with some important differences observed between shallow and deep seeps (>200 m; Tarasov *et al*, 2005; Dando, 2010). In general, it seems that in shallow seeps the benthic communities are relatively diverse, often showing many similarities to fauna from background sediments (Dando, 2010). With depth increment, while standing stocks might still be increased in relation to background sediments, composition often differs from background sediments and diversity tends to be de lower, with often present endemic and/or symbiotic fauna specialized in inhabiting reduced conditions (Sibuet and Olu, 1998; Sahling *et al*, 2003; Dando, 2010). For example, on the Sakhalin shelf and slope, a comparison of seep sites was made, from around 160 m to 2500 m water depth. Herewith symbiotic macro-infaunal taxa were only found at depths below 370 m. One piece of evidence that the seep communities might be depth-dependent is based on the presence of endemic fauna, such as frenulate tubeworms, bathymiolid mussels, and *vesicomys* bivalves are often limited to depths below ~400 m (Hashimoto *et al*, 1993; Sibuet and Olu, 1998; Sahling *et al*, 2003). One of the possible explanations for the depth-related differences in seep communities likely relates to the quality and availability of organic matter that reaches the seafloor, as the increase in depth often translated into degradation and lower availability of food leaving the deep-sea benthic community, with almost only local organic matter production, where the chemosynthesis start playing an important role (Levin, 2005; Dando, 2010).

Current knowledge of seep associated fauna in the Arctic

The Arctic Ocean is known for extremely cold-water temperatures, with the presence of both permanent and seasonal ice coverage areas and a strong seasonal variation of the photoperiod, which directly affect primary production in the ecosystem (Clark & Peck, 1991; Clarke & Harris, 2003).

Gas seepage in the Arctic is a widespread seafloor phenomenon affecting local seafloor conditions (Vogt *et al*, 1999), including community structure, diversity, and ecosystem functioning (Åström *et al*, 2016). Nevertheless, faunal investigation of cold seeps in the Arctic, remains scarce, with most studies focused on the Håkon Mosby mud volcano (HMMV) in the Barents Sea (Gebruk *et al*, 2003; Niemann *et al*, 2006; Van Gaever *et al*, 2006; Van Gaever *et al*, 2009; Decker *et al*, 2012; Galkin *et al*, 2013), and more recently in the pockmark fields located in the Vestnesa ridge, off Svalbard (Åström *et al*, 2016; Åström *et al*, 2018; Dessandier *et al*, 2019). Further south studies on fauna in the Norwegian margin, are limited to the Storegga and Nyegga pockmark fields (Van Gaever *et al*, 2009; Portnova *et al*, 2014).

General faunal patterns in cold-seep environments in the Arctic region, including the Norwegian margin, are still difficult to establish due to the limited number of investigated sites. Areas investigated seem to display a varying coverage of bacterial mats, from a few centimeters to hundreds of meters, from the genera *Beggiatoa* spp, *Thioploca* spp., and subgenera *Leucothrix* spp. and *Thiothrix* spp. (Decker *et al*, 2012; Van Gaever *et al*, 2006; Niemann *et al*, 2006; Foucher *et al*, 2009). It is common to find patches of frenulate tubeworms surrounding these mats (Fig.6) as the dominating sessile megafauna with the sparse presence of bivalves, anemones, carnivorous sponges, small gastropods, pycnogonids, and other crustaceans (Gebruk *et al*, 2003; Niemann, *et al*, 2006; Åström *et al*, 2016; Åström *et al*, 2018). Contrary to what seems to be the rule in other areas in the world, megafauna coverage of seeps in the Arctic is sparse and devoided by seep endemic taxa, i.e., frenulates tubeworms of the species *Oligobranchia haakonmobiensis* and *Scleronlinum contortum*, that can also be found in other reduced sedimented area and even on hydrothermal sites along the Arctic mid-ocean ridge (AMOR) (Gebruk *et al*, 2003; Pedersen *et al*, 2010; Georgieva *et al*, 2015). Often present in the vicinities are heterotrophic fauna, such as ophiuroids, crinoids, hydrozoans, and bryozoans (Åström *et al*, 2018). Vagrant megafauna, like the case of Zoarcid

fishes, is usually abundant around all seep sites investigated (Gebruk *et al*, 2003; Bergmann *et al*, 2011; Meyer *et al*, 2013; Åström *et al*, 2018).

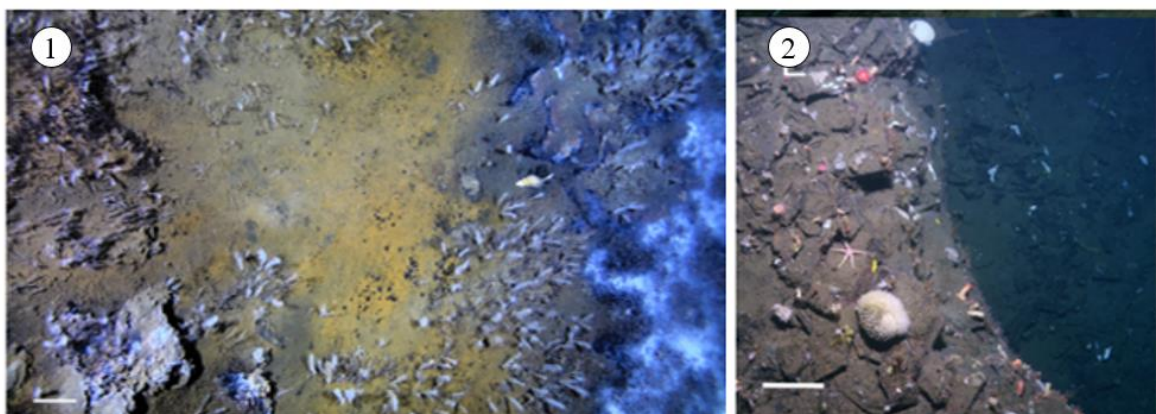


Figure 6: (1) Carbonate outcrops, microbial mats, and filamentous bacteria covering frenulate tubes at the Lofoten canyon seeps. **From:** Åström *et al*, 2020; (2) Epifaunal organisms on a rocky slope into one of the craters at the Bjørnøyrenna crater seeps. **From:** Åström *et al*, 2020.

With macro- and megafauna taxa being the main studied benthic groups in the Arctic Ocean seeps, there is a high gap in knowledge on the marine metazoan meiofauna from these ecosystems. Research on this subject is still very scarce and limited to the HMMV in the Barents Sea and two pockmarks' sites located much further south in the Norwegian margin, the Storegga and Nyegga pockmarks (Van Gaever *et al*, 2006, 2009; Portnova *et al*, 2014).

Marine Meiofauna

Meiofaunal organisms ($\geq 32\mu\text{m}$ up to 1mm) are the most widespread and abundant faunal groups in marine sediments. Among the several groups present in meiofauna, nematodes typically constitute the dominant fraction (more than 90%), followed by harpacticoid copepods, being these two groups the most studied components of meiofauna (Giere, 2009). Following decreasing order ostracods, kinorhynchs, and several other groups are often found in meiofaunal communities (Giere, 2009). Meiofauna, and particularly nematodes, play an important role in the ecosystem functioning, such as the biogeochemical cycle in the sediments, including the mineralization of carbon (Alkemade *et al*, 1992; Heip *et al*, 1992; Schratzberger & Ingels, 2018). Meiofauna is also a key component in the marine

food webs, by being an intermediary in the relationship between primary production and decomposition of organic matter to higher trophic levels (Schratzberger & Ingels, 2018). Since meiofaunal communities, especially nematodes, are ubiquitous and present in all marine habitats, these animals are incredibly diverse, morphologically, and physiologically, with some groups able to easily sustain extreme environmental conditions (Schratzberger & Ingels, 2018). Their small size, limited mobility, lack of pelagic life stages, and relatively short reproductive cycles, often make them good indicators of changes in environmental conditions, pollution, and even climate-driven changes, while relatively easy to sample (Bellard *et al*, 2012; Zeppilli *et al*, 2018).

Meiofauna from cold seeps in the Arctic region

Metazoan meiofauna from cold seeps in high-latitude regions, and especially in the Arctic Ocean, have remained unstudied, despite their known role in the ecosystem functioning. Although still limited, some information is available regarding meiofaunal assemblages from seeping sites in the Barents Sea (i.e., Håkon Mosby mud volcano (Van Gaever *et al*, 2006, 2009), and two Pockmarks sites within the Norwegian margin, the Storegga, and Nyegga pockmarks (Van Gaever *et al*, 2009; Portnova *et al*, 2014).

Along with these three sites, meiofaunal communities are predominantly composed of nematodes, accounting generally for more than 90% of all observed major taxa (Van Gaever *et al*, 2006, 2009; Portnova *et al*, 2014). Three main micro-habitats were generally distinguished within these cold seeps in this region: **1)** crater (only investigated at HMMV), **2)** reduced sediment covered by bacterial mats, and **3)** sediment colonized by frenulate tubeworms. For comparison usually, a fourth location was investigated for reference conditions outside the influence of seepage and with no obvious presence of seep-associated presence of meiofauna (Van Gaever *et al*, 2009; Portnova *et al*, 2014). Overall, meiofaunal standing stocks (density and biomass), community composition, and diversity strongly differ between the different micro-habitats investigated but also, to some extent, between regions, driven by differences in fluid emissions and consequently sediment geochemical conditions, as well as trophic conditions (Van Gaever *et al*, 2009; Portnova *et al*, 2014). Meiobenthic communities and nematode density in seeps were generally higher when compared with non-seep-related sediments, particularly within microbial mats microbiotope and frenulate

tubeworms fields. Interestingly, in the crater location, Harpacticoid copepods became dominant, in opposition to nematodes (Van Gaever *et al*, 2009; Portnova *et al*, 2014). An important observation was that differences between sites might be related to the presence and density of macro-and megafauna, which may negatively affect meiofaunal density, an assumption observed at Storegga where macrofaunal densities were twice as high when compared with the other two studied cold seeps (HMMV and Nyegga), while meiofaunal density recorded was the lowest (Van Gaever *et al*, 2009). This can be explained by the high abundance of non-frenulate polychaetes, that might predate meiofaunal taxa (Van Gaever *et al*, 2009). Diversity patterns decrease generally towards bacterial mats and reduced sediments, with high taxonomic diversity usually recorded at frenulate tubeworms fields, within the margin values of the reference locations (Van Gaever *et al*, 2009; Portnova *et al*, 2014). Reduced environmental conditions created a genera-poor nematode community, dominated by one to two species (Van Gaever *et al*, 2006, 2009), with the example of the *Halomonhystera disjuncta* as the only species present in bacterial mats of the HMMV, with the highest ever recorded density in seep sites ($>11.000 \text{ ind.}10\text{cm}^{-2}$), and strongly presence at bacterial mats at Nyegga sites, alongside *Thalassomonhystera* sp. and *Terschellingia longicaudata* (Van Gaever *et al*, 2009; Portnova *et al*, 2014).

High densities of single nematode species observed in these cold seeps could have resulted from evolutionary adaptations developed to survive and even thrive in such conditions (Zeppilli *et al*, 2018). Behavioral or physiological adaptations of nematodes to extreme environmental conditions are known in several regions of the globe and can include alterations in metabolism and symbiosis, body size incre-/decrement, alteration in reproductive strategies, and a wide range of life span (Giere *et al*, 1995; Seibel and Drazen, 2007; Giere, 2009; Van Gaever *et al*, 2006). Some studies suggest that to survive in reduced conditions some species with longer and thinner body shapes can better be adapted as they can use “oxygen islands” in sediment, created by bioturbation of meio and macrofauna, to refuge (Ritt *et al*, 2010). Some nematodes can harbor a bacterial coat as a protective layer against toxic hydrogen sulfide (Jensen *et al*, 1992; Giere *et al*, 1995) or develop endosymbiotic relationships to obtain food (Giere *et al*, 1995). A clear case of species evolutionary adaption to seep conditions were found at HMMV, where the uncommon ovoviviparous reproduction strategy, by the dominant nematode species, was observed

Halomonhystera disjuncta, allowing the offspring to survive toxic conditions during the developing stages (Van Gaever *et al*, 2006). Nematode adaptation to cold seep can also be associated with high food supply or trophic specialization and lower competition or predation pressure, which allowed the establishment of this group and consequent adaptation (Zeppilli *et al*, 2018). The high presence and dominance of *Halomonhystera disjuncta* at HMMV, and *Terschellingia longicaudata* at Nyegga pockmark, both cosmopolitan species are known from anoxic sediments in saltmarshes and mangrove mudflats, praising the capacity of these nematodes to adapt to extreme conditions in both shallow and deep-sea habitats (Van Gaever *et al*, 2009).

References

- Alkemade, R., Wielemaker, A., De Jong, S., & Sandee, A. J. J. (1992). Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina anglica* detritus. *Marine Ecology Progress Series*, 90(2),149-155. doi: 10.3354/meps090149
- Åström, E. K. L. (2018). Benthic Communities at High-Arctic Cold Seeps: Faunal Response to Methane Seepage in Svalbard. Ph.D. thesis, UiT-The Arctic University of Norway, Tromsø. <https://hdl.handle.net/10037/12795>
- Åström, E. K. L., Carroll, M. L., Ambrose Jr, W. G., & Carroll, J. (2016). Arctic cold seeps in marine methane hydrate environments: impacts on shelf macrobenthic community structure offshore Svalbard. *Marine Ecology Progress Series*, 552, 1-18. doi: 10.3354/meps117773
- Åström, E. K. L., Carroll, M. L., Ambrose Jr, W. G., Sen, A., Silyakova, A., & Carroll, J. (2018). Methane cold seeps as biological oases in the high-Arctic deep sea. *Limnology and Oceanography*, 63(1), 209-S231. doi: 10.1002/lno.10732
- Åström, E. K. L., Carroll, M. L., Sen, A., Niemann, H., Ambrose Jr, W. G., Lehmann, M. F., & Carroll, J. (2019). Chemosynthesis influences food web and community structure in high-Arctic benthos. *Marine Ecology Progress Series*, 629, 19-42. doi: 10.3354/meps13101
- Åström, E. K. L., Sen, A., Carroll, M. L., & Carroll, J. (2020). Cold seeps in the warming Arctic: Insights for benthic ecology. *Frontiers in Marine Science*, 7, 244. doi: 10.3389/fmars.2020.00244
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F. (2012) Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15(4):365–377. doi: 10.1111/j.1461-0248.2011.01736.x

- Bergmann, M., Soltwedel, T., & Klages, M. (2011). The interannual variability of megafaunal assemblages in the Arctic deep sea: Preliminary results from the HAUSGARTEN observatory (79 N). *Deep Sea Research Part I: Oceanographic Research Papers*, 58(6), 711-723. doi: 10.1016/j.dsr.2011.03.007
- Boetius, A. (2005). Microfauna–macrofauna interaction in the seafloor: lessons from the tubeworm. *PLoS biology*, 3(3), 102. doi: 10.1371/journal.pbio.0030102
- Boetius, A., & Wenzhöfer, F. (2013). Seafloor oxygen consumption fuelled by methane from cold seeps. *Nature Geoscience*, 6(9), 725-734. doi: 10.1038/ngeo1910
- Boetius, A., & Suess, E. (2004). Hydrate Ridge: A natural laboratory for the study of microbial life fueled by methane 31 from near-surface gas hydrates. *Chemical Geology*, 205, 291–310. doi: 10.1016/j.chemgeo.2003.12.034
- Bowden, D. A., Rowden, A. A., Thurber, A. R., Baco, A. R., Levin, L. A., & Smith, C. R. (2013). Cold seep epifaunal communities on the Hikurangi Margin, New Zealand: composition, succession, and vulnerability to human activities. *PLoS One*, 8(10), 76869. doi: 10.1371/journal.pone.0076869
- Clarke, A., & Harris, C. M. (2003). Polar marine ecosystems: major threats and future change. *Environmental Conservation*, 30(1), 1-25. doi: 10.1017/S0376892903000018
- Cordes, E. E., Cunha, M. R., Galeron, J., Mora, C., Olu-Le Roy, K., Sibuet, M., Van Gaever, S., Vanreusel, A., & Levin, L. A. (2010a). The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. *Marine Ecology*, 31(1), 51-65. doi: 10.1111/j.1439-0485.2009.00334.x
- Cordes, E. E., Becker, E. L., Hourdez, S., & Fisher, C. R. (2010b). Influence of foundation species, depth, and location on diversity and community composition at Gulf of Mexico lower-slope cold seeps. *Deep Sea Research Part II: Topical Studies in Oceanography*, 57(21-23), 1870-1881. doi: 10.1016/j.dsr2.2010.05.010
- Dando, P.R. (2010). Biological Communities at Marine Shallow-Water Vent and Seep Sites. In: Kiel S. (eds). *The Vent and Seep Biota. Topics in Geobiology*, Springer, Dordrecht, 33, 333-378. doi: 10.1007/978-90-481-9572-5_11
- Decker, C., Morineaux, M., Van Gaever, S., Caprais, J. C., Lichtschlag, A., Gauthier, O., Andersen, A. C., & Olu, K. (2012). Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin. Part 1: Macrofaunal community structure. *Marine Ecology*, 33(2), 205-230. doi: 10.1111/j.1439-0485.2011.00503.x
- Dessandier, P. A., Borrelli, C., Kalenitchenko, D., & Panieri, G. (2019). Benthic foraminifera in arctic methane hydrate bearing sediments. *Frontiers in Marine Science*, 6, 765. doi: 10.3389/fmars.2019.00765
- Ferré, B., Mienert, J., & Feseker, T. (2012). Ocean temperature variability for the past 60 years on the Norwegian-Svalbard margin influences gas hydrate stability on human

- time scales. *Journal of Geophysical Research: Oceans*, 117(C10). doi: 10.1029/2012JC008300
- Fischer, D. (2010). Cold seeps: marine ecosystems based on hydrocarbons. *Science in School*, 16, 60-64.
- Fisher, C., Roberts, H., Cordes, E., & Bernard, B. (2007). Cold seeps and associated communities of the Gulf of Mexico. *Oceanography*, 20(4), 118-129. doi: 10.5670/oceanog.2007.12
- Foucher, J. P., Westbrook G. K., Boetius, A., Ceramicola, S., Dupré, S., Mascle, J., Mienert, J., Pfannkuche, O., Pierre, C., & Praeg, D. (2009). Structure and drivers of cold seep ecosystems. *Oceanography* 22(1):92–109. doi: 10.5670/oceanog.2009.11
- Galkin, S., Bergmann, M., Soltwedel, T., & Gebruk, A. (2013). Density and distribution of megafauna at the Håkon Mosby mud volcano (the Barents Sea) based on image analysis. *Biogeosciences*, 10(5), 3359-3374. doi: 10.5194/bg-10-3359-2013
- Gebruk, A. V., Krylova, E. M., Lein, A. Y., Vinogradov, G. M., Anderson, E., Pimenov, N. V., Cherkashev, G. A., & Crane, K. (2003). Methane seep community of the Håkon Mosby mud volcano (the Norwegian Sea): composition and trophic aspects. *Sarsia*, 88(6), 394-403. doi: 10.1080/00364820310003190
- Georgieva, M. N., Wiklund, H., Bell, J. B., Eilertsen, M. H., Mills, R. A., Little, C. T., & Glover, A. G. (2015). A chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan species. *BMC Evolutionary Biology*, 15(1), 1-17. doi: 10.1186/s12862-015-0559-y
- Giere, O. (2009). *Meiobenthology: the microscopic motile fauna of aquatic sediments*. (2nd ed.). Springer-Verlag, Heidelberg (526 p.)
- Giere, O., Windoffer, R., & Southward, E. C. (1995). The bacterial endosymbiosis of the gutless nematode, *Astomonema southwardorum*: ultrastructural aspects. *Journal of the Marine Biological Association of the United Kingdom*, 75(1), 153-164. doi: 10.1017/S0025315400015265
- Glover, A. G., Gooday, A. J., Bailey, D. M., Billett, D. S. M., Chevaldonné, P., Colaço, A., Copley, J., Cuvelier, D., Desbruyères, D., Kalogeropoulou, V., Klages, M., Lampadariou, N., Lejeune, C., Mestre, N. C., Paterson, G. L. J., Perez, T., Ruhl, H., Sarrazin, J., Soltwedel, T., Soto, E.H., Thatje, S., Tselepides, A., Van Gaever, S., & Vanreusel, A. (2010). Temporal change in deep-sea benthic ecosystems: a review of the evidence from recent time-series studies. *Advances in Marine Biology*, 58, 95. doi: 10.1016/B978-0-12-381015-1.00001-0
- Grünke, S., Felden, J., Lichtschlag, A., Girnth, A. C., de Beer, D., Wenzhöfer, F., & Boetius, A. (2011). Niche differentiation among mat-forming, sulfide-oxidizing bacteria at cold seeps of the Nile Deep Sea Fan (Eastern Mediterranean Sea). *Geobiology*, 9(4), 330-348. doi: 10.1111/j.1472-4669.2011.00281.x

- Hashimoto, J., Miura, T., Fujikura, K., & Osaka, J. (1993). Discovery of vestimentiferan tube-worms in the euphotic zone. *Zoological science*, *10*(6), 1063-1067. doi: 10.34425/zs001172
- Heip, C., Huys, R., & Alkemade, R. (1992). Community structure and functional roles of meiofauna in the North Sea. *Netherland Journal of Aquatic Ecology*, *26*(1), 31-41. doi: 10.1007/bf02298026
- Hunter, S. J., Goldobin, D. S., Haywood, A. M., Ridgwell, A., & Rees, J. G. (2013). Sensitivity of the global submarine hydrate inventory to scenarios of future climate change. *Earth and Planetary Science Letters*, *367*, 105-115. doi: 10.1016/j.epsl.2013.02.017
- Jensen, P., Aagaard, I., Burke Jr, R. A., Dando, P. R., Jorgensen, N. O., Kuijpers, A., Laier, T., O'Hara, S. C. M., & Schmaljohann, R. (1992). 'Bubbling reefs' in the Kattegat: submarine landscapes of carbonate-cemented rocks support a diverse ecosystem at methane seeps. *Marine Ecology Progress Series*, *83*, 102-112. doi: 10.3354/meps083103
- Levin, L. A. (2005). Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanography and marine biology* (pp. 11-56). CRC Press. doi: 10.1201/9781420037449-3
- Levin, L. A., Baco, A. R., Bowden, D., Colaço, A., Cordes, E. E., Cunha, M. R., Demopoulos, A. W. J., Gobin, J., Grupe, B. M., Le, J., Metaxas, A., Netburn, A. N., Rouse, G. W., Thurber, A. R., Tunnicliffe, V., Dover, C. L. V., Vanreusel, A., & Watling, L. (2016). Hydrothermal Vents and Methane Seeps: Rethinking the Sphere of Influence. *Frontiers in Marine Science*, *3*, 72. doi: 10.3389/fmars.2016.00072
- Levin, L. A., & Michener, R. H. (2002). Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: The lightness of being at Pacific methane seeps. *Limnology and Oceanography*, *47*, 1336–1345. doi: 10.4319/lo.2002.47.5.1336
- Levin, L. A., Mendoza, G.F. (2007). Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). *Marine Ecology* *28*, 131–151. doi: 10.1111/j.1439-0485.2006.00131.x
- Levin, L. A., Mendoza, G. F., Konotchick, T., & Lee, R. (2009). Macrobenthos community structure and trophic relationships within active and inactive Pacific hydrothermal sediments. *Deep Sea Research Part II: Topical Studies in Oceanography*, *56*(19-20), 1632-1648. doi: 10.1016/j.dsr2.2009.05.010
- Levin, L.A., Sibuet, M. (2012). Understanding Continental Margin Biodiversity: A New Imperative. *Annual Review of Marine Science* *4*, 79–112. doi: 10.1146/annurev-marine-120709-142714
- Menot, L., Galéron, J., Olu, K., Caprais, J. C., Crassous, P., Khripounoff, A., & Sibuet, M. (2010). Spatial heterogeneity of macrofaunal communities in and near a giant pockmark area in the deep Gulf of Guinea. *Marine Ecology*, *31*(1), 78-93. doi: 10.1111/j.1439-0485.2009.00340.x

- Meyer, K. S., Bergmann, M., & Soltwedel, T. (2013). Interannual variation in the epibenthic megafauna at the shallowest station of the HAUSGARTEN observatory (79 N, 6 E). *Biogeosciences*, *10*(6), 3479-3492. doi: 10.5194/bg-10-3479-2013
- Niemann, H., Lösekann, T., De Beer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E. J., Schluter, M., Klages, M., Foucher, J. P., & Boetius, A. (2006). Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature*, *443*(7113), 854-858. doi: 10.1038/nature05227
- Olu, K., Lance, S., Sibuet, M., Henry, P., Fiala-Médioni, A., & Dinét, A. (1997). Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. *Deep Sea Research Part I: Oceanographic Research Papers*, *44*(5), 811-841. doi: 10.1016/S0967-0637(96)00123-9
- Panieri, G., Bünz, S., Fornari, D. J., Escartin, J., Serov, P., Jansson, P., Torres, M. E., Johnson, J. E., Hong, W., Sauer, S., Garcia, R., & Gracias, N. (2017). An integrated view of the methane system in the pockmarks at Vestnesa Ridge, 79 N. *Marine Geology*, *390*, 282-300. doi: 10.1016/j.margeo.2017.06.006
- Paull, C. K., Hecker, B., Commeau, R., Freeman-Lynde, R. P., Neumann, C., Corso, W. P., Golubic, S., Hook, J. E., Sikes, E., & Curray, J. (1984). Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. *Science*, *226*(4677), 965-967. doi: 10.1126/science.226.4677.965
- Pedersen, R. B., Rapp, H. T., Thorseth, I. H., Lilley, M. D., Barriga, F. J., Baumberger, T., Flesland, K., Fonseca, R., Fruh-Green, G. L., & Jorgensen, S. L. (2010). Discovery of a black smoker vent field and vent fauna at the Arctic Mid-Ocean Ridge. *Nature communications*, *1*(1), 1-6. doi: 10.1038/ncomms1124
- Portail, M., Olu, K., Dubois, S. F., Escobar-Briones, E., Gelin, Y., Menot, L., & Sarrazin, J. (2016). Food-web complexity in Guaymas Basin hydrothermal vents and cold seeps. *PLoS one*, *11*(9), 162263. doi: 10.1371/journal.pone.0162263
- Portnova, D. A., Mokievsky, V. O., Haflidason, H., & Todt, K. (2014). Metazoan meiobenthos and nematode assemblages in the Nyegga Region of methane seepage (Norwegian Sea). *Russian Journal of Marine Biology*, *40*(4), 255-265. doi: 10.1134/S1063074014040075
- Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., Levin, L. A., Arbizu, P. M., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., & Vecchione, M. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences*, *7*(9), 2851-2899. doi: 10.5194/bg-7-2851-2010
- Ritt, B., Sarrazin, J., Caprais, J. C., Noël, P., Gauthier, O., Pierre, C., Pierre, H., & Desbruyeres, D. (2010). First insights into the structure and environmental setting of cold-seep communities in the Marmara Sea. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(11), 1711-1724. doi: 10.1016/j.dsrp.2010.08.006

- Oceanographic Research Papers*, 57(9), 1120-1136. doi: 10.1016/j.dsr.2010.05.011
- Sahling, H., Galkin, S. V., Salyuk, A., Greinert, J., Foerstel, H., Piepenburg, D., & Suess, E. (2003). Depth-related structure and ecological significance of cold-seep communities-a case study from the Sea of Okhotsk. *Deep Sea Research Part I: Oceanographic Research Papers*, 50(12), 1391-1409. doi: 10.1016/j.dsr.2003.08.004
- Sahling, H., Rickert, D., Lee, R. W., Linke, P., & Suess, E. (2002). Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, 231, 121-138. doi: 10.3354/meps231121
- Schratzberger, M., & Ingels, J. (2018). Meiofauna matters: the roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology*, 502, 12-25. doi: 10.1016/j.jembe.2017.01.007
- Seibel, B. A., & Drazen, J. C. (2007). The rate of metabolism in marine animals: environmental constraints, ecological demands, and energetic opportunities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1487), 2061-2078. doi: 10.1098/rstb.2007.2101
- Sibuet, M., & Olu, K. (1998). Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep Sea Research Part II: Topical Studies in Oceanography*, 45(1-3), 517-567. doi: 10.1016/S0967-0645(97)00074-X
- Smith C.R., De Leo F.C., Bernardino A.F., Sweetman A.K., & Arbizu P.M. (2008). Abyssal food limitation, ecosystem structure and climate change. *Trends in Ecology and Evolution*, 23(9), 518-28. doi: 10.1016/j.tree.2008.05.002
- Suess, E. (2014). Marine cold seeps and their manifestations: geological control, biogeochemical criteria and environmental conditions. *International Journal of Earth Sciences*, 103(7), 1889-1916. doi: 10.1007/s00531-014-1010-0
- Tarasov, V. G., Gebruk, A. V., Mironov, A. N., & Moskalev, L. I. (2005). Deep-sea and shallow-water hydrothermal vent communities: two different phenomena?. *Chemical Geology*, 224(1-3), 5-39. doi: 10.1016/j.chemgeo.2005.07.021
- Van Dover, C. L., German, C. R., Speer, K. G., Parson, L. M., & Vrijenhoek, R. C. (2002). Evolution and biogeography of deep-sea vent and seep invertebrates. *Science*, 295(5558), 1253-1257. doi: 10.1126/science.1067361
- Van Gaever, S., Moodley, L., De Beer, D., & Vanreusel, A. (2006). Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series*, 321, 143-155. doi: 10.3354/meps321143

- Van Gaever, S., Olu, K., Derycke, S., & Vanreusel, A. (2009). Metazoan meiofaunal communities at cold seeps along the Norwegian margin: Influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep Sea Research Part I: Oceanographic Research Papers*, 56(5), 772-785. doi: 10.1016/j.dsr.2008.12.015
- Vanreusel, A., Anderson, A. C., Boetius, A., Connelly, D., Cunha, M. R., Decker, C., Hilario, A., Kormas, K. A., Maignien, L., Olu, K., Pachiadaki, M., Ritt, B., Rodrigues, C., Sarrazin, J., Tyler, P., Van Gaever, S., & Vanneste, H. (2009). Biodiversity of cold seep ecosystems along the European margins. *Oceanography*, 22(1), 110-127. doi: 10.5670/oceanog.2009.12
- Vogt, P. R., Gardner, J., Crane, K., Sundvor, E., Bowles, F., & Cherkashev, G. (1999). Ground-truthing 11-to 12-kHz side-scan sonar imagery in the Norwegia–Greenland Sea: Part I: Pockmarks on the Vestnesa Ridge and Storegga slide margin. *Geo-Marine Letters*, 19(1-2), 97-110. doi: 10.1007/s003670050098
- Zeppilli, D., Leduc, D., Fontanier, C., Fontaneto, D., Fuchs, S., Gooday, A. J., Goineau, A., Ingels, J., Ivanenko, V. N., Kristensen, R. M., Neves, R. C., Sanchez, N., Sandulli, R., Sarrazin, J., Sørensen, M. V., Tasiemski, A., Vanreusel, A., Autret, M., Bourdonnay, L., Claireaux, M., Coquillé, V., De Wever, L., Rachel, D., Marchant, J., Toomey, L., & Fernandes, D.(2018). Characteristics of meiofauna in extreme marine ecosystems: a review. *Marine Biodiversity*, 48(1), 35-71. doi: 10.1007/s12526-017-0815-z

Aims of the study

The global aim of this thesis is to characterize two, so far unstudied, shallow, and deep methane-rich seep sites, located off Svalbard (78°N), concerning metazoan meiofaunal assemblages, with a focus on free-living marine nematodes, a group that has remained unstudied in the Arctic seeps. The thesis is incorporated in the scope of the “AKMA” – “Advancing knowledge on methane in the Arctic”, where aspects related to the geology, biogeochemistry, and biology of several seep areas in the Arctic were investigated. The specific objectives of this thesis are:

- i.** Compare in terms of composition and structural and functional diversity, the metazoan meiofaunal assemblages, with a focus on nematodes, between methane-rich sediments, covered by bacterial mats, and reference sediment sites.
- ii.** Determine differences in meiofauna and nematode assemblages between both shallow and deep-water seepage sites

The following null hypotheses will be tested:

- i.** Meiofaunal abundances and nematode community composition and structural and functional diversity will not differ between the bacterial mats and reference sediments investigated in each seep site.
- ii.** Shallow and deep-water seep sites will not differ in terms of the same metrics investigated (i.e., density, composition, structural and functional diversity).

Scientific article

Meiofauna assemblages from cold seeps in the Arctic

Abstract

Cold seeps are unique habitats characterized by the flow of reduced chemical compounds, often rich in methane, sulfide, and even crude oil, exuding at the seafloor. These habitats are considered energy hotspots on the seafloor, establishing singular conditions, for endemic fauna to thrive. Studies conducted in cold seeps in the Arctic Ocean have mainly investigated their geological and geochemical setting, focusing the biological studies focusing on the large-sized organism, such as macro- and megafauna, with an unsettling lack of research on smaller-sized organisms, such as meiofauna ($\geq 32\mu\text{m}$ up to 1mm), and more precisely, nematodes. Under the scope of the AKMA project, two depth-differing seep sites were sampled, both in both active venting sediment areas covered with bacterial mats, and reference areas where no active venting was observed for a detailed study of meiofaunal assemblages, with focus on the nematodes. Meiofaunal and nematode abundances were, as found in other studies, higher in the bacterial mats at the shallow seep station but composed of two genera dominated community, one from the family Monhysteridae (*Halomonhystera* sp.) and Chromadoridae (Chromadoridae msp1). At the deeper superstation overall communities' density at reduced sediments, was lower compared to the reference, but keeping the same trend of a decreasing nematode diversity in the bacterial mat areas, where meiofauna composition was exclusively predominantly composed of copepods. Functional traits differed between micro-habitats and indicated a stronger presence of epistrate feeders and usually small slit-like and circular amphids at reduced sediments, indicating that nematodes' morphological change according to the habitat conditions. Ovoviviparous reproduction, an important adaptation of parents to secure their brood survival, was identified in three different nematode genera, *Halomonhystera* sp., *Halanonchus* sp., and one from the family Cyatholaimidae (Cyatholaimidae msp1), likely allowing these groups to survive and even thrive in the reduced environmental conditions in the bacterial mat sediments.

Keywords: deep-sea, chemosynthetic environments, Nematoda, community structure, structural and functional diversity

1. Introduction

Cold seeps are a widespread phenomenon, known to all the world's oceans, found from shallow to hadal areas (Suess, 2014). These are unique habitats characterized by the flow of reduced chemical compounds, predominantly methane and other hydrocarbons, including crude oil, boosted by a range of geophysical processes. Methane emissions to the atmosphere are a growing concern regarding cold seeps, a problem fuelled by climate-induced changes (i.e., ocean warming, acidification, de-oxygenation), particularly worrying in polar regions such as the Arctic, where several gas flares have been detected in the last decades, turning cold seeps into a common seabed feature in the region (Vogt *et al*, 1999; Judd *et al*, 2002).

Cold seeps are considered energy hotspots on the seafloor, establishing unique conditions that promote high biological productivity (Boetius & Suess, 2004). These are also characterized by an enhanced influence of habitat heterogeneity, which mainly depends on the fluid flow regimes and depth, altering the Physico-chemical, geological, and even biological conditions of the environment, ultimately promoting the formation of micro-habitats (zonation) and increasing by itself habitat complexity (Fisher *et al*, 2007; Cordes *et al*, 2010; Levin & Sibuet, 2012). Although little studied, and even though it is not yet possible to determine clear faunal patterns across the investigated seep sites in the high latitude regions, very few biological studies were carried out, with most research conducted on the Håkon Mosby Mud Volcano (HMMV) in the Barents Sea (Gebruk *et al*, 2003; Niemann *et al*, 2006; Van Gaever *et al*, 2006; Van Gaever *et al*, 2009; Decker *et al*, 2012; Galkin *et al*, 2013). From endemic to directly dependent on the commonly found CO²-fixing symbiotic bacteria, fauna associated with cold seeps in the high latitude regions near the Arctic is usually composed of the establishment of sparse bacterial mats (*Beggiatoa* and *Thioploca*), patches of frenulate tubeworms, as well as the presence of bivalves, anemones, and carnivorous sponges associated with small gastropods, pycnogonids, other crustaceans, and the vagrant zoarcid fishes (Gebruk *et al*, 2003; Van Gaever *et al*, 2006; Niemann *et al*, 2006; Foucher *et al*, 2009; Decker *et al*, 2012; Åström *et al*, 2016; Åström *et al*, 2018).

Marine metazoan meiofauna ($\geq 32\mu\text{m}$ up to 1mm), is the most widespread and abundant faunal group in marine sediments, normally dominated by Nematodes (more than

90%), followed by harpacticoid copepods, ostracods, and kinorhynchs (Grove *et al*, 2006; Giere, 2009; Schratzberger & Ingels, 2018). Meiofaunal organisms, and particularly nematodes, play an important role in the ecosystem functioning such as the biogeochemical cycle in sediments or being a key component in the marine food webs, and since they are ubiquitous and present in all marine habitats, show an incredible diversity and are easily found in extreme environmental conditions, having the advantage of developing several adaptations (Alkemade *et al*, 1992; Heip *et al*, 1992; Bellard *et al*, 2012; Schratzberger & Ingels 2018; Zeppilli *et al*, 2018). Despite their crucial role in the ecosystem, and their ubiquitous occurrence in marine sediments, studies regarding metazoan meiofauna from cold seeps in high-latitude regions, and particularly in the Arctic Ocean, are yet to be done, with only two areas near the Norwegian margin, of Storegga, and Nyegga pockmarks (Van Gaever *et al*, 2009; Portnova *et al*, 2014) and the Håkon Mosby mud volcano in the Barents sea (Van Gaever *et al*, 2006, 2009). Nematodes accounted for more than 90% of all observed taxa in all these three sites (Van Gaever *et al*, 2006, 2009; Portnova *et al*, 2014), and often present adaptations like alterations in metabolism and symbiosis, body size incre-/decrement, alteration in reproductive strategies (i.e., ovoviviparous reproduction) and wide range of life span (Giere *et al*, 1995; Van Gaever *et al*, 2006; Seibel and Drazen, 2007; Giere, 2009). Opposing the other areas, Harpacticoid copepods were found dominant in the crater location at HMMV (Van Gaever *et al*, 2009b). Furthermore, possible evidence related to the negative effect of the presence and density of macrofauna compared to meiofauna was observed at the Storegga site macrofaunal densities, which were twice as high when compared with the other two studied cold seeps. Three main micro-habitats were generally distinguished within these cold seeps in this region: **1)** crater (only investigated at HMMV), **2)** Reduced sediment covered by bacterial mats, and **3)** sediment colonized by frenulate tubeworms, supporting again the existence of habitat heterogeneity at cold seeps habitats. Overall, meiofaunal standing stocks (density), community composition, and diversity strongly differed between the different micro-habitats investigated but also to some extent between sites, driven by differences in fluid emissions and consequently sediment geochemical conditions, as well as trophic conditions (Van Gaever *et al*, 2009b; Portnova *et al*, 2014).

This study is incorporated into the scope of the AKMA – “Advancing knowledge on methane in the Arctic”, targeting the characterization of two depth differing and so far,

unstudied, seep sites, located off Svalbard, regarding metazoan meiofaunal assemblages, by focusing on free-living nematodes. With the use of all collected material from the microhabitats (bacterial mats and reference sediment) of both shallow and deep seeps, without evidence of seepage, the following null hypotheses were tested: (1) Meiofaunal communities and nematode structural and functional diversity will not differ between the bacterial mats and reference sediments investigated in each seep site, and (2) Shallow and deep-water seep sites will not differ in terms of the same metrics investigated (i.e., density, composition, structural and functional diversity).

2. Material and methods

2.1 Study area

The study was conducted in two seep sites (Fig 2.1), located offshore the western Svalbard archipelago, where evidence of seeping fluids was spotted: the South Vestnesa Ridge (SS02) at ~870m water depth and in Prins Karls Forland (SS01) at 112m water depth. The Vestnesa ridge (78°35'N, 73°50'E), belongs to the Western continental margin of the Svalbard archipelago, part of the eastern spreading segments of the Molloy ridge (Schneider *et al*, 2018). The ridge is mostly elongated (~ 100 km long), has a >2 km thick oceanic crust, and is known for holding deep-water methane seepage pockmarks (Bünz *et al*, 2012; Hong *et al*, 2016). Even though is an ultra-slow spreading ridge (<12 mm/year), with a valley depth of around 3200 to 3400 m, the region is notorious for exhibiting enhanced volcanic activity, and for the presence of seamounts (Okino *et al*, 2002; Dick *et al*, 2003). Several fluid flow structures have been previously identified by surveys conducted by CAGE - Centre for Arctic Gas Hydrate, Environment and Climate, in the region during the CAGE15-5 and CAGE16-6 expeditions. The shallow shelf west off the island Prins Karls Forland (78°50'N, 10°30'E), is distinguished by the strong presence of methane seeps, based on the numerous gases spotted in the region, where a huge area is shallower than the upper limit of methane-hydrate stability zone. Is also defined by irregular bathymetry, presenting a vast number of substantial depressions (Landvik *et al*, 2005; Grunder *et al*, 2021). Hydrodynamics in the area complex with a strong influence by warm Atlantic water masses and sediment is composed of a heterogeneous coarse mix of gravel, sand, and ice-rafted debris from terrigenous origins (Grunder *et al*, 2021).

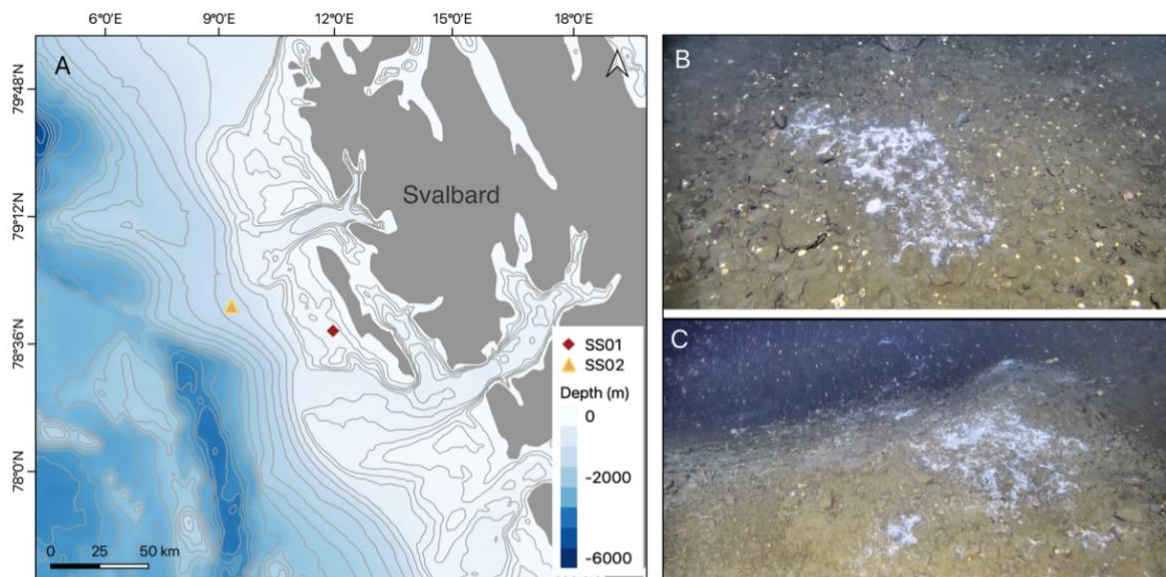


Figure 2.1: Study area. (A) bathymetric map showing sampling sites, with an example of the seafloor during sampling of (B) bacterial mats on Prins Karl Forland (SS01) and (C) south Vestnesa ridge (SS02).

2.2 Sampling strategy

Meiofaunal and macrofaunal sediment samples were collected at both study sites, during the “AKMA” cruise (May – June 2021), onboard the RV Kronpins Haakon. The two sampling areas, designated by superstation (SS), were located in the Prins Karl Forland (SS01) at 155 m deep, and South Vestnesa Ridge (SS02) at 875 m water depth. In each superstation, replicate sediment samples were collected with the aid of ROV ÆGIR 6000, with the help of either a blade-corer (BLAC - total surface of 83.3 cm²) or push-corer (PUSC - total surface of 50.3 cm²). These replicates were sampled in both sediments with clear evidence of methane venting and covered by bacterial mats, and in sediment without seepage evidence named as a reference, for both environmental characterization and faunal analysis (Fig 2.2, Table 2.1). At each site, three replicates were collected. Once onboard, each core was sliced into four different layers down to the 5 cm (0-1 cm; 1-2 cm; 2-3 cm; 3-5 cm). With the support of ROV ÆGIR 6000, visual observations of each sampling site were also made to characterize the area in both terms of sediment composition and mega-epifauna presence. All the samples were fixed in 4% formalin buffered in seawater.

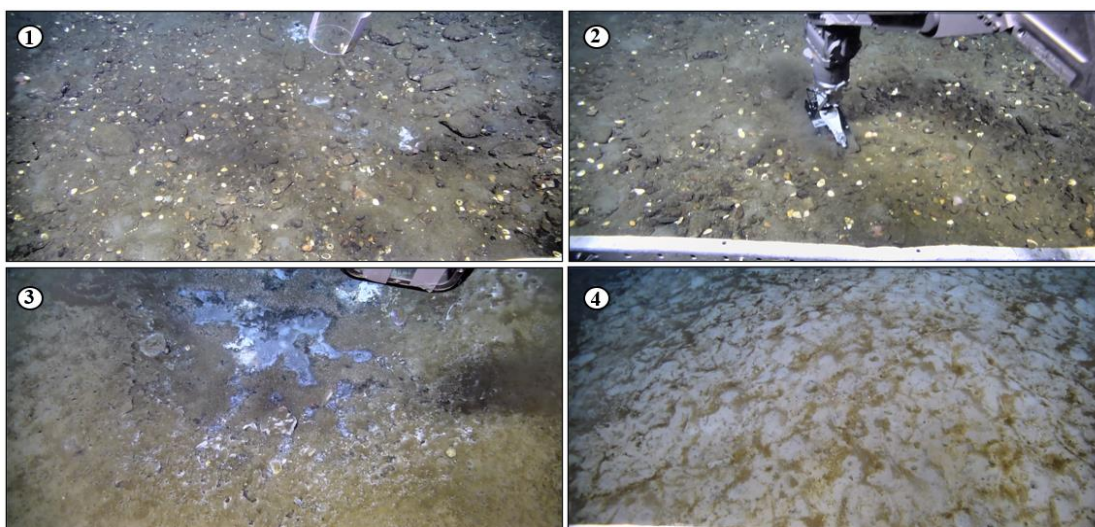


Figure 2.2: Images of sediment sampling, from different cores in different micro-habitats and sites, collected by the ROV ÆGIR 6000. (1) bacterial mat and (2) reference sediments in the Prins Karl Forland. (3) bacterial mat and (4) reference in the south Vestnesa ridge

Table 2.1: Sample collection metadata

Area	Superstation	Date	Dive	Time	Sample	Latitude	Longitude	Micro-habitat	Depth (m)
Prins Karls Forland	KH01	5/31/21	Dive12	11:19:14	PusC03	78° 49.27' N	10° 52.33' E	Bacterial Mat	112
				11:32:55	PusC05	78° 49.27' N	10° 52.34' E	Bacterial Mat	112
				11:38:35	PusC06	78° 49.27' N	10° 52.33' E	Bacterial Mat	112
				11:49:10	BlaC1A	78° 49.27' N	10° 52.35' E	Reference	112
				11:49:10	BlaC1B	78° 49.27' N	10° 52.35' E	Reference	112
				11:59:15	BlaC02	78° 49.27' N	10° 52.33' E	Reference	112
South Vestnesa ridge	KH02	5/31/21	Dive13	18:14:10	PusC09	78° 68.45' N	08° 27.89' E	Reference	877
				18:20:08	PusC10	78° 68.45' N	08° 27.79' E	Reference	877
				18:29:26	PusC11	78° 68.43' N	08° 27.71' E	Bacterial Mat	874
				18:42:56	BlaC03	78° 68.41' N	08° 28.07' E	Bacterial Mat	872
				18:48:17	BlaC04	78° 68.41' N	08° 28.07' E	Bacterial Mat	872

2.3 Sample treatment and laboratory analysis

The density gradient solution (Heip *et al*, 1985) was performed for meiofauna and nematode analyses (Fig 2.3). The fixed sediment samples were rinsed at the laboratory through a 1000-µm mesh, followed by a 32-µm sieve. The fraction trapped in the 1000-µm sieve, was screened and collected, using a decantation technique (3 times), and stored in 96% ethanol for macrofauna analysis and to check for larger nematodes. The remnant sediment retained in the 32-1000 µm fraction was washed and centrifuged four times using LUDOX HS 40 (specific density 1.21 g.cm⁻³) at 3000 rpm for 10 min. After each centrifugation, the

supernatant was collected, delicately poured over the 32 μ m sieve, rinsed into a flask, preserved in buffered 4% formalin, and stained with Rose Bengal. All metazoan meiofauna and macrofauna were counted and identified at higher taxa levels following Higgins & Thiel (1988), Giere (2009), and Schmidt-Rhaesa (2020), under a Leica M250C stereomicroscope (50 x magnification).

For further nematode identification, about 150 nematodes (or all nematodes, in the samples with less than 150), were picked arbitrarily from each sample vertical level up to 3 cm. The selected nematodes were mounted on permanent glycerin slides, after stepwise slow dehydration using the formalin-ethanol-glycerol method (De Grisse, 1965, 1969) with a laboursaving adjustment of Seinhorst (1959) method. After that, all nematodes were identified at the genera level, using pictorial keys (Platt & Warwick, 1988) and online keys and guides on Nemys database (www.nemys.ugent.be). All nematodes were identified using a compound microscope (Olympus BX-50 and Leica DM 2500 LED). When the identification of the genus was not possible, the nematodes were identified at the family level.

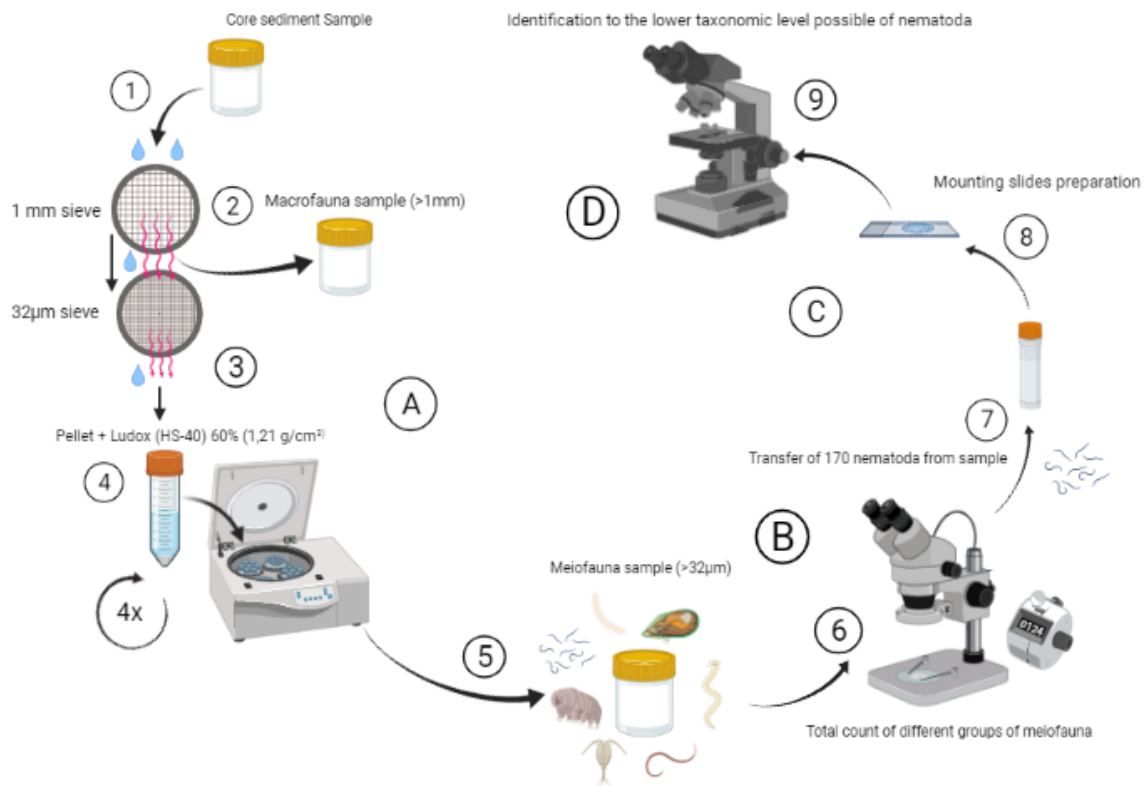


Figure 2.9: Schematic representation of the laboratory methodology used in this thesis. **A-** Fauna extraction from sediments; **B-** metazoan meiofauna count; **C-** permanent slides preparation; **D-** Nematoda identification. **1-** sediment washing through 1mm and 32 μ m mesh sieves; **2-** separation of macrofauna sized fauna (>1000 μ m) to an individual flask; **3-** transfer of the retained sediment in 32 μ m mesh sieves to centrifugation tubes **4-** pellet centrifugation (4x); **5-** supernatant (with meiofauna) collection and storage in formaldehyde 4% with Bengal rose; **6-** metazoan meiofauna count; **7-** Picking of 170 nematodes; **8-** mounting nematodes on a permanent slide preparation; **9-** Nematoda identification to the lower taxonomic level possible (**Created in BioRender.com**)

Various functional characters or traits of free-living marine nematodes are related to important ecological functions and help detect environmental variability in the ecosystem, possibly mirroring adaptations that species can display at the morphological, physiological, phenological, and behavioral levels (Mcgill *et al*, 2006; Williams *et al*, 2010; Semprucci *et al*, 2018). For a better perception of the role of these organisms in the ecosystem, and their functional differences in the studied sites, all identified Nematoda taxa were grouped into four different functional traits (buccal cavity, amphid shape, tail shape, and cuticle pattern types) (Table 2.2). To comprehend the trophic composition and role of the community, nematodes were divided into four feeding-type or buccal cavity morphological structures categories: selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A), predators/scavengers (2B) according to the Wieser classification (1953). Nematode amphid shape, the principal and most intricate chemosensory organ, associated with the search for food and partners (Decraemer *et al*, 2014), was classified into eight categories based on the structure: Indistinct (1), slit-like (2), pocket-like (3), spiral (4), rounded or elongated loop (5), circular (6), blister-like (7), and longitudinal slit (8). The tail shape, which can be related to locomotion, reproduction, and retention in the sediment (Thistle & Sherman, 1985; Thistle *et al*, 1995) was divided into four categories: short/ round (1), elongated/ filiform (2), clavate conical cylindrical (3), and conical (4). Lastly, the body cuticle, a flexible and resilient exoskeleton, was also distinguished, and distributed into five groups: smooth (1), with desmens (2), with a bacteria covering (3), punctuated or annulated with or without lateral differentiation (4), and with wide body annules and longitudinal ridges (5).

Table 2.2: Functional trait types and their correspondent functions descriptor for marine nematodes.

Functional traits	Type	Illustration
Buccal cavity	1. Selective deposit feeders (1A)	1.
	2. Non-selective deposit feeders (1B)	2.
	3. Epistrate feeders (2A)	3.
	4. Predators/Omnivores (2B)	4.
Amphid	1. Indistinct	
	2. Slit-like	2.
	3. Pocket-like	3.
	4. Spiral	4.
	5. Rounded loop	5.
	6. Circular or oval	6.
	7. Blister-like	7.
	8. Longitudinal slit	8.
Tail	1. Short/round	
	2. Elongated/filiform	1.
	3. Clavate conical	2.
	4. Conical	3.
Cuticle	1. Smooth	4.
	2. With desmens	5.
	3. With a bacteria covering	1.
	4. Punctuated or annulated; or with lateral differentiation	2.
	5. With wide body annule and longitudinal ridges	3.

2.4 Environmental data

The substrate composition and cover were estimated based on images collected by the ROV ÆGIR 6000 for each sampling site with the aid of the image processing software, ImageJ. The characterization of the main mega-epifauna present was also described. Additionally, during the ROV dives other parameters like water temperature (°C) and dissolved methane concentration ($\mu\text{mol/l}$), were also measured at the sampling moment while additional environmental variables concerning the sediment and pore-water geochemistry have been already conducted for the Prins Karl Forland site (SS01) by other scientific teams from UiT – The Arctic University of Norway, the dataset for South Vestnesa ridge (SS02) is yet to be completed and therefore those parameters were not yet included in this work due to consistency reasons.

2.5 Data analysis

All analyses, apart from correlations (done with R), were achieved using Primer v6 and PERMANOVA+ (Anderson *et al*, 2008). Environmental data were normalized and calculated for the Euclidean distance as a resemblance measure. This data was then explored with the support of a principal component analysis (PCA), to investigate for any spatial variability patterns. The meiofauna and nematode density and communities' composition among two main factors, sites (SS01 and SS02) and micro-habitats (Bacterial mats and Reference), were explored with the use of PERMANOVA and PERMDISP analysis for significant differences, after standardization of data, with square-root transformation and Bray-Curtis similarity used for calculating the resemblance matrix. The correlation between meio- and macrofaunal taxa density, was calculated using Spearman's rank correlation test in R software. A multidimensional scaling (MDS) was plotted to investigate spatial patterns based on nematode community composition among sites and micro-habitats. To define the relative contribution of each genus to both similarities and dissimilarities between the two factors, a similarity percentage analysis procedure (SIMPER; cut-off percentage: 90%) was executed. The species richness, (S) Shannon-Wiener diversity index (H') (Shannon-Wiener *et al*, 1949), and species evenness, Pielou evenness measure (J) (Pielou, 1966) were applied, to understand the structural diversity, respectively at the genera level. Rarefaction curves

were plotted based on Hulbert's expected number of genera (EG) calculated in PRIMER, for each micro-habitat, site, and overall study. Nematode functional composition was also investigated and the trophic index (Heip *et al*, 1998), and maturity index (MI) were calculated as the weighted mean of the individual mean densities of each trophic group (Wieser, 1953) and CP class (Bongers. T, 1990). Multidimensional scaling (MDS) was plotted to verify spatial patterns between sites and microhabitats built on the combination of the four functional traits studied. Nematode life stage relative density (%) was investigated to check differences between investigated seep sites.

3. Results

3.1 Environmental results

The bacterial mat and reference areas of the SS01 were mainly constituted of coarse sediment, namely pebbles and boulders and a few large dropstones, sporadically interrupted by fine sediment patches. Fragments of bivalve shells were also present on the seafloor (Table 3.1). Mega-epifauna present was similar between the two micro-habitats at this depth, with sparse anemones and sponges spotted. At the SS02, the substrate composition at both micro-habitats was mainly composed of fine sediments with few boulders found at the seabed near the bacterial mat, but with almost no expression ($1.4 \pm 1.41\%$). Epifauna at this depth was only detected near the bacterial mat, with the presence of tubeworms, anemones, and sea urchins (Table 3.1).

Table 3.1: Environmental description of each studied site.

Superstation	Micro - habitats	Mega-epifauna	General description	Substrate cover (%)			Water temperature (°C)	Methane conc. [$\mu\text{mol/l}$]
				Soft sediment	Coarse sediment	Dropstones		
Prins Karl Forland (SS01)	Bacterial mat	-Anemones -Sponges	- Mainly pebbles and boulders	11.5 ± 3.02	77.4 ± 5.67	11.1 ± 2.86	1.81 ± 0.045	0.033 ± 0.0023
	Reference	-Anemones -Sponges	- Mainly pebbles and boulders	5.2 ± 2.42	71.9 ± 1.98	22.9 ± 0.44	1.81 ± 0.018	0.024 ± 0.0003
South Vestnesa ridge (SS02)	Bacterial mat	-Tubeworms -Anemones -Sea urchins	- Mainly fine sediment (silt) - A few sparse boulders	93.5 ± 5.54	5.1 ± 4.15	1.4 ± 1.41	0.07 ± 0.025	0.003 ± 0.0015
	Reference	-No epifauna	- Entirely constituted of fine sediment	100.0 ± 0.00	-	-	-0.02 ± 0.009	0.002 ± 0.0010

The outcome of the PCA ordination (Fig 3.1) supported the distinction in environmental conditions between the superstations, with a high influence of depth and sediment composition, but not necessarily between micro-habitat at the SS02. In the SS01, bacterial mat sites seemed to differ from reference sediments mostly because of the higher methane concentrations by comparison to the reference sites, while dropstones were more common in the reference of SS01 accounting for $22.9 \pm 0.44\%$ of the total seafloor coverage.

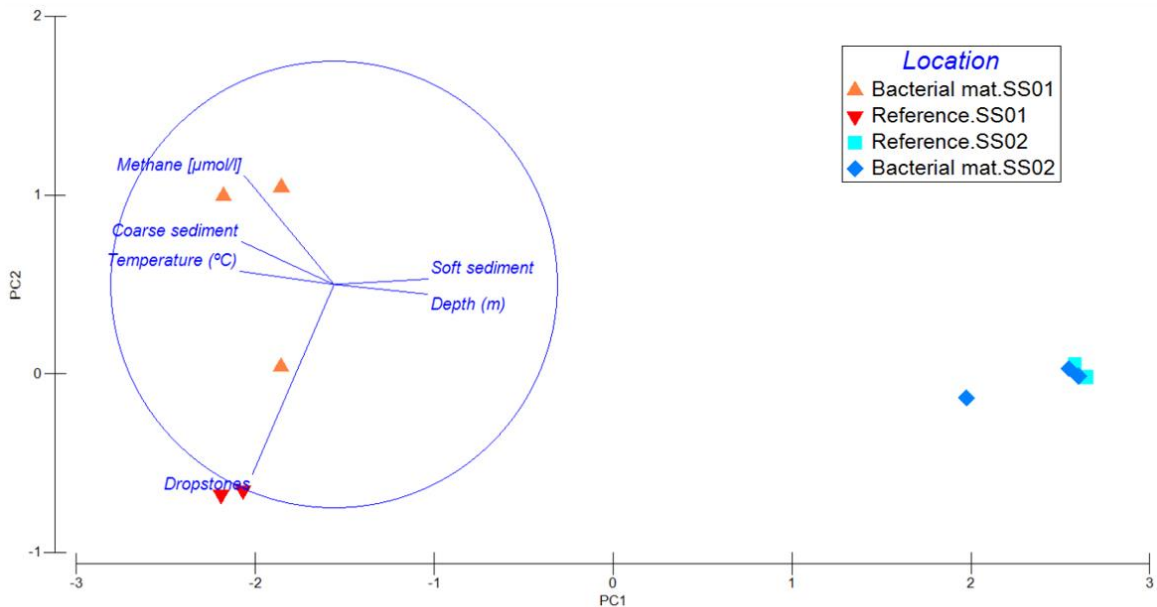


Figure 3.1: Principal component analysis (PCA) created based on environmental data per micro-habitat (bacterial mat and reference) and site (superstations SS01 and SS02).

3.2 Metazoan meiofauna and macrofauna

In total 24 taxa of the meiofauna (Table 3.2) were recorded and identified to the highest taxonomical level, with similar taxa richness (14) observed between the bacterial mat and reference sites at the shallow superstation. At the deeper superstation, bacterial mat areas recorded 15 taxa in comparison with the 12 taxa at the reference site, the lowest of all locations. Total metazoan meiofaunal density ranged between 51.45 ± 30.430 ind. 10cm^{-2} at the bacterial mat in SS02 to 1156.99 ± 323.927 ind. 10cm^{-2} at the bacterial mat site in SS01. Although not significant (Table A1 and A2), possibly due to a strong natural variability of the data, substantial differences between superstations were detected, having the shallow-water site a total meiofauna density of more than 3 times higher than the deep-water one (1040.5 ± 185.66 ind. 10cm^{-2} at SS01 and 326.1 ± 58.76 ind. 10cm^{-2} at SS02, Table 3.2). The most common phylum was Nematoda, across all stations (77% - 94%), except for the bacterial mat samples collected in SS02, where nematodes represented only 15% of total meiofauna, after Harpacticoida (40%) and Nauplii larvae (25%) (Table 3.2).

Table 3.2: Total average densities (\pm standard error; ind.10cm⁻²) per meiofauna taxa identified, per each site and micro-habitat.

Superstation	Prins Karl Forland (SS01)		South Vestnesa ridge (SS02)	
	Bacterial mat	Reference	Bacterial mat	Reference
Amphipoda	-	-	0.03 \pm 0.027	-
Bivalvia	0.33 \pm 0.175	2.80 \pm 0.280	0.58 \pm 0.345	348 \pm 1.704
Ciliophora	-	-	-	0.10 \pm 0.081
Cladocera	-	0.04 \pm 0.040	0.04 \pm 0.040	-
Copepoda	61.10 \pm 6.531	24.41 \pm 4.510	20.45 \pm 14.978	88.37 \pm 30.274
Cumacea	0.07 \pm 0.066	-	-	-
Gastropoda	-	0.24 \pm 0.139	0.07 \pm 0.035	-
Gastrotricha	-	-	0.07 \pm 0.066	-
Halacarida	0.27 \pm 0.175	0.64 \pm 0.423	0.05 \pm 0.053	0.10 \pm 0.081
Isopoda	0.07 \pm 0.066	-	-	0.10 \pm 0.081
Kinorhyncha	-	0.60 \pm 0.250	-	-
Loricifera	-	-	0.16 \pm 0.023	0.10 \pm 0.081
Nauplii larvae	16.63 \pm 4.235	5.52 \pm 1.040	13.08 \pm 11.471	28.43 \pm 11.687
Nematoda	1044.73 \pm 323.838	868.23 \pm 40.259	7.57 \pm 4.108	465.11 \pm 48.292
Nemertea	-	-	0.03 \pm 0.027	-
Oligochaeta	0.99 \pm 0.994	1.24 \pm 0.313	-	1.59 \pm 1.136
Ophiuroidea	.	0.44 \pm 0.262	-	-
Ostracoda	2.05 \pm 0.861	4.72 \pm 1.290	0.27 \pm 0.265	3.88 \pm 0.568
Polychaeta	30.42 \pm 4.793	13.73 \pm 2.028	6.85 \pm 5.697	8.25 \pm 3.165
Priapulida	0.07 \pm 0.066	0.56 \pm 0.223	-	-
Sipuncula	0.07 \pm 0.066	0.92 \pm 0.472	0.05 \pm 0.053	-
Tanaidacea	-	-	2.16 \pm 2.101	1.19 \pm 0.162
Tardigrada	0.07 \pm 0.066	-	-	-
Turbellaria	0.13 \pm 0.133	-	-	-
Total (ind.10cm⁻²)	1156.99 \pm 323.927	924.09 \pm 47.383	51.45 \pm 30.430	600.70 \pm 87.088

Vertical variation of nematodes seemed to be similar across all investigated sites, following the pattern of the meiofauna overall densities, nematode density was higher in the top 3 cm of sediment. The highest and lowest nematode density discrepancy between sediment layers (0-3 and 3-5 cm), were recorded at the SS02, with a difference of 97% at the bacterial mat and 76% at the reference site, respectively.

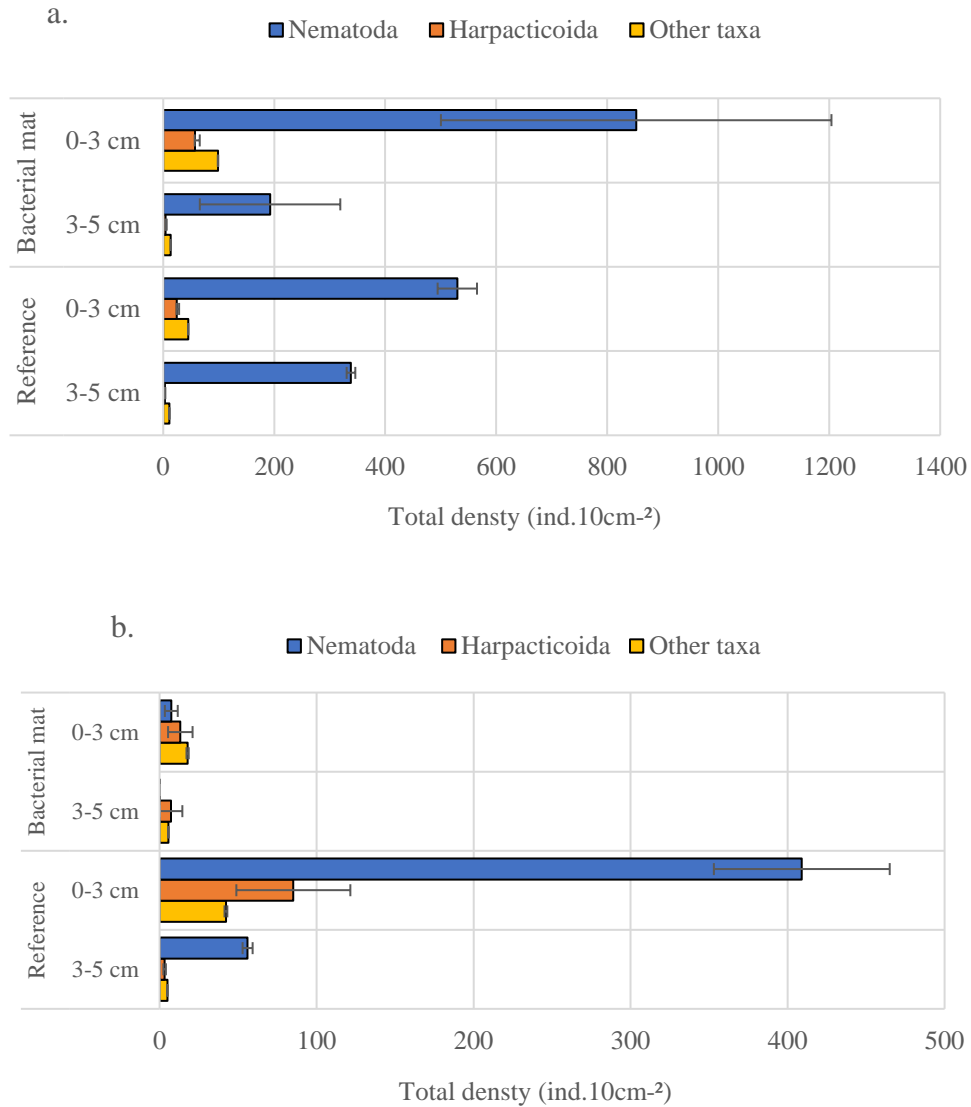


Figure 3.2: Total nematode density (ind.10cm⁻²) per sediment layer (0-3 cm and 3-5 cm). (a) Vertical density distribution at Prins Karl Forland (SS01) and (b) south Vestnesa ridge (SS02).

The highest average macrofaunal densities (Table A3) were recorded at the bacterial mat from the SS01 (9.41 ± 3.688 ind.10cm⁻²), identical to meiofauna, and the lowest at the reference of SS02 (2.09 ± 0.895 ind.10cm⁻²), different from the meiofauna, that was at the bacterial mat, contrasting with macrofauna that reported the second highest overall density (5.29 ± 4.794 ind.10cm⁻²) at this micro-habitat, albeit with great variability. Average densities were almost twice as high in the bacterial mat when compared to the reference

sediments regarding both depths (4.80 ± 1.232 to 9.41 ± 3.688 ind.10cm⁻² in SS01 and 2.09 ± 0.895 to 5.29 ± 4.794 ind.10cm⁻² in SS02), similar result as observed at Prins Karl Forland site for meiofauna, but different as the one found south Vestnesa ridge , where the reference sediments recorded an overall density 11 times higher than in the bacterial mat. To denote that the presence of macrofaunal Gastropoda, was only observed at bacterial mats at SS02, with a relatively high density of 2.84 ± 2.662 ind.10cm⁻². Macro and meiofaunal total average densities were positively correlated ($r= 0.57$; p -value = 0.07, Fig 3.3)

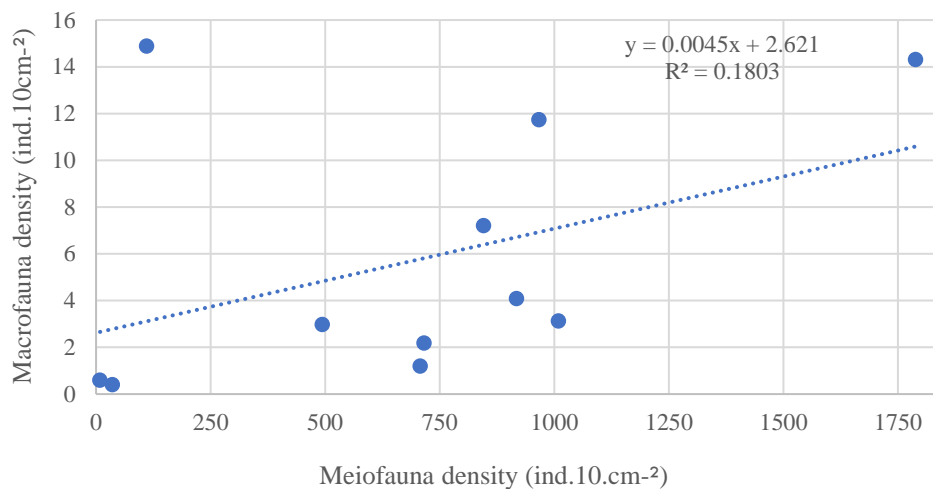


Figure 3.3: Correlation scatter plots comparing macro- and meiofauna total density.

3.3 Nematode community composition and structural diversity

A total of 1492 nematodes were examined throughout all sites and microhabitats, being of these 35 considered impossible to identify, due to bad body conditions, or due to the impossibility to find key morphological structures. The total number of nematodes was distributed to a total of 27 different families, corresponding to a total of 60 different genera (Table A4). Overall, the most common families across all stations studied, were Comesomatidae (24.8%), Chromadoridae (23.1%), Monhysteridae (19.3%), Linhomoidae (6.8%), and Microlaimidae (4.0%). The highest genera richness was observed in the reference site of SS01, with 46 genera identified, and the lowest in the bacterial mat at the same superstation, with 19, a number that didn't differ much from the bacterial mat of SS02 (20). The most common genera identified across all the investigated sites were *Halomonhystera* sp. (18.9%), *Sabatieria* spp. (18.5%), Chromadoridae msp1 (15.2%), and

Dorylaimopsis sp. (6.2%). Even if not showing significant differences due to possible high natural variability, or insufficient statistical support (Table A5 and A6), it was clear to observe differences between micro-habitat and superstations, regarding Nematoda genera composition as the multidimensional scaling plot (MDS, Fig 3.4) shows. The samples from the bacterial mat in the deep-water site differed in terms of compositions from the rest of the samples, having a high weight in the discrepancies between superstations and being mainly composed of one genus, *Dichromadora* spp. (64%, Fig 3.5). The two reference sites displayed more even compositions through several genera, having in common among others a high proportion of *Sabatieria* spp, *Halalaimus* spp, and *Microlaimus* sp. The bacterial mat of the shallow-water site, although variable across replicates, was primarily composed of *Halomonhystera* sp. (39%, Fig 3.5), and Chromadoridae msp1 (32%, Fig 3.5).

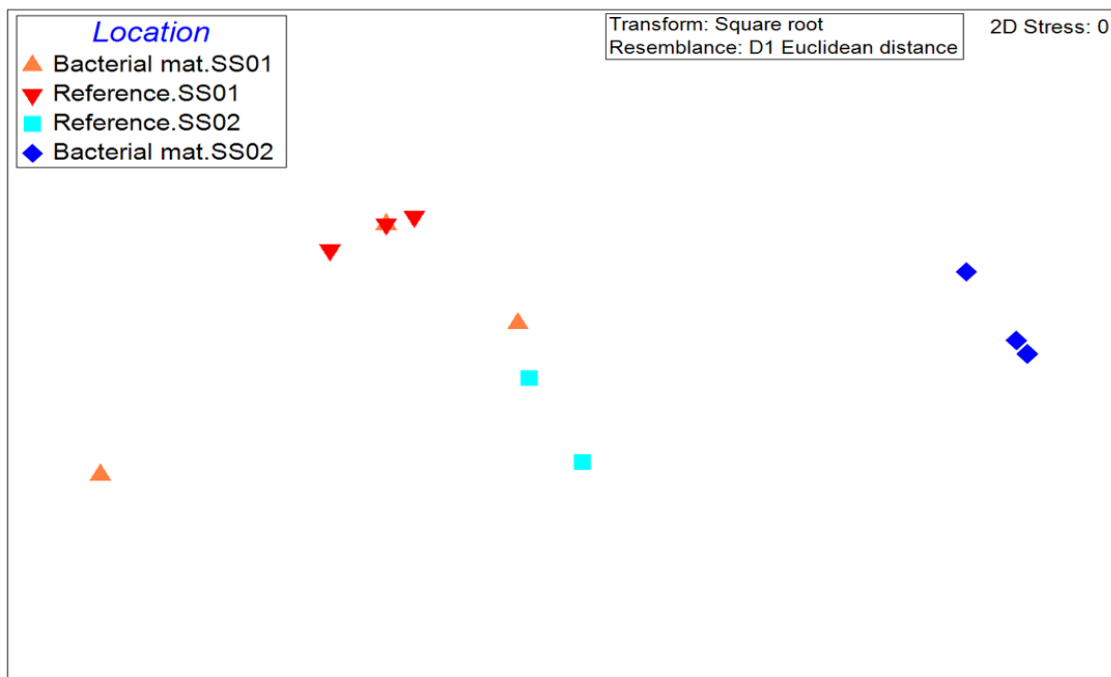


Figure 3.4: Multi-dimensional scaling (MDS) based on nematode genera composition per investigated site

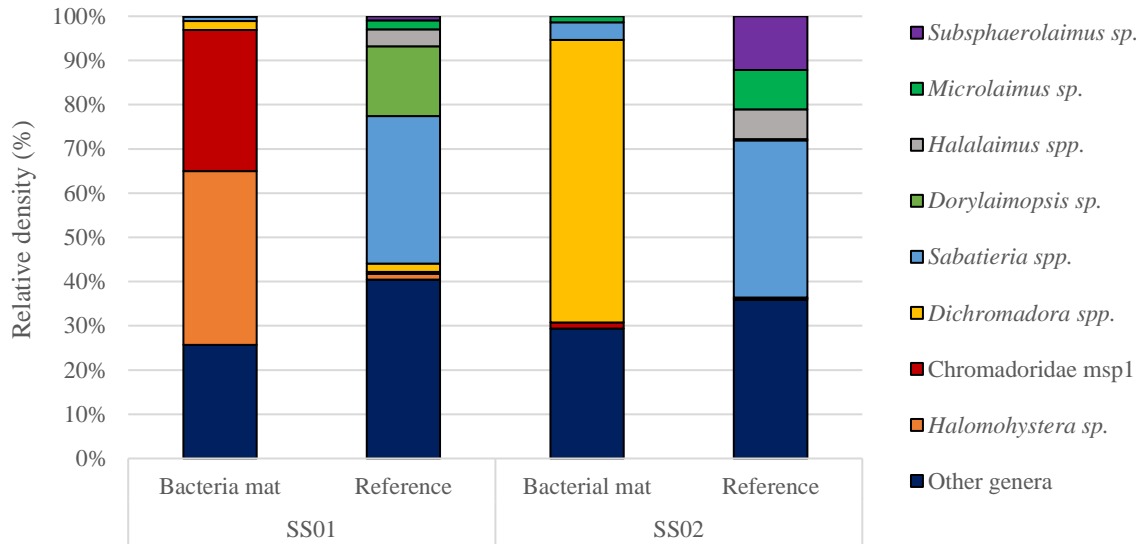


Figure 3.5: Relative density (%) of the most abundant genera at the different sites investigated.

SIMPER results (Table 3.3) showed a high dissimilarity between micro-habitats (78,44%) and superstations (75,82%), explained mainly by the high variability among replicates, namely due to the contribution of *Sabatieria* spp. (6.8%), and *Halalaimus* spp. (5.01%), especially for their presence at reference sediment sites and absence at the bacterial mats, but also *Halomonhystera* sp. (10.75%) and Chromadoridae msp1 (10.13%) for their almost exclusive presence in one of the superstations (SS01). Results on the low similarity among bacterial mats (39.18%) and high among references (61.69%) seemed to be in line with the observations made in the MDS. The low similarity between bacterial mats was mainly caused by the high contribution of Chromadoridae msp1 (19.39%) and *Halomonhystera* sp. (17.12%) in the shallow bacterial mat. Shallow-water superstation in general shared more genera in common between the micro-habitats, and so it registered a similarity of 63.96%, much different from the deeper superstation, which recorded a very low similarity (24.52%), just as shown by easily denoted in MDS (Fig 3.4).

Table 3.3: Results of similarities and dissimilarities (bold), and genera contribution (%) (SIMPER analysis) between micro-habitats and sites (superstations). Individual genera cut-off level for similarity and dissimilarity was 4%.

	Bacterial mat	Reference	SS01	SS02
	39.18%	78.44%		
Bacterial mat	Chromadoridae msp1 (19.39) Halomonhystera sp. (17.12) Cyatholaimidae msp1 (11.14) Dichromadora spp. (9.98) Chromadorita sp. (9.37) Linhomoeus spp. (8.98) Sabatieria spp. (4.6) Leptolaimus sp. (4.21)	Sabatieria spp. (6.8) Halalaimus spp. (5.01) Subsphaerolaimus sp. (4.46)		
Reference		61.69% Sabatieria spp. (11.97) Halalaimus spp. (7.2) Dorylaimopsis sp. (6.75) Terschellingia spp. (6.28) Microlaimus sp. (5.32) Metalinhomoeus sp. (5.2) Subsphaerolaimus sp. (4.21) Trefusia sp. (4.12) Phanodermatidae msp3 (4.07)		
SS01			63.96%	75.82%
			Chromadoridae msp1 (12.51) Halomonhystera sp. (11.22) Chromadorita sp. (7.75) Cyatholaimidae msp1 (6.83) Dichromadora spp. (6.18) Linhomoeus spp. (5.5) Sabatieria spp. (5.01) Dorylaimopsis sp. (4.34)	Halomonhystera sp. (10.75) Chromadoridae msp1 (10.13) Cyatholaimidae msp1 (6.99) Chromadorita sp. (5.78) Linhomoeus spp. (5.49) Chromadoridae msp1 (4.91)

SS02

24.52%

Sabatieria spp. (21.56)

Desmoscolex sp. (12.43)

Leptolaimus sp. (10.1)

Dichromadora spp. (8.49)

Subsphaerolaimus sp. (7.94)

Camacolaimus sp. (7.91)

Sphaerolaimus spp. (7.27)

Halalaimus spp. (6.87)

Aponema sp. (5.38)

Microlaimus sp. (4.52)

The diversity measures revealed consistent results across the different indices used. Shannon-Wiener index (H') was found to be higher at the reference sediments when compared to bacterial mats, in both shallow and deep-water sites, that when accounted for individually only had a negligible difference (Table 3.4). The highest diversity value was observed at the reference of Prins Karl Forland (SS01). Pielou evenness index (J) values were in accordance with the diversity results and recorded lower values at the reduced sediments (Table 3.4), which links to the higher genera dominance in the sediments covered with bacterial mats, due to higher variability in genera abundances. Although slightly, was possible to observe a lower evenness value at the shallow site (SS01), when compared with the deeper one (SS02).

The rarefaction curves (Fig A1) plotted individually for each studied micro-habitat, showed that only the reference of SS02 and the bacterial mat of SS01 were close to reaching a good representation of the total number of genera present in the community, by starting to reach an asymptote. A much steeper curve rise was detected in the reference of SS01, due to a quicker accumulation of genera. When plotting the pooled observations from each site and the overall study, the rarefaction curve indicated that sampling was sufficient to grasp a good representation of the total number of genera present in the community (Table 3.4).

Table 3.4: Mean and standard error, of structural indices, per micro-habitat (bacterial mat and reference), across both depths, H' : Shannon-Wiener index, EG (60): expected number of genera, J : Pielou evenness index, trophic diversity, and maturity index.

Site	Micro-habitat	S	N	Pielou (J)	EG (60)	Shannon-Wiener (H')	Trophic diversity	Maturity index
Prins Karl Forland (SS01)	Bacterial mat	11 ± 2.3	183 ± 33.9	0.64 ± 0.048	8.0 ± 1.36	1.5 ± 0.14	0.52 ± 0.03	2.2 ± 0.14
	Reference	31 ± 2.2	138 ± 4.4	0.74 ± 0.039	19.9 ± 1.72	2.5 ± 0.18	0.36 ± 0.03	2.5 ± 0.06
	Total	48	963	0.69	18.0	2.7	0.44 ± 0.042	2.4 ± 0.09
South Vestnesa ridge (SS02)	Bacterial mat	8 ± 3.0	32 ± 12.1	0.68 ± 0.213	8.3 ± 2.96	1.5 ± 0.55	0.62 ± 0.11	2.3 ± 0.31
	Reference	19 ± 3.5	197 ± 20.0	0.73 ± 0.048	12.3 ± 1.11	2.1 ± 0.00	0.37 ± 0.03	2.5 ± 0.01
	Total	36	489	0.72	16.9	2.6	0.52 ± 0.088	2.4 ± 0.18
Total		60	1452	0.72	21.0	3.0	0.48 ± 0.045	2.4 ± 0.09

3.5 Nematode functional diversity

The trophic diversity lightly differed between micro-habitats inside each superstation, but similar values were observed between both reference sites (Table 3.4). The lower trophic diversity was observed at the reference of SS01 (0.36 ± 0.03), followed by the other reference station (0.37 ± 0.03), and on the other hand, the highest belonged to the bacterial mat of SS02 with a value of 0.62 ± 0.11 . In general, trophic diversity was higher in the bacterial mat areas and slightly superior at the deeper superstation, comparing depths (Table 3.4). Similar patterns of trophic groups were observed between the studied sites, with a predominance of epistrate feeders in bacterial mats, and non-selective deposit feeders in reference sediments, at both depths (Fig 3.6). Note that, the highest incidence of predators/omnivores was recorded at reference sediments in SS02 ($26.7 \pm 7.45\%$) and the highest incidence of selective deposit feeders (1A) was observed at bacterial mat at the same depth ($23.9 \pm 13.32\%$). The same distribution was discovered, across the locations, for the tail type, with a prevalence of conical tails in the bacterial mats across both depths and clavate conical cylindrical being more common in reference sediments (Fig 3.6). Both bacterial mat sites had a higher frequency of slit-like amphid type, closely followed by circular type at the bacterial mat in SS02 (Fig 3.6). In comparison, in reference sediments of the SS01, spiral amphid was the prevailing shape ($53.7 \pm 4.07\%$) and the circular type overcame at the deeper superstation ($46.7 \pm 10.33\%$), proving here a difference possibly related to the depth. Nematodes, across every micro-habitat at both depths, were largely mainly constituted with punctuated or annulated cuticles, representing always more than 58% of cuticle type (Fig 3.6). Nevertheless, smooth cuticles recorded a much higher occurrence at SS01.

A combination of the four investigated functional traits, resulted in 43 different combinations. A Multidimensional scaling (MDS) was plotted and revealed a substantial difference between the deep-water bacterial mat and the other studied sites for the combination of traits (Fig A2), showing how morphologically distinct the nematodes from that micro-habitat were, even with high variability between replicates. Both MDS plots for genera composition and combination of traits showed a very similar distribution of the studied locations.

The maturity index (MI) didn't show evident variability between the investigated sites (Table 3.4). At the bacterial mat of SS01, the nematodes identified were predominantly of the colonizer-persister (cp) scale class 3 ($57.1 \pm 8.61\%$) but is important to highlight the high presence of nematodes with a cp class of 1 ($35.3 \pm 6.56\%$). The remaining micro-habitats had a prevalence of nematodes from a cp class of 2.

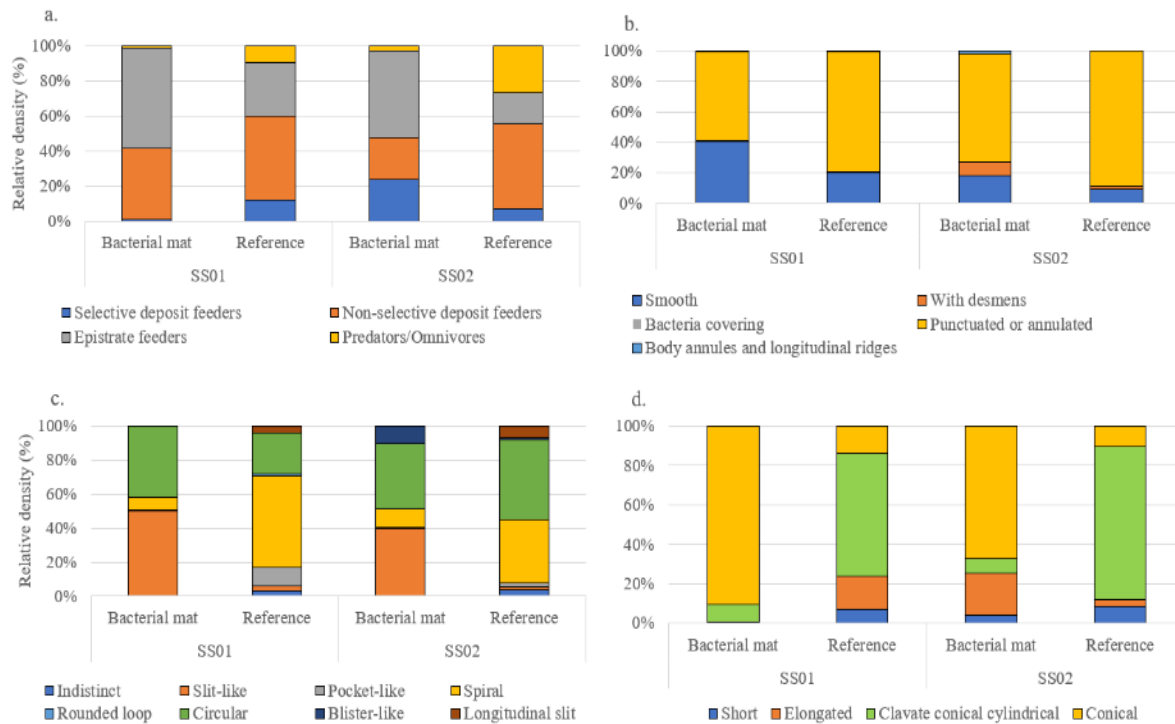


Figure 3.6: Relative density (%) of each functional trait per micro-habitat and site (superstation). **(a)** Trophic group **(b)** Cuticle type **(c)** Amphid type **(d)** Tail type

The juvenile nematodes were the most detected life stage, at both bacterial mat (32.4% in SS01 and 36.9% in SS02) and reference sediments (49% in SS01 and 47.6% in SS02) (Fig 3.7). There was a higher incidence of females carrying eggs and juveniles at the SS01, predominantly at the bacterial mat sites (Fig 3.7). No evidence of females carrying juveniles was spotted at the SS02, with only a few females with eggs being recorded at reference sites (Fig 3.7). The female nematodes with what it indicated to be an ovoviviparous reproduction adaptation belong to the genera *Halomonhystera* sp., *Cyatholaimidae* msp1, and

Halanonchus sp., the first two found at the bacterial mat and the last in reference sediments (Fig 3.8).

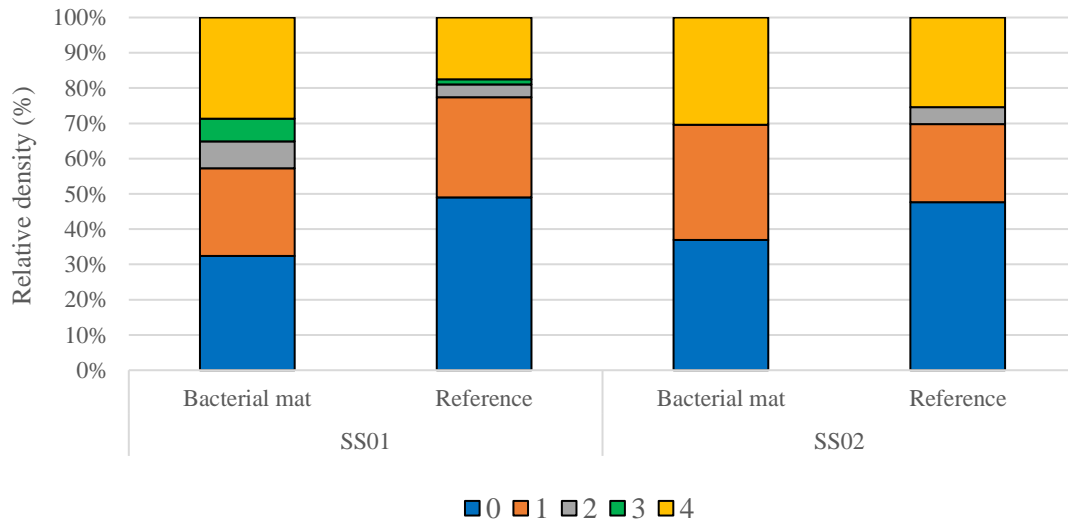


Figure 3.7: Relative density (%) of each life stage across the two micro-habitats (bacterial mat and reference) at both sites (SS01 and SS02). Life stages: **0** (Juvenile or Immature), **1** (Female), **2** (Female with eggs), **3** (Female with juveniles), and **4** (Male)

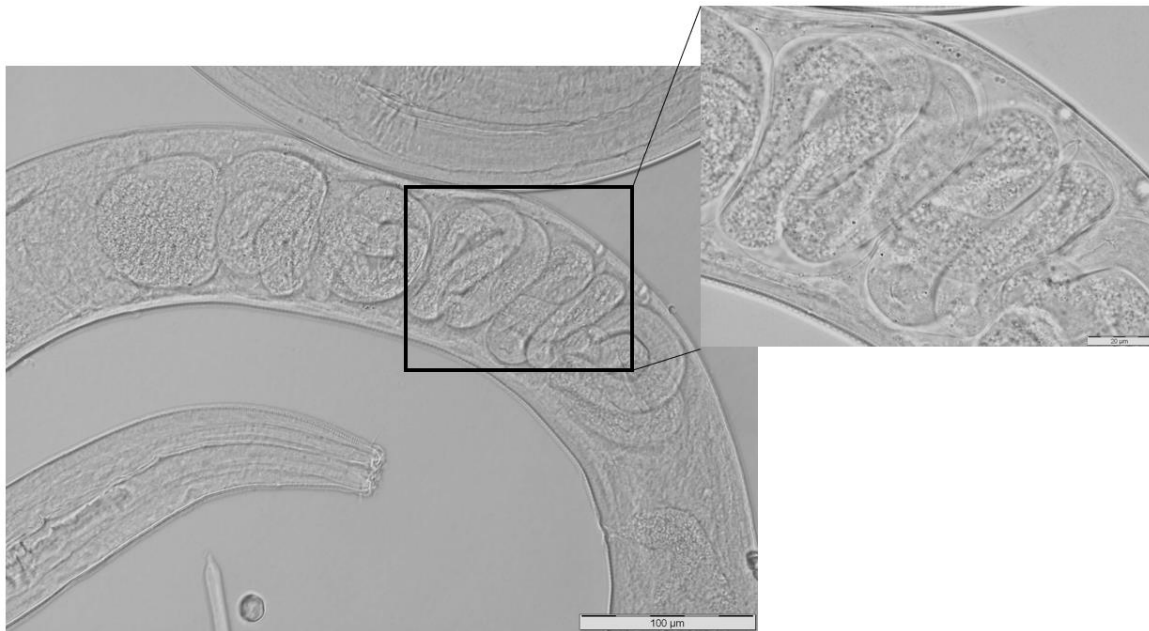


Figure 3.8: Ovoviviparous reproduction adaptation: Microscope photograph of a female individual from the genera from the Cyatholaimidae family (*Cyatholaimidae msp1*), carrying juveniles in the uterus.

4. Discussion

4.1 Metazoan meiofauna density and composition

Metazoan meiofauna assemblages were driven and influenced by different factors, such as environmental parameters or the macrofaunal community present, which usually define the variability and distribution of these communities. It was clear that both superstations were affected by different environmental factors, which may explain the considerable differences observed between them regarding meiofaunal densities. A key factor for the difference between superstations was depth, and consequently softer sediments, colder temperature, and higher pressure, resulting in a lower meiofaunal and nematode density at the south Vestnesa ridge. This fact can be highly linked to lower food availability, derived from the sediment concentration of organic matter and overall oxygen concentration (Mokievsky, 2009), subsequent to a more elevated primary production commonly greater at lower depths in the ocean. Highly influenced by oil and methane concentration, reduced sediments, covered by bacterial mats at the Prins Karl Forland site (SS01), recorded the highest meiofaunal and nematode standing stock, when compared to the surrounding sediments, even if just slightly. The increase of meiofaunal and nematode density with increasing reduced chemicals concentrations was also observed in several other works, being on average 2-5 greater at reduced sediments, in comparison with the surrounding sediments (Powell *et al*, 1981; Buck *et al*, 1998; Bernhard *et al*, 2000), mainly due to the enhanced availability of organic matter produced by the bacteria established at these seep sites, which also can signify a vital food source for some species of nematodes, thriving in such conditions (Moens & Vincx, 1997). Nematodes accounted for the majority of metazoan meiofauna taxa discovered at both micro-habitats in the shallow superstation, followed by copepods, their Nauplii larvae, and Polychaetes, a pattern that is observed in several other cold seep habitats (Van Gaever *et al*, 2009b; Lampadariou *et al*, 2013). This remarkably high density of nematodes may not only be related to the ubiquitous distribution of this taxa, or their preference for bacteria at reduced sites but also suggests that a sulfide-rich environment can protect against their predation (Van Gaever *et al*, 2006). The abrupt decrease of meiofauna and nematode abundances at the bacterial mat of the south Vestnesa ridge site (SS02), may be linked to the negligible difference in methane concentration found between the two micro-habitats. This fact supports the hypothesis that active seepage at the sediments covered with

bacterial mats, was through a period of less fluid flow activity and as such, possibly going through a recolonization process. It should be noted that differences in fluid regimes are characteristic in cold seeps, which may vary in time or may even be linked to the methane storage itself, which strongly contributes to greater habitat heterogeneity, and more instability in the communities (Ferré *et al*, 2020). Reduced meiofaunal and nematode standing stocks, in relation to the background sediments were also reported in similar biochemical conditions from deep-sea vents at North Fiji (Vanreusel *et al*, 1997), but more studies are required at deep-sea seep sites, still far behind shallow seep meiofauna knowledge (Tarasov *et al*, 2005). Among others, there are possible explanations for the fact that nematodes were not the most abundant taxa in this site, like by the first settling of copepods, their nauplii larvae, and polychaetes in this area due to the development of specific adaptations, or by a local lack of food resources, but all these possibilities are still not clear. The prevalence of copepods at the vent site is a phenomenon already observed at the crater of HMMV (Van Gaever *et al*, 2006), and was linked to a possible feeding specialization on bacterial mats or symbiosis with vent obligated animals (e.g., vestimentiferans and shrimps) (Heptner & Ivanenko, 2002). As the copepods show a greater facility in swimming, which allows them to move up and down in the sediment, may be another explanation for this fact since the nematodes are not so motile. Geochemical activity and sediment outburst are other possible explanations not to exclude (Portnova *et al*, 2014), along with an elevated density of meiofaunal tanaidacea, almost exclusive in this micro-habitat, which may have influenced the nematode presence by possible predation (Giere, 2009).

A completely different result regarding the macro- and meiofauna relationship was found at the HMMV seep and Nyegga and Storegga sites, with a negative correlation (Van Gaever *et al*, 2009b). A higher or lower correlation between macro and meiofauna is normally related to predation and competition effects, mainly caused by the greater or lower polychaete assemblages as found in other studies (Van Gaever *et al*, 2009b), acting as structuring agents of meiofauna communities, either negatively with sediment disruption or positively with sediment aeration and creation of oxic micro-environments (Ronn *et al*, 1988; Tita *et al*, 2000; De Troch *et al*, 2000). The density of macrofaunal polychaetes was in general higher than the other macrofaunal taxa, nevertheless, it seemed to have a positive impact on the total meiofauna assemblages present in this study.

The vertical variability of meiofauna and nematodes showed to be consistent along the studied areas, always with higher densities recorded at the top 3cm, due to increased availability of oxygen and food, which usually decrease with water and sediment depth (Soltwedel *et al*,2003; Hoste *et al*,2007). Similar vertical patterns were also reported by Van Gaever *et al*, (2006) and Portnova *et al* (2011). Vertical distribution of meiofauna and nematodes in the sediment can be influenced by several causes, such as inter-specific relations, tolerance to sulphiridic environment, and trophic preferences (Portnova *et al*,2011) but a further assessment of the sediment biogeochemical conditions is needed to further explore this issue.

4.2 Nematode community composition

Habitat type was revealed to be a crucial factor for nematode composition, exposing the characteristic habitat heterogeneity usually found at cold seeps. When comparing micro-habitats at both depths, it was clear to denote a contrast in genera composition, where reduced sediments, covered with bacterial mats, presented a decreasing diversity, tending to a community dominated by one or two nematode genera, replacing a more diverse and rich community typical of the surrounding sediments. This is a tendency already observed in other studies performed at cold seeps (Mokievsky *et al*, 2005; Van Gaever *et al*, 2006; Van Gaever *et al*,2009b; Portnova *et al*,2011), and supported by the SIMPER results with a high dissimilarity between micro-habitats. Looking at the diversity indices for each investigated location separately, the bacterial mat sediments always showed lower values for diversity and evenness exposing some genera dominance. These outputs are corroborated by the high presence of the successful genera *Halomonhystera* sp. (previously referred to as *Geomonhystera*) and a genus from the family Chromadoridae (Chromadoridae msp1), accounting together 61% of all nematodes identified in the bacterial mat of Prins Karl Forland, characterized by extreme conditions (exudation of oil and methane). *Halomonhsytera* sp., was surprisingly absent in this bacterial mat area, nevertheless, its high prevalence in the shallow reduced sediments, is a common result described in other works, especially in bacterial mats in HMMV (Van Gaever *et al*,2006; Portnova *et al*,2011), since this is a cosmopolitan, bacterivorous nematode, recognized for its high resistance to

environmental stress (Vranken *et al*,1989). Before this study, genera from the family Chromadoridae have already been reported to inhabit reduced environments, just as in hydrothermal vents off the coast of Japan at the Lheya ridge (Shirayama, 1992), and showing a similar dominant behavior at bacterial mats in Nyegga region (Portnova *et al*,2014). Another Chromadoridae genus (*Dichromadora* spp.) was dominant at the bacterial mat of the deep-water site, suggesting that even in a low fluid flow regime, this specific genus could have been established faster than others by a quicker adaptation. At the deep-sea vents of North Fiji (Vanreusel *et al*,1997), it was found that *Monhystera* dominated the hydrothermal inactive ridge sites, a genus from the family Monhysteridae, that was only recorded at the bacterial mat of south Vestnesa ridge, which showed similar environmental conditions in the present study. Due to the extremely low nematode densities, these site samples were handled and analyzed with caution. Bigger and overall, less-specialized nematode genera, such as *Sabatieria* spp., *Halalaimus* spp., or *Subsphaerolaimus* sp., composed the reference sediment sites and constituted the major contributors to the dissimilarity between the two micro-habitats, following SIMPER results. The genera *Sabatieria* spp. included the first and most prominent nematode genera at both reference sites, since it normally tolerates long periods of oxygen deficiency (Portnova *et al*,2014), especially at the south Vestnesa ridge, although being surprisingly absent from the reduced sediments in the present study, as observed in other works at Storegga in North Atlantic (Van Gaever *et al*,2009b), Darwin mud volcano seep in the central Atlantic (Pape *et al*,2011) or the REGAB cold seep in the Gulf of Guinea (Van Gaever *et al*,2009a). Depth-related dissimilarities regarding nematode genera composition, were very high, with the major contributors being the genera *Halomonhystera* sp., Chromadoridae msp1, and Cyatholaimidae msp1, with all genera tending to show a preference for inhabiting the shallower superstation. These results cannot be properly compared to the deeper superstation because may not serve as a reliable term of comparison, due to the limitation in nematode low availability of samples. Not all species were discovered in this study, as the rarefaction curves demonstrate, and so there was not an overall good compositional representation of all samples (Schneider & Culver, 2004). Nevertheless, when analyzing each studied location individually, the bacterial mat of SS01 and reference of SS02, showed to be reliable samples, with almost a good compositional representation. The fact that the bacterial mat of SS02 is assumed to be a less active cold seep, and possibly more

unstable, and the reference of SS01 was expected to have huge diversity, are explanations not to exclude from these results. Great habitat heterogeneity and the variability within samples at each site are also possibilities since the replicates were always dependent on two key factors, depth and reduced chemical concentrations (Culver *et al*,2004).

4.3 Nematode functional composition and adaptations to seep conditions

In a general way because nematodes have short generation times, low mobility, often direct benthic development, and high densities when compared to other benthic groups, this phylum is often used to study the responses to both natural and anthropogenic induced changes in the ecosystem (Semprucci *et al*, 2022). Even with low genera diversity, the bacterial mats investigated displayed a higher trophic diversity, which indicated the occurrence of a broader variety of food sources (Vanaverbeke *et al*, 2011). Selective epistrate feeders at the bacterial mat sediments and non-selective deposit feeders at the reference constituted the majority of the trophic guilds found in this study, since their capability of feeding and thriving from a broad variety of food items, from organic deposits, bacterial biofilm, or microphytobenthos (Misto *et al*,2002; Semprucci *et al*,2022). The high proportion of epistrate feeders at the bacterial mats, both rich in oil and methane, support the fact that nematodes inhabiting these sediments largely depend on the biofilm formed and other sources of organic matter. For example, it was demonstrated for *Chromadorina germanica*, an epistrate feeder, can choose a mix of microbiota and particulate and dissolved organic matter to feed if the food is adsorbed on the substratum (Alongi & Tietjen, 1980). Additionally, amphid type of chromadorids (slit-like) and monhysterids (circular) found at the oil and methane seepage sites, were in general smaller and inconspicuous compared with non-seep sites, as well as known to be present in nematodes normally more exposed to contaminants, and in areas rich in food sources, which together with their feeding strategy allowed them to thrive in the extreme environment more easily (Wakkaf *et al*,2020; Semprucci *et al*,2022). Epistrate feeders are also widely found in coarse-grained, shallow-water sediments (Moens *et al*,2005), substrate characteristic mainly of the bacterial mat in the Prins Karl Forland site. By contrast, reference sediments were constituted by nematodes with substantially larger circular and spiral amphids, depending on depth, which usually

allows the nematodes to detect and avoid a wider range of toxic conditions, including contaminated sediments by oil and methane (Wakkaf *et al*,2020). Conversely to feeding mode and amphid shape, tail types seem to be rather associated with the sites different in environmental conditions than the micro-habitat itself. Muddy/silt sediments at the south Vestnesa ridge site favored the presence of clavate conical tails, known to be common in this type of substrate (Armenteros *et al*,2009; Semprucci *et al*,2022).

Although without major differences, bacterial mat sediments showed consistently a lower MI (maturity index), and the highest percentage of extreme colonizers (c-p 1), which is usually interpreted with the increased pollution/contamination of the sediments (sewage waste, oil, heavy metals), but also the increase of degraded organic matter as noted in Bongers (1990). The high percentage of juvenile nematodes present at each location (always >30%), could be related to these MI and cp values, given that cp classes were generally low and characteristic of nematodes with short generation time and high reproduction rate (Bongers & Bongers, 1998). A similar result was noticed by Portnova *et al*, (2014).

In addition to observed life-style differences in the nematode communities between bacterial mats and reference sediments, during this study, three different nematode genera (*Halomonhystera* sp., Cyatholaimidae msp1, and *Halanonchus* sp.), showed ovoviviparous reproductive adaptation, an unusual strategy for marine nematodes, known for only a few free-living marine nematode species inhabiting under extreme conditions (e.g., *Metachromadora vivipara* De Man, 1907; *Daptonema matrona* Neres, Fonseca-Genevois, Torres, Cavalcanti, Castro, Da Silva, Rieger & Decraemer, 2010), and only one being described for a cold seep site, at the Hakon Mosby mud volcano in the Barents sea for the nematode *Halomonhystera disjuncta* (Van Gaever *et al*, 2006). The ovoviviparous reproductive behavior is stimulated as a stress reaction upon exposure to for example pollutants or reduced chemical compounds, in which female nematodes hold and carry their brood internally, promoting the internal development of juveniles and ensuring their survival and proper growth, to ultimately release the new recruits with increased motility that will allow them to migrate more easily, vertically in the sediment across the anoxic areas (Van Gaever *et al*,2006; Grzelak *et al*,2016). The fact that this adaptation was also observed in the reference sediments (in *Halanonchus* sp.), may indicate that either there was larger mobility between micro-habitats than expected, or the reference sediments sampled may still be under

the influence of some degree of seepage from its surroundings, which is expected due to the great habitat heterogeneity observed in these ecosystems and fluid regimes.

5. Conclusion

The results strongly suggest that the distribution of metazoan meiofauna and nematodes was influenced by micro-habitat heterogeneity, as observed in other cold seeps worldwide. In general metazoan meiofauna, specifically nematode composition and structural and functional diversity revealed differences not only between and within the micro-habitat of the two investigated sites, suggesting a strong dependency on the local environmental conditions. A higher prevalence of a few nematode genera was recorded at the seeping sites representing a high proportion of the whole community, such as the presence of *Halomonhystera* sp. and Chromadoridae msp1 as the main taxa present at the shallow seep in Prins Karl Foreland, while *Dichromadora* spp. was the most abundant taxa at the deeper seep in the South Vestnesa ridge. Even with lower taxonomical diversity, reduced sediments recorded a higher trophic diversity, which exemplifies a broader variety of strategies (e.g., feeding strategies) of the genera to survive. Furthermore, this study identified ovoviviparous reproduction behavior in three different genera, supporting that besides the other phenotypical adaptations (trophic mode, amphid shape), also changes at the reproductive level seem to allow this phylum not only to survive but also to thrive in these extreme environments where many other benthic groups are unable to survive.

In conclusion, this study emphasizes the impact that reduced environmental conditions at the small spatial scale level (micro-habitat), can influence the infauna communities both in shallow and deep-water seep sites. As the first study on meiofauna and nematode assemblages in cold seeps in the Arctic, this study helps us to have a better understanding of the role of this phylum in this extreme habitat, that is on the verge of being threatened by climate change.

6. References

Alkemade, R., Wielemaker, A., De Jong, S., & Sandee, A. J. J. (1992). Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella*

- dievengatensis* in stimulating the mineralization of *Spartina anglica* detritus. *Marine Ecology Progress Series*, 90(2),149-155. doi: 10.3354/meps090149
- Alongi, D. M., & Tietjen, J. H. (1980). Population growth and trophic interactions among free-living marine nematodes. *Marine benthic dynamics*, (11), 151.
- Anderson, M., Gorley, R. N., & Clarke, R. K. (2008). *Permanova+ for primer: Guide to software and statisticl methods*. Plymouth, UK.
- Armenteros, M., Ruiz-Abierno, A., Fernández-Garcés, R., Pérez-García, J. A., Díaz-Asencio, L., Vincx, M., & Decraemer, W. (2009). Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. *Estuarine, Coastal and Shelf Science*, 85(2), 179-189. doi: 10.1016/j.ecss.2009.08.002
- Åström, E. K., Carroll, M. L., Ambrose Jr, W. G., & Carroll, J. (2016). Arctic cold seeps in marine methane hydrate environments: impacts on shelf macrobenthic community structure offshore Svalbard. *Marine Ecology Progress Series*, 552, 1-18. doi: 10.3354/meps11773
- Åström, E. K., Carroll, M. L., Ambrose Jr, W. G., Sen, A., Silyakova, A., & Carroll, J. (2018). Methane cold seeps as biological oases in the high-Arctic deep sea. *Limnology and Oceanography*, 63(1), 209-S231. doi: 10.1002/lno.10732
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15(4):365–377. doi: 10.1111/j.1461-0248.2011.01736.x
- Bernhard, J. M., Buck, K. R., Farmer, M. A., & Bowser, S. S. (2000). The Santa Barbara Basin is a symbiosis oasis. *Nature*, 403(6765), 77-80. doi:10.1038/47476
- Boetius, A., & Suess, E. (2004). Hydrate Ridge: A natural laboratory for the study of microbial life fueled by methane 31 from near-surface gas hydrates. *Chemical Geology*, 205, 291–310. doi: 10.1016/j.chemgeo.2003.12.034
- Bongers, T., & Bongers, M. (1998). Functional diversity of nematodes. *Applied soil ecology*, 10(3), 239-251. doi: 10.1016/s0929-1393(98)00123-1
- Bongers, T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83(1), 14-19. doi: 10.1007/bf00324627
- Cordes, E. E., Cunha, M. R., Galeron, J., Mora, C., Olu-Le Roy, K., Sibuet, M., Van Gaever, S., Vanreusel, A., & Levin, L. A. (2010). The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. *Marine Ecology*, 31(1), 51-65. doi: 10.1111/j.1439-0485.2009.00334.x
- Culver, D. C., Christman, M. C., Sket, B., & Trontelj, P. (2004). Sampling adequacy in an extreme environment: species richness patterns in Slovenian caves. *Biodiversity & Conservation*, 13(6), 1209-1229. doi: 10.1023/b:bioc.0000018153.49280.89

- De Troch, M., Fiers, F., & Vincx, M. (2000). Range extension and micro-habitat of *Lightiella incisa* (Cephalocarida). *Journal of Zoology*, 251(2), 199-204. doi: 10.1111/j.1469-7998.2000.tb00604.x
- Decker, C., Morineaux, M., Van Gaever, S., Caprais, J. C., Lichtschlag, A., Gauthier, O., Andersen, A. C., & Olu, K. (2012). Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin. Part 1: Macrofaunal community structure. *Marine Ecology*, 33(2), 205-230. doi: 10.1111/j.1439-0485.2011.00503.x
- Dick, H. J. B., Lin, J., Schouten, H., (2003). An ultra-slow spreading class of ocean ridge. *Nature*, 426(6965) 405–412. doi: 10.1038/nature02128
- Ferré, B., Jansson, P. G., Moser, M., Serov, P., Portnov, A., Graves, C. A., Panieri, G., Grundger, F., Berndt, C., Lehmann, M. F., & Niemann, H. (2020). Reduced methane seepage from Arctic sediments during cold bottom-water conditions. *Nature Geoscience*, 13(2), 144-148.
- Foucher, J. P., Westbrook G. K., Boetius, A., Ceramicola, S., Dupré, S., Mascle, J., Mienert, J., Pfannkuche, O., Pierre, C., and Praeg, D. (2009). Structure and drivers of cold seep ecosystems. *Oceanography* 22(1):92–109. doi: 10.5670/oceanog.2009.11
- Galkin, S., Bergmann, M., Soltwedel, T., & Gebruk, A. (2013). Density and distribution of megafauna at the Håkon Mosby mud volcano (the Barents Sea) based on image analysis. *Biogeosciences*, 10(5), 3359-3374. doi: 10.5194/bg-10-3359-2013
- Gebruk, A. V., Krylova, E. M., Lein, A. Y., Vinogradov, G. M., Anderson, E., Pimenov, N. V., Cherkashev, G. A., & Crane, K. (2003). Methane seep community of the Håkon Mosby mud volcano (the Norwegian Sea): composition and trophic aspects. *Sarsia*, 88(6), 394-403. doi: 10.1080/00364820310003190
- Giere, O. (2009). *Meiobenthology: the microscopic motile fauna of aquatic sediments*. (2nd ed.). Springer-Verlag, Heidelberg (526 p.)
- Giere, O., Windoffer, R., & Southward, E. C. (1995). The bacterial endosymbiosis of the gutless nematode, *Astomonema southwardorum*: ultrastructural aspects. *Journal of the Marine Biological Association of the United Kingdom*, 75(1), 153-164. doi: 10.1017/S0025315400015265
- Grove, S.L., Probert, P.K., Berkenbusch, K., Nodder, S.D. (2006). Distribution of bathyal meiofauna in the region of the Subtropical Front, Chatham Rise, south-west Pacific. *Journal of Experimental Marine Biology and Ecology*, 330, 342–355. doi: 10.1016/j.jembe.2005.12.038
- Grzelak, K., & Kotwicki, L. (2016). *Halomonhystera disjuncta*—a young-carrying nematode first observed for the Baltic Sea in deep basins within chemical munitions disposal sites. *Deep Sea Research Part II: Topical Studies in Oceanography*, 128, 131-135. doi: 10.1016/j.dsr2.2014.12.007

- Heip, C., Herman, P. M., & Soetaert, K. (1998). Indices of diversity and evenness. *Oceanis*, 24(4), 61-88.
- Heip, C., Huys, R., & Alkemade, R. (1992). Community structure and functional roles of meiofauna in the North Sea. *Netherlands Journal of Aquatic Ecology*, 26(1), 31-41. doi: 10.1007/bf02298026
- Heip, C., Vincx, M., & Vranken, G. (1985). The ecology of marine nematodes. *Oceanography and Marine Biology: an annual review*.
- Hong, W. L., Sauer, S., Panieri, G., Ambrose Jr, W. G., James, R. H., Plaza-Faverola, A., & Schneider, A. (2016). Removal of methane through hydrological, microbial, and geochemical processes in the shallow sediments of pockmarks along eastern Vestnesa Ridge (Svalbard). *Limnology and Oceanography*, 61(S1), S324-S343. doi: 10.1002/lno.10299
- Heptner, M. V., & Ivanenko, V. N. (2002). Copepoda (Crustacea) of hydrothermal ecosystems of the World Ocean. *Arthropoda Selecta*, 11(2), 117-134.
- Higgins, R. P., & Thiel, H. (1988). *Introduction to the study of meiofauna*. Smithsonian Institution Press (458 p.)
- Hoste, E., Vanhove, S., Schewe, I., Soltwedel, T., & Vanreusel, A. (2007). Spatial and temporal variations in deep-sea meiofauna assemblages in the Marginal Ice Zone of the Arctic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 54(1), 109-129. doi: 10.1016/j.dsr.2006.09.007
- Judd, A. G., Hovland, M., Dimitrov, L. I., Garcia Gil, S., & Jukes, V. (2002). The geological methane budget at continental margins and its influence on climate change. *Geofluids*, 2(2), 109-126. doi: 10.1046/j.1468-8123.2002.00027.x
- Landvik, J. Y., Ingolfsson, O., Mienert, J., Lehman, S. J., Solheim, A., Elverhoi, A., & Ottesen, D. A. G. (2005). Rethinking Late Weichselian ice-sheet dynamics in coastal NW Svalbard. *Boreas*, 34(1), 7-24. doi: 10.1111/j.1502-3885.2005.tb01001.x
- Levin, L.A., & Sibuet, M. (2012). Understanding Continental Margin Biodiversity: A New Imperative. *Annual Review of Marine Science* 4, 79–112. doi: 10.1146/annurev-marine-120709-142714
- McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in ecology & evolution*, 21(4), 178-185. doi: /10.1016/j.tree.2006.02.002
- Moens, T., & Vincx, M. (1997). Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, 77(1), 211-227. doi: 10.1017/S0025315400033889
- Moens, T., Bouillon, S., & Gallucci, F. (2005). Dual stable isotope abundances unravel trophic position of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, 85(6), 1401-1407. doi: 10.1017/S0025315405012580

- Mokievsky, V. (2009). Ekologiya morskogo meiobentosa. *Ecology of Marine Meiobenthos*, Moscow: KMK.
- Mokievsky, V., Filippova, K. A., & Chesunov, A. V. (2005). Nematode fauna associated with detached kelp accumulations in the subtidal zone of the White Sea. *Oceanology*, 45(5), 689-697.
- Niemann, H., Lösekann, T., De Beer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E. J., Schluter, M., Klages, M., Foucher, J. P., & Boetius, A. (2006). Novel microbial communities of the Håkon Mosby mud volcano and their role as a methane sink. *Nature*, 443(7113), 854-858. doi: 10.1038/nature05227
- Okino, K., Curewitz, D., Asada, M., Tamaki, K., Vogt, P., Crane, K., (2002). Preliminary analysis of the Knipovich Ridge segmentation: influence of focused magmatism and ridge obliquity on an ultraslow spreading system. *Earth and Planetary Science Letters* 202(2), 275–288. doi: 10.1016/S0012-821X(02)00790-2
- Pape, E., Bezerra, T. N., Vanneste, H., Heeschen, K., Moodley, L., Leroux, F., Van Breugel, P., & Vanreusel, A. (2011). Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz). *Marine Ecology Progress Series*, 438, 71-83. doi: 10.3354/meps09278
- Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *Journal of theoretical biology*, 13, 131-144.
- Portnova, D. A., Mokievsky, V. O., Haflidason, H., & Todt, K. (2014). Metazoan meiobenthos and nematode assemblages in the Nyegga Region of methane seepage (Norwegian Sea). *Russian Journal of Marine Biology*, 40(4), 255-265. doi: 10.1134/S1063074014040075
- Portnova, D., Mokievsky, V., & Soltwedel, T. (2011). Nematode species distribution patterns at the Håkon Mosby mud volcano (Norwegian Sea). *Marine Ecology*, 32(1), 24-41. doi: 10.1111/j.1439-0485.2010.00403.x
- Powell, E. S., & Bright, T. J. (1981). A Thiobios Does Exist-Gnathostomulid Domination of the Canyon Community at the East Flower Garden Brine Seep. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 66(5), 675-683. doi: 10.1002/iroh.19810660504
- Schmidt-Rhaesa, A. (Ed.). (2020). *Guide to the identification of marine meiofauna*. Pfeil-Verlag, Dr. Friedrich. Munich (607 p.). doi: 10.1111/ivb.12304
- Schneider, A., Panieri, G., Lepland, A., Consolaro, C., Crémière, A., Forwick, M., Johnson, J. E., Plaza-Faverola, A., Sauer, S., & Knies, J. (2018). Methane seepage at Vestnesa Ridge (NW svalbard) since the last glacial maximum. *Quaternary Science Reviews*, 193, 98-117. doi: 10.1016/j.quascirev.2018.06.006
- Schneider, K., & Culver, D. C. (2004). Estimating subterranean species richness using intensive sampling and rarefaction curves in a high density cave region in West Virginia. *Journal of Cave and Karst Studies*, 66(2), 39-45.

- Schratzberger, M., & Ingels, J. (2018). Meiofauna matters: the roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology*, 502, 12-25. doi: 10.1016/j.jembe.2017.01.007
- Seinhorst, J.W., (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, 4, 67–69. doi: 10.1163/187529259x00381
- Semprucci, F., Cesaroni, L., Guidi, L., & Balsamo, M. (2018). Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity?. *Marine environmental research*, 135, 114-122. doi: 10.1016/j.marenvres.2018.02.001
- Semprucci, F., Grassi, E., & Balsamo, M. (2022). Simple Is the Best: An Alternative Method for the Analysis of Free-Living Nematode Assemblage Structure. *Water*, 14(7), 1114. doi: 10.3390/w14071114
- Shannon-Wiener, C. E., Weaver, W., & Weater, W. J. (1949). The mathematical theory of communication. *The Mathematical Theory of Communication*. EUA: University of Illinois Press, Urbana.
- Shirayama, Y. (1992). Studies of meiofauna collected from the Iheya Ridge during the dive 541 of the " SHINKAI 2000". *JAMSTEC Deep Sea Res.*, 8, 287-291.
- Soltwedel, T., Miljutina, M., Mokievsky, V., Thistle, D., & Vopel, K. (2003). The meiobenthos of the molloy deep (5 600 m), Fram Strait, Arctic Ocean. *Vie et Milieu/Life & Environment*, 1-13.
- Suess, E. (2014). Marine cold seeps and their manifestations: geological control, biogeochemical criteria and environmental conditions. *International Journal of Earth Sciences*, 103(7), 1889-1916. doi: 10.1007/s00531-014-1010-0
- Tarasov, V. G., Gebruk, A. V., Mironov, A. N., & Moskalev, L. I. (2005). Deep-sea and shallow-water hydrothermal vent communities: two different phenomena?. *Chemical Geology*, 224(1-3), 5-39. doi: 10.1016/j.chemgeo.2005.07.021
- Thistle, D., & Sherman, K. M. (1985). The nematode fauna of a deep-sea site exposed to strong near-bottom currents. *Deep Sea Research Part A. Oceanographic Research Papers*, 32(9), 1077-1088. doi: 10.1016/0198-0149(85)90063-9
- Thistle, D., Lambshead, P. J. D., & Sherman, K. M. (1996). Nematode tail-shape groups respond to environmental differences in the deep sea. *Oceanographic Literature Review*, 2(43), 181.
- Tita, G., Desrosiers, G., Vincx, M., & Nozais, C. (2000). Predation and sediment disturbance effects of the intertidal polychaete *Nereis virens* (Sars) on associated meiofaunal assemblages. *Journal of Experimental Marine Biology and Ecology*, 243(2), 261-282. doi: 10.1016/S0022-0981(99)00116-1

- Vanaverbeke, J., Merckx, B., Degraer, S., & Vincx, M. (2011). Sediment-related distribution patterns of nematodes and macrofauna: Two sides of the benthic coin?. *Marine Environmental Research*, 71(1), 31-40. doi: 10.1016/j.marenvres.2010.09.006
- Van Gaever, S., Galéron, J., Sibuet, M., & Vanreusel, A. (2009a). Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea. *Deep Sea Research Part II: Topical Studies in Oceanography*, 56(23), 2259-2269. doi: 10.1016/j.dsr2.2009.04.008
- Van Gaever, S., Moodley, L., De Beer, D., & Vanreusel, A. (2006). Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series*, 321, 143-155. doi: 10.3354/meps321143
- Van Gaever, S., Olu, K., Derycke, S., & Vanreusel, A. (2009b). Metazoan meiofaunal communities at cold seeps along the Norwegian margin: Influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep Sea Research Part I: Oceanographic Research Papers*, 56(5), 772-785. doi: 10.1016/j.dsr.2008.12.015
- Vanreusel, A., Van den Bossche, I., & Thiermann, F. (1997). Free-living marine nematodes from hydrothermal sediments: similarities with communities from diverse reduced habitats. *Marine Ecology Progress Series*, 157, 207-219. doi: 10.3354/meps157207
- Wakkaf, T., Allouche, M., Harrath, A. H., Mansour, L., Alwasel, S., Ansari, K. G. M. T., Beyrem, H., Sellami, B., & Boufahja, F. (2020). The individual and combined effects of cadmium, polyvinyl chloride (PVC) microplastics and their polyalkylamines modified forms on meiobenthic features in a microcosm. *Environmental Pollution*, 266, 115263. doi: 10.1016/j.envpol.2020.115263
- Wieser, W. (1953). Sexual dimorphism of the Enchelidiidae (free-living marine nematodes) as a taxonomic problem. *Zoologischer Anzeiger*, 150(7/8), 152-170.
- Williams, N. M., Crone, E. E., T'ai, H. R., Minckley, R. L., Packer, L., & Potts, S. G. (2010). Ecological and life-history traits predict bee species responses to environmental disturbances. *Biological Conservation*, 143(10), 2280-2291. doi: 10.1016/j.biocon.2010.03.024
- Zeppilli, D., Leduc, D., Fontanier, C., Fontaneto, D., Fuchs, S., Gooday, A. J., Goineau, A., Ingels, J., Ivanenko, V. N., Kristensen, R. M., Neves, R. C., Sanchez, N., Sandulli, R., Sarrazin, J., Sørensen, M. V., Tasiemski, A., Vanreusel, A., Autret, M., Bourdonnay, L., Claireaux, M., Coquillé, V., De Wever, L., Rachel, D., Marchant, J., Toomey, L., and Fernandes, D.(2018). Characteristics of meiofauna in extreme marine ecosystems: a review. *Marine Biodiversity*, 48(1), 35-71. doi: 10.1007/s12526-017-0815-z

Appendix

Table A1: Results of PERMANOVA (factors: micro-habitat and site (superstation)) concerning meiofauna density and composition. p -value < 0.05 (*), p -value < 0.01 (**).

Factors	df	SS	MS	Pseudo-F	P (perm)	Perms
<i>Meiofauna</i>						
<i>Density</i>						
Superstation	1	906.95	906.95	4.4046	0.0001**	12
Micro-habitat (Superstation)	2	400.04	200.02	8.2029	0.0214*	8954
Residual	7	170.69	24.384			
Total	10	1477.7				
<i>Composition</i>						
Superstation	1	3409.8	3409.8	1.8132	0.1691	6
Micro-habitat (Superstation)	2	3795.5	1897.8	3.7326	0.003*	8953
Residual	7	3559	508.43			
Total	10	11807				

Table A2: Results of PERMADISP (factors: micro-habitat and site (superstation)) concerning meiofauna density and composition. p -value < 0.05 (*), p -value < 0.01 (**).

Factors	F	df1	df2	P (perm)
<i>Meiofauna</i>				
<i>Density</i>				
Superstation	5.0994	1	9	0.1953
Micro-habitat	15.977	1	9	0.0046*
<i>Composition</i>				
Superstation	46.197	1	9	0.0025*
Micro-habitat	25.16	1	9	0.0063*

Table A3: Density of all metazoan macrofaunal taxa (ind.10cm⁻²; average ±standard error) for each superstation and micro-habitat.

Site (superstation)	Prins Karl Foreland (SS01)		South Vestnesa ridge (SS02)	
	Bacterial mat	Reference	Bacterial mat	Reference
Amphipoda	-	-	0.05 ± 0.053	-
Bivalvia	-	0.16 ± 0.040	-	-
Copepoda	0.53 ± 0.289	0.08 ± 0.040	0.07 ± 0.066	-
Gastropoda	-	-	2.84 ± 2.662	-
Isopoda	-	-	-	-
Nematoda	2.05 ± 0.435	2.00 ± 0.878	0.07 ± 0.035	0.50 ± 0.298
Oligochaeta	-	-	0.03 ± 0.027	-
Ophiuroidea	-	0.04 ± 0.040	-	-
Polychaeta	6.83 ± 3.025	2.52 ± 0.318	2.20 ± 2.101	1.59 ± 0.596
Total (ind.10cm⁻²)	9.41 ± 3.688	4.80 ± 1.232	5.29 ± 4.794	2.09 ± 0.895

Table A4: Average density (± standard error; ind.10cm⁻²) of all nematode genera identified, per site (superstation) and micro-habitat.

Family	Genera	Prins Karl Foreland (SS01)		South Vestnesa ridge (SS02)	
		Bacterial mat	Reference	Bacterial mat	Reference
Aegialoalaimidae	<i>Aegialoalaimus sp.</i>	-	-	-	0.94 ± 0.935
Anticomidae	<i>Anticoma sp.</i>	-	8.74 ± 4.372	-	-
Axonolaimidae	<i>Axonolaimus spp.</i>	-	7.90 ± 5.013	-	0.94 ± 0.935
Chromadoridae	<i>Acantholaimus msp1</i>	-	1.91 ± 1.910	-	-
Chromadoridae	Chromadoridae msp4	62.1 ± 37.09	-	-	2.96 ± 2.962
Chromadoridae	<i>Chromadorita sp.</i>	53.2 ± 22.82	8.49 ± 2.388	0.11 ± 0.105	2.96 ± 20962
Chromadoridae	<i>Dichromadora spp.</i>	21.1 ± 15.99	16.59 ± 5.592	4.84 ± 4.780	0.94 ± 0.935
Chromadoridae	Chromadoridae msp1	334.1 ± 18.71	4.07 ± 2.049	0.11 ± 0.105	1.48 ± 1.481
Chromadoridae	<i>Prochromadora sp.</i>	2.5 ± 2.52	-	-	-
Comesomatidae	<i>Dorylaimopsis sp.</i>	-	138.16 ± 6.718	-	1.48 ± 1.481
Comesomatidae	<i>Laimella sp.</i>	-	1.91 ± 1.910	-	-
Comesomatidae	<i>Sabatieria spp.</i>	10.1 ± 10.09	292.45 ± 51.706	0.30 ± 0.166	165.25 ± 0.618
Cyatholaimidae	Cyatholaimidae msp2	-	12.01 ± 5.456	-	-
Cyatholaimidae	Cyatholaimidae msp1	68.9 ± 25.51	3.82 ± 3.821	-	1.87 ± 1.871
Cyatholaimidae	<i>Pomponema sp.</i>	-	6.03 ± 3.322	-	-

Desmodoridae	<i>Desmodorella sp.</i>	-	-	0.11 ± 0.105	-
Desmoscolecidae	<i>Desmoscolex sp.</i>	2.5 ± 2.52	2.16 ± 2.164	0.25 ± 0.194	7.25 ± 1.637
	<i>Desmoscolex</i>				
Desmoscolecidae	<i>(Prototricomoides) sp.</i>	-	1.91 ± 1.910	0.22 ± 0.218	-
Diplopeltidae	<i>Campylaimus sp.</i>	-	2.16 ± 2.164	-	-
Diplopeltidae	<i>Diplopeltula</i>	-	4.12 ± 2.075	-	-
Diplopeltidae	<i>Southerniella spp.</i>	-	12.65 ± 7.671	-	-
Diplopeltoididae	<i>Diplopeltoides sp.</i>	-	2.16 ± 2.164	-	-
Enchelidiidae	Enchelidiidae msp3	-	2.16 ± 2.164	-	-
Enchelidiidae	<i>Ledovitia sp.</i>	-	4.12 ± 2.075	-	-
Enchelidiidae	<i>Pareurystomina sp.</i>	-	4.07 ± 2.049	-	-
Leptolaimidae	<i>Camacolaimus sp.</i>	-	-	0.14 ± 0.092	-
Leptolaimidae	<i>Leptolaimus sp.</i>	-	1.91 ± 1.910	0.19 ± 0.097	-
Linhomoidae	<i>Desmolaimus sp.</i>	5.1 ± 5.05	6.28 ± 0.278	-	-
Linhomoidae	<i>Linhomoeus spp.</i>	38.6 ± 15.95	6.49 ± 6.492	0.19 ± 0.191	1.48 ± 1.481
Linhomoidae	<i>Linhomoidae msp2</i>	-	-	0.04 ± 0.038	-
Linhomoidae	<i>Metalinhomoeus sp.</i>	2.5 ± 2.52	31.49 ± 17.364	0.04 ± 0.038	7.79 ± 4.053
Linhomoidae	<i>Terschellingia spp.</i>	5.1 ± 5.05	47.20 ± 8.685	0.04 ± 0.038	3.90 ± 2.026
Microlaimidae	<i>Aponema sp.</i>	6.3 ± 3.30	9.85 ± 6.812	0.08 ± 0.077	38.19 ± 26.968
Microlaimidae	<i>Microlaimus sp.</i>	0.9 ± 0.93	18.25 ± 6.390	0.11 ± 0.105	41.31 ± 35.698
		409.9 ±			
Monhysteridae	<i>Halomonhystera sp.</i>	201.23	10.86 ± 7.790	-	-
Monhysteridae	<i>Monhystera sp.</i>	-	-	0.57 ± 0.574	-
Monhysteridae	Monhysteridae msp4	-	-	0.11 ± 0.105	8.42 ± 8.419
Oncholaimidae	<i>Adoncholaimus sp.</i>	-	7.94 ± 5.007	-	1.87 ± 1.871
Oncholaimidae	<i>Viscosia sp.</i>	10.1 ± 10.09	4.37 ± 2.186	-	-
Oxystominidae	<i>Halalaimus spp.</i>	-	33.11 ± 6.819	-	31.26 ± 1.324
Oxystominidae	<i>Oxystomina spp.</i>	-	3.82 ± 3.821	-	1.87 ± 1.871
Oxystominidae	<i>Thalassoalaimus sp.</i>	-	-	-	3.74 ± 3.742
Oxystominidae	<i>Wieseria sp.</i>	-	-	-	0.94 ± 0.935
Phanodermatidae	<i>Micoletzkyia sp.</i>	2.5 ± 2.52	5.73 ± 5.731	-	-
Phanodermatidae	<i>Phanodermatidae msp2</i>	-	1.91 ± 1.910	-	-
Phanodermatidae	<i>Phanodermatidae msp3</i>	-	34.33 ± 13.571	-	-
Richtersiidae	<i>Richtersia sp.</i>	6.0 ± 4.65	6.24 ± 3.757	0.08 ± 0.077	-
Selachinematidae	<i>Halichoanolaimus sp.</i>	-	6.28 ± 0.278	-	-
Siphonolaimidae	<i>Siphonolaimus sp.</i>	-	4.37 ± 2.186	-	-
Sphaerolaimidae	<i>Sphaerolaimus spp.</i>	-	-	-	42.40 ± 4.987

Sphaerolaimidae	<i>Subsphaerolaimus sp.</i>	-	7.94 ± 5.007	-	56.59 ± 3.276
Thoracostomopsidae	<i>Enoplolaimus spp.</i>	-	8.79 ± 5.849	-	-
Thoracostomopsidae	<i>Paramesacanthion sp.</i>	-	-	-	15.90 ± 15.902
Trefusiidae	<i>Halanonchus sp.</i>	-	30.30 ± 12.396	0.04 ± 0.038	-
Trefusiidae	<i>Trefusia sp.</i>	-	23.17 ± 4.706	-	1.87 ± 1.871
Trypiloididae	<i>Bathylaimus sp.</i>	-	2.21 ± 2.208	-	-
Xyalidae	<i>Amphimonhystrella sp.</i>	-	-	-	0.94 ± 0.935
Xyalidae	<i>Daptonema spp.</i>	2.5 ± 2.52	13.67 ± 10.534	0.04 ± 0.038	17.77 ± 17.771
Xyalidae	<i>Elzalia sp.</i>	-	-	-	2.81 ± 2.806
Xyalidae	<i>Steinera sp.</i>	-	4.07 ± 2.049	-	-
		1044.7 ±			
Total		323.84	868.23 ± 40.259	7.57 ± 4.108	465.11 ± 59.145

Table A5 : Results of PERMANOVA (factors: micro-habitat and site (superstation)) concerning Nematoda composition. *p*-value < 0.05 (*), *p*-value < 0.01 (**).

Factors	df	SS	MS	Pseudo-F	P (perm)	Perms
<i>Nematoda</i>						
<i>Composition</i>						
Superstation	1	5858.9	5858.9	0.94168	0.3334	12
Micro-habitat (Superstation)	2	12177	6088.5	3.8237	0.0001**	8932
Residual	7	11146	1592.3			
Total	10	29182				

Table A6 : Results of PERMADISP (factors: micro-habitat and site (superstation)) concerning Nematoda composition. *p*-value < 0.05 (*), *p*-value < 0.01 (**).

Factors	F	df1	df2	P (perm)
<i>Nematoda</i>				
<i>Composition</i>				
Superstation	13.098	1	9	0.0027**
Micro-habitat	8.2558	1	9	0.0743

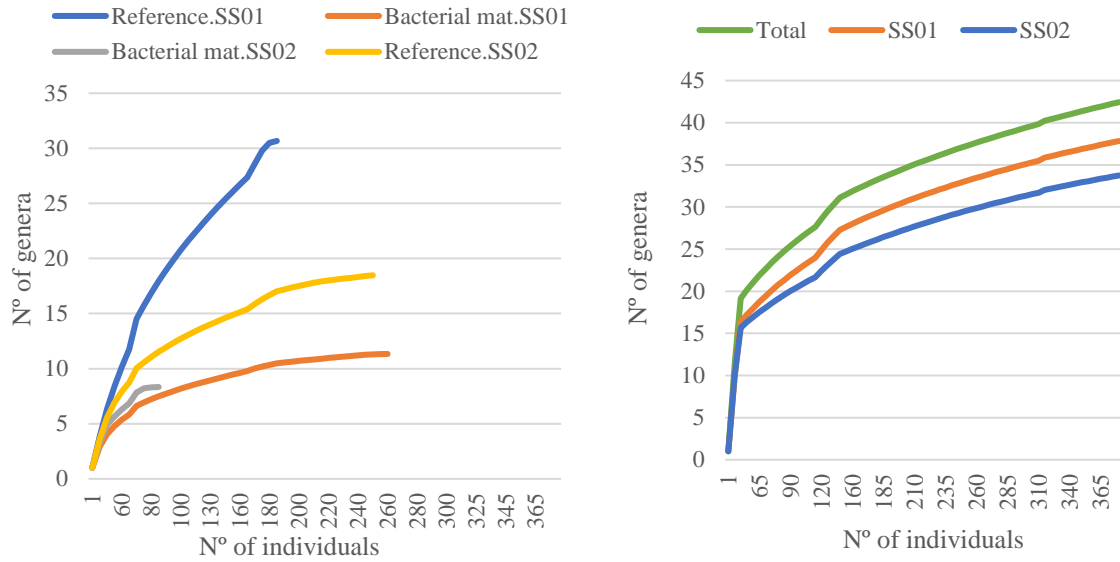


Figure A1: Left: Rarefaction curve based on Hulberts' estimated number of genera (EG) per combination of site-microhabitat. **Right:** per superstation and all sites.

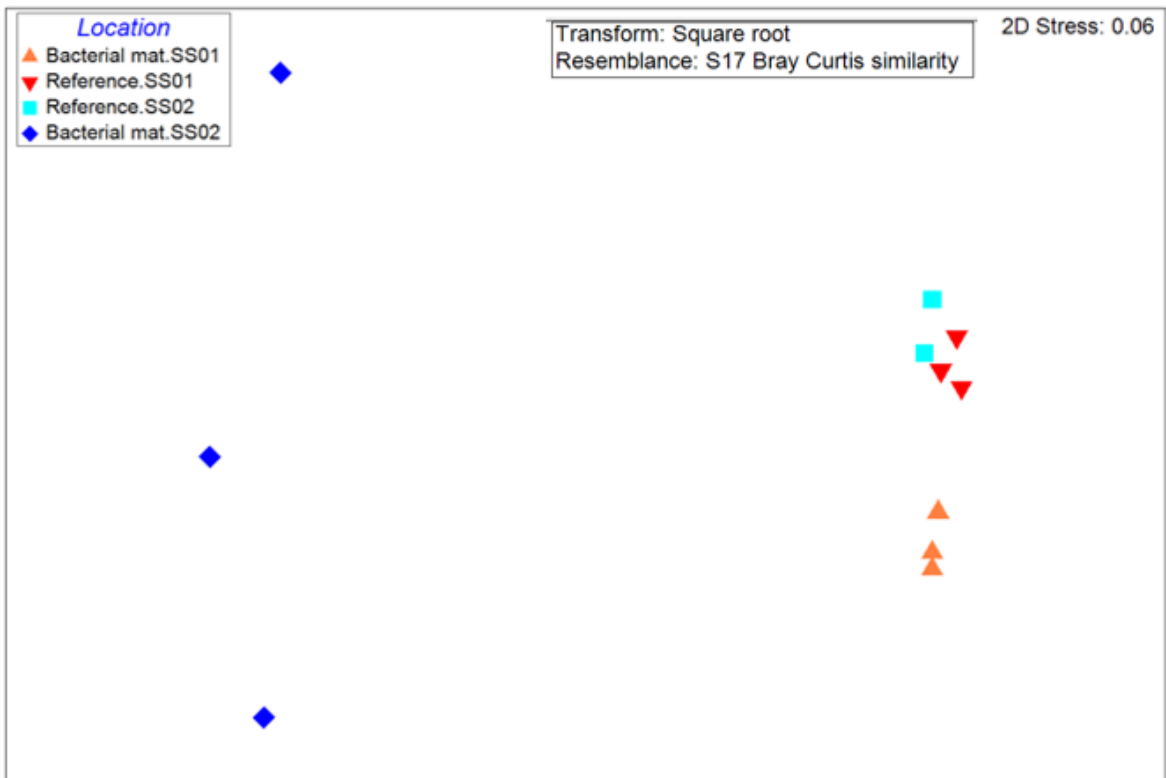


Figure A2: Multi-dimensional scaling (MDS) based on the combination of traits from each micro-habitat from both depths.