Trophic ecology of peripheral fauna at the Fåvne hydrothermal vent field on the Arctic Mid-Ocean Ridge

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Abstract

Fåvne vent field is one of the most recently discovered vents on the Arctic Mid-Ocean Ridge (AMOR) and consists of both active and inactive vents. It was discovered in 2018, and there are only a few published papers on the area to date. Due to the increasing interest in deep-sea mining of rare earth metals and other minerals at AMOR, there is a race to reduce the knowledge gaps in vent areas to better understand how mining will affect life in the surrounding areas. This thesis aims to understand the food web structure of active hydrothermal vent´s background fauna, investigate how connected and dependent they are to the active vents, and examine how decisions are made when knowledge is lacking. To achieve this, specimens and environmental samples collected from Fåvne were tested for Carbon and Nitrogen stable isotopes to create a food web. In addition, DNA sequences were run to identify if there were any cryptic species among the data set. We collected 29 specimens divided into six groups based on morphological classification: *Asconema*, Demospongiae, *Cladorhiza*, Actiniaria, Asteroidea, and Amphipoda. The food web was created and compared to the expected trajectory of a deep-sea habitat. There was no clear indication that the background fauna had any direct connection to the active vents or that the sea floor was a part of the nutrient chain. But there was a large gap between the primary food source and the primary consumer, indicating that the food web lacks one or more trophic levels. This baseline knowledge can be useful for assessing the impact of deep-sea mining as part of a more extensive knowledge base. There will always be knowledge gaps concerning deep-sea ecology and mining hydrothermal vents, and it is, therefore, essential to enlighten and communicate these uncertainties in a transparent manner. And when making decisions, it is important to assess the situation against the best available knowledge. A good principle to use is the precautionary principle, which allows choices to be made based on the best available knowledge and the awareness that the knowledge base may be incomplete and allows for reevaluation if the situation or knowledge should change.

Sammendrag

Fåvne ventilasjonsfelt er en av de sist oppdagede ventilene på den Arktiske Midthavsryggen (AMOR) og består av aktive og inaktive ventiler. Den ble oppdaget i 2018, og det er bare noen få publiserte artikler om området til nå. På grunn av den økende interessen for dyphavsgruvedrift av sjeldne jordmetaller ved AMOR, er det et kappløp for å redusere kunnskapshullene i ventilasjonsområder og for å bedre forstå hvordan gruvedrift vil påvirke livet i områdene rundt. Målet for denne oppgaven er å forstå næringsnettstrukturen til aktiv hydrotermiske ventilers bakgrunns fauna, undersøke hvor tilknyttet og avhengig de er til de aktive ventilene, og undersøke hvordan beslutninger tas når kunnskap mangler. For å oppnå dette ble eksemplarer av dyr og miljøprøver samlet inn fra Fåvne og testet for karbon- og nitrogenstabile isotoper for å lage et næringsnett. I tillegg ble DNA-sekvenser kjørt for å identifisere om det var noen kryptiske arter. Vi samlet inn 29 individer fordelt på seks grupper basert på morfologisk klassifisering: *Asconema*, Demospongiae, *Cladorhiza*, Actiniaria, Asteroidea og Amphipoda. Det ferdige næringsnettet ble sammenlignet opp mot den forventede banen til et dyphavshabitater. Det var ingen klare indikasjoner på at bakgrunns faunaen hadde noen direkte tilknytning til de aktive ventilene eller at havbunnen var en del av næringskjeden. Men det var et stort gap mellom den primære matkilden og primær konsumenten, noe som indikerer at næringsnettet mangler ett eller flere trofiskenivåer. Denne grunnleggende kunnskapen kan være nyttig for å vurdere virkningen av dyphavsgruvedrift som en del av en mer omfattende kunnskapsbase. Det vil alltid være kunnskapshull når det kommer til dyphavs økologi og hydrotermiske ventiler og det er derfor viktig å belyse og kommunisere disse usikkerhetene på en transparent måte. Ved beslutningstaking er det viktig å vurdere situasjonen opp mot den best tilgjengelige kunnskapen. Et godt prinsipp å bruke er føre var prinsippet som lar valg bli tatt på grunnlag av den beste tilgjengelige kunnskapen og med bevissthet om at kunnskapsgrunnlaget kan være både ufullstendig og tillater reevaluering om situasjonen eller kunnskapen skulle endre seg.

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1 Introduction

In this thesis, I will look at food webs in the deep sea, more specifically in areas surrounding the Fåvne hydrothermal vent field on the Arctic Mid-Ocean Ridge as part of the Eco-Safe project, to see how connected the ecosystem is to the output and chemosynthesis from hydrothermal vents. This is currently a crucial topic as the Norwegian government and the International Seabed Authority are considering opening for exploration and exploitation for deep-sea mining. I will, therefore, reflect on the possible effects of mining in the research area and discuss how to make decisions when the uncertainties are high. For me, deep-sea ecology is probably the most intriguing part of biology, as there will always be so much unknown, and it is interesting to see how the process of discovering new species and trying to understand the effects of human interactions and different knowledge can contribute to decision-making at different points in time.

1.1 The deep-sea

My master's thesis focuses on investigating the trophic ecology and food web interactions of deep-sea fauna residing near the Fåvne hydrothermal vent field located on the Arctic Mid-Ocean Ridge. The deep sea, which constitutes 95% of the ocean, remains one of the least explored biomes on the planet, with only approximately 5% of it studied thus far (Levin, 2019; Danovaro et al., 2020). In biological terms, the deep sea encompasses the seabed and water column below 200 meters (Pedersen et al., 2021). Contrary to previous assumptions of its homogeneity, research has revealed that the deep sea is characterized by a diverse range of topographic features, including seamounts, hills, ridges, canyons, and more (Levin, 2019).

Advancements in technology, such as remotely operated vehicles (ROVs), have significantly contributed to our understanding of deep-sea life. ROVs enable us to capture high-resolution images, which can be live-streamed to the surface, providing visual insights into the environment and species inhabiting these depths. Equipped with tools like manipulator arms, ROVs allow pilots to interact with the environment and collect samples in a low- or non-destructive manner, minimizing the impact on the studied habitats (Macreadie et al., 2018;

Levin et al., 2019). This capability facilitates the study of biological community structures within these habitats.

The deep sea, characterized by lower food availability compared to other oceanic regions, has given rise to adaptations among its inhabitants to thrive in low-energy environments, resulting from extreme conditions (Tunnicliffe, Juniper, and Sibuet, 2003; Ramirez-Llodra et al., 2010). Over the past several decades, extensive research efforts have transformed our understanding of the deep sea, shedding light on this previously unknown realm (Levin, 2019). The biodiversity in the deep sea is one of the highest on the planet, and the discovery rate of species and habitats is high for most areas, and the biodiversity has a high evenness (Ramirez-Llodra *et al.*, 2010). However, in extreme environments like hydrothermal vents, biodiversity is generally lower, with a few dominant species contributing to high biomass (Paulus, 2021). While exclusive phyla are not found exclusively in deep waters, the fauna composition at lower taxonomic levels significantly differs from that observed in the upper ocean (Ramirez-Llodra et al., 2010).

In the deep sea, where food and primary production are limited, filter feeders or suspension feeders such as sponges, corals, crinoids, and anemones dominate most habitats. These organisms rely on the deposition of particulate organic matter originating from higher regions of the water column (Gollner et al., 2017), including plankton and carcasses (Danovaro, Snelgrove, and Tyler, 2014). Furthermore, sponges possess the remarkable ability to utilize dissolved organic matter and convert it into forms that can be utilized at higher trophic levels (Olinger et al., 2021). This adaptive mechanism allows them to exploit available resources in the deep-sea environment efficiently.

Chemosynthesis-based ecosystems (CBEs) are an exception in the deep sea, where bacteria utilize reduced compounds found in seeps or vent fluid as an energy source to produce organic carbon. In the absence of sunlight, certain chemosynthetic microorganisms have adapted to convert inorganic CO_2 into organic carbon, analogous to the process of photosynthesis in plants and algae (Smith, 2012). Initially, chemosynthesis was not considered significant in the deep sea until the discovery of hydrothermal vents (Smith, 2012). Chemosynthesis occurs in specific locations where there is readily available chemical energy, such as whale carcasses, wood falls, cold seeps, and hydrothermal vents (Baco and Smith, 2003; Tunnicliffe, Juniper, and Sibuet, 2003). This process attracts higher organisms that establish symbiotic relationships with the

chemosynthesizing microorganisms, leading to the formation of highly specialized ecosystems that are exclusive to these deep-sea areas (Sweetman et al., 2013).

The presence of different substrates and their properties plays a crucial role in determining the fauna that inhabits them. Soft sediments can host infauna, organisms that live within the sediments, while hard substrates provide attachment sites for species such as corals and sponges (Baco and Smith, 2003). Examples of hard substrates that can be found in various deep-sea habitats include hydrothermal vents, cold seeps, whale carcasses, manganese nodules, reef areas, and rock formations. Due to the substantial variations in substrate properties, geographical distribution, and chemical conditions, the fauna composition can vary significantly between different sites with similar substrates. However, it is important to note that different substrate types do not imply a complete separation of fauna. Relationships and connections can be traced between habitats and through food webs, highlighting the interconnectivity and complexity of deep-sea ecosystems (Tunnicliffe, Juniper, and Sibuet, 2003).

1.2 Hydrothermal vents

As mentioned above, hydrothermal vents are a highly specialized ecosystem and contain high concentrations of metals and minerals, making them an attractive area for researchers and the mining industry. Hydrothermal vents are found in the deep sea, specifically at the spreading zones of the seafloor, such as mid-ocean ridges and subduction zones (Martin et al., 2008; Jamieson and Gartman, 2020). Seawater seeps into the cracks and gets geothermally heated and enriched with sulfide complexes with minerals and metals that are discharged under high pressure and get mixed in the water column when pumped out as hydrothermal fluid (Jamieson and Gartman, 2020). The first hydrothermal vent systems were discovered in 1977, and since then, hundreds of vents have since been visually recorded, with many more inferred from physicochemical traces of hydrothermal plumes in the water column. Globally, the active vents system is estimated to occupy less than $50km^2$ of the seabed and are oases of life in the deep sea (van Dover *et al.*, 2002, 2018). The rare and exclusive environment creates unique species compositions on and around the vents, and the environment highly differs between active and inactive vents.

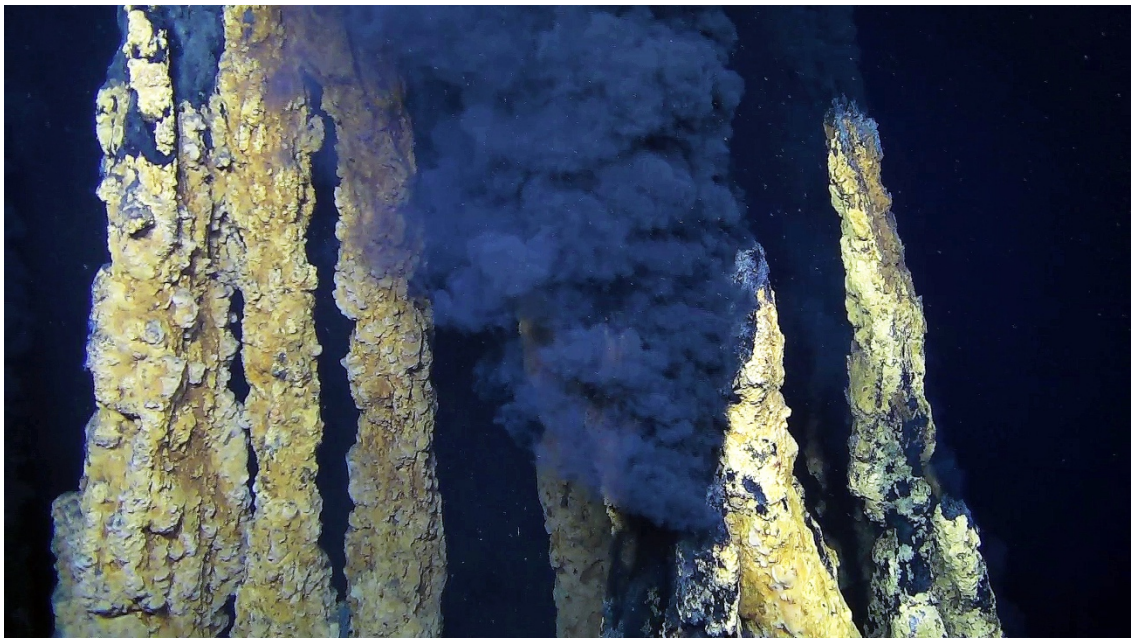

Figure 1.1: Black smoker at Fåvne vent field (credit: Centre for Deep-Sea Research at UiB).

Although active vents may be relatively small, they play a significant ecological role by supporting extensive ecosystems not found in other environments (van Dover *et al.*, 2018). For a vent to mineralize and create chimneys, they need temperatures over 250°C. When the warm water mixes with cold water, the output materials will settle and build chimneys over time. The height and width of the chimney will depend on the temperature of the output water, currents, and the mineral and metal composition of the output water. Certain hydrothermal vents can reach extreme temperatures, up to 350-400°C (Tunnicliffe, Juniper and Sibuet, 2003; van Dover *et al.*, 2018). These vents will discharge water with high concentrations of dissolved metals and minerals and are called black smokers. These vents can be found at the Fåvne vent field as seen in Figure 1.1. When the discharge from black smokers reaches the cold water surrounding the vents, the dissolved substances precipitate and settle on or below the sea floor, forming mineral deposits such as seafloor massive sulfides (SMS) (Gollner *et al.*, 2017; van Dover *et al.*, 2018). The SMS deposits are of great interest commercially due to the high concentration of essential metals, such as gold, silver, copper, and zinc (van Dover *et al.*, 2020). The accumulated volume of SMS will vary from site to site and depends on the active vent's lifetime and the output of water from the vents. The Mid-Atlantic Ridge is an ultra-slow spreading ridge and can therefore persist for hundreds of thousands of years and create great sources of mineral and metal deposits (Tunnicliffe, Juniper and Sibuet, 2003; Pedersen *et al.*, 2021).

All hydrothermal vents will eventually become inactive, which happens when all fluid venting stops. However, the duration of vent activity can be substantial, with vents at slow-spreading ridges remaining stable and active for thousands of years (Jamieson and Gartman, 2020). When there is no chance for reactivation of vents, these inactive vents will then be called extinct (van Dover *et al.*, 2020). Some hydrothermal vents can be temporarily inactive by clogging conduits in the vent or covering the vent. In such cases, there might be some links between different vents through fluid flow in pipes to the underlying heat source, and the vents can be reactivated. Reactivation of vents can happen naturally through tectonic activity or as an effect of human interactions like drilling. Nevertheless, all vents will eventually become permanently inactive, and the creation of new SMS deposits will cease in the area. This will happen when the vent becomes disconnected from the heat source through migration of the ocean floor away from the spreading zone.

1.2.1 Hydrothermal vent biota

Hydrothermal vents are important ecological features. The absence of sunlight in these environments makes it impossible for photosynthesizing organisms like algae to live there (Tunnicliffe, Juniper and Sibuet, 2003; Eilertsen *et al.*, 2017), and possibly making life surrounding vents dependent on chemosynthesis. Most species living on and around vents are endemic species that must adapt to an extreme and highly varying environment (Desbruyères, Hashimoto and Fabri, 2006; van Dover *et al.*, 2018). These ecosystems are considered rare and vulnerable biodiversity hotspots with intrinsic value for genetic diversity and the potential to discover new marine genetic resources (van Dover *et al.*, 2018, 2020). Active hydrothermal vent systems often support dense populations of microbial life and invertebrates. Fluid from these vents is the most chemically complex of reducing solutions in the marine environment and contains many substances that support chemosynthesis (Tunnicliffe, Juniper and Sibuet, 2003).

The presence of cold water surrounding the chimney walls creates a temperature gradient resulting in a diverse environment suitable for a large variety of animal species (Tunnicliffe, Juniper and Sibuet, 2003; Pedersen et al., 2021). Each vent field can have up to hundreds of openings, like chimneys or porous flow through the sediment, creating a mosaic of colonies. Hydrothermal vents in the Arctic are inhabited by specialized fauna that depends on symbiosis with microbes living on the vents (Pedersen *et al.*, 2021). On active vents, we find endemic species of gastropods and amphipods, and the sedimented areas with diffuse venting are dominated by tubeworm forests with other associated fauna. And when the vent sites become inactive, the specialized fauna will be replaced by typical species of the surrounding deep sea, such as sponges, anemones, and crinoids.

There is still some work to be done to define the difference between active, inactive, and extinct vents. Therefore, it is essential to understand what type of biological communities are found at the different sites and how these communities change depending on venting activity. A proposed definition for these venting stages is active vents currently display a flow of hydrothermal fluid, inactive vents have no current flow but can become active again, and extinct vents are not expected to become active again (Jamieson and Gartman, 2020). A clear distinction between inactive and extinct vents is essential to establish criteria for management and conservation in the context of seabed mining, as mining will happen on extinct vents.

1.3 Research on hydrothermal vents in the Arctic mid-ridge (AMOR)

The Arctic mid-ocean ridge (4000km long) is the boundary of the North American and Eurasian plates in the Eurasian basin (Pedersen and Bjerkgård, 2016). Currently, there are nine known active hydrothermal vent fields along the Norwegian part of AMOR, shown in Figure 1.2. Close to Jan Mayen, there are four shallow vent systems Seven Sisters (130m), Soria Moria (700m), Trollveggen (500m), and Perle & Bruse (580m). On the Mohns Ridge, there are four deeper vents systems, Ægir's Kilde (2500m), Mohn's Treasure (2600m), Loki's Castle (2400m), and the focus area for my thesis, Fåvne (3000m). Lastly, one active hydrothermal vent field, Jøtul, was recently discovered on the Knipovich Ridge at 3000m depth. All these fields are currently active, but some of them also have inactive vents.

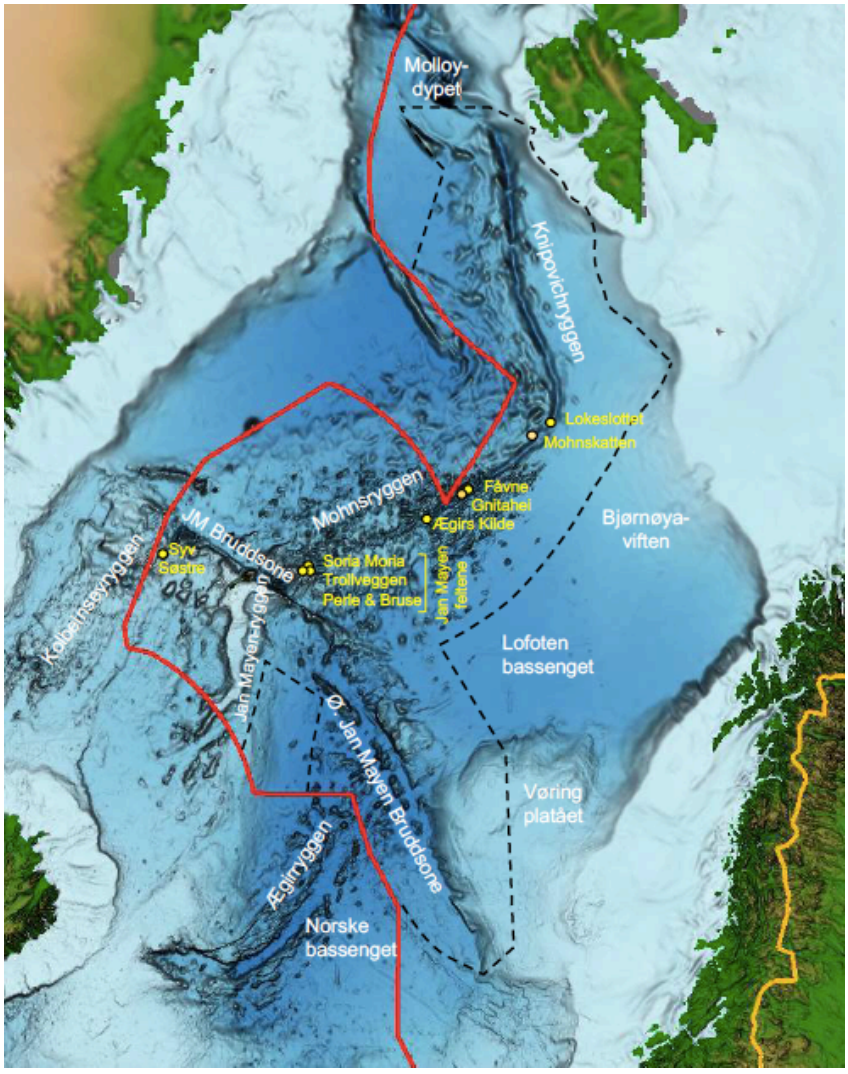

Figure 1.2: Map of the Norwegian continental shelf from Pedersen et al. (2021) with the outer limit of the Norwegian extended continental shelf marked with the red line. The yellow text represents the known hydrothermal vent sites; all vent sites are active except for Gnitahei.

On the Mohn's Ridge, it has been documented that 30% of the sea floor in the axis valley of the ridge is covered by newly formed volcanic masses that are still not covered in sediment (Pedersen *et al.*, 2021). Most of the benthic surveys conducted on the hard substrate in the area have been done at Mohn's Treasure and the Schulz Bank. At Mohn's Treasure, there is a mixture of soft sediments and hard substrates with fauna that represents typical Arctic deep-sea fauna (Lim *et al.*, 2019; Ramirez-Llodra *et al.*, 2020), with various taxonomic groups such as Porifera, Amphipoda, Echinodermata, and Polychaeta (Bluhm et al., 2005). The Schulz Bank is not an active vent site but a seamount with significant variations in depth, water currents, and substrate composition, making it habitable for a large variety of biological communities, including arctic sponge grounds (Meyer *et al.*, 2023).

1.3.1 Fåvne hydrothermal vent field

The vent area at Fåvne, located on the Mohn's Ridge section of the Arctic Mid-Ocean Ridge (AMOR), is the primary focus of this thesis. Notably, Fåvne is one of the region's most recently discovered hydrothermal vents. Its discovery took place in 2018 during a research cruise organized by the Norwegian Petroleum Directorate (Pedersen *et al.*, 2021). While there have been several research cruises to investigate the Fåvne vent area, no research findings from these expeditions have been published to date. This underscores the current lack of scientific knowledge and understanding regarding the biological communities associated with this vent site. Given the relatively recent discovery of Fåvne, conducting research and investigation in this area will contribute to filling the knowledge gap and expanding our understanding of the unique hydrothermal vent ecosystems on the Mohn's Ridge section of the AMOR. The findings from this thesis will provide valuable insights into the trophic ecology and food web interactions of deep-sea fauna in this specific vent field.

The Fåvne vent field is located at 72°45'N, 3°50'E, at a depth of 3000m below the surface, and it is a part of the Mohns Ridge (Pedersen *et al.*, 2021). The seafloor consists of lava covered in approximately half a meter of sediments. The vent field spans about 40 000m^2, with the active part located within an area of 1000m^2, consisting of nine hydrothermal vent structures with varying activity, from black smokers emitting fluids up to 280°C to low-temperature activity with shimmering and inactive vents.

1.4 The economic push for deep sea mining for rare earth elements and metals

Seafloor massive sulfide deposits at hydrothermal vents have gained significant political and economic interest as potential new sources of valuable minerals and metals (Merrie *et al.*, 2014; Sjursen, Bjerga and Nyvoll, 2022). Some vent systems have been active for hundreds to thousands of years, and the long accumulation makes them uniquely rich in metals and, therefore, of high interest to the deep-sea mining industry (van Dover *et al.*, 2018).

Volcanic activity transports geothermic energy from the earth's mantle towards the sea floor and releases heat, creating hydrothermal circulation with seawater under the ocean floor crust (Pedersen et al., 2021). This results in the precipitation of minerals that can create SMS deposits (Van Dover et al., 2020; Jamieson and Gartman, 2020). These deposits can grow over time to such high concentrations that they can have economic value for deep-sea mining. Some can lead to the formation of chemical energy carriers such as hydrogen (H_2), methane (CH_4), sulfide (S_2-), and ferrous iron (Fe_2^+), which microbes can consume through metabolism (Pedersen et al., 2021). Other mineral sources in the deep sea with interest for mining are manganese nodules on abyssal plains and cobalt crust at seamounts (Petersen *et al.*, 2016).

Deep sea mining activities are often justified as contributions to the potential green transition. However, this is very questionable. The use of rare earth elements and metals is not sustainable as extracting these finite metals is assumed to be very destructive to local ecosystems (Childs, 2018; Hallgren and Hansson, 2021). In addition, there are huge concerns about depleting the stock of rare metals as well as the environmental impact of terrestrial (as well as marine) mining (Hallgren and Hansson, 2021). However, deep-sea mining is an industry with a lot of economic power, and the wishful discourses around supporting technological advancements are central to political discourses. In addition, it is assumed that the many technological innovations are relying on batteries for cars and solar and wind power storage that need rare earth metals and therefore drive the prices up, which means that the demand for deep-sea mining will continue to increase (Heffernan, 2019). But a report by SINTEF suggests that new technology will be developed in a way that will have low or no need for critical minerals and that recycling can reduce the need for mineral extraction, reducing the need for new mineral sources (Simas et al., 2022).

1.4.1 The environmental impacts of deep-sea mining and the role of uncertainties in decision-making

Deep-sea mining in international waters is projected to start in the next decade as licenses for exploration have been granted and innovative technology is being developed (Wiltshire, 2017; Drazen *et al.*, 2020), making the need for regulation urgent. Regulation for deep-sea mining in the area beyond national jurisdiction is controlled by the International Seabed Authority (ISA). ISA is a UN body selected to manage mineral resources on the seabed beyond the jurisdiction of the continental shelf of the coastal states, the Area. Although a regulatory framework for the exploitation of seabed minerals in the Area is being negotiated, there is a risk that licenses for mineral exploitation are granted before regulations can be finalized. This is due to the triggering of the "two-year rule" by the Pacific nation of Nauru (Singh, 2021). Simultaneously, there is a growing trend among several countries and large multinational corporations to advocate for a ban or precautionary moratorium on deep-sea mining (Van Dover, 2018). This reflects increasing recognition of the potential environmental risks associated with this activity and the need to protect vulnerable deep-sea ecosystems.

In June 2021, the island republic of Nauru sent a letter requesting the Council of the International Seabed Authority to adopt regulations under section 1, paragraph 15 of the 1994 Agreement relating to the Implementation of Part XI of the United Nations Convention on the Law of the Sea (Ardito and Rovere, 2022). This request triggers the approval process for a plan of work to exploit the mineral resources of the Area, which must be completed within two years. This process has also triggered national governments, such as Norway, to decide on whether they should open for mining. Currently, the government has opened a hearing for exploration for deep sea mining in Norway (Olje- og energidepartementet, 2022). From the hearing, they will decide if mining exploration and exploitation rights will be given to private actors. The deadline for submitting a consultation response to the Impact Assessment was 27 January 2023, and a political decision will be made during the spring of 2023.

In Norway, the government regulates marine protected areas and how marine areas are utilized, including fishing and oil extraction (Havforskningsinstituttet, 2021; *Norges kyst og havområder*, 2021). However, the legal framework around deep-sea mining is not fully developed yet. There are significant uncertainties around the impact of deep-sea mining and how national and global guidelines will align (Thompson *et al.*, 2018). Therefore, an urgent

discussion on how to value and protect hydrothermal vent ecosystems has started (Van Dover *et al.*, 2018). The proposed mining of sulfide deposits on the seabed will have some similarities to open-cut mining on land, where ore is removed down to 20-30 meters into the seabed. However, the physical, biological, and ecological consequences of seabed mining are difficult to compare and highly uncertain (Olje- og energidepartementet, 2022), both for the immediate and the long-term system's ability to recover from mining (Van Dover *et al.*, 2018). It is a political process to decide on the legal guidelines for exploitation based on scientific knowledge. According to Thissen et al. (2017), uncertainties in these heated debates, which are loaded with economic and political interests, can be either downplayed or overemphasized to push one or the other decision. The biggest argument for starting mining is the green transition and the critical need for more minerals, which also creates geopolitical tensions. It is an issue between security and progress, and environmental disaster and precaution (Childs, 2018). The mineral availability has influenced discourses of future recourse security, framing deep-sea mining as necessary to secure future resource flow (Hallgren and Hansson, 2021).

To date, scientific drilling on hydrothermal vents has been the primary form of exploration, which is considerably less damaging compared to large-scale mining operations. However, even this limited activity provides valuable insights into the potential impact of industrial mining and offers a glimpse into the ecosystem's capacity to recover from disturbances (van Dover et al., 2018). Mining will undoubtedly alter the seafloor by changing the structures and textures of the substratum (Van Dover, 2011). Changes in the seafloor structure play a crucial role in determining the species composition and distribution, particularly considering many organisms are substrate-specific (Gollner *et al.*, 2017). A case study conducted at the Iheya North hydrothermal field in the Okinawa Trough of the Pacific Ocean demonstrated how drilling activities could alter the soft sediments surrounding vent areas to a more rigid crust with higher temperatures, resulting in a decline in clam populations and an increase in bacterial mats and squat lobsters (Nakajima *et al.*, 2015). And in contrast, at the TAG (Trans-Atlantic Geotraverse) hydrothermal vents, scientific drilling seemed to have no effect on the shrimp population. An experiment on mining plumes on sea mounts by Sperman et al. (2020) shows that the plume consecration is greater than the natural variation in turbidity but is mainly localized to the mining sites.

There are numerous uncertainties regarding the effects of full-scale mining operations, but it is presumed that hydrothermal systems will not cease but rather alter the distribution of venting activity locally as a result of mining (Van Dover, 2011; Jamieson and Gartman 2020). All areas with mining interest are at risk, but regions with active hydrothermal vents raise even greater concerns due to the potential impact, such as the removal of habitats, release of toxic plumes, and changes in vent fluid (Blanchard and Gollner, 2022). The knowledge we currently have about the deep sea, and the impacts of scientific drilling on these ecosystems, gives us an indication of the potential effects of bulk mining. However, accurately predicting how these stressors will alter the species and ecosystem on a large scale remains impossible.

There are significant knowledge gaps when it comes to hydrothermal vents and their fauna in AMOR. The most researched area on Mohn's Ridge is Loki's Castle, which was discovered in 2008 (Pedersen *et al.*, 2010). There is still much work to describe species in the area, and there is no published species list to date. This means that AMOR has only been included to a small extent in the biogeographic analysis of the global vent fauna (Pedersen *et al.*, 2021). The lack of a complete species list from most of the hydrothermal vent sites along AMOR makes it challenging to describe the variations in the fauna regionally. To better understand AMOR's biogeographic placement, there is a need for a biogeographical analysis of the deep hydrothermal vents, like Fåvne and Loki's Castle.

Only through more scientific research will it be possible to understand these ecosystem's uniqueness, richness, and multiple roles in supporting life. The ability to predict responses from ecosystems to environmental disturbance from mining and other stressors is limited due to this lack of knowledge and research (Blanchard and Gollner, 2022). It is important to note that these ecosystems are complex and hard to reach, and it will therefore take a lot of resources and time to acquire a solid knowledge base of the diversity and ecology of this ecosystem. The long lifetime of these species makes them more vulnerable, and the regeneration time will therefore be long after disturbance (Danovaro et al., 2017). In addition, we cannot claim to be able to predict the full range of short and long-term impacts of mining because these practices are also complex and uncertain. However, filling scientific knowledge gaps will reduce uncertainty to the degree that it enables understanding, assessing, and managing environmental risk.

However, gaining insights into the richness and functioning of these deep-sea ecosystems is essential, as it is also a way to give them value and protect them. Therefore, my thesis addresses one specific knowledge gap relating to understanding possible trophic connections between organisms in different habitats and looking at the food web structure, particularly in my thesis on the Fåvne hydrothermal vent field. Gaining this knowledge will advance our understanding of possible links between vents and fauna living in their periphery and how disturbance on hydrothermal vents along the AMOR may affect benthic communities in the surrounding area.

1.4.2 Legal Framework and Protection

Seabed activity and mining are regulated by national jurisdiction by coastal States and by the International Seabed Authority (ISA) beyond national jurisdiction (Thompson *et al.*, 2018). United Nations Law of the Sea Convention and Sustainable Development Goal 14 demands international calls for the conservation and sustainable use of the sea and mineral resources (van Dover, 2019). Not all inactive vents will need such measurements as some areas are low in sulfide and not of commercial interest. These areas will experience little to no disturbance from anthropogenic activity and they might not hold rare or highly specific fauna. While in areas rich in sulfides and close to active sites or other vulnerable ecosystems, there is a possible elevated risk of mining, and regulations must be in place to ensure effective preservation of these areas.

All coastal states have an Exclusive Economic Zone (EEZ) that reaches a maximum of 200 nautical miles off their shoreline, where they have exclusive rights to manage and utilize the area (Andreone, 2016, p.162). In some areas where a continental shelf is geologically connected to the EEZ, there can be grants for an extension of this zone, becoming an extended exclusive economic zone. Norway has an extended exclusive economic zone according to Article 76 of UNCLOS (Secretary-General of the United Nations, 1982; Brekke, 2020). In some coastal states, measures are in place to protect the hydrothermal vent ecosystem on Extended Continental Shelf and through regional sea conventions (Andreone, 2016, p.164; van Dover *et al.*, 2020). Since there are limited studies on inactive and extinct vent sites, it is difficult to determine the connection of habitats to surrounding non-hydrothermal habitats, making it difficult to predict the impact of mining on local biodiversity (van Dover, 2019; van Dover et al., 2020). Inactive vent systems receive less attention from researchers than active vents

making it even harder to predict the outcome of mining in these areas (Collins, Kennedy and van Dover, 2012; van Dover, 2019).

In Norway, the government decides what areas get protection and how to utilize the resources in the Norwegian extended EEZ (Havforskningsinstituttet, 2021). Through the Natural Diversity Act (Naturmangfoldloven, 2009) or the Marine Resources Act (Havressurslova, 2009) particular and vulnerable species and nature can get protection. Through the Natural Diversity Act, the government can allow for the protection of the seabed and water column. And the act on mineral activities on the continental shelf tells where and who can do mining on the Norwegian continental shelf (Havbunnsmineralloven, 2019). According to §1-6, the state must give permission for mineral activity.

1.5 Thesis aim

Against the heavy political and economic background, my thesis aims to investigate if there is an influence on nutrient sources from active vents for the background fauna and predict how changes or destruction to the active vents can potentially affect the surrounding fauna in terms of food supply. I used stable carbon and nitrogen isotope analysis on selected invertebrate species from inactive chimneys and background areas of Fåvne, together with sediment and water samples, to identify food web structures. In addition, I did DNA barcoding to investigate if there are any cryptic species, as species lists are missing. The results of this study will be discussed in the context of the ecology of Arctic hydrothermal vents, the impact of uncertainty in decision-making, and the sustainability of deep-sea mining.

2 Research method

2.1 Study site

The fieldwork for this study was conducted during a two-week research cruise on the G.O. Sars in July of 2022 at Fåvne hydrothermal vent field in the framework of the Eco-Safe project. Fåvne is located at 3,000 meters depth in the Mohn's Ridge section of the Arctic Mid-Ocean Ridge (Figure 2.1). Since its discovery in 2018, Fåvne has been regularly visited for scientific studies by UiB research cruises on RV G.O. Sars. Multiple studies regarding its geology, geochemistry, biodiversity, and ecology are in progress and remain unpublished to date. Data collected for the Eco-Safe project (funded by the Research Council of Norway and coordinated at the University of Bergen) will contribute to a risk analysis for deep sea mining on Mohn's Ridge.

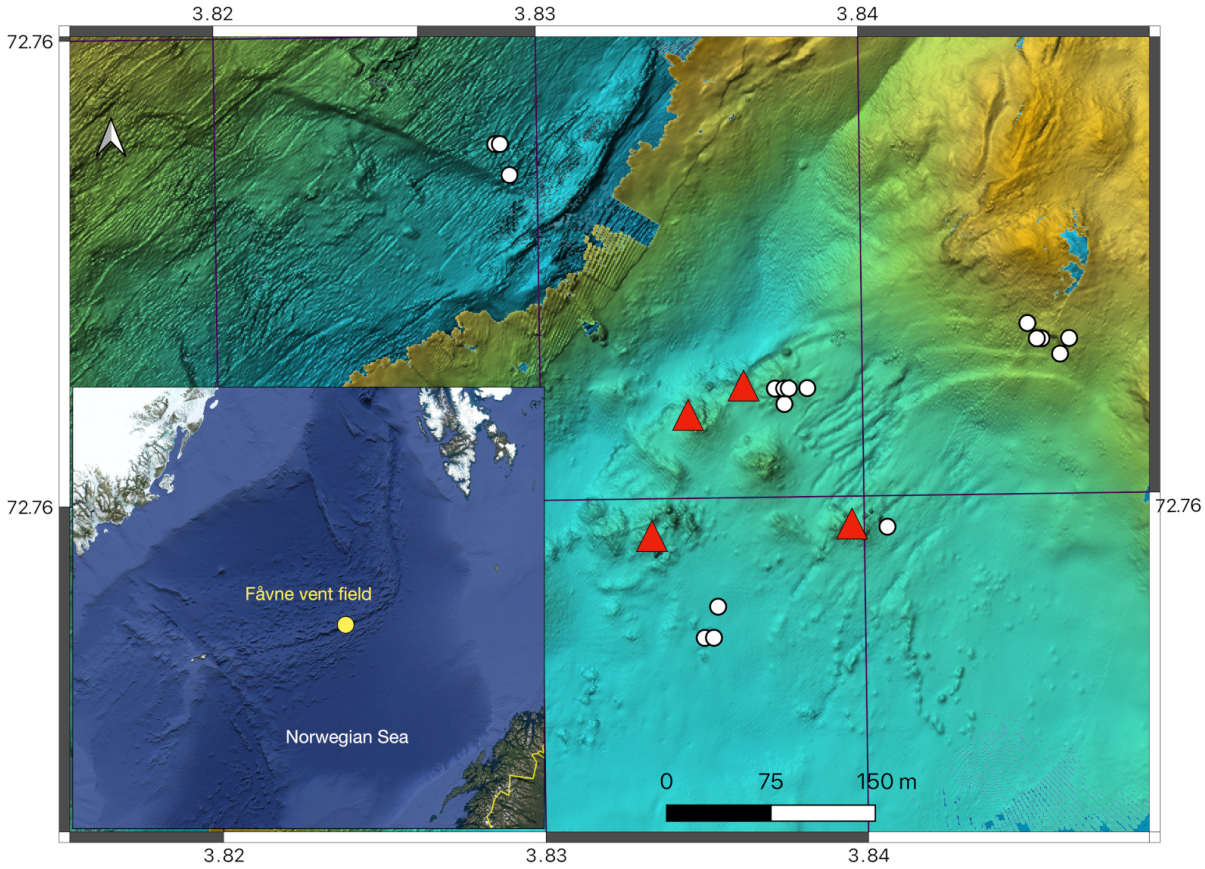

Figure 2.1: Map of Fåvne. The red triangles represent the active vents, and the red circles represent fauna sampling areas

2.2 Sampling

All hydrothermal vent sites will have differences, but we can expect to see some similarities in the fauna. The differences in the habitat structures and temperatures will impact the specie´s composition and distribution. There is no species list for Fåvne, as samples collected previously are still being processed (Pedersen *et al.*, 2021).

Specimens of the most representative background fauna covering as many trophic levels as possible were collected during the 2022 cruise with the ROV Ægir6000, managed by the University of Bergen, as shown in Figure 2.2. The ROV is equipped with arms called manipulators that can pick up or hold samples or equipment and a suction sampler. Other sampling equipment used on the ROV was a blade core to collect sediment and an amphipod trap that was left on the sea floor for a few hours. Fauna was sampled opportunistically, selecting from what was seen on the video transect. *Asconema*, *Cladorhiza* sp., Actiniaria sp.1, Demospongiae sp.1, and Asteroidea were collected from hard substrates, while Actiniaria sp.2 were collected from soft sediment and Amphipoda in a trap on soft sediment.

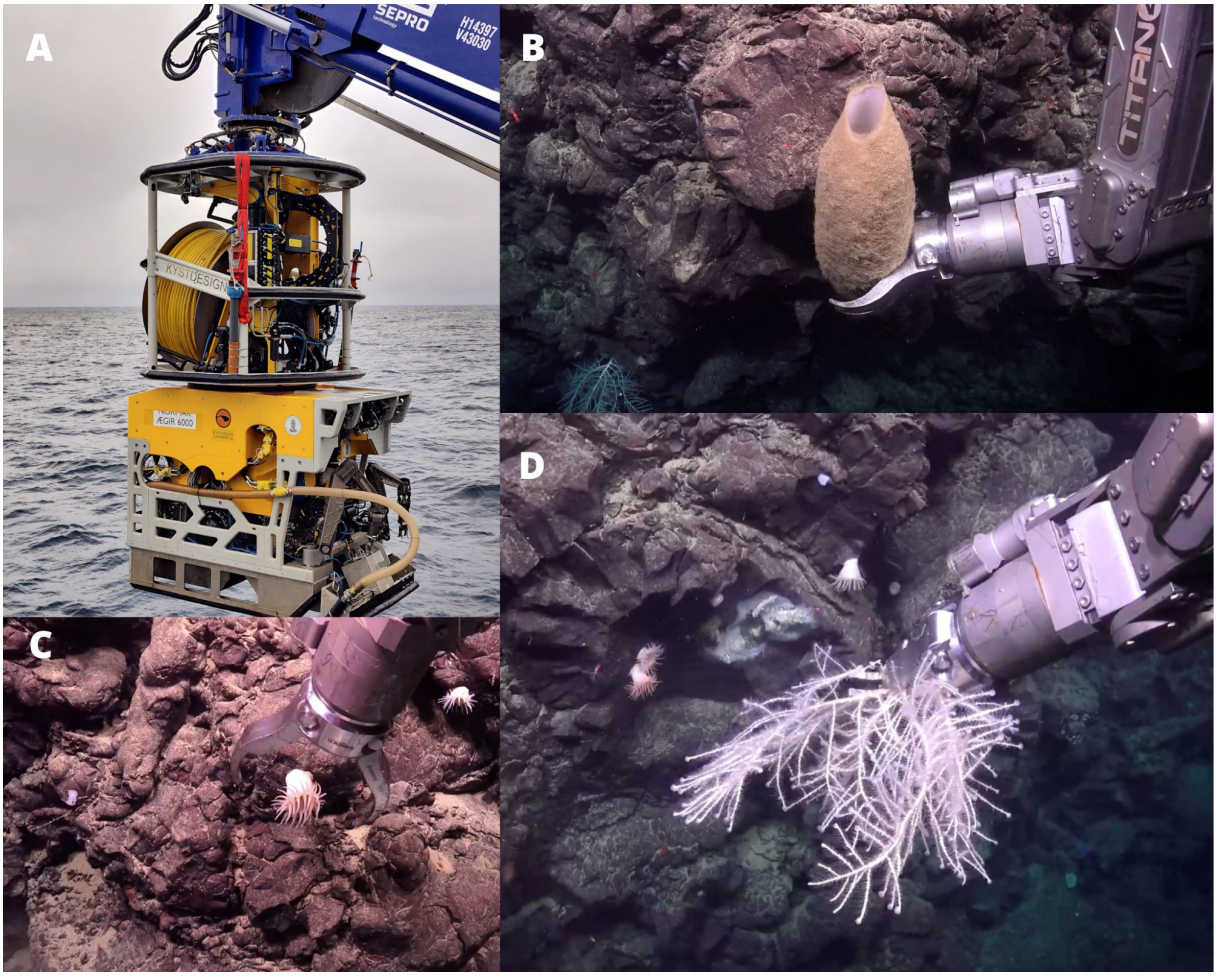

Figure 2.2: A – ROV being deployed from the vessel. B – Collection of Asconema megaatrialia with the manipulator arm. C – Collection of Actiniaria. D – Collection of Cladorhiza sp.

Fauna specimens collected were measured and weighed before being cut into suitable pieces for DNA and stable isotope analysis. Some of these specimens are shown in Figure 2.3. For the larger sponges, cube portions or branches were subsampled for DNA and isotope analysis, and the remaining part of the animal was preserved whole. And for the smaller specimens, legs were sampled for DNA, and the remaining was preserved for isotope analysis. On board the vessel, the samples were fixed in ethanol for DNA barcoding and frozen at -20°C for stable isotope analysis.

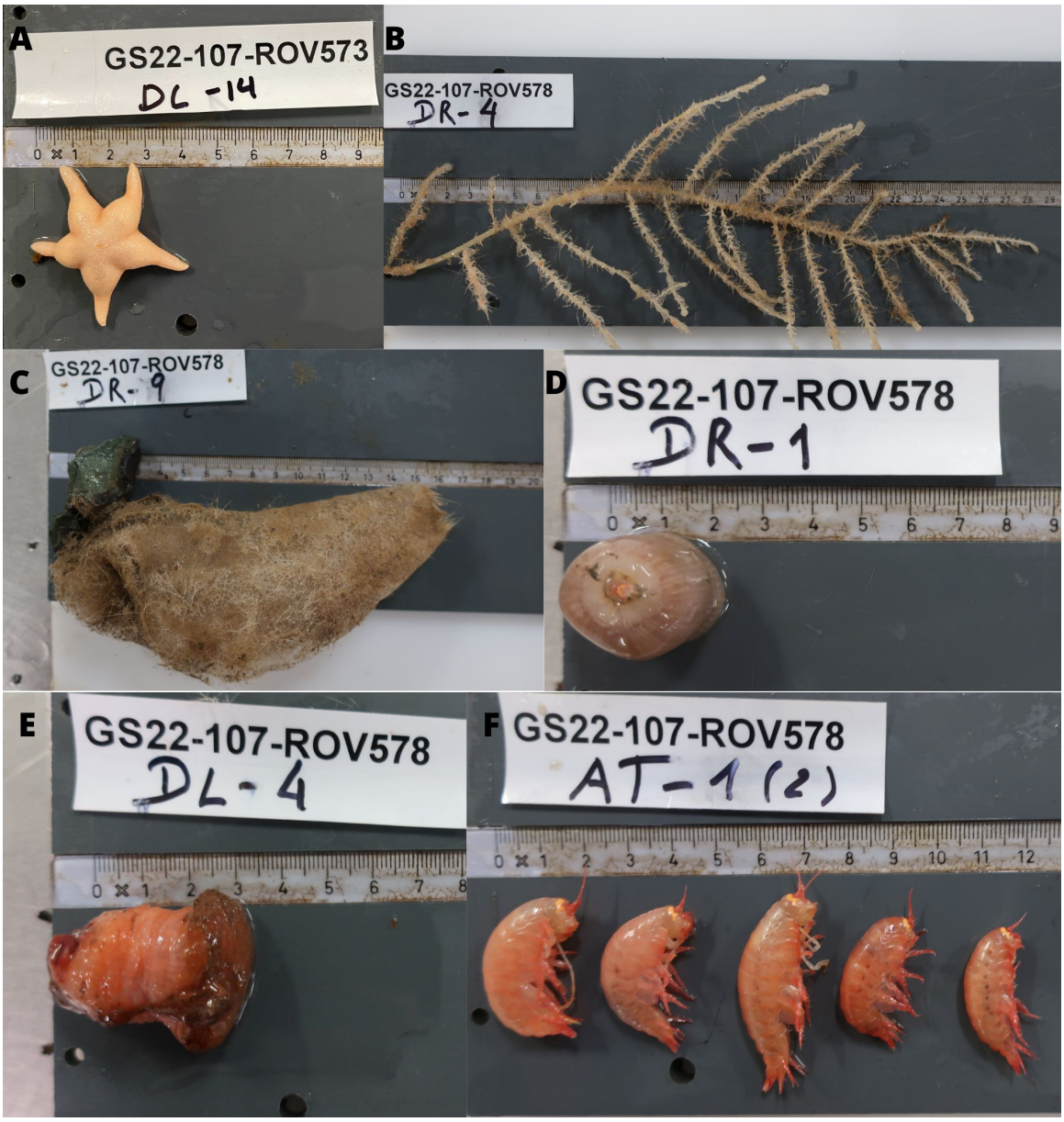

Figure 2.3: A – Asteroidea. B – Cladorhiza.C – Asconema. D – Actiniaria sp.1. E – Actiniaria sp.2. F – Amphipoda

CTD (conductivity, temperature, depth) rosette casts (Figure 2.4A) were done to collect water samples at about 25m depth (at the chlorophyll maximum) and 3000m depth (as close to the bottom as possible). When brought on board, the seawater was filtered through a pre-weighed combusted GFF (glass fiber filter) (Figure 2.4B) and stored at -20°C. The filters were analyzed for stable isotope analysis for nitrogen and carbon isotopes. For the surface water, we used 2*5L, and for the bottom water, we used 2*10L. The filters were stored at -20°C until processing and isotope analysis.

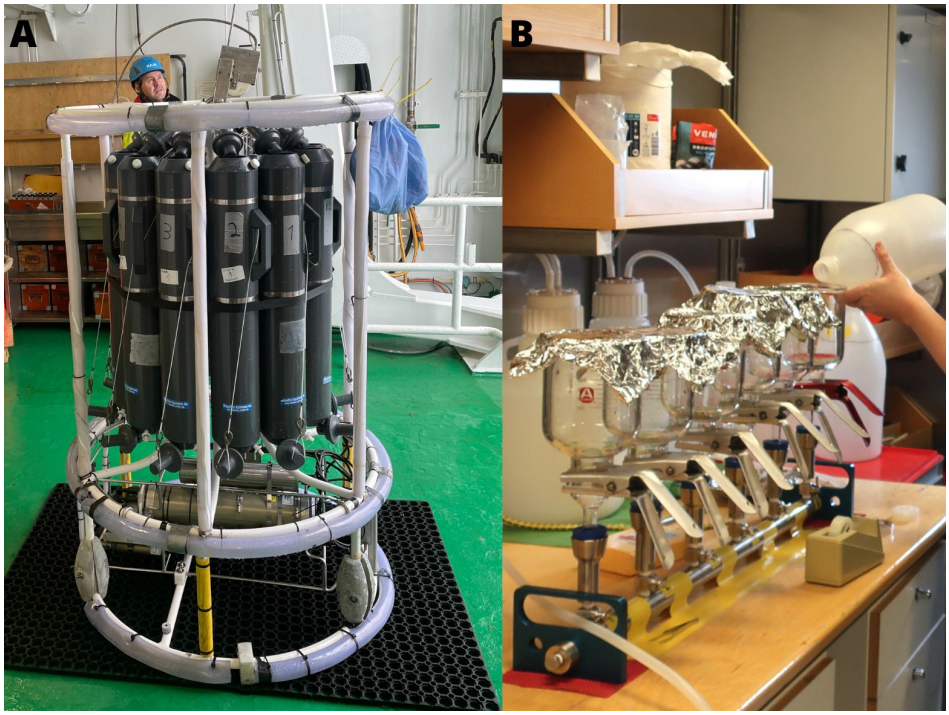

Figure 2.4: A – CTD rosette with tubes for collecting water B – Filtering of water from CTD through GFF filters

Sediment samples were collected with a 4-tube multicore (MUC) that was deployed from the ship over soft sediment areas in the vicinity of Fåvne, as seen in Figure 2.5A, and pulled back on deck with the core samples after reaching the seabed. When the multicore reached the seabed, a trigger mechanism was activated, and core lids were closed so that the sediment would stay in the core tube during recovery. When the MUC (Figure 2.5B) was on board the vessel, we first removed the top layer of water and sampled a small portion of the top 1cm of the core from two cores.

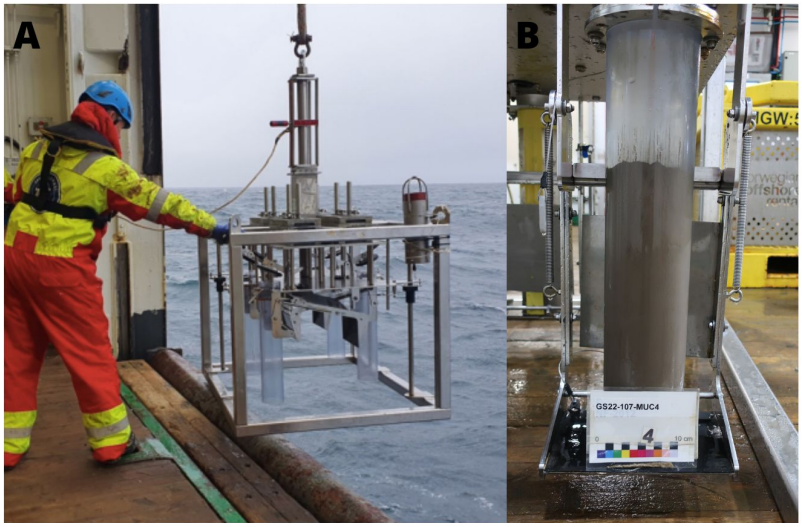

Figure 2.5: A - Multicore before deployment B - Multi tube with sediment from Fåvne

2.3 Specimen identification through DNA barcoding

I performed DNA barcoding analysis on selected specimens to confirm their identification of specimens listed in Table 2.1. This was to ensure that no cryptic species were present, as morphological identification is not entirely reliable for deep-sea fauna, particularly sponges. For the extraction of genomic DNA, I used Qiagen's "QIAwave DNA Blood and Tissue kit" and the kit's protocol for "Purification of total DNA from Animal Tissue." More details are provided in Appendices A.

Table 2.1: Specimens sampled for DNA

Specimen ID.	**Location**	**Date**	**Taxon I**	**Taxon II**
GS22107ROV573 DL-03	Fåvne	12.07.2022	Porifera	*Asconema sp.1*
GS22107ROV573 DL-08	Fåvne	12.07.2022	Porifera	*Cladoriza sp.1*
GS22107ROV573 DL-14	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1
GS22107ROV573 DL-15	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1
GS22107ROV573 DL-16	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1
GS22107ROV578 DR-01	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1
GS22107ROV578 DR-02	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1
GS22107ROV578 DR-03	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1
GS22107ROV578 DR-04	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*
GS22107ROV578 DR-05	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*
GS22107ROV578 DR-06	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*

GS22107ROV578 DR-07	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DR-08	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DR-09	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DR-10	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DR-11	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DR-12	Fåvne	16.07.2022	Porifera	Demospongiae sp.1
GS22107ROV578 DR-13	Fåvne	16.07.2022	Porifera	Demospongiae sp.1
GS22107ROV578 DL-01	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DL-02	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DL-03	Fåvne	16.07.2022	Porifera	*Asconema sp.1*
GS22107ROV578 DL-04	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2
GS22107ROV578 DL-05	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2
GS22107ROV578 DL-06	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2
GS22107ROV578 AT1-4	Fåvne	16.07.2022	Crustacea	Amphipoda sp.1
GS22107ROV578 AT1-5	Fåvne	16.07.2022	Crustacea	Amphipoda sp.2

For DNA barcoding, I amplified a fragment of the 16S rRNA mitochondrial gene using primers 16S1FW (Watkins and Beckenbach, 1999) and 16SH_mod (Adl *et al.*, 2019). The PCR mix consisted of 2.5uL of 10x Taq buffer, 0.15uL of TaKaRa Taq polymerase, 2uL of dNTP mix, 1 uL of each primer, 1uL of DNA and 17.35 uL of ddH2O, making up a final reaction volume of 25 uL. Conditions for PCR amplification are described in Table 2.2.

Table 2.2: Condition for PCR amplification.

Denaturing	Touchdown (x10)	Denaturing	Additional amplification (x25)	Extension
94°C- 3 min	95°C - 45 sec	94°C - 5 min	94°C - 45 sec	72°C - 7 min
	54°C - 30 sec (-0,5 sec per cycle)		48°C - 30 sec	
	72°C – 60 sec		72°C - 60 sec	

In preparation for DNA sequencing, PCR products were cleaned with ExoSAP-IT (Thermo Fisher Scientific) and mixed with sequencing primers, which are the same as the amplification primers. Sequencing was performed in both forward and reverse directions at UiB.

2.3.1 DNA sequence analysis

To better understand the food web and species interaction, I did DNA barcoding to investigate if there are any cryptic species. Cryptic species are morphologically indistinguishable species but are placed in different lineages and therefore are molecularly different (Bickford et al., 2007).

For the analysis of DNA barcoding sequences, I used Geneious Prime 2023.0.4 (Biomatters Ltd.), a program for bioinformatics analysis of DNA sequences. Before inserting the sequences into Geneious, I trimmed the unreadable nucleotide sequence´s ends. Sequence files (fasta) for each species were uploaded separately into Geneious. The reverse sequence for each individual was reverse-complemented to enable alignment with the forward sequence. Then the forward and reverse sequences were aligned. A consensus sequence for each individual was created,

with manual corrections for miss readings or unreadable nucleotides (N) in the sequences whenever possible. All consensuses within each presumed species got aligned and compared to examine if one or more species were present with morphological resemblances.

To check for species identification, the consensus sequences were also compared against the online NCBI nucleotide database using the BLAST tool.

2.3.2 Stable isotope analysis

There is an increase in our knowledge of deep-sea feeding ecology, but there is still a considerable lack of knowledge of the food web structures (Iken *et al.*, 2001). Traditionally in food web studies, we analyze the gut content. But in the deep sea, this is challenging as it is difficult to obtain, and gut content can be damaged during sampling and depressuring effects. An alternative is, therefore, stable isotope analysis. Stable isotope analysis is a valuable tool in areas where direct observations of feeding and samples are difficult to obtain (Erickson, Macko and van Dover, 2009; Hanz *et al.*, 2022). Stable carbon and stable nitrogen can give us much insight into sources of organic matter and trophic relocation in the food web (Middelburg, 2014). The unique $\delta^{13}C$ signatures exhibited by vent microbes serve as excellent biomarkers for chemosynthesis due to the diverse range of metabolic and carbon fixation pathways they employ (Hügler & Sievert, 2011). Hydrothermal vents offer a variety of potential food sources that exhibit distinct $\delta^{13}C$ ratios, including organic matter derived from photosynthesis at the sea surface typically exhibits $\delta^{13}C$ ratios ranging from -24‰ to -22‰, chemo-autotrophs that utilize the Calvin-Benson-Bassham (CBB) cycle to fix carbon through sulfide oxidation typically display $\delta^{13}C$ ratios ranging from -36‰ to -30‰ and carbon fixation process fueled by energy derived from sulfide oxidation often leads to relatively light $\delta^{13}C$ values, reaching as low as approximately -40‰ (Kennicutt et al., 1992; Roohi et al., 2022).

The preparation of fauna and environmental samples started at UiB, and the isotope analysis was conducted at the Royal Netherlands Institute for Sea Research (NIOZ).

2.3.3 Fauna

Samples (Table 2.3) were dried in a stove at 60°C for 2-3 days to remove water, and when dried, the samples were then weighed before being homogenized with mortar and pestle. Then each sample was measured in a silver cup and weight according to the template for isotope analysis (Appendices B). The samples were acidified with 2M HCl in the silver cup to remove all inorganic carbon and left in a stove at 60°C overnight for the acid to evaporate completely.

Table 2.3: Specimens for stable isotope analysis

Specimen ID	**Location**	**Date**	**Phylum**	**Species**	**Weight (mg)**
GS22107ROV573 DL-03	Fåvne	12.07.2022	Porifera	*Asconema* sp.1	4,839
GS22107ROV573 DL-08A	Fåvne	12.07.2022	Porifera	*Cladoriza* sp.1	5,950
GS22107ROV573 DL-08B	Fåvne	12.07.2022	Porifera	*Cladoriza* sp.1 (duplicate)	5,169
GS22107ROV573 DL-14	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1	1,540
GS22107ROV573 DL-15	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1	1,548
GS22107ROV573 DL-16	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1	1,278
GS22107ROV573 DL-16 (duplicate)	Fåvne	12.07.2022	Echinodermata	Asteroidea sp.1	1,575
GS22107ROV578 DR-01A	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,646
GS22107ROV578 DR-01B	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,562
GS22107ROV578 DR-02A	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,821
GS22107ROV578 DR-02B	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,598
GS22107ROV578 DR-03A	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,195
GS22107ROV578 DR-03B	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.1	1,540
GS22107ROV578 DR-04A	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	6,027

GS22107ROV578 DR-04B	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	5,395
GS22107ROV578 DR-05A	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	5,853
GS22107ROV578 DR-05B	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	6,228
GS22107ROV578 DR-05B (Duplicate)	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	4,829
GS22107ROV578 DR-06A	Fåvne	16.07.2022	Porifera	*Cladorhiza sp.1*	5,532
GS22107ROV578 DR-06B	Fåvne	16.07.2022	Porifera	*Cladorhiza sp1*	4,930
GS22107ROV578 DR-07A	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,788
GS22107ROV578 DR-07B	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	6,629
GS22107ROV578 DR08-A	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,548
GS22107ROV578 DR-08B	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,303
GS22107ROV578 DR-08B (Duplicate)	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	4,719
GS22107ROV578 DR-09	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	6,078
GS22107ROV578 DR-10	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	4,868
GS22107ROV578 DR-11A	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	6,266
GS22107ROV578 DR-11B	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	6,033
GS22107ROV578 DR-12	Fåvne	16.07.2022	Porifera	Demospongiae sp.1	5,242
GS22107ROV578 DR-13A	Fåvne	16.07.2022	Porifera	Demospongiae sp.1	5,162
GS22107ROV578 DR-13B	Fåvne	16.07.2022	Porifera	Demospongiae sp.1	6,555
GS22107ROV578 DL-01	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,314
GS22107ROV578	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,826

DL-01 (Duplicate)					
GS22107ROV578 DL-02	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	5,106
GS22107ROV578 DL-03	Fåvne	16.07.2022	Porifera	*Asconema sp.1*	4,767
GS22107ROV578 DL-04	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2	1,449
GS22107ROV578 DL-05A	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2	1,791
GS22107ROV578 DL-05B	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2	1,520
GS22107ROV578 DL-06A	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2	1,443
GS22107ROV578 DL-06B	Fåvne	16.07.2022	Cnidaria	Actiniaria sp.2	1,230
GS22107ROV578 AT1-1	Fåvne	16.07.2022	Arthropoda	Amphipoda sp.3	1,328
GS22107ROV578 AT1-2	Fåvne	16.07.2022	Arthropoda	Amphipoda sp.4	1,448
GS22107ROV578 AT1-3	Fåvne	16.07.2022	Arthropoda	Amphipoda sp.5	1,803
GS22107ROV578 AT1-4	Fåvne	16.07.2022	Arthropoda	Amphipoda sp.1	1,251
GS22107ROV578 AT1-5	Fåvne	16.07.2022	Arthropoda	Amphipoda sp.2	1,244

2.3.4 Sediment

Two sediment samples from off-vent sites were freeze-dried to remove water and homogenized with mortar and pestle. Then the samples were subsampled to 0.5-1.0g per sample with two samples from each location. The subsamples were then acidified with 2M HCl to remove organic carbon. The acid was added until there were no more reactions in the solution. When there was no reaction, acid was added so it covered 1cm over the sediment and transferred to a shaking table and left overnight. The sediment was then rinsed with distilled water until the pH was about 7, before being freeze-dried and homogenized again. The homogenized samples were then weighed and compacted into silver cups (Table 2.4).

2.3.5 Water filtering for SPOM analysis

The samples GFF filters were freeze-dried to remove the moisture and weight, and then the filters were subsampled into quarters (25m depth) or half (3000m depth), depending on the depth from which the samples were collected. The subsamples were put into a desiccator with 37% HCl gas and left overnight to remove inorganic carbon, then moved to an oven and left at 60°C overnight before each filter was folded and compacted into silver cups (table 2.4).

Table 2.4: Water and sediment samples for stable isotope analysis.

Cruise and dive	**Location**	**Date**	**Depth**	**Amount collected**	**Sample nr.**	**Weight (mg)**
GS22107-2 CTD-2	Fåvne	13.07.2022	24 m	5 L	FB46	17,661
GS22107-2 CTD-3	Fåvne	13.07.2022	2751 m	10 L	FB57	41,081
GS22107 CTD-3	Fåvne	13.07.2022	25 m	4 L	FB60	23,005
GS22107 CTD-4	Fåvne	13.07.2022	3016m	10 L	FB59	41,250
-	-	-	-	Blank	FB50	30,187
GS22107MUC	Fåvne	13.07.2022	-	0,508 g	MUC-1A	63,148
GS22107MUC	Fåvne	13.07.2022	-	0,660 g	MUC1-B	70,995
GS22107MUC	Fåvne	13.07.2022	-	0,809 g	MUC-2A	67,411
GS22107MUC	Fåvne	13.07.2022	-	0,629 g	MUC-2B	59,520

2.3.6 Isotope analysis

All the samples were analyzed for concentration of total nitrogen ($\delta^{15}N$) and total organic carbon ($\delta^{13}C$) on a Delta V Advantage isotope ratio MS coupled online to an Elemental analyzer (Flash 2000 EA-IRMS). Mass spectrology separates the different ions by using an electromagnetic field and measures the abundance of each ion present (Murayama et al., 2009). The silver cups prepared in the previous step got transferred to the Delta V analyzer. The first step is the combustion of the solid material, which transforms into a gaseous product, N_2, and CO_2. Then the δN and δC get determined in the isotope mass-spectrometer, where they get separated based on their mass and measured for three different masses (g/mol), 28, 29, and 30 for N_2, and 44, 45, and 46 for CO_2. Acetanilide is used as a correction standard, and Casein and Urea are used as a control standard. The precision of replicate measurements for both δ13C and δ15N was ±0.15‰.

3 Results

3.1 DNA barcoding

As shown in Table 2.1, 26 specimens were subsampled for DNA barcoding, which we separated into six groups based on morphological classification: *Asconema* sp., Demospongiae, *Cladorhiza* sp., Actiniaria, Asteroidea, and Amphipoda.

3.1.1 *Asconema* sp. 1

DNA barcoding analysis supports the presence of only one species, most likely *A. megaatrialia* (see Neighbour-Joining tree in Figure 3.1).

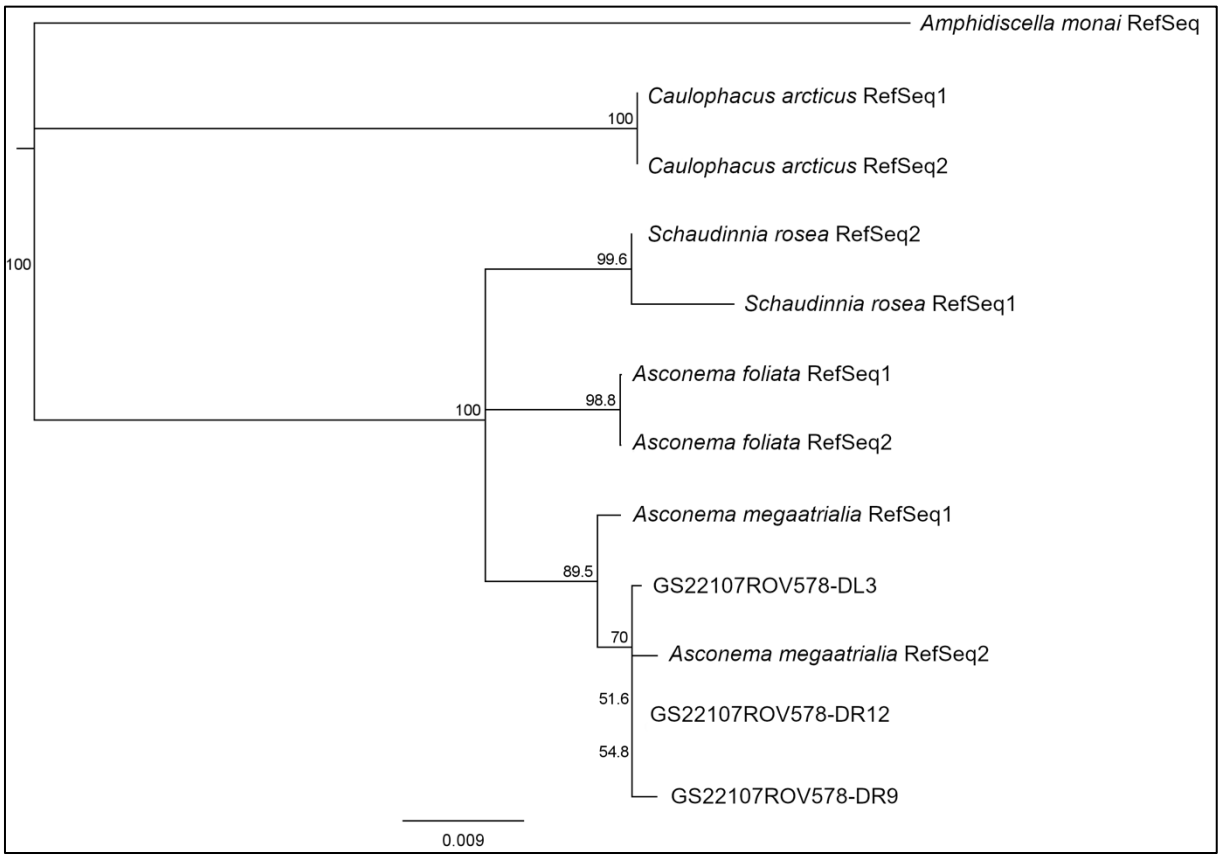

Figure 3.1: Neighbour-joining phylogenetic tree based on the Tamura-Nei genetic distance model obtained from 16S DNA sequence alignment of putative A. megaatrialia specimens collected for this thesis and reference sequences for some of the more closely related glass sponge species found in the study region. Bootstrap support values (1,000 replicates) are indicated, support threshold was set to 50%. RefSeq: reference sequence (unpublished).

3.1.2 Demospongiae sp.1

Out of two samples processed, only one came back as applicable. The DNA sequences did not return any hits due to a lack of references for the 16S sequence.

3.1.3 *Cladorhiza* sp. 1

The 16S DNA sequences for the three specimens are identical, indicating they belong to the same species. We could not build an NJ tree due to lacking reference 16S sequences. For this reason, there are also no relevant hits on the NCBI Blast database. Specimens are therefore classified as *Cladorhiza sp.* based on visual examination until DNA sequences from another marker become available (either 28S or 18S).

3.1.4 Actiniaria

Both morphology and DNA barcoding indicate the presence of two species. DNA sequences from Actiniaria sp.1 cluster together with a reference sequence from *Anthosactis janmeyeni* (Danielssen, 1890) in the Neighbour Joining tree (Figure 3.2), providing strong evidence that this is the correct species identification. Actiniaria sp.2 grouped in the same clade as three deep-sea species belonging to the family Kadosactinidae. However, it was not possible to reach species-level identification.

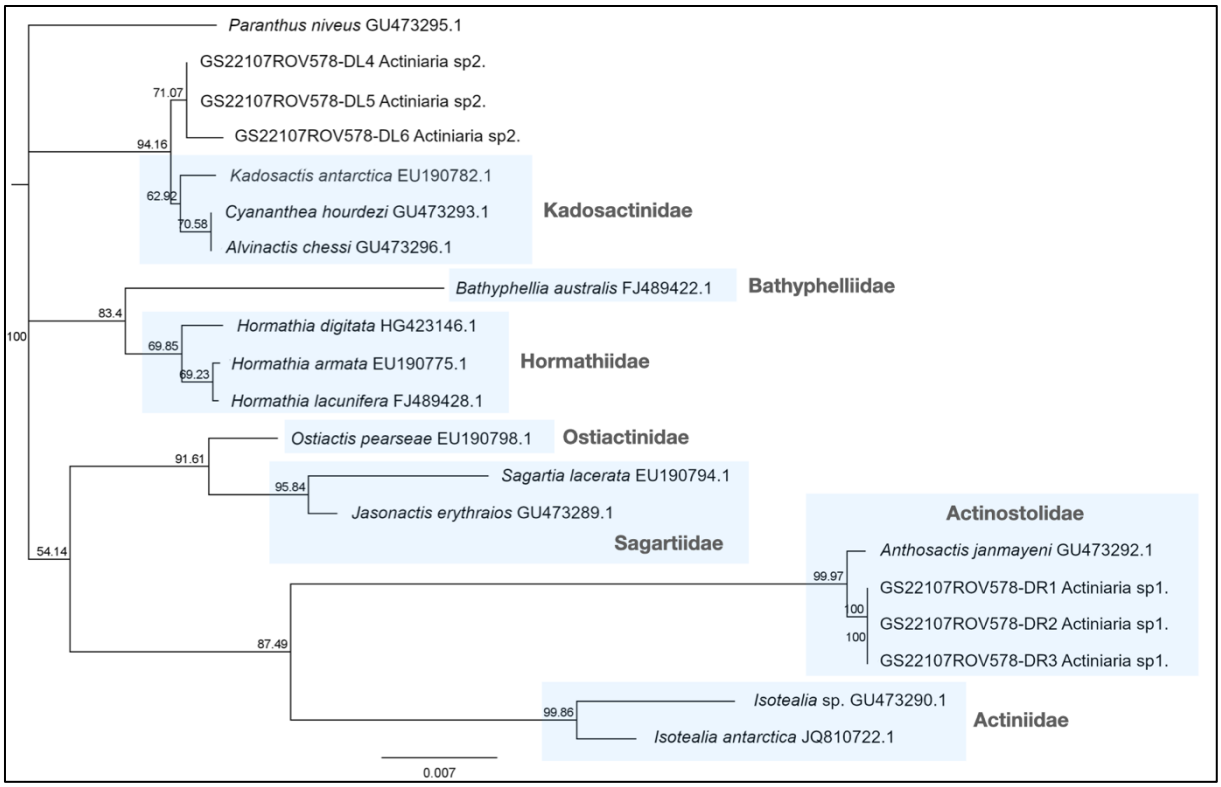

Figure 3.2: Neighbour-joining phylogenetic tree based on the Tamura-Nei genetic distance model obtained from 16S DNA sequence alignment of actiniarian specimens collected for this thesis and reference sequences downloaded from NCBI Genbank. Selection of reference sequences was based on Rodriguez et al. (2014). Bootstrap support values (1,000 replicates) are indicated, support threshold was set to 50%.

3.1.5 *Tylaster willei*

Preliminary visual examination of the collected specimens indicated that these belong to the family Poraniidae, and to only one species. The Neighbour-Joining phylogenetic tree built from the 16S sequences of these specimens with reference sequences of Poraniidae obtained from NCBI Genbank confirmed the presence of only one species but was inconclusive about its identity (Figure 3.3). Based on external morphology and existing occurrence records for the Norwegian Sea, Asteroidea sp.1 can be one of two possible species: *Poraniomorpha tumida* (Stuxberg, 1878) and *Tylaster willei (*Danielssen & Koren, 1881). However, the absence of large blisters on the upper side (characteristic of *P. tumida* – see Figure 3.4B), as well as the distant placing from *Poraniomorpha* in the phylogenetic tree strongly suggests that Asteroidea sp.1 may be *Tylaster willei* (Figure 3.4A). Full confirmation of species identity will require access to validated *T. willei* specimens for further taxonomic and molecular work.

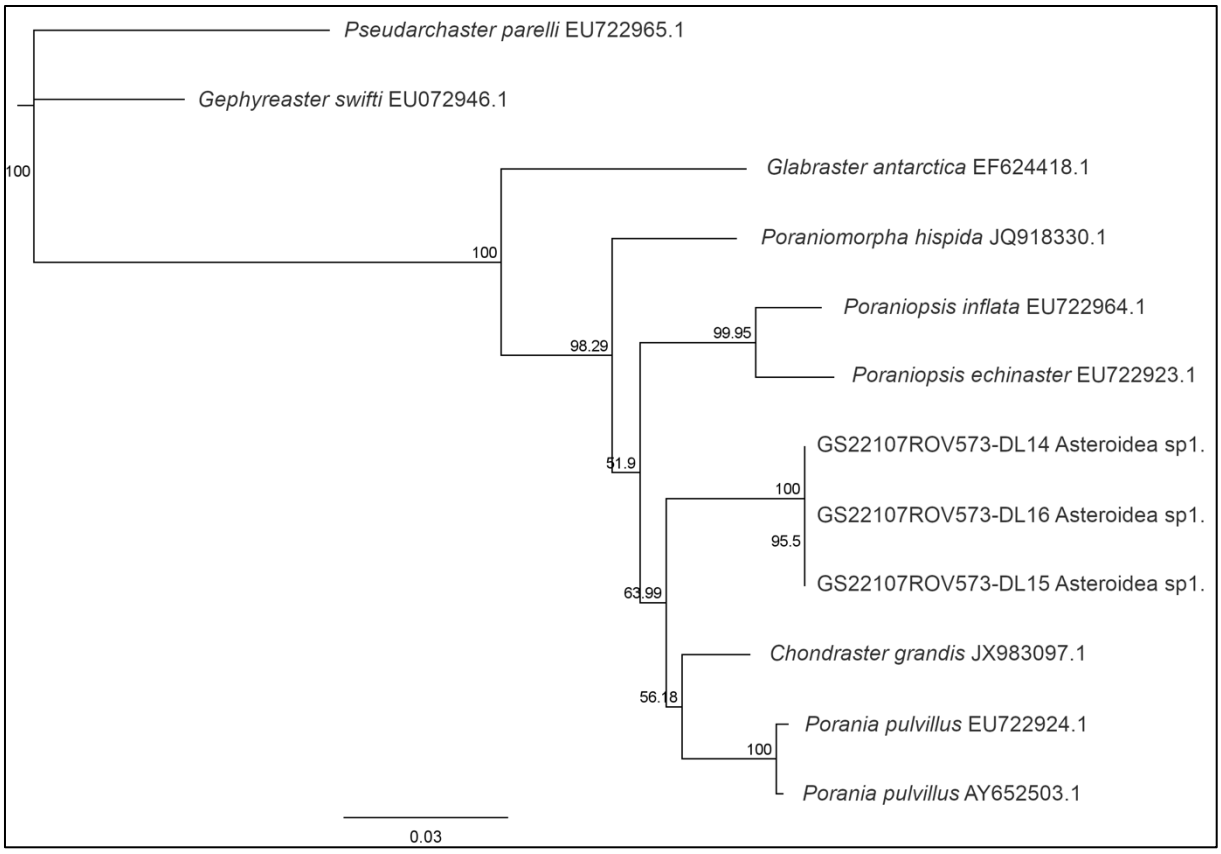

Figure 3.3: Neighbour-joining phylogenetic tree based on the Tamura-Nei genetic distance model obtained from 16S DNA sequence alignment of asteroidea specimens collected for this thesis and reference sequences from Poraniid species (Mah & Foltz 2014). Bootstrap support values (1,000 replicates) are indicated, support threshold was set to 50%.

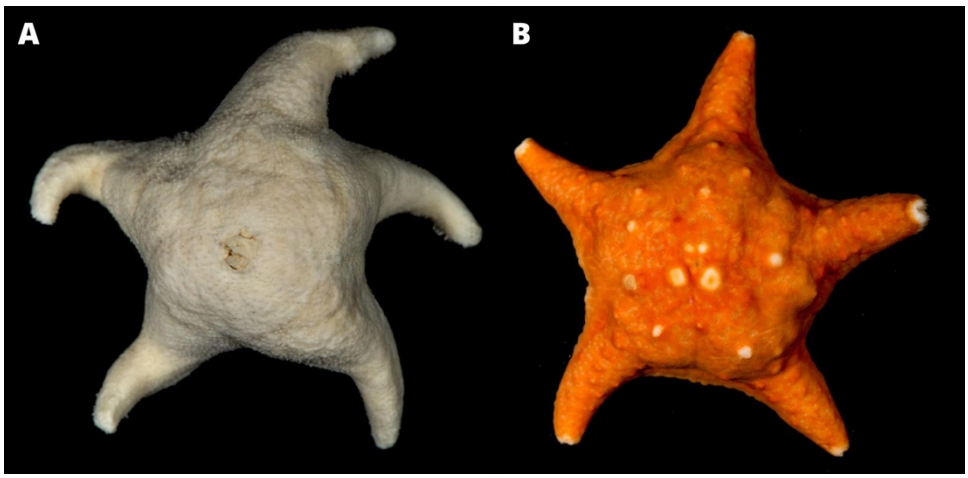

Figure 3.4: A - Tylaster willei (Danielssen & Koren, 1881) B - Poraniomorpha tumida (Stuxberg, 1878). Pictures by Espen Rekdal from the Artsdatabanken website.

3.1.6 Amphipoda

Morphologically the Amphipoda subsampled for DNA barcoding, all look like distinct species, which is confirmed by the DNA barcoding. Due to the lack of replicates, cryptic species cannot be ruled out. Specimens could not be identified to species level with the 16S marker.

3.2 Stable carbon and nitrogen isotope analysis

All samples from the stable isotope analysis came out with good values except for the bottom water samples which were too low to be used in a food web. Organic particles in the surface water ranged between 2.80‰ and 3.15‰ for δ^{15}N, –26.82‰ and –26.31‰ for δ^{13}C (shown in figure 3.5). We got such low readings for organic particles in the bottom filters that the results were unreliable. Sediment particles collected with the multicore had values of δ^{15}N between 5.17‰ to 6.08‰ and for δ13C between –23.08‰ to –22.05‰. For the Amphipoda (sp.1, sp.2, sp.3, and sp.4), the primary consumer had a range of 9.76‰ to 12.71‰ of δ^{15}N and –45.29‰ –24.15‰ of δ^{13}C. The *Cladorhiza* sp.1 ranged from 11.34‰ to 15.06‰ of δ^{15}N –23.57‰ to –21.83‰ of δ^{13}C. *Anthosactis janmeyeni* varied between 12.57‰ to 13.73‰ of δ^{15}N and –23.05‰ to –19.50‰ of δ^{13}C. Actiniaria sp.2 is different from *Anthosactis janmeyeni* and ranges from 14.92‰ to 15.94‰ of δ^{15}N and –21.70‰ –18.52‰ of δ^{13}C. *Asconema* sp.1 overlaps with multiple species and have a range of δ^{15}N from 14.13‰ to 16.36‰ and for δ^{13}C from –21.81‰ to –20.47‰. Demospongiae sp.1 is more grouped together than other species and has a range of 17.29‰ to 17.64‰ of δ^{15}N and –20.60‰ to –20.20‰ of δ^{13}C. The top predator in the system is *Tylaster willei* which has a range of 15.38‰ to 18.37‰ for δ^{15}N and –21.62‰ to –19.38‰ for δ^{13}C.

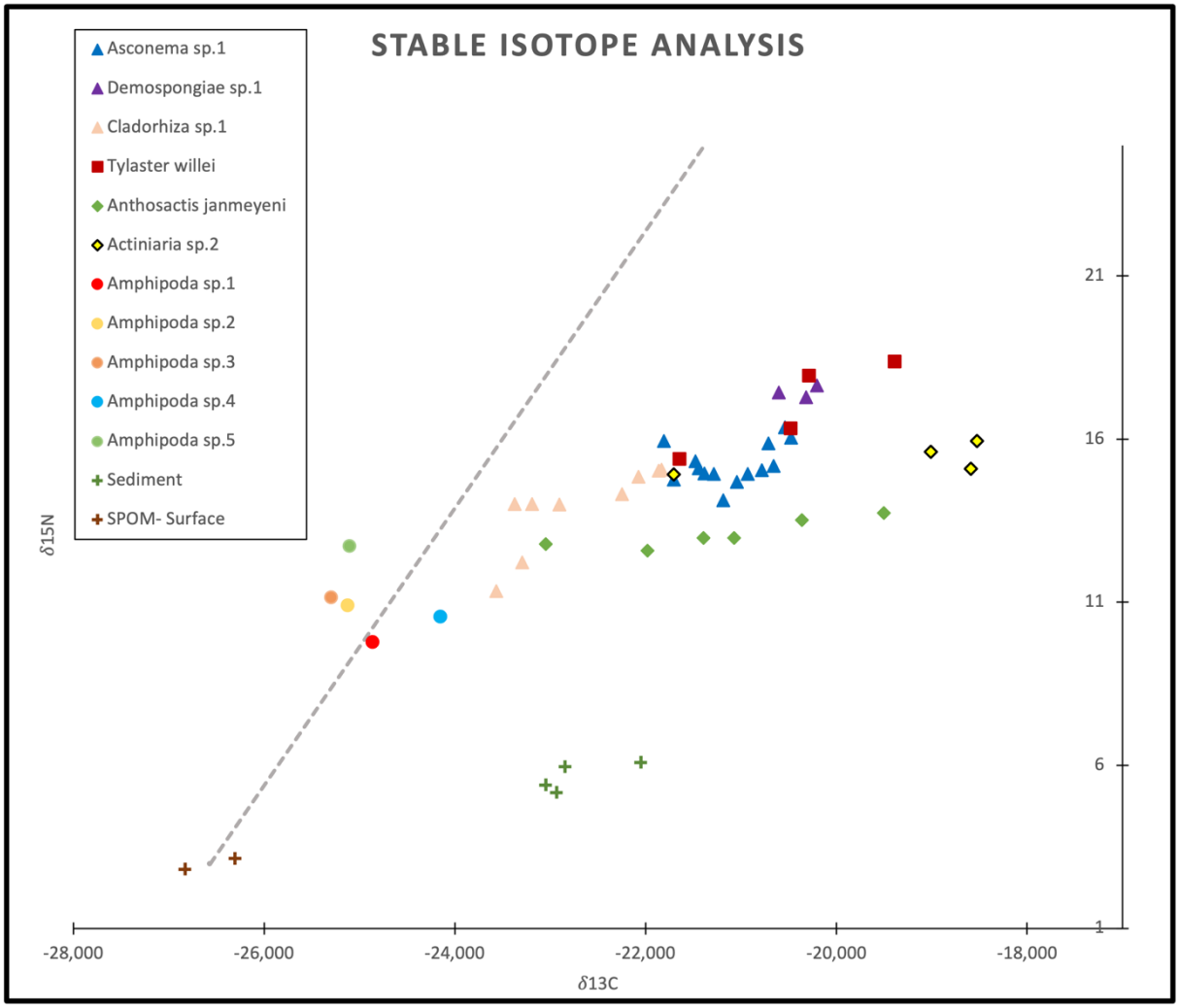

Figure 3.5: Carbon and Nitrogen stable isotope of fauna, sediment, and SPOM-surface water. The dotted line indicates the anticipated enrichment in marine food webs of 3,4‰ $\delta^{15}N$ and 0,8‰ $\delta 13C$ (Zanden and Rasmussen, 2001) *with SPOM as the primary food source.*

Figure 3.5 is a food web based on the stable isotope analysis. In the figure, we have the SPOM-surface water as the primary food source, and from the mean of the SPOM filters, the anticipated enrichment in the system is indicated. We see a connection between surface water with algae and the system's primary consumer, Amphipoda. From the primary consumer, the food web shifts towards the right, meaning a more significant increase of carbon than nitrogen in the food web. The sediment collected with multicore is way outside of the expected area for the food web and has no direct connection to the food web.

The carnivorous sponges, *Cladoriza* sp., are the secondary consumers in the system, likely feeding on the amphipods. They are close to the expected line for the food web. The sponges, Actiniaria, and Asteroidea are outside the expected food web, and they seem to have a higher uptake of carbon and nitrogen than what is expected. At the top of the food web, we have Demospongiae sp.1 and *Tylaster willei*.

Anthosactis janmeyeni has a large variation in the levels of ^{13}C but is almost consistent in the levels of ^{15}N. Actiniaria sp.2 has three samples grouped together, while one sample is closer to other sponges. The Asteroidea are also spread out, and there does not appear to be a pattern. Analysis from deep water samples gave no results, indicating little particulate organic matter in the system.

4 Discussion

4.1 DNA barcoding

To gain a deeper understanding of the species diversity within my samples, I investigated if there were any cryptic species among the species that were assessed morphologically to be the same. To achieve this, I did DNA barcoding. The results of the analysis revealed that there were no cryptic species identified among the collected specimens

In some instances, I encountered difficulties obtaining readable results for the DNA analysis of some samples. Considering the time constraints, I decided not to rerun those particular samples, as most of the samples yielded informative outcomes for the planned purpose. However, it is important to note that species confirmation could not be obtained for all specimens, as the primer used (16S) was not optimal for all taxa. In future research, these specimens should be reanalyzed using alternative DNA markers for a more comprehensive investigation.

To enhance the reliability of DNA barcoding for species identification, it is crucial to have a comprehensive and robust database of DNA sequences available for comparison. Currently, there is a shortage of published data for many deep-sea species, limiting our ability to make accurate comparisons. However, this situation is expected to improve in the near future due to ongoing biodiversity studies conducted at UiB. These studies will contribute to expanding the available data and significantly enhance our understanding of deep-sea species through DNA barcoding.

4.2 Food web structure in the periphery of the Fåvne vent field

The primary objective of my thesis was to examine the impact of active hydrothermal vents on nutrient sources for peripheral fauna and assess the potential consequences of changes or

damage to these vents on the surrounding fauna's food supply. Based on the findings, the analysis of the food web in Figure 3.5 indicates that the primary food source for the peripheral fauna is likely the plankton from surface water. This suggests that the feeding habits of the peripheral fauna may not rely significantly on active hydrothermal venting.

When examining the food web, it is evident that the collected sediment shows no connection to the food web. This suggests that the resuspension of sediments from the seafloor may not serve as a food source for the peripheral fauna, or it could be due to low currents that are insufficient to resuspend the sediments. It is important to note that the collected sediment samples may not entirely represent the overall conditions, as only two samples were obtained from locations away from the primary collection sites for fauna. Variations in sediment composition and characteristics between different locations may contribute to these observations.

It is possible that there is a missing trophic level between the sediment/surface water and the species we have collected, or the significant gap could be resulting from feeding interactions higher up in the food web. It is worth considering the presence of sediment-dwelling species, such as holothurians, which might play a crucial role in the food web. Additionally, water from other levels in the water column or plume water from active vents could represent missing links. Future research should investigate these aspects to gain a more comprehensive understanding of ecosystem dynamics.

Amphipoda, from what we see in Figure 3.5, is the primary consumer. Even though we have four different species, they are relatively close together, and we can therefore assume they have a similar feeding pattern. Deep sea Amphipoda is typically detritivores or scavengers (Guerra-García *et al*., 2014) and based on the method of capture (baited trap), we can assume that all of these are scavengers. This also matches the food web, as sediments are not a part of the nutrient chain.

Cladorhiza sp. is carnivorous sponges that feed on small planktonic species and crustaceans as they get stuck to their adhesive surface (Hestetun *et al.*, 2016). From this, we can assume that *Cladorhiza* sp. will be in the middle of the food web, which matches what we see in Figure 3.5.

None of the prey seems to be derived from outside of the research area or from the active vents for *Cladrohiza* sp. due to the alignment in the food web.

Filter-feeding sponges, such as *Asconema* sp., are usually expected to be placed low in the food web but, as we see in Figure 3.5, have an elevated position here. This is expected, as seen in other research papers such as Hanz et al. (2022). This is due to the internal recycling of nutrients in sponges, and they also have the ability to take up dissolved organic carbon (Yahel *et al.*, 2003; Hoer *et al.*, 2018) which and can release it as particulate organic carbon (de Goeij *et al.*, 2013; McMurray *et al.*, 2018). This forms a source of particulate organic matter obtainable for the associated fauna (e.g., anemones).

As for the rest of the deep sea, the feeding habitats of Actinaria are little researched. But a study by Sun et al. (2022) shows that *Actinostola callosa*, which are in the same family as the *Anthosactis janmeyeni*, have a diverse diet, including sediments and direct predation on sponges. The gut content analysis from Sun et al. (2022) showed sponge spicules, gastropods, amphipods, and copepods in 50-17% of the specimens they analyzed. This variation in gut content can explain the varied placement of the *Anthosactis janmeyeni* in the food web. They have approximately the same amount of δ^{15}N but vary more in δ^{13}C which can indicate that they feed on *Asconema* sp.

For Actiniaria sp.2, we do not know the family and, therefore, can't compare the feeding patterns with closely related species. As we see in the food web, the two species we have of Actiniaria are clearly separated. *Anthosactis janmeyeni* is found on hard substrate, and Actiniaria sp. 2 is found on soft sediment, and we can from that assume that they have different feeding patterns. Actiniaria sp. 2 also has a higher position in the food web than *Anthosactis janmeyeni* and might have food sources that we don't see in the food web, such as sediment-dwelling species.

Demospongiae sp.1 is with *Tylaster willei*, the top predator in the food web. Demosponges are filter feeders such as *Asconema* but have a higher position in the food web. This can mean that both species feed off the discharge from *Asconema* and other species lower in the food web. There has been restricted research on *Tylaster willei* as only a few specimens have been found

and examined, but most *Tylaster willei* are predators and will eat sponges, microalgae, snails, and other small animals (Rahman *et al.*, 2018).

The analysis of the bottom water has too low values for the result to be reliable. We can assume that there are little dissolved organic particulates in the area and larger amounts of water should be collected and filtered to get a better result from these samples.

From the food web, we can see no apparent connection between active vents and the peripheral fauna. With plankton from the surface water as the primary food source, the primary production from chemosynthesis does not appear to be critical for the surrounding ecosystem. But without further research on food web structures, it is impossible to say anything for certain due to the significant gap between the primary food source and the primary consumer. In addition, stable isotope analysis of vent fauna could help to interpret the food web.

To get elaborate and get a better picture of the food web I could have included amino and fatty acids. Amino acids can unravel trophic positions and give an even more precise picture of the food web's appearance in addition to the stable isotopes (Chikaraishi *et al.*, 2009). Fatty acids can provide a better understanding of trophic relationships, particularly the transfer of organic matter from sponges to the food web (Colaço, Desbruyères and Guezennec, 2007; Hanz *et al.*, 2022).

Another method for creating a food web could be to use gut content analysis, this method allows for an understanding of the relative dietary composition and prey selection for each specimen (Amundsen and Sánchez-Hernández, 2019). But this is not possible for sponges as they do not have a true digestive system but rather intravacuolar digestion, meaning they digest food in their cells (Godefroy *et al.*, 2019).

4.3 Limitations of the study method

This study relies on laboratory methods, but it is not possible to conduct this kind of lab work without some field research. Laboratory studies are highly controlled and can easily be repeated

(Aziz, 2017). Field research is expensive and time-consuming and can be invasive to the research environment. To conduct good field research, planning it well in advance is essential. In this section, I will look at some of the limitations and challenges with research cruises, such as the one I participated in for this thesis. Most of the limitations we can expect in this kind of work are being planned for, and mitigations are in place to reduce the consequences of them happening.

The first and maybe the biggest limitation when researching at sea is the time available. For this cruise, we were set to visit multiple sites at the Mohn's Ridge and each location, therefore, had a limited time slot. This makes this kind of research vulnerable to unforeseen circumstances such as weather changes. Some equipment, such as the ROV, is sensitive to bad weather and big waves. There are, of course, no ways to prevent harsh weather, but mitigations for such instance is to either use other types of equipment that are not as weather dependent (can tolerate bigger waves) or to relocate and return at a later time. For our purpose, we collected all the planned samples and got good results from all the specimens.

Another limitation of this type of work is the possibility of not collecting a large enough variety of species or enough samples for each species. For my thesis to make a complete food web, I need to collect the most abundant species and have such a quantity that we can replicate and compare individuals for each species. Therefore, we decided to collect the most common species and have at least three replicates for each species. We also had to choose species based on our ability to collect them with the ROV, as some species, such as bivalves, could not be sampled due to the fragile bodies that broke when trying to collect them. Another involuntary limitation of the selection was that we could not cover the whole area due to time (limiting our chance to encounter and sample rare species), and lights from the ROV can attract or scare off species, influencing our ability to sample them.

A third limitation of the sampling method is the framing of the project. We chose to focus on peripheral benthic megafauna, meaning smaller species and sediment-dwelling species were excluded from the sampling. This can result in holes or gaps in a food web, something I got in my food web sampling. Whether this is from a missing species or environmental sample can only be answered by collecting the missing species and rerunning some of the environmental

samples. Thanks to my study, potential sampling gaps were identified and will be addressed in future cruises and research.

For environmental samples, it is essential to collect two or more duplicates to check the reproducibility of the results and to see if they give different results, which can indicate errors in the testing. And for analysis, such as the food web, it provides additional data points, and we can detect inconsistencies in the system. For my project, we collected two duplicates of each environmental sample. But even then, we did not get a result from the bottom water sample. The water sampled from the deep did not contain enough organic matter to get results for stable isotopes, which also means there is not much suspended particulate organic matter at the sampling depth.

It is also important to consider the impact the research has on the ecosystem as some interventions can cause serious harm. But the use of ROV also has a low impact on the habitats, allowing for selective sampling. And the multicore will affect such small areas that we can assume that the systems will quickly regenerate.

4.4 Possible effects of deep-sea mining on vent background fauna

As mining most likely will not happen at active sites, we must look at inactive and extinct sites and their ability to regenerate after disturbances. Due to the insufficient understanding of species diversity, species distribution, connectivity, and settlement behavior, it is impossible to

determine the potential for recovery in inactive vents and vent periphery regions after mining disruptions (Gollner *et al.*, 2017).

Deep-sea mining activity is potentially associated with a number of environmental risks. The first step to extract seafloor massive sulfides (SMS) would be to crush the minerals on the ocean floor, turning them into a slurry that can then be transported to the surface through a riser pipe (Liu *et al.*, 2016). During this processing stage, the newly crushed sulfide surfaces from both the bulk deposit and fine particulate debris will be exposed to seawater that contains oxygen. The populations of invertebrates that live on mineable surfaces and have limited or no mobility will be eliminated (Van Dover, 2019). Extracting minerals from the inactive sulfide habitat could reduce the overall available habitat, leading to a decrease in abundance and diversity due to the reduced surface area or lower quality of the habitat (Cuvelier *et al.*, 2018). The ability to recover fixed or sessile invertebrate species will rely on their larval recruitment. If unique species endemic to the inactive sulfide habitat are identified, local recovery of those species might not be feasible if no inactive sulfides of adequate quality are left.

Mining of SMS deposits will more likely than not create plumes of sediment in the water column. And mining plumes will affect not only the bottom habitats but also midwater organisms (Drazen et al., 2020). The plume will go through three phases as mining happens: discharge, buoyancy, and passive transport (Peacok and Ouillon, 2023). The discharge is the initial and turbulent process where the sediment is discharged into the water. In the buoyancy-driven phase, the sediment interacts with the surroundings, and in the passive transport phase, the sediments get passively transported with the currents and get dispersed. Near the seafloor, the majority of deep midwaters exhibit extremely low concentrations of naturally suspended sediment (Drazen et al., 2020). Plumes can cause distress, such as clogging of respiratory systems and olfactory sense and absorbing light disturbing communication and bioluminescence signals.

In the 90s, there were multiple studies by the USA, Germany, Japan, and India to test the effect of deep-sea mining (Sharma, 2015). The studies tested for disturbance and relocation and benthic impact, and the results showed changes in fauna, reduction in abundance, and the process of restoration. An experiment conducted by Nakajima et al. (2015) found that drilling can cause changes in the sea floor from soft sediment to hard substrate. They regularly measured the changes over 40 months. The study showed that the sediment was soft and shimmering in

the beginning, reaching temperatures up to at least 160°C. Over time the shimmering was reduced, and the sediment hardened. These are events that will change the species composition of vent systems more rapidly, and the time it takes to regenerate is highly unpredictable if recovery is at all to be expected. These studies are quite sparse and date back to the 90s, but these are supported by more recent studies, as mentioned in the introduction. The change in the sea floor structure will alter species composition and possibly the temperature gradients. There are, of course, a lot of political and economic interests at play around mining activities and studies that indicate that deep-sea mining might have irreversible effects that could seriously undermine the legitimacy of proceeding with these activities. Therefore, it is unsurprising that such studies are hard to find so that some stakeholders can use the uncertainties about the potential (irreversible) effects to justify going ahead with them.

It is not only the mining itself that can harm the deep sea and the ecosystems there, lights and noise might also create stress (Weaver, Billett and Van Dover, 2018). Sound and vibration can be emitted from the mining tools, the transfer of minerals from the sea bottom to the vessel, and from the vessel through different equipment and the motor, meaning that the whole water column in an area can be disturbed. Sound can affect marine animals by creating discomfort, interfering with communication, and reducing their ability to detect prey. Lights will most likely be emitted from the mining tool and many organisms living in the depths of the ocean exhibit either partially or fully diminished eyesight or light-sensitive organs. Despite this, there are numerous fish and invertebrate species that possess fully developed eyes that are likely highly responsive to the extremely faint levels of bioluminescence in the deep-sea environment (Christiansen et al., 2020).

4.5 Incomplete knowledge for decision-making?

According to Gollner et al. (2017), we must avoid “serious harm” during deep sea mining. This indicates seriously harming species or ecosystems in a potentially irreversible way. But with the limited knowledge of the deep-sea, serious harm is hard to define. And this limited knowledge base, and associated uncertainties, is also what will be variously used in the decision

for opening deep-sea mining. Deep sea mining activities are surrounded by many uncertainties regarding their potential impact on the local ecosystems and the surrounding environment, as well as the potential economic and social benefits that are often put forward to justify such activities. Decision-making around deep-sea mining is characterized by heated debates, where the different actors involved (including policymakers, the industry, researchers, and environmental activists) all have different interests at play and value different things differently (nature's intrinsic value, economic profit, and growth, and job opportunities). Therefore, uncertainties related to the future environmental, social, and economic impacts of deep-sea mining need to be dealt with in a transparent manner.

One way to engage in decision-making processes when knowledge is characterized by so many uncertainties and incompleteness, and when the issue is so complex and connected is to follow the precautionary principle. It can be interpreted as a "better safe than sorry" approach (Gollier and Treich, 2003), but also, and perhaps more importantly, as making a decision, at a certain point in time, based on the best available knowledge. This means that there is an awareness of the uncertain and incomplete nature of the knowledge base at the time the decision will be made. In the case of deep-sea mining, decision-making should consider potential long-term environmental effects and irreversibility, social, and economic effects, and uncertainties of the knowledge base (Gollier and Treich, 2003). Indeed, we will not know the full consequences of mining until it happens, if it ever happens, and as of now, the knowledge base for the deep sea is too poor to even make a justified knowledge-based decision. There needs to be a certain level of knowledge before a knowledge-based decision can be made and justified. I have shed light on some important initial results in my thesis, but they are accompanied by uncertainties, as I have discussed above. But as a part of a larger knowledge base, it can bring some insights into how the ecosystems at hydrothermal vents are connected and who will be affected if one or more species gets reduced or removed.

Decision-making around deep-sea mining activities, as the situation is today, will be based on highly uncertain knowledge. This can prove challenging as decision-makers might expect complete, certain, or 'perfect' knowledge to base their decisions on. Even though the knowledge base can be extended through more research, there will always be unknowns and uncertainties relative to the potential impacts of deep-sea mining activities. There will always be new questions and emerging uncertainties. That is when the precautionary principle and adaptive management is useful. Acting under the precautionary principle is, as I said above,

acting based on the best available knowledge at a particular point in time and being transparent about the fact that the knowledge base is incomplete. And adaptive management allows for management simultaneously as new knowledge is created (Williams, 2011). Therefore, it is important to be transparent about what is uncertain, what is left out of the study, and what is unknown in a clear and apprehensible manner. As the knowledge base grows, the precautionary principle allows for re-evaluation so that the best action is taken based on the knowledge at hand, and to understand if the knowledge at hand is enough to base a decision on. My master thesis is in this way, contributing to the knowledge base as of now, but it is part of a 'moving' knowledge base. It is therefore important to accompany my findings with the considerations of uncertainties and limitations for the method and data sampling I have made above.

To relay the uncertainties, it is important to have a good level of communication between scientists and decision-makers, based on openness and trust in showing the uncertain nature of the knowledge base. But there are some barriers that can make this difficult. For instance, stakeholders from different backgrounds can speak and communicate in such diverse ways that understanding each other might be difficult. A scientist might focus on communicating in a professional and 'objective' way, using technical language aimed at a specific audience. At the same time, political discourses and rhetoric might allow for a more conversational approach, allowing moral judgments, values, interests, pragmatism, and even emotions to come through (Madsen, 2007). In natural sciences, my experience is that we often want to give one single, correct answer for a given problem. Still, the issues around deep-sea mining is so complex and characterized by so many uncertainties that this does not seem like a suited and relevant approach in this case. There is no one 'good' way to go about this, but rather many different values, opinions, and interests to take into account. And these different opinions on what 'should' be done are legitimated precisely because there are uncertainties around deep sea mining. Ultimately, for now, no one really knows what would happen if it took place (van der Sluijs et al., 2008). For policymaking, this raises more questions about whether the information available is reliable enough to guide decisions. It is a matter of balancing out different risks, potentially harmful effects and loss of ecosystem services, and potential socio-economic gains or losses. Even though scientists would ideally like to provide a single, agreed-upon perspective on those issues, it is crucial that we also focus on the limitations and uncertainties in our research so that discussions and debates can take them into account as best as possible. But again, because decision-making processes are often thriving for certainty and simplicity, even for complex issues, this can lead to challenges when policy and science try to talk to each other.

Another barrier that can cause issues in communication is the fundamentally different values or interests in the issue at stake. With regards to deep sea mining, for biologists, the awareness of the intrinsic value of the ecosystem, and its conservation, might be their biggest motivation, while the politicians or the industry sector will value job opportunities, economic growth, technological development, and profits. This opposition between environmental aspects, on the one hand, and socio-economic ones on the other, often characterizes debates around sustainability issues I found. But from a sustainability perspective, these aspects should support each other. There are no thriving societies in an environment that is totally depleted. There are, however, in deep-sea mining projects, very high-power structures, where some aspects get more value, attention, and weight in debates than others. These power dynamics are also very striking in sustainability issues, and maybe, being aware of them, like being aware of the uncertain and incomplete nature of the knowledge base, might make for a more inclusive and reasonable decision-making process.

5 Conclusion

There is no apparent connection between the active hydrothermal vents and the peripheral fauna in the food web. All the species I looked into seemed to be connected through the food web, while the sediment samples appear to have no connection. But this is just a small part of understanding the ecosystem and connectivity between the biotic and abiotic factors of the deep

sea. But even if there is no connection through feeding, the vents can still have important ecosystem functions. They create substrates for many sessile species to live on, and the removal of this can remove these species permanently. This can then have cascading effects on the food web, reducing the ecosystem.

It is clear that more research is needed on hydrothermal vents and surrounding areas. Further research to improve the knowledge of my study will involve adding fatty acids and amino acids to the food web analysis and expanding the number of species in the data set. More bottom water will be collected to improve results for the isotope analysis, and more sediment samples from varying areas in and around the area for specimen sampling. Other elements that can be interesting to see are how sponges react to minerals in the water through sulfide-stable isotopes and to investigate if they have any ability to utilize the minerals. In addition, more specimens should be sampled such as infauna and fauna from active vents.

For seabed mining, it will be important to study more inactive and extinct vent systems as these are the areas of interest for mining. As we see in Figure 1.2 only one inactive site is known on the Mohn´s Ridge as these areas is more difficult to find than active vents. There will also be necessary to do more exploration to find these areas. And to understand the ability of inactive and extinct vents to regenerate themselves, it is crucial to understand the connection between different sites. For that, we would have to look at the species composition and larva dispersal between sites.

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Appendices

A. DNA barcoding protocol

QIAGEN DNeasy Blood & Tissue Kits - Purification of Total DNA from Animal Tissues (Spin-Column Protocol)

This protocol is designed for purification of total DNA from animal tissues, including rodent tails.

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- For fixed tissues, refer to the pretreatment protocols "Pretreatment for Paraffin Embedded Tissue", page 46, and "Pretreatment for Formalin-Fixed Tissue", page 48.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- **Optional:** RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Blood & Tissue Kit (see "Copurification of RNA", page20).

Things to do before starting

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath or rocking platform to 56°C for use in step 2. If using frozen tissue, equilibrate the sample to room temperature(15–25°C).
- Avoid repeated thawing and freezing of samples, because this will lead to reduced DNA size.

Procedure

1. Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces, and place in a 1.5 ml microcentrifuge tube. For rodent tails, place one (rat) or two (mouse) 0.4–0.6 cm lengths of tail into a 1.5 ml microcentrifuge tube. Add 180 µl Buffer ATL. Earmark the animal appropriately.

   Ensure that the correct amount of starting material is used (see "Starting amounts of samples", page 15). For tissues, such as spleen, with a very high number of cells for a given mass of tissue, no more than 10 mg starting material should be used.

   We strongly recommend cutting the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen* before addition of Buffer ATL and Proteinase K. Alternatively, tissue samples can be effectively disrupted before Proteinase K digestion using a rotor–stator homogenizer, such as the TissueRuptor II, or a bead mill, such as the TissueLyser II (see ordering information starting on page 59). A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the TissueLyser II can be obtained by contacting QIAGEN Technical Services (see back cover). For rodent tails, a maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

2. Add 20 µl Proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample or place in a thermomixer, shaking water bath or on a rocking platform. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h or, for rodent tails, 6–8 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.
   After incubation, the lysate may appear viscous, but should not be gelatinous as it may clog the DNeasy Mini spin column. If the lysate appears very gelatinous, see the "Troubleshooting Guide", page 52, for recommendations.
   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C) before continuing with step 3.
   Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA, which will copurify with genomic DNA. For tissues that contain low levels of RNA, such as rodent tails, or, if residual RNA is not a concern, RNase A digestion is not necessary.

3. Vortex for 15 s. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing. It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.
   A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen, lung) may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

4. Pipet the mixture from step 3 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.*

5. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.*

6. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube. It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.
   Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through
   since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000rpm).

7. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥ 6000 x g (8000 rpm) to elute.
   Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate but also decreases the overall DNA yield (see Figure 2, page 23).

8. **Recommended**: For maximum DNA yield, repeat elution once as described in step 7. This step leads to increased overall DNA yield. A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

PCR prep.

Table 1: Mastermix – total volume 25µl per sample

Vann	17,35µl
Buffer	2,5µl
dNTP	2µl
Primer 1	1µl
Primer 2	1µl
Enzym	0,15µl
DNA	1µl

PCR-machine

Table 2: Condition for PCR amplification.

Denaturing	Touchdown (x10)	Denaturing	Additional amplification (x25)	Extension
94°C- 3 min	95°C - 45 sec	94°C - 5 min	94°C - 45 sec	72°C - 7 min
	54°C - 30 sec		48°C - 30 sec	

	(-0,5 sec per cycle)			
	72°C – 60 sec		72°C - 60 sec	

Test of PCR product
Agarose mixture:

- 2g agarose powder
- 200ml TAE buffer

Gel:

- Agarose mixture
- gel red

Mix in the electrophoresis tub and let sit for 25 min with a contraption for making wells in.
PS: important to use thick glows when handling gel red as it can attach to DNA

PCR product mixed with loading dye

- 4µl DNA
- 1,3µl dye

Fill 5,4µl DNA in each well in the gel
Let's sit with the current on 80V for 25min

- Us a UV camera to read the gel and see which PCR product is good and can be sent for sequencing.

Washing PCR product
Master mix for PCR washing ad up for the number of samples to run:

- Exo - 0,1µl
- SAP - 1,0 µl
- ddH2O - 0,9 µl

Washing:

- master mix - 2µl
- DNA - 8 µl

Start the PCR machine for the ExoSAP program with the PCR product and washing mixture.

Sanger sequestering
BigDye protocol – per sample

BigDye	1µl
Buffer	1µl
Primer	1µl
H2O+DNA	7µl

The amount of DNA will depend on the exposure read of the UV photo. One mix for each primer (forward and backward)

Run the PCR in the PCR machine on a BigDye program, when finished ad 10 µl distilled water and send for sequencing.

B. Table for freeze-drying grinding weighing isotope samples (NIOZ)

Group	Treatment		
	Freeze drying	Grinding	Weighing
Standard (Ace/Casein)	no	no	0,5 - 1 mg
Urea	no	no	>0,5 mg

Fish	yes	yes, morter and pastel / ball mill	0,4 - 0,8 mg
Zeenadel	yes	yes, morter and pastel / ball mill	0,4 - 0,8 mg
Crustacean	yes	yes, morter and pastel / ball mill	1,2 - 1,6 mg **
Filter	yes	yes, pincher / punch-kit / inweeg	No / 1 mg
Zooplankton	yes	yes, morter and pastel / ball mill	1,2 - 1,6 mg **
Jellyfish	yes	spatula *	5 - 10 mg
Worm	yes	yes, morter and pastel / ball mill	0,4 - 0,8 mg
Bivalve	yes	yes, morter and pastel / ball mill	0,4 - 0,8 mg **
Algae	yes	yes, morter and pastel / ball mill	1,0 / 2,0 mg
Starfish	yes	yes, morter and pastel / ball mill	1,2 - 1,6 mg **
Otolite**	yes	yes, morter and pastel / ball mill	15 - 20 mg ***

* Jellyfish needs to be grind in 'weighing step' before aluminum tin cup is filled.
** Acidification needs to be done after silver cup is filled. In case of crustacean and bivalve only whole ones!
*** Fuming in silver cups for approximately 24 hours and then acidification.

C. Stable isotope results for 15N and 13C

Standard: Acetanilide

d15N = 1,18
N % = 10,36

Sequence Line	Weight (mg)	N-isotopes		
		Area	d15N	factor K
4	0,594	37,474	2,034	0,1642
5	0,529	32,844	1,676	0,1669
6	0,604	42,842	1,996	0,1461

17	0,682	42,623	1,708	0,1658
29	0,619	38,44	1,754	0,1668
41	0,650	40,996	1,964	0,1643
53	0,735	43,815	1,757	0,1738
65	0,766	46,036	1,488	0,1724
77	0,633	38,178		0,1718
85	0,589	35,38	1,910	0,1725
86	0,648	39,332	1,738	0,1707
		Average:	**1,803**	**0,167**
		Stdev:	**0,17**	**0,01**
		Difference:	**0,623**	

d13C = -29,53
C % = 71,08

C-isotopes		
Area	d13C	factor K
65,076	-29,538	0,6488
57,066	-29,487	0,6589
74,977	-29,566	0,5726
76,306	-29,556	0,6353
69,514	-29,581	0,6329
76,254	-29,480	0,6059
79,709	-29,792	0,6554
85,501	-29,740	0,6368
70,138	-29,890	0,6415
65,228	-30,519	0,6418
72,756	-30,22	0,6331
Average:	-29,761	0,633
Stdev:	0,33	0,02
Difference:	-0,231	

Standard: Urea

d15N = 20,17
N % = 46,65

Sequence		**N-isotopes**			
Line	Weight (mg)	Area	d15N	d15N (corr.)	N (%)
18	0,608	172,285	21,264	20,642	47,27
54	0,779	215,579	21,356	20,734	46,17
66	0,743	207,598	21,340	20,718	46,61
87	0,801	219,103	21,363	20,741	45,63
88	0,764	161,897	21,291	20,669	35,35

			Average:	20,700	44,207
			Stdev:	0,04	4,99

d13C = -8,02
C % = 20,00

C-isotopes			
Area	d13C	d13C (corr.)	TOC (%)
18,328	-9,911	-9,680	19,08
23,346	-9,943	-9,712	18,97
23,094	-10,921	-10,690	19,68
24,397	-10,336	-10,105	19,28
18,004	-11,028	-10,797	14,92
	Average:	-10,197	18,385
	Stdev:	0,53	1,96

Standard: Casein

d15N = 5,94
N % = 13,32

Sequence		N-isotopes			
Line	Weight (mg)	Area	d15N	d15N (corr.)	N (%)
30	0,485	37,228	6,678	6,056	12,81
42	0,530	41,598	6,578	5,956	13,09
78	0,513	41,036	6,772	6,150	13,34
83	0,580	45,478	6,732	6,110	13,08
84	0,786	15,8	6,594	5,972	3,35

			Average:	6,048	11,136
			Stdev:	0,08	4,35

d13C = -26,98

C % = 46,50

C-isotopes			
Area	d13C	d13C (corr.)	TOC (%)
33,469	-27,547	-27,316	43,68
38,32	-27,620	-27,389	45,77
37,418	-27,988	-27,757	46,17
42,067	-27,837	-27,606	45,91
14,168	-27,912	-27,681	11,41
	Average:	-27,550	38,589
	Stdev:	0,19	15,23

Samples

Sequence Line	Sample	Weight (mg)	Area	d15N	d15N (corr.)	N (%)	Area	d13C	d13C (corr.)	TOC (%)	
7	*Asconema* sp.1	4,839	15,102	16,557	15,935	0,52	19,85	-22,039	-21,808	2,60	C Too low but accaptable
8	Cladorhizasp. 1	5,950	92	12,837	12,215	2,58	136,023	-23,527	-23,296	14,47	
9	Cladorhiza sp.1	5,169	97,855	11,966	11,344	3,16	151,284	-23,797	-23,566	18,53	
10	Asteriodea sp.1	1,540	59,152	16,003	15,381	6,41	86,272	-21,873	-21,642	35,46	
11	Asteriodea sp.1	1,548	71,533	16,933	16,311	7,71	91,134	-20,708	-20,477	37,27	
12	Asteriodea sp.1	1,278	50,623	18,557	17,935	6,61	66,885	-20,513	-20,282	33,13	
13	*Anthosactis janmeyeni*	1,575	69,329	18,991	18,369	7,34	83,216	-19,614	-19,383	33,45	
14	*Anthosactis janmeyeni*	1,646	82,045	13,598	12,976	8,32	96,214	-21,622	-21,391	37,00	
15	*Anthosactis janmeyeni*	1,562	64,264	13,402	12,780	6,86	107,706	-23,278	-23,047	43,65	
16	*Anthosactis janmeyeni*	1,821	90,128	13,195	12,573	8,26	116,43	-22,212	-21,981	40,47	
19	*Anthosactis janmeyeni*	1,598	82,798	13,586	12,964	8,64	91,043	-21,305	-21,074	36,06	
20	*Anthosactis janmeyeni*	1,195	65,44	14,144	13,522	9,14	68,983	-20,590	-20,359	36,54	
21	*Anthosactis janmeyeni*	1,540	95,628	14,349	13,727	10,36	93,13	-19,733	-19,502	38,28	
22	Cladorhiza sp.1	6,027	70,399	14,928	14,306	1,95	90,788	-22,478	-22,247	9,54	
23	Cladorhiza sp.1	5,395	76,737	14,613	13,991	2,37	113,22	-23,133	-22,902	13,28	
24	Cladorhiza sp.1	5,853	50,081	15,466	14,844	1,43	66,477	-22,308	-22,077	7,19	
25	Cladorhiza sp.1	6,228	44,625	15,680	15,058	1,20	54,131	-22,064	-21,833	5,50	
26	Cladorhiza sp.1	4,829	40,441	15,660	15,038	1,40	49,152	-22,091	-21,860	6,44	
27	Cladorhiza sp.1	5,532	68,555	14,625	14,003	2,07	115,223	-23,422	-23,191	13,18	
28	Cladorhiza sp.1	4,930	78,924	14,635	14,013	2,67	140,032	-23,606	-23,375	17,98	
31	*Asconema* sp.1	5,788	36,039	15,556	14,934	1,04	43,112	-21,160	-20,929	4,71	
32	*Asconema* sp.1	6,629	42,337	15,675	15,053	1,07	51,331	-21,013	-20,782	4,90	
33	*Asconema* sp.1	5,548	36,35	15,731	15,109	1,09	45,189	-21,676	-21,445	5,16	
34	*Asconema* sp.1	5,303	31,027	15,384	14,762	0,98	38,651	-21,937	-21,706	4,61	

35	*Asconema* sp.1	4,719	27,418	15,559	14,937	0,97	33,265	-21,517	-21,286	4,46	
36	*Asconema* sp.1	6,078	17,65	15,572	14,950	0,48	21,739	-21,613	-21,382	2,26	
37	*Asconema* sp.1	4,868	38,311	15,803	15,181	1,31	44,208	-20,889	-20,658	5,75	
38	*Asconema* sp.1	6,266	48,795	16,984	16,362	1,30	58,072	-20,771	-20,540	5,87	
39	*Asconema* sp.1	6,033	50,408	16,660	16,038	1,39	55,449	-20,705	-20,474	5,82	
40	Demospogiae sp.1	5,242	101,298	18,051	17,429	3,22	159,373	-20,834	-20,603	19,25	
43	Demospogiae sp.1	5,162	60,976	18,262	17,640	1,97	84,402	-20,432	-20,201	10,35	
44	Demospogiae sp.1	6,555	103,754	17,908	17,286	2,64	146,605	-20,547	-20,316	14,16	
45	*Asconema* sp.1	5,314	26,475	14,752	14,130	0,83	43,92	-21,418	-21,187	5,23	
46	*Asconema* sp.1	5,826	25,293	15,305	14,683	0,72	31,474	-21,276	-21,045	3,42	
47	*Asconema* sp.1	5,106	23,944	15,940	15,318	0,78	29,59	-21,707	-21,476	3,67	
48	*Asconema* sp.1	4,767	38,967	16,491	15,869	1,36	46,041	-20,945	-20,714	6,11	
49	Actiniaria sp.2	1,449	91,332	16,227	15,605	10,52	81,618	-19,239	-19,008	35,66	
50	MUC1A	63,148	78,937	6,581	5,959	0,21	151,484	-23,076	-22,845	1,52	
51	Actiniaria sp.2	1,791	110,294	15,358	14,736	10,27	110,173	-19,878	-19,647	38,94	Nitrogen has a carry over effect from precious sample
52	Actiniaria sp.2	1,520	99,527	15,718	15,096	10,92	86,219	-18,818	-18,587	35,91	
55	Actiniaria sp.2	1,443	99,301	16,568	15,946	11,48	84,326	-18,754	-18,523	36,99	
56	Actiniaria sp.2	1,230	57,08	15,545	14,923	7,74	65,741	-21,934	-21,703	33,83	
57	Amphipoda sp.3	1,328	37,867	11,765	11,143	4,76	125,86	-25,527	-25,296	59,99	
58	Amphipoda sp.4	1,448	68,953	11,166	10,544	7,94	114,863	-24,380	-24,149	50,21	
59	Amphipoda sp.5	1,803	47,933	13,327	12,705	4,44	132,94	-25,335	-25,104	46,67	
60	Amphipoda sp.1	1,251	45,844	10,383	9,761	6,11	92,587	-25,089	-24,858	46,85	
61	Amphipoda sp.3	1,244	47,64	11,517	10,895	6,39	86,074	-25,351	-25,120	43,80	
62	MUC 1B	70,995	83,763	6,706	6,084	0,20	173,653	-22,281	-22,050	1,55	
63	MUC 2A	67,411	38,767	5,793	5,171	0,10	81,2	-23,165	-22,934	0,76	
64	MUC2B	59,520	33,271	6,024	5,402	0,09	67,051	-23,279	-23,048	0,71	

67	CTD2 FB46 24 m	17,661	23,497	3,772	3,150	0,22	34,483	-26,539	-26,308	1,24	
69	CTD3 FB60 25 m	23,005	23,16	3,428	2,806	0,17	32,055	-27,059	-26,828	0,88	
71	Blank filter	30,178			-0,623	0,00	2,223	-34,243	-34,012	0,05	
73	CTD3 FB57 2751 m	41,081	3,539	7,172	6,550	0,01	10,853	-28,114	-27,883	0,17	C Too low
75	CTD4 FB59 3016 m	41,250	4,886	6,478	5,856	0,02	10,406	-28,221	-27,990	0,16	C Too low
77	Ace	0,633	38,178	4,140	3,518	10,06	70,138	-29,890	-29,659	70,14	