

Developing Molecular Tools to study Trophic Interactions in zooplankton and their implementation in a vent system

-Norwegian Sea Hydrothermal Vents

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Scientific environment

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Summary

Understanding hydrothermal plumes in the Arctic and Nordic Seas is an important objective for ecosystem assessments of these habitats. Very little is known about what role the hydrothermal plumes play in the Nordic Seas. Recent visual observation and echo-sound imaging suggest that this habitat is densely inhabited by larger zooplankton compared to the surroundings. In this thesis I focused particularly on the diet of a pelagic predator that is commonly found in plumes of the Nordic Seas, the amphipod *Themisto abyssorum* (**Paper III**). The diet of *T. abyssorum* in the deep-sea and at hydrothermal vents was previously unknown and new tools were needed to access this information. Therefore, we developed a molecular assay to help us find and identify predator-prey interactions that are difficult to detect using classical microscopy methods (**Paper I and II**). We also conducted a genetic survey of the pelagic eukaryote microorganism community at the Jan Mayen Vent Field, Loki's Castle and the Håkon Mosby Mud Volcano (**Paper IV**) to understand the vent impact on these communities.

Molecular tools have become important in predator-prey interaction studies where dissection can be inadequate. The most common approach is to use specific primers that target known prey taxa. However, to study the complete range of prey items consumed we needed to work with universal primers. We therefore developed an application for the Denaturing High-Performance Liquid Chromatograph (DHPLC) for separating amplicons in a mixture, in order to study the total prey DNA from stomach contents. This technique was developed as part of a study of predator-prey interactions of the copepod *Limnocalanus macrurus* from the Bothnian Bay in the Northern part of the Baltic Sea (**Paper I and II**). The DHPLC assay was efficient, unbiased and could be used for any predator-prey interaction (**Paper II**). More than 30 taxa (at different taxonomic levels) were identified from the samples suggesting highly carnivorous feeding preferences. The assay development in **Paper I and II** confirmed that it was possible to explore stomach DNA using universal primers without significant bias from predator DNA.

Until now, dissection and microscopy have been the most common tools used to study trophic interactions of *T. abyssorum*. However, sole use of such tools may have caused an underestimation of prey diversity, particularly among those prey items not visible in the microscope. *Themisto* spp. are very useful as target species in deep-sea studies with long haul times because they have long gut passage time, so prey items are not digested as quickly as in some other species. The novel technique for excluding predator amplicons developed in **Paper II** was used to analyse the gut content of *T. abyssorum*. Specimens from three localities; The Jan Mayen Vent Fields, The Loki's Castle Vent Field and The Håkon Mosby Mud Volcano, were analysed using a 3-500 bp long 18S rRNA sequence. The number of Operational Taxonomical Units (OTUs) reported in **Paper III** was highest in *T. abyssorum* guts from Loki's Castle while the Jan Mayen samples had the lowest numbers of prey. The most abundant prey was calanoid copepods, supporting previous findings. However, the molecular approach has also revealed soft-bodied prey and possibly detritus. In summary, our study showed much higher diversity of prey than previous studies and the wide range of taxa found also indicates that *T. abyssorum* is likely highly omnivorous.

In **Paper III**, our data indicates that the localities were quite different from each other in terms of prey diversity and we therefore hypothesised that biodiversity of hydrothermal vents might be more closely linked to the general water masses of the Nordic Seas than to the hydrothermal activity alone. A molecular diversity assay was used to describe the eukaryote community of the hydrothermal vents in the Norwegian Sea (**Paper IV**). Water samples from five localities and from three different depths were collected. The five localities were Jan Mayen Vent Fields with a reference station, Loki's Castle with a reference station and the Håkon Mosby Mud Volcano. The results obtained from **Paper IV** support the hypothesis from **Paper III**, where water masses gave the strongest effect followed by depth, and thirdly a differentiation between Loki's Castle and the reference station indicated an effect of the hydrothermal vent.

Papers I, II and III illustrate the power of molecular markers in dietary studies, generating a more in-depth understanding of predator-prey interactions. In addition, both the stomach content analysis in **Paper III** and the genetic survey of the water column in **Paper IV** suggests that the plankton taxa composition was not vent specific. The results illustrate the need to go deeper and further into a complete inventory of the plankton community surrounding vents and seeps. Furthermore, several technical challenges regarding sampling for stomach content analysis are yet to be solved and an even better understanding of predator-prey interactions depends on that development.

List of publications

- I. Dahlgren K, Olsen BR, Troedsson C, Båmstedt U (2012) Seasonal variation in wax ester concentration and gut content in a Baltic Sea copepod [*Limnocalanus macrurus* (Sars, 1863)]. *Journal of Plankton Research* 34(4): 286-297
- II. Olsen BR, Dahlgren K, Schander C, Båmstedt U, Rapp HT, Troedsson C (2012) PCR-DHPLC assay for the identification of predator-prey interactions. *Journal of Plankton Research* 34 (4): 277-285
- III. Olsen BR, Troedsson C, Hadziavdic K, Pedersen RB, Rapp HT (2013) A molecular gut content study of *Themisto abyssorum* (Amphipoda) from Arctic hydrothermal vent and cold seep systems. *Molecular Ecology* DOI: 10.1111/mec.12511
- VI. Olsen BR, Troedsson C, Hadziavdic K, Pedersen RB, Rapp HT (2014) The influence of vent systems on pelagic eukaryotic microorganism composition in the Nordic Seas. Manuscript submitted to *Polar Biology*

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1. Introduction

“For decades an oceanographer has studied the deep-sea floor where no sunlight penetrate. Where the temperatures is just above freezing. Where pressure is enormous and life is thinly scattered. Because there is no sunlight and green plants can't grow to form the basis of a food chain, scientists has had a good reason to believe that the deep-sea is like a desert. But in 1977 scientists were amazed, to discover in the Pacific warm water springs or vents with temperatures up to 60 degrees Fahrenheit. And around the vents a myriad of creatures no one had ever seen before. Biologists were astounded. What were these animals doing there? What could they find to eat in that desert world? Geologists were equally confounded. A mile and a half down they had discovered four vents in a small area. Was this a freak oasis or were there even more vents. Could such vents even be a worldwide phenomenon? Now a second expedition sets out to explore the vent area to seek answers to these questions. The answers they find will revolution our concept of the deep sea (...)”

Dive to the edge of Creation (directed by James Lipscomb and narrated by Leslie Nielsen, 1980)

Hydrothermal vents and organic falls are ephemeral habitats that are colonized by organisms relatively fast Vrijenhoek (1997). Their temporal nature is dependent on the underlying geology and some vents are recent while others are old. Organisms living there have adapted to the low stability and the ephemeral nature of the hydrothermal vents (and other reducing habitats) (e.g. Smith et al. 1989, Vrijenhoek 1997). Many invertebrates have short lifecycles, and what we consider as temporal in geological scale is for them very long from a reproduction and colonization perspective. Hydrothermal vents are of different age; while older ones are slowing and cooling down, new ones begin and a new colonisation process starts. Each vent can be considered a stepping-stone in the colonisation process, and e.g. Smith et al. (1989) and Tandberg et al. (2013) also include fall biota among the stepping-stones because of the similar reducing conditions. The temporal overlap between these stepping-stones provides sufficient stability of reduced habitats to support deep-sea reduced ecosystems in spite of the relatively short lifetime for each “stone”. The stability of communities linked to reduced habitats is made possible by some key factors. First is the temporal overlap mentioned above, i.e. a new vent or fall is colonized before the first has declined. Second, the reduced habitats are wide spread even though hydrothermal vents are located along mid ocean ridges. Thirdly, many of the organisms have life history strategies and adaptations supporting pelagic transport, making it possible to colonize new sites and complete and thereby maintain the life cycle regardless of the instability within individual vent sites. The distinct similarities in biogeographic regions (Rogers et al. 2012), endemism and rapid

colonization are indications of stability. The key to this stability is the large number of temporally diverse hydrothermal vents and the continuous supply of new large falls such as e.g. whales and tree trunks. Thus, if we can consider the nature of vent biology to be truly stable, we then need to ask, what role do vents play in a broader perspective such as e.g. plankton life history and seasonal cycles? Are hydrothermal vents and other reduced habitats housing unique, isolated niches for small and fragile compilations of organisms, or are they part of a larger, vital community that is able to provide the stability needed over time for genetic adaptation (opposed to plasticity) and endemism? Are these systems indicators that the marine ecosystem is resilient to rapid environmental changes or are they an exception? These are important scientific questions and in this thesis I will provide more information to underline why we should be investigating them by showing that it is critical for our understanding of the deep-sea (biologically and geologically) to view hydrothermal vents and their complex surrounding ecosystems as something more than a curiosity.

Food webs are the focus of this thesis, with a particular emphasis on the molecular trophic interactions in the pelagic realm around hydrothermal vents. To our knowledge this is the first study to use molecular approaches to understand the feeding of the deep-sea *Themisto abyssorum* in the Nordic Seas. Furthermore, the environmental perspective, better understanding of hydrothermal vents and the deep-sea ecosystems is particularly important today to establish baselines since the deep-sea technology that is providing us with more biological insights, is also providing economic interests with the tools to exploit new deep-sea natural resources.

1.1 Hydrothermal vents

The pioneer spirit of deep-sea researchers has somewhat diminished as people had come to believe that not much life was found there. Then, in 1977, life was found at hydrothermal vents in the Pacific Ocean (Lonsdale 1977). Renewed interest for the benthic deep-sea, deep pelagic ecosystems and their associations with hydrothermal vents followed. This discovery of vents suggested that the deep-sea was far from fully explored. New technologies made it possible to explore extreme depths, and

corresponding developments in technology-based methodologies opened up better possibilities for studying small, rare and previously undescribed fauna. Advanced identification tools including electron microscopy and DNA barcoding have during the last two decades become commonly available. Thus, since 1977 more than 600 new species have been described from hydrothermal vent ecosystems and increasing numbers of new vent sites are being found throughout the world (Desbruyères et al. 2006). The deep-sea will never again be considered as a solely detritus-dependent environment. It is now understood as being a highly productive habitat with hundreds of endemic species connected to chemosynthetic primary production (e.g. Ramírez-Llodra et al. 2010). The discovery of hydrothermal vents in the Nordic Seas further renewed interest for continuous exploration of deep and offshore parts of the Nordic Seas. This has resulted in the description of new species, and has led to increased knowledge about the diversity of these ecosystems. Inventories will continue to be a significant part of deep-sea research. However, with more knowledge of the fauna in these systems, we are now asking questions relating to food web and trophic interactions, ecosystem functions, the role of hydrothermal vents vs. non-vent (both vertical and horizontally), and natural resources (biotic, oil, gas and minerals) as well as potential anthropogenic impacts.

Sites with chemosynthetic primary production are referred to as reducing habitats such as hydrothermal vents and cold seeps (Hügler and Sievert 2011). Organic falls (e.g. whale- and wood-falls), which may share related taxa with hydrothermal vents, are also reducing environments. These latter are established by sulphate reducing bacteria coming from decomposition instead of inorganic input from the Earth's crust (Van Dover 2000, Smith and Baco 2003, Tandberg et al. 2013). As mentioned before, renewed interest in the deep-sea was followed by additional discoveries of hydrothermal fields in the Pacific Ocean, and in 1985 the first Atlantic hydrothermal vent was discovered at the Trans-Atlantic Geotraverse (TAG) on the Mid-Atlantic Ridge (MAR) (Rona et al. 1984) (Figure 1). This discovery showed that it was possible to sustain hydrothermal activity and specialized biota even on slow-spreading ridges. Initially, scientists were unsure whether slow or ultraslow-spreading ridges ($<20 \text{ mm yr}^{-1}$) could sustain hydrothermal activity due to their low magmatic

heat budget (Baker and German 2004, Pedersen et al. 2010a). However, the first hydrothermal vent field in an ultraslow-spreading ridge, the Arctic Mid-Ocean Ridges (AMOR) was localized in 2005 (Pedersen et al. 2005, Pedersen et al. 2010a, 2010b). Hydrothermal vents are now recognised as being a worldwide phenomenon.

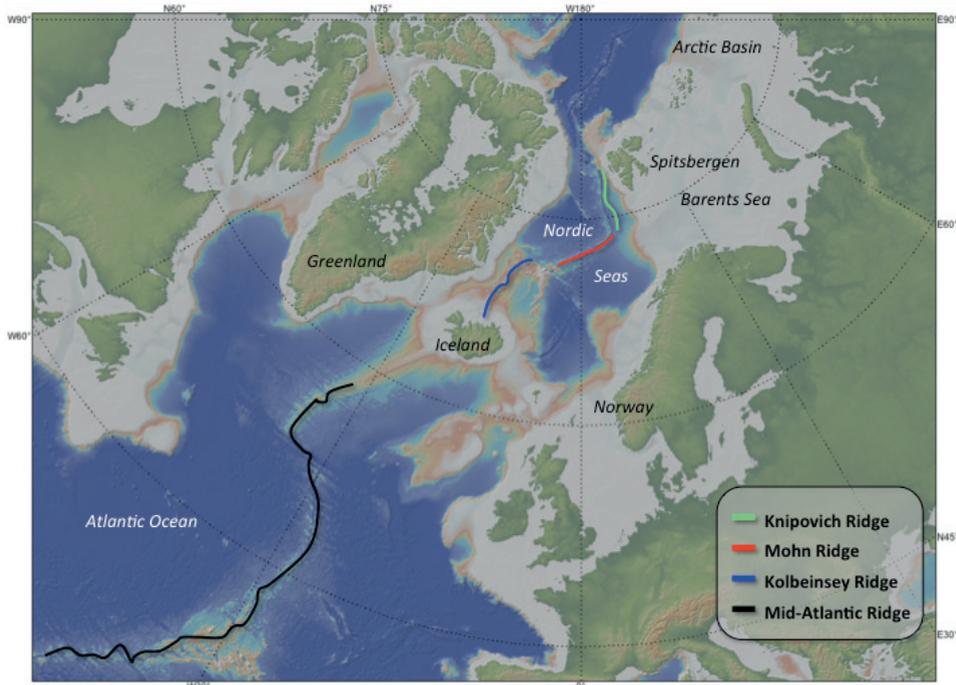


Figure 1. The Arctic Mid-Ocean Ridge (AMOR) constitutes the Kolbeinsey Ridge, the Mohn Ridge and the Knipovich Ridge in the Nordic Seas. It is a continuation of the Atlantic Mid-Ocean Ridge (MAR). Map source: GeoMapApp 3.3.9

The Jan Mayen Vent fields were discovered in 2005 during a cruise with the R/V G.O. Sars (Pedersen et al. 2005). These two vent fields, Troll Wall and Soria Moria are located about 5 km from each other, in rather shallow water at the southern part of the Mohn Ridge (~550 meter), northeast of the volcanic island, Jan Mayen. The second vent field on AMOR was found in 2008 in the bend between the Mohn's and the Knipovich Ridge at 2350 meters depth. This was named Loki's Castle (Pedersen et al. 2010a). In 2013 two more vent fields were found on the Mohn's Ridge (Cruise Report 2013), one close to the Troll Wall vent field and one associated with a volcano at the Kolbeinsey Ridge west of the island Jan Mayen. This latter is a true shallow water vent area with quite low fluid temperatures (~100 °C). The top of the volcano is

only 20 meters below sea level and the vents are situated in the upper parts of the slope from the caldera. CTD (Conductivity, Temperature and Density) transects along the Mohn's Ridge, towards Loki's Castle and the Knipovich Ridge, have indicated that there may be even more hydrothermal vents between Loki's Castle and the Jan Mayen Vent Fields (Cruise report 2013). This discovery of geothermal activity in the Nordic Seas during the last decade is likely to be only the beginning of identifying such activity in this region. On a global scale it is hypothesized that only a fraction of the world's hydrothermal vents have been found (e.g. Baker and German 2004). Indeed, the search for more vents is not only motivated by basic research and explorative purposes. Hydrothermal vents can also function as natural laboratories, e.g. researchers are studying the Jan Mayen vent fields as a natural laboratory for studying natural CO₂ leakage. They hope to learn more about the potential effects of sub-seafloor CO₂ storage and ocean acidification. Bioprospecting is important new bio-industry. Organisms that endure extreme environments, such as that around hydrothermal vents, can have useful enzymes with commercial value. Natural laboratory concepts and bioprospecting are bridges between applied and basic research. Commercial interests such as deep-sea mining for abundant, rare and important minerals associated with hydrothermal vents are also strong motivators for research on hydrothermal vents.

A characteristic feature of hydrothermal vents (hot springs) on the sea floor is the warm-water buoyant plume, which was first confirmed geochemically in 1976 by Weiss (1977). Hydrothermal fluids vary in chemistry, temperature and density from vent to vent. The fluid chemistry varies according to the mineral composition of the crust, while the temperature depends on crust thickness and density, as well as water depth of the vent site. Along the AMOR (Figure 1) we find both black and white smokers diffuse flow sites and gas hydrates as well as bubble plumes (Pedersen et al. 2005, 2010a). Loki's Castle is a typical black smoker field with sulphide chimneys (Figure 2).

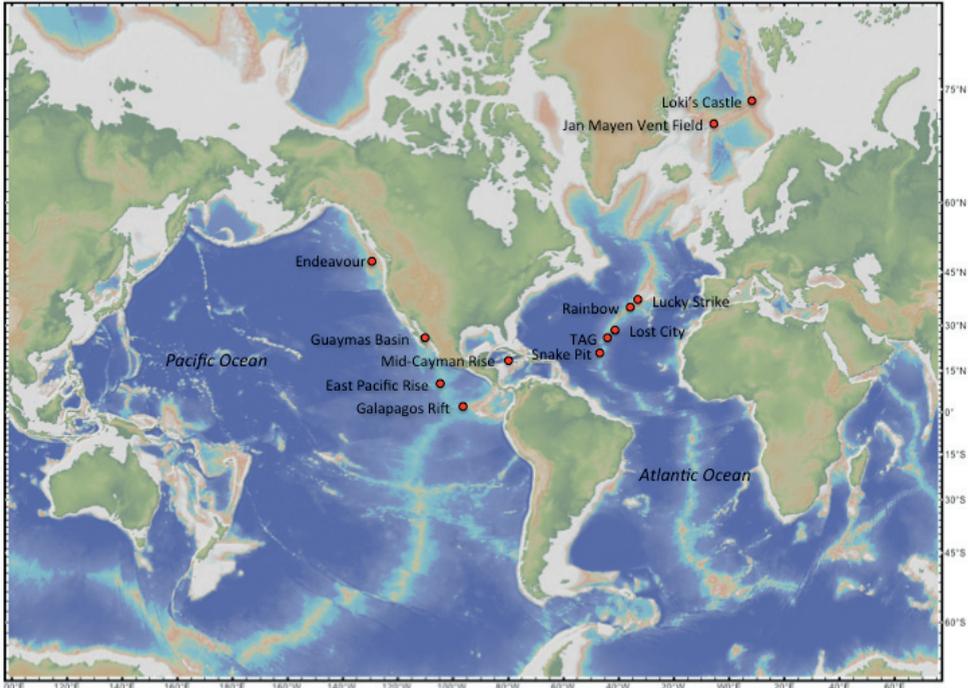


Figure 2. Hydrothermal vents are found throughout the world oceans. The hydrothermal vents in this map represent a selection of more than 200 known vents. Map source: GeoMapApp 3.3.9

Unique to Loki's Castle are the high methane (CH_4), hydrogen (H_2) and ammonium (NH_4) levels, which are similar to sediment-influenced vents found in the Pacific rather than to other vent sites along the MAR (Baumberger 2011). The Jan Mayen Vent Fields have characteristics of MAR sites (270°C) (Figure 2). They are also low in methane and hydrogen, but high in CO_2 (Baumberger 2011). Preliminary data suggest that there are higher levels of inorganic carbon around the vents compared to reference stations (Pedersen et al. 2012). Bubble plumes are a unique feature and recent surveys found that these plumes could be traced to the surface (Pedersen et al. 2012), while the plume water is neutral buoyant at approximately 250 meters depth and transported along currents (Baumberger 2011, Stensland 2013) (Figure 3).

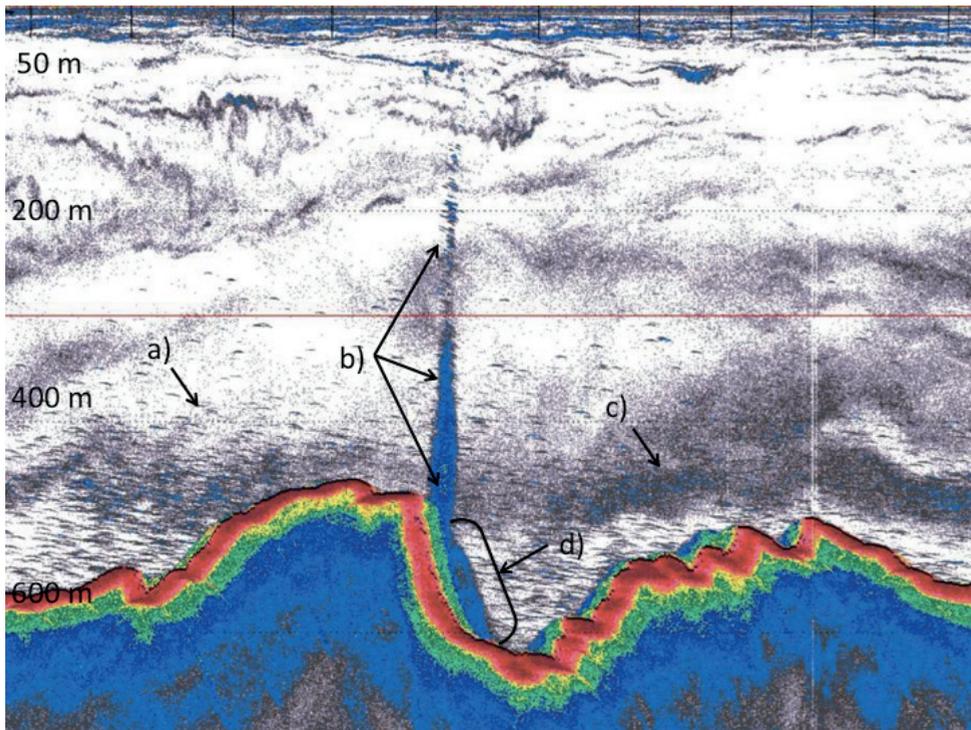


Figure 3. The dark blue scatter above the seabed on the echo-sounder profile (38kHz, Simrad ER60) indicates dense aggregations of biological activity, which is large zooplankton and meso-pelagic fish. The image was taken from the Soria Moria vent at the Jan Mayen Vent Fields. A) Fish, b) gas bubbles ($\sim 1 \text{ cm}^3 \sim 0.5 \text{ ms}^{-1}$), c) plankton and meso-pelagic fish and d) vent field.

Hydrothermal plumes are considered to be pelagic hot spots (Bennet et al. 2013), although the contrast with “non vent” environments is not as distinctive as is found for benthic communities. In the Pacific, Winn et al. (1986) found more bacteria in the plume than in the surrounding water mass. Furthermore, Berg and Van Dover (1987) found similar results for mesozooplankton in the Guaymas Basin (Figure 2). Burd and Thomson (1995) found additional support for higher pelagic production at the Endeavour vent sites, while Skebo et al. (2006) did not find support for a large increase in zooplankton abundance over the vent fields (Figure 2). However, the findings of abundant vent-specific amphipods in the Pacific at the East Pacific Rise ($104^\circ\text{W } 10^\circ\text{E}$), supports the hypothesis that plumes do induce increased biological activity (Kaartvedt et al. 1994). Atlantic studies have been more divergent in their conclusions and in general there may not be sufficient evidence for a hydrothermal vent-induced zooplankton communities at Snake Pit, TAG, Lost City, Rainbow and

Lucky Strike (Vinogradov et al. 2003), while Bennet et al. (2013) clearly demonstrated an increased productivity near the plume at the Mid-Cayman Rise (Von Damm vent site) (Figure 2). Increased planktonic abundances above the Jan Mayen Vent Fields were also found using echo sound imaging to visualize plankton in the water column (Aarbakke 2007). Preliminary sampling and observation using a Remote Operated Vehicle (ROV) supported the echo sound image of high densities of mesozooplankton. Among the larger species observed were the two *Themisto* species, *T. abyssorum* and *T. libellula*. The observed high density of plankton and the observation of the *Themisto* spp. at this site was the first incentive to undertake this thesis (Figure 3).

1.2 Vent fauna of the Nordic Seas

The Nordic Seas comprise three main basins (the Norwegian Sea, the Greenland Sea and the Iceland Sea) that are separated by ridges (Skjoldal 2004). The topography of the Nordic Seas is highly variable with continental shelves, slopes, abyssal plains, seamounts, volcanoes, rifts/canyons, ridges and large elevation differences. This variability gives rise to an immense diversity of habitats that supports a high diversity of fauna (e.g. Skjoldal 2004, Schander et al. 2010) and approximately 200 species have been found at the Nordic Seas hydrothermal vents alone. These numbers are likely to increase since much of the collected material from Loki's Castle has not yet been investigated or published and additional material will be collected in the years to come. Presently, the knowledge of benthic vent fauna in the Nordic Seas is from Jan Mayen communities (Schander et al. 2010), from the seep communities of Håkon Mosby Mud Volcano (Gebruk et al. 2003) and from cold seeps of Nyegga (Vanreusel et al. 2009). In addition, Pedersen et al. (2010a), Tandberg et al. (2011, 2013), Kongsrud and Rapp (2012) and Skarsvåg (2013) have investigated the amphipod, gastropod and polychaete fauna of Loki's Castle.

The first Nordic Sea vents were found north of Iceland in 1974. These were shallow water vents (revealed by surface bubbles), and did not have the characteristics of a vent fauna (Fricke et al. 1989). Expectations were therefore high when the Jan Mayen

Vent Fields were discovered in 2005 (Pedersen et al. 2005). However, in spite of the dense microbial mats, including methane-oxidizing bacteria, and the fact that this vent met all the criteria expected for vent fauna; most of the species found originated from “non-vent” environments (Øvreås et al. 2007, Schander et al. 2010). The general fauna at the Jan Mayen Vent Fields consists of large structural fauna such as echinoderms, anthozoans, gastropods and hydroids. Unique for the Jan Mayen Vent Fields is the high abundance of calcareous sponges in spite of high levels of CO₂. Cladorhizid sponges are also highly abundant on the chimney structures and examples of a new species of *Alexandromenia* (Mollusca) were found inside hard and porous iron-hydroxide deposits (unpublished data).

Presently the only species found in common between Jan Mayen and Loki’s Castle, are the gastropods *Skenea profunda* and *Pseudosetia griegi*, where the latter is also found at the Nyegga cold seeps (Pedersen et al. 2010a, Schander et al. 2010). The two gastropods have also been found on wood falls indicating that they are confined to reduced habitats (i.e. primary production based on energy from reduced inorganic chemicals such as e.g. H₂S and CH₄) (Pedersen et al. 2010a, Tandberg et al. 2013). There are also some polychaetes, brittle stars and gastropods in common between the Jan Mayen Vent Fields and the Håkon Mosby Mud Volcano (Gebruk et al. 2003, Schander et al. 2010). The Håkon Mosby Mid Volcano and Loki’s Castle have, however, symbiotrophic vent organisms such as the polychaete *Scerolium contortum* in common (Gebruk et al. 2003, Schander et al. 2010, Kongsrud and Rapp 2012). Furthermore, the polychaete *Nicomache (Loxochona) lokii*, originally described from Loki’s Castle, belongs to a group associated with hydrothermal activity in the Pacific Ocean (Schander et al. 2010, Kongsrud and Rapp 2012). Skarsvåg (2013) found additional support for vent specific fauna at Loki’s Castle in the amphipod *Monoculodes cf. anophthalma* that was found at both the MAR site, Lucky Strike, and Loki’s Castle. Loki’s Castle is nevertheless different from the Jan Mayen Vent Field because of the presence of true vent fauna (Tandberg et al. 2011, Kongsrud and Rapp 2012, Skarsvåg 2013). There are even subtle indications that Loki’s Castle may represent part of a link between the Atlantic and the Pacific Ocean. However, final conclusions can only be made when all the fauna material from the

Loki's Castle has been identified and further sampling along the AMOR and the Arctic Basin has been conducted.

The mesozooplankton composition of the upper 600 meters of the Nordic Seas has been well studied because of its importance as a trophic link between phytoplankton and higher trophic levels. Included in the commonly studied groups are copepods, amphipods and krill due to their importance for some of the commercially important fish species. Plankton inventories of deep parts of the Nordic Seas are rarely part of regular plankton studies, however, and the few existing studies that have detailed species lists from the deep-sea are old (e.g. Østvedt 1955). More recent studies are primarily focused on very abundant species such as *Calanus finmarchicus* (e.g. Gaardsted et al. 2010). However, the Nordic Seas do not have a unique mesozooplankton fauna. For example, some species that were only reported below 1000 meter in the Nordic Seas were all known Atlantic species found elsewhere further south, also in deeper layers (e.g. Østvedt 1955). Another study showed that species found below 600 meters in the Nordic Seas (Østvedt 1955) were found in the upper layers in the Arctic Basin (Kosobokova and Hirche 2000). These results may provide information about the importance of water masses in terms of biogeographic patterning in planktonic systems (Dalpadado et al. 1998, Dvoretzky and Dvoretzky 2013). The only vent-specific study from the Nordic Seas is, however, a master thesis where 18 species of planktonic crustaceans (mostly copepods) were found in or near the plume at the Jan Mayen Vent Field (Aarbakke 2007, Schander et al. 2010). Information about such populations is therefore an important gap in knowledge of the Nordic Seas.

1.3 Benthic-pelagic coupling

Some dead organic particles sink towards the bottom where they are an important energy source for the benthic community and part of the benthic-pelagic coupling in an ecosystem. Sinking particles range from microscopic faecal pellets, and phytoplankton-detritus to dead whales (Raffaelli et al. 2003). The sinking particles can be utilized by e.g. zooplankton, particle feeders and scavengers (e.g. Alldredge

and Silver 1988, Jackson and Checkley 2011). Actually only a small fraction (approximately 1%) reaches the seabed and most of the surface production is remineralized and decomposed in the upper 2-300 meters. Thus zooplankton function as gatekeepers, preventing particles from sinking, and transporting the chemical energy contained in the particles upwards (Jackson and Checkley 2011). However, a large portion of the Particulate Organic Matter (POM) is buoyant and represents an upward flux (Smith et al. 1989). The organic material that does finally reach the bottom does not accumulate and as even deep-sea benthic fauna respond rapidly to any input of dead debris or organic matter (Graf 1989). A similar result was also shown in the Arctic Basin where the macro-benthos there responds to fall-out from ice algae (Boetius et al. 2013). However, the benthic can also be important to the pelagic and Raffaelli et al. (2003) describes three ways that benthic animals contribute directly to the pelagic community; 1) ontogenetic movement (i.e. active movement due to life-form shifts), 2) active movement and 3) passive movement by e.g. re-suspension. In addition, larger moving predators such as fish can also contribute to move energy from the benthic to the pelagic. Benthic-pelagic coupling is a process of significance (Raffaelli et al. 2003).

In general the marine food web is presented as a phytoplankton-based food web where all new energy is produced in the upper layers (euphotic zone). However, we now know that not all energy is produced in the upper layers through photosynthesis. Hydrothermal vents, gas seeps and sub-sea volcanoes contribute with new energy from the seabed through chemosynthetic primary production (Van Dover 2000). This energy is important at a global scale but difficult to measure (Sievrt and Vetriani 2012). Estimates have suggested 0.02% of the surface production (e.g. McCollom and Shock 1997). Different from the surface production is the fact that chemolithoautotrophic metabolism is less seasonal. Accurate estimates of the chemosynthetic primary production are nevertheless uncertain, particularly because we do not know the true extent of venting. Still, it is clear that hydrothermal vents cause aggregations of zooplankton close to the plumes compared to non-venting areas, i.e. biological hotspots (e.g. Kaartvedt et al. 1994, Burd et al. 2002). Swarms of the amphipod *Halice hesmonectes* were observed above vents in the Pacific

(Kaartvedt et al. 1994), and aggregations of *Themisto* spp. in deep waters have been observed in the arctic (Vinogradov 1999, personal observation), although the swarming behaviour was not confirmed as being specifically connected to vents. Additionally, Vinogradov (1999) did not find a specific vent effect at the Lucky Strike or Broken Spur vent fields in the Atlantic and suggests that aggregation observed with submersibles were an artefact due to possible attraction to the submersibles lights. Echo-sound imaging results can nevertheless be considered as showing support for aggregation caused by hydrothermal activity (figure 3).

1.4 Marine Food Webs

Predators within an ecosystem shape the community by affecting prey population abundance and distribution (Begon et al. 1996, Verity and Smetacek 1996). Thus, investigating trophic interactions within an ecosystem is of great importance to understand its structure and function (Valentini et al. 2009, Carreon-Martinez and Heath 2010). Marine food web studies can be both a top-down and bottom-up perspective. However, both perspectives should be considered simultaneously because resource limitation in a food web has not been able to explain the processes of the marine plankton communities' alone (Verity and Smetacek 1996). The concept of top down control was introduced through the “*Green World Hypothesis*” developed by Hairston et al. (1960), illustrated that terrestrial herbivores were not resource limited but predator controlled, while secondary predators were resource limited. These observations led to the understanding of trophic cascades. Trophic cascades, i.e. trophic links that influence each other (e.g. Carpenter et al. 1986), and top-down control theories from terrestrial ecosystems were later adapted to limnic ecosystems and more recently to marine systems (Verity and Smetacek 1996, Terborgh and Estes 2010 and references therein). The adaption towards the marine system was strongly motivated from a fishery management perspective. Even though international conventions and national laws regulate fishery activities, negative and even severe effects on the ecosystem are common, and we are still lacking good predictive tools that include the whole ecosystem. Indeed, studies of cascades in

fishery indicate consequences over several trophic levels in the food web (Verity and Smetacek 1996, Casini et al. 2008).

Cascading effects are difficult to identify in pelagic systems because of food web complexity and perturbations (excessive disturbance) of several trophic levels (Andersen and Pedersen 2010). Also, the potential of marine systems for structural redundancy can prevent cascading effects (i.e. multiple species covering the same niche). For example, increased abundances of *Themisto* spp. correlated to a reduction by its main predator, capelin (Dalpadado et al. 2001). However, since there was less capelin available for other predators (cod, seals and birds) there was an increased grazing on *Themisto*, possibly preventing cascading effects on the next trophic levels (e.g. copepods). Furthermore, advection contributes to preventing cascading effects caused by human impact or natural changes (Terborgh and Estes 2010). Thus, cascading effects in rich shelf areas like the North Sea have been difficult to address (Reid et al. 2000). However, there are other examples of cascading effects in the marine environment. E.g. cod stocks in Georges Bank off Nova Scotia (NW Atlantic) collapsed during the late 1980s and early 1990s (Frank et al. 2005). Time series of nutrients, zooplankton, benthic fish, pelagic fish, grey seal and some invertebrates indicated that overfishing had led to cascading effects (Frank et al. 2005). Recovery of this ecosystem is still on going, and it may be limited by increase in e.g. the seal population causing high cod mortality, temperature decline and bycatch in other fisheries (Frank et al. 2005, Shelton et al. 2006, O'Boyle and Sinclair 2012). Another example is from the Baltic Sea where eutrophication has, until recently been explained by a bottom-up control mechanism due to increased nutrients load (e.g. historical overview by Voss et al. 2011). Studies by Casini et al. (2008) suggest that cod fisheries in the Baltic Sea caused cascading effects. Reduction of cod led to growth of planktivorous sprat that indirectly contributed to a reduction of herbivorous zooplankton. Thus, eutrophication and phytoplankton growth could be explained by a top-down mechanism. Although somewhat controversial, this indicates that we have to consider both top-down and bottom-up control to understand cause and effect in ecosystems. Common from both examples mentioned here was the availability of

time series and knowledge of predator-prey interactions within the respective ecosystems.

Several trophic links in the marine ecosystem are well studied and have yielded substantial amounts of data. Nevertheless, there are still gaps of knowledge and studies of the marine environment are complicated due to the inaccessibility of this environment and species diversity. This is especially the case for the identification and quantification of trophic interactions where direct observation is seldom an option and gut content analyses may be biased towards hard parts, which are not easily digested, and as a result the frequency of e.g. soft-bodied prey often remains unknown in these studies. Thus, further progress and understanding of ecosystem mechanisms would benefit from alternative methods that can provide high-resolution species-specific data. Alternative methods should also be able to investigate lower trophic levels and smaller organisms as predators that can be difficult to dissect and investigate with microscopy. Methods previously used in *Themisto* foraging studies are stable isotopes, fatty acids and microscopy (e.g. Auel et al. 2002, Søreide et al. 2006, Dalpadado et al. 2008). In addition feeding experiments have been conducted in the laboratory (Sheader and Evans 1975).

The use of stable isotopes (nitrogen (^{15}N) and carbon (^{13}C)) as trophic markers expresses ratios between the natural form (standard) and the isotope. There is an increase in the ^{13}C content ($^{13}\text{C}/^{12}\text{C}$ ratio) and ^{15}N content ($^{15}\text{N}/^{14}\text{N}$ ratio) of the organism due to selective metabolic loss of ^{12}C and ^{14}N during food assimilation and growth. Carbon is suitable to determine the carbon source, i.e. the primary production source, which can tell us whether a species' food web base is e.g. algae (phytoplankton) or of hydrothermal vent origin (chemolithotroph). Nitrogen can also distinguish trophic levels because predators accumulate ^{15}N and each trophic level will be enriched in ^{15}N compared to the previous level (Petursdottir et al. 2008).

Another commonly used method involves fatty acids (Graeve et al. 1997). However, similar to stable isotopes, it rarely identifies species-specific relationships. The use of fatty acids in foraging ecology is based on the fact that some groups have specific

fatty acid patterns and these patterns are stable throughout the food chain (Petursdottir et al. 2008). However, fatty acids can also have a taxon-specific component (Olsen et al. 2009). This component is small relative to the overall fatty acid component ingested through feeding and is often masked Graeve et al. (1997). The taxon-specific component can be linked to specific tissues and it is therefore possible to adapt the study accordingly (e.g. Birkely et al. 2003). Due to these factors fatty acids therefore work best with well-established and known fatty acid patterns, and with taxa that have specific biomarkers; hence it is restricted to specific systems.

Since neither fatty acids nor stable isotopes can adequately identify species-specific predator-prey interactions, other methods are needed. Traditionally, the most commonly used species-specific diet and stomach investigation tool is microscopy. Microscopy can provide results directly from the field, it is a relatively low cost analysis (but also high cost, see below) and most laboratories have adequate equipment. More advanced microscopy methods e.g. (electron microscopy) can supplement and significantly improve the visual resolution of prey items. However, there remain detection limits for stomach content and faecal pellets using microscopy. The Method is also limited to organisms that we could dissect stomachs from; hence a size restriction. In addition, microscopy is often laborious and requires high taxonomical expertise.

Species-specific molecular based tools can complement techniques such as stable isotopes, fatty acids and microscopy. The early, species-specific dietary investigations included protein electrophoresis and use of monoclonal and polyclonal antibodies. However these were laborious and costly (Symondson 2002). The use of proteins as target was primarily applied in terrestrial research fields in order to identify predators' impact on crops. The next step was the use of DNA-PCR techniques that were applied successfully already in the late 90's and early 2000's (e.g. Chen et al. 2000, Rosel and Kocher 2002). The DNA-PCR approach was cost efficient and required a minimal of laboratory infrastructure (Symondson 2002).

Advantages of DNA-PCR trophic interaction analysis are many. Firstly it has the potential of taxonomical identification of prey even with no visible traces in the gut. Furthermore, a molecular approach can be predator specific or universal, i.e. it can target one specific prey or give a complete overview of prey particles. The latter is particularly useful for analysis of generalist predators. It is further non-invasive because it can be applied in situ and will therefore represent feeding under natural behaviour patterns. Molecular tools are high throughput and cost efficient, which enable us to analyse the high number of samples that are often required in ecological studies. Finally, the size of the predator analysed is not important and it identifies unknown prey. For these reasons, molecular tools are attractive and can be promising within a number of different fields and environments investigating trophic interactions (e.g. Symondson 2002, King et al. 2008, Carreon-Martinez and Heath 2010, Pompanon et al. 2012).

In general there are two methods to identify predator-prey relationships using a DNA based approach. The first targeting prey by using specific primers based on *a priori* knowledge of the prey field, while the second uses universal primers targeting all, or a group of prey particles (Deagle et al. 2006). However, template bias from the predator is a significant challenge when applying universal primers because prey target genes are few compared the predator. When using faeces we avoid tissue from the predator, however studying faeces of small invertebrates can be challenging. One approach to overcome this is the use of blocking probes in the initial PCR (e.g. Vestheim and Jarman 2008). The use of blocking probes (primers) in combination with non-specific amplification primers is a promising development. A weakness of blocking primers is efficient exclusion of closely related species and is limited to the phylogenetic resolution of the region and target gene (Hadziavdic et al. 2014). Another challenge with blocking primers is its limited flexibility and the need to develop new blocking primers for each predator in a system.

1.5 Molecular taxonomy

A challenge with using molecular tools is that a given DNA sequence does not always reflect a specific species. The use of "Operational Taxonomical Units" (OTUs) or MOTUs (MOlecular Taxonomical Units) as proxies for taxonomical ranks is therefore instrumental because it enables scientists to work with environmental data efficiently (Caron 2013). For example it is possible to extract and amplify DNA from a sediment sample from the seabed instead of processing the sample with microscopy, and the DNA sequences then mimic classical ecological datasets where the OTU represents the taxon. Abundance, diversity and taxonomy depend on sample representativeness, DNA quality (storage/fixation/age), extraction kits, contamination and PCR- and template bias. Proper laboratory and sampling protocols can help to minimize these challenges. However, primer and gene choice are linked to the taxonomy and phylogeny (evolution) of each taxon and cannot always be solved through protocol adjustments in the lab. For example 18S rRNA in copepods is variable and can be used to separate two families even with relatively short gene fragments. In contrast, Porifera and Choanoflagellata have low variability in parts of 18S rRNA gene leading to confusion although they are from two different kingdoms. Other regions of the 18S rRNA gene should therefore be chosen as targets if these groups are specifically studied in an ecosystem. Thus, gene and primer choice can affect the results. Other problems of taxa assignment are connected to barcoding activity and database quality. The quality of each sequence in NCBI (National Centre for Biotechnology Information) depends on the labels that researchers use when they deposit sequences, and can generate erroneous conclusions if they are wrong.

The capacity of sequence technology has developed from a few hundred sequences (or less) to hundreds of thousands in recent years. Environmental samples from soil or water may also contain numerous unknown single and multicellular organisms (e.g. benthic meiofauna). Unidentified sequences (unclassified environmental sequence) have therefore increased exponentially with this technological development. The unclassified environmental sequence labels are not very useful as identifiers, but should nevertheless be uploaded to databases. However, it is important

that they are not given names based on other database sequences because those could be wrong. It is pivotal that labels are exclusively given based on assured information about the sequence that is deposited. Consequently, we accept that data are submitted without taxonomical specific labels. As the growth of “unclassified environmental sequences” will inevitable be larger than quality assured barcoding, one should complement the labelling with detailed environmental information like depth/altitude, position, location name, physical characteristics and source. This will ease later interpretation of these sequences.

The final output of molecular data for ecological interpretation is particularly sensitive to the species cut-off level. We can generate different results with varying cut-off values using the same data set. A cut-off value refers to percentage similarity value between sequences, and is based on the assumption that similar sequences are taxonomically closer than less similar ones. The use of OTUs can therefore provide an overview and estimation of the diversity. I.e. OTUs can generate a biodiversity estimate, but the choice of cut-off level will influence this estimate (Hadziavdic et al. 2014). The prokaryote 16S rRNA cut-off values are consistent at 97-99% similarity for species limitation (Stackebrandt 2011). Consensus contributes to make studies more or less comparable. Eukaryote consistency does not exist and several genes and regions are used. The lack of agreement leads to continuous use of different gene fragments and cut off levels. The trend is nevertheless the use of similarity cut-off values between 95 and 99% in many regions of the 18S rRNA gene.

2. Aim of study

The overall aim of this study was to increase our knowledge about the pelagic ecosystem of the Nordic Seas hydrothermal plumes. The project aimed at developing a molecular tool to study the trophic interactions of a key mesozooplankton component in the Baltic using universal primers, application of these tools on one mesozooplankton component in the hydrothermal plume ecosystem and finally to make a description of the pelagic eukaryote micro organisms at the base of the heterotrophic food web in hydrothermal vents of the Arctic Mid-Ocean Ridge. The work was divided into the following sub-projects:

- Development of molecular tools and protocols to screen eukaryote microorganisms in the water mass and gut of crustaceans
- Study and compare the diet of *Themisto abyssorum* at two hydrothermal plumes and a methane seep in the Nordic Seas
- Describe and compare the eukaryote microorganism genetic biodiversity from the water column above two hydrothermal vents, two reference stations and one cold seep

3. Results and discussion

This thesis aimed to investigate the food web of hydrothermal plumes recently discovered in the Nordic Seas (**Paper III** and **IV**). We knew very little about the feeding behaviour of the targeted species *Themisto abyssorum* at this depth and in proximity to the vents. We wanted to investigate the diet in-depth and dissection would likely result in an overview of the prey that leave traces of hard parts. Molecular tools were likely to yield a more complete overview of the interactions, including soft tissue prey. Development of a PCR-DNA technique was needed before it was possible to study the gut content without large interference of predator DNA (**Paper II**). Deep-sea studies have challenges regarding sampling and handle-time of the organisms before DNA extraction. Thus, the molecular method was developed on a common copepod species from the Baltic Sea, *Limnocalanus macrurus* (**Paper I** and **II**).

Using universal eukaryote primers alone, normal PCR amplification would have exclusively generated predator amplicons in the analysis. We therefore had to develop a method to exclude predator DNA. An application for investigating prey genomic DNA in copepod gut using Denaturing High Performance Liquid Chromatograph (DHPLC) was successfully developed in **Paper II**. The DHPLC has predominately been used to identify single nucleotide polymorphism (Xiao and Oefner 2001). The ability to identify single nucleotide differences in amplicons and separate them was particularly attractive for ecological questions. An additional feature that makes the DHPLC very useful was the possibility to collect the separated amplicons in a fragment collector. However, the amplicon mix needs to be in even ratios (Troedsson et al. 2008). Troedsson et al. (2008) further developed a DHPLC assay with universal 18S rRNA primers combined with a blocking probe (peptide nucleic acid probe specific for the host/predator) that reduced the host amplification to achieve even ratios between parasite amplicons from the hemolymph of blue crab (*Callinectes sapidus*). Thus, instead of one chromatographic peak representing the host, the chromatogram then contained several parasite peaks that were collected and

sequenced. This assay could be used for detection of trophic interactions as well. However, instead of using a blocking primer/probe to remove dominant amplicons from the predator we introduced a second DHPLC run excluding the predator amplicons in the chromatogram. This exclusion process involved subsampling the DHPLC eluate using the fragment collector at fixed intervals excluding the predator eluate and thereby generating more homogenous ratios of the amplicons. Each of these was re-amplified and separated on the DHPLC. This identified prey amplicons since most of the predator dominance was reduced and it enabled us to combine universal primers with no use of blocking probes. The DHPLC method developed here is therefore a high throughput method that can be efficiently applied on several predators in an ecosystem with a relatively low running cost. The optimization process, that is obligatory for the quality of the results, is considered the most time consuming process. The optimization pipeline has however been streamlined. Since the DHPLC excludes the abundant amplicons and targets the rare amplicons, there is also less need for high sequencing effort. The bioinformatics is, compared to other high throughput sequencing technologies, therefore simplified with no need of specialized software or adapted computer pipelines.

Paper I was the first molecular study of trophic interactions for *L. macrurus*. Even though *L. macrurus* dominates the mesozooplankton community in the Baltic Sea only a few studies have investigated this species (Dahlgren et al. 2012). As a first analysis it was therefore particularly important to study the diet of this dominating predator because they have a key role in shaping the ecosystem (e.g. Estes and Palmisano 1974). In **Paper I**, *L. macrurus* was sampled during the ice-free period from April to December, and this was the first study that included seasonal field data on population cycle, energy status, feeding and food composition. This study was also part of developing a novel technique of molecular trophic interactions (**Paper II**) and DNA was extracted from 32 specimens per sampling event. Furthermore, oil sac length was measured on 30 specimens per sampling time and converted to Wax Ester (WE) content. Diameter of largest egg size was also measured from the same 30 specimens and gut fullness was evaluated (**Paper I**).

The diet of *L. macrurus* has predominantly been identified as other copepods, but large phytoplankton have also been found and *L. macrurus* was previously considered as omnivore (Warren 1985). In our study Crustaceans dominated all but one of the stomach samples (**Paper I**). In that sample we found one diatom represented by one sequence, which correlated to the peak abundance of phytoplankton in July. The low frequency of algae sequences in our samples could reflect secondary predation (Sheppard et al. 2005). Also, the large number of metazoan prey may bias the results due to higher tissue mass combined with hard parts that take longer to digest when compared to single cellular algae. This however could potentially apply for both visual and molecular methods. Nevertheless, our results indicated a variety of taxa within Crustacea suggesting that the method was able to pick up different DNA signals. However, co-amplification of both 18S rRNA and 16S rRNA genes caused a significant reduction in resolution of the stomach analysis (**Paper I**). In addition, predator and possibly parasite tissue constituted a high number of the taxa found. This illustrated that the exclusion of predator tissue was not absolute and further that any taxa associated with the predator, not necessarily in the gut, will be detected. Nevertheless, **Paper I** and **II** represented a proof of concept that the use of universal primers in combination with the DHPLC in gut content investigations of whole animals could be conducted, yielding ecologically relevant data.

The carnivorous behaviour suggests that *L. macrurus* assimilated WE directly through feeding, similar to other high latitude copepods (**Paper I**). An alternative hypothesis was that lipids could have been synthesized directly from essential fatty acids from phytoplankton. Our study cannot exclude the latter hypothesis and the degree of lipid synthesis in addition to direct assimilation through carnivorous feeding would require additional investigations. However, our study did suggest that the direct assimilation was the primary source of WE. Furthermore, a low number of mature eggs were found during our sampling (April to December) suggesting that spawning occurred in the ice covered period. In addition, WE increased until November and was subsequently reduced in December, suggesting that *L. macrurus* used the stored lipids from this time, possibly allocated towards reproduction. A high abundance of nauplii in April also supported the hypothesis that *L. macrurus*

spawned once during winter; i.e. a univoltine reproductive cycle. The carnivorous behaviour illustrates the complexity of an ecosystem and the need to study direct predator-prey interactions instead of assuming links associated with classical trophic levels.

The method developed in **Paper I** and **II** was further improved in **Paper III** in order to investigate stomach content of the deep-sea hyperiid amphipod *T. abyssorum* from three different deep-sea localities in the Nordic Seas (Figure 4). The methodological improvements were 1) simplified optimization procedure in the DHPLC and 2) new primers that did not co-amplify the 16S rRNA gene. In addition, we increased the sequencing depth. Furthermore, in **Paper III** single specimens were analysed instead of pooled specimens. All together 16 *T. abyssorum* specimens were analysed, of which six were from the Loki's Castle, six from the Håkon Mosby Mud Volcano and four from the Jan Mayen Vent Fields. The advantage of analysing single specimens was that we were able to investigate a more complete prey field. However, future analyses with e.g. next generation sequencing, pooled samples would increase the numbers of specimens in an analysis and would be advantageous for screening the most common prey particles.

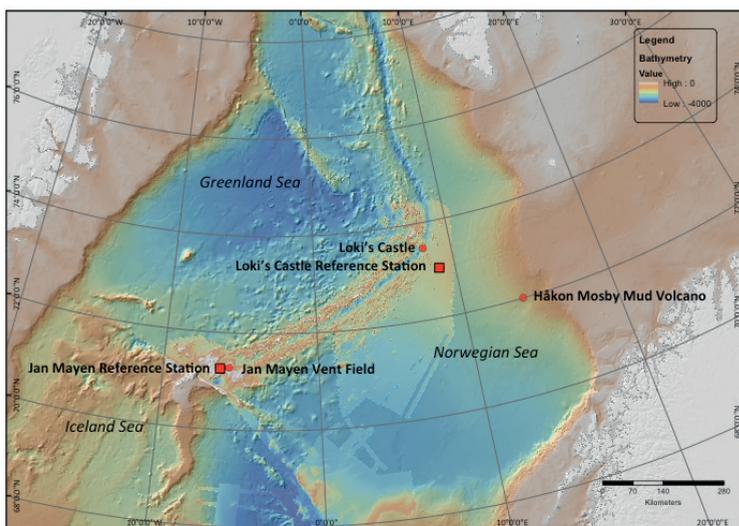


Figure 4. The Nordic Seas constitutes three basins; the Greenland Sea, the Norwegian Sea and the Iceland Sea. The stations that were sampled for **Paper III** are marked with circles and overlaps with the stations for **Paper IV**. **Paper IV** has in addition two more stations marked as squares. Map source: Alden et al. unpublished.

Because of the long haul time (sampling time) predators might feed during the haul and digest prey. However, *Themisto* is known to have a long gut passage time (up to 72 h at 4° C), and suggested to be semi-selective (Sheader and Evans 1975). This suggests that if food was eaten during the haul, it was likely part of their existing diet, and it was unlikely that all specimens had emptied their stomach completely for the original prey before they reached the surface. Consequently, it is likely that our data represents true feeding. *Themisto* spp. is known to be carnivorous and our data identified a dominance of calanoid copepods. Different from other studies, our study revealed that the diet was much more diverse. Specifically, the taxonomical ranges within calanoid copepods were wide compared to recent microscopy studies of the *T. abyssorum* diet (Dalpadado et al. 2008, Kraft et al. 2013).

In **Paper IV** we investigated whether the prey field of *T. abyssorum* is influenced by the vent system or if other factors such as water mass and depth had a stronger influence. This study was also part of a general inventory of the planktonic community surrounding hydrothermal vents in the Nordic Seas. The same localities as in **Paper III** were used, and in addition the Loki's Castle and the Jan Mayen Vent Fields were complemented with reference stations (Figure 4). From each sampling point, five litres of water (sampled with Niskin bottles connected to the CTD) were passed through three filter sizes (5, 10 and 63/125 µm). This filter fraction combination was repeated for each of the three depths from the 5 localities of this study. In total, 45 filters (different locality/depth/filter fraction) were extracted for genomic DNA, amplified using universal eukaryote 18S rRNA primers, cloned and sequenced (96 sequences per sample). Samples were condensed by merging the three filter size fractions part of the same water sample. This resulted in 3719 sequences generating 482 OTUs; representing 5 Kingdoms, 32 Phyla, 74 Classes and 121 Orders. OTUs were constructed based on a 99% similarity, producing a high proportion of single sequence OTUs (almost 60%). At class level the number of singletons was reduced to 20%, with a significant loss of taxa compared to the OTU level at 99% cut off (**Paper IV**). The class level would be similar to 87% cut off level and can be considered similar to taxonomical surrogacy (Bertrand et al. 2006). However, short DNA fragments, as well as the quality of the database itself, limited

us and generated uncertain BLAST results. The use of surrogacy was therefore evaluated, and viewed correct for this analysis given the existing database.

The eukaryote microorganisms of hydrothermal vents in the Nordic Seas have not been studied previously, and an overview of what is present was needed. Questions such as “*what is present*”, “*are vents biologically more similar between themselves or does the background water determine the species composition*” and “*can we explain the patterns that we see*” were discussed in **Paper IV**. Our conclusion was that the communities from vents were similar to the surrounding waters. Hydrothermal vents normally host endemic macro faunal benthic species and the diversity and species composition change across biogeographic regions (Van Dover 2000). In the Pacific this specialization also extends to the mesozooplankton (Kaartvedt et al. 1994). In the Atlantic, even though there is a specialized and endemic benthic vent fauna, no vent-specific mesozooplankton have been found so far. The Jan Mayen Vent Fields have a benthic fauna very similar to the general Nordic Sea fauna from the same region and depth. Our results underline the lack of a unique vent signature at the Jan Mayen Vent Field (Troll Wall) also in the planktonic community. It was, however, less clear at Loki’s Castle since the plume sample was interpreted as different from background samples. This was coherent with preliminary conclusions regarding the benthic fauna at Loki’s Castle, indicating that some species were either endemic to Loki or that some have still not been found elsewhere in the Nordic Seas. The Loki’s Castle hosts species linking fauna to the MAR site Lucky Strike (Skarsvåg 2013), and to the Pacific (Pedersen et al. 2010a, Kongsrud and Rapp 2012). In addition, a new amphipod was described, *Exitomelita sigynae*, at Loki’s Castle (Tandberg et al. 2011).

The difference between Loki’s Castle and the reference station suggest that the surrounding water mass was not the only factor determining species composition within the hydrothermal plumes. Further, depth and chemical conditions might favour some species over others (i.e. competitive advantages). The chemistry is however not likely to be an important factor for the overall biodiversity since there was significant transport of water that diluted the plume. Also, gas concentrations were quickly

reduced to background levels (Stensland 2013), and the vent was small in comparison to the water masses. More specific sampling in and around the plume may yield more in-depth data for short-term influence by the vent. In our study we concluded that the absence of surface taxa and depth was the two most important factors causing the observed difference between vent and reference stations at Loki's Castle. Such a difference could not be found at Jan Mayen. At the Jan Mayen Vent Field a substantial part of surface production reaches the bottom (Sweetman et al. 2013) and the difference caused by increased nutrient availability at the vent may be shaded by the surface production. At the depths of Loki's Castle a much smaller fraction of the surface production reaches the bottom (and the deeper water layers) and therefore the gradient between vent and non-vent was stronger at Loki's Castle compared to Jan Mayen. In addition, Loki's Castle had higher concentrations of energy rich compounds (CH_4 and H_2) than the Jan Mayen Vent Field. This could explain the difference in planktonic species composition between the Loki's Castle and the reference station.

Molecular tools have become important in predator-prey interaction studies and we successfully developed an assay using the DHPLC and universal primers to explore stomach content of an important predator of the Baltic mesozooplankton community. We demonstrated that *L. macrurus* had a predominant carnivorous feeding behaviour, suggesting an assimilation of lipid reserves from the diet over time. The assay developed for stomach analysis of *L. macrurus* was further optimized and applied on *T. abyssorum* and revealed a more extensive prey range than previously reported. The prey field also indicated that the hydrothermal localities were different. The difference between hydrothermal localities based on *T. abyssorum* diet was further supported by data from eukaryote microorganism 18S rRNA diversity at the same localities. It is, however, premature to conclude about the mechanisms behind these findings, but it was clear that the biodiversity was primarily affected by the prevailing water mass rather than the hydrothermal vents. Secondary effects like depth and hydrothermal conditions were indicated by the eukaryote microorganisms, but not by the prey range of *T. abyssorum*. This thesis increased our knowledge about an important pelagic predator but also provided the first results on the deep-water

pelagic community of the Nordic Seas and arctic hydrothermal vents. This thesis has also developed a method to investigate the food web dynamics in these remote systems. Our data and method development is an important step towards the exploration and study of remote ecosystems.

4. Future perspectives

Through the following chapter I will look at some of the scientific challenges and possibilities related to future research and shed some light on the environmental perspective.

4.1 Methodology- the new frontier

Development of molecular biological/ecological tools is driven by a number of scientific disciplines. The recent development in Next Generation Sequencing (NGS) technology represents the near future and there will be a growth of new applications and uses because platforms for NGS provided by e.g. Ion Torrent and Illumina MySeq are made available for a broad range of users. At the same time the costs are reduced and the amplicon lengths possible to sequence increased (Wetterstrand 2013). Thus, the major challenge of deep-sea research is more dependent on sub-sea technology development, sampling technology and sampling frequency (i.e. funding and ship time).

The use of ROV has become a standard sampling technique, and both micro- and macro-biological samples are retrieved easily at many thousand meters depth. Still, there is much more to learn (Van Dover 2011). The future challenge involves time series (monitoring), in situ fixation of samples and in situ experiments. Deep-sea expeditions are expensive, far apart in time and logistically demanding. Even though sites are revisited, the seasonal aspect is rarely possible and basically left out. For instance one has observed that organisms can change from abundant at first visit to absent the next. Gastropods were observed abundantly at the Jan Mayen Vent Fields (Troll Wall) in 2008, virtually almost absent in 2011 and observed again in moderate numbers in 2012. Without means of continuous monitoring, we can only speculate on the mechanisms behind these fluctuations. Time series are used to study seasonal fluctuations and considered important in order to find biological patterns and how they are affected by e.g. physical factors over time. The Continuous Plankton Recorder from the North Sea (CPR, Sir Alister Hardy Foundation for Ocean Science,

SAHFOS) is one of few examples of marine decade-long time series (Reid et al. 2003). The CPR is towed, while others are station based like the Narragansett Bay long-term monitoring of phytoplankton and zooplankton (Rhode Island, USA). Time series or baseline studies have become increasingly important due to the focus on climate change and resource depletion (anthropogenic effects). Data from such studies are invaluable to ecosystem-based management. Gasini et al. (2008) demonstrated the importance of multi-species monitoring over time, because inter-species dynamic may be equally important as physical factors. In coastal areas we can repeat sampling throughout the seasons, while in more inaccessible areas like e.g. the Arctic various moorings have been used to collect physical data instead of year around visits. However, at present they cannot sample biological data. Some sampling conditions like the Arctic winter and ice-covered waters can however often only be maintained for a short period or parts of the year (e.g. Weydmann et al. 2013, Dahlgren et al. 2012). Some of these challenges can be overcome using automate remote sampling buoys like e.g. the Environmental Sample Processor (ESP) that sample water, perform chemical and DNA analysis in situ (Scholin et al. 2009). This system can be used down to 4000 meters depth (Deep-ESP) and has been tested successfully in the Santa Monica Basin (Ussler et al. 2013). The ESP concept uses a sandwich hybridization assay to identify organisms, which imply a priori knowledge of organisms present in the sample. Therefore, ESP is adapted for monitoring, but not optimal in an exploratory context. Still, ESP represents a great potential and similar concept is likely to be part of future research of deep-sea biology, and perhaps a requirement to be able to include time as a factor. The Deep-Ocean Environmental Long-term Observatory System (DELOS) (Vardaro et al. 2013) is another approach that is promising for long term monitoring. The DELOS is an example of how oil, gas and mineral exploration may provide infrastructures necessary for monitoring and sampling seasonal data. Collaboration with industrial partners may be considered controversial, but it may also be one of few possible ways to perform the needed long-term sampling. The industry represents an environmental challenge for the deep-sea that can only be addressed through scientific effort and communicated to the

public by the scientific community. However, environmental challenges can function as the incentive for collaborations (Collins et al. 2013).

4.2 Deep-sea in an environmental perspective

The deep-sea is exposed to threats from bottom trawling and sub-sea mining in addition to oil and gas exploration, submarine cables and pipelines, waste disposal and energy production (Allen 2001). Deep-sea management is challenging due to the legal aspects in international waters, and due to political disagreements within the Exclusive Economical Zone (EEZ). Deep-sea outside national jurisdiction is largely unregulated and subjected to the U.N. Convention on the Law of the Sea of 1982 (UNLOSC), the Implementation of part XI of the 1994 Agreement (Allen 2001), and the U.N. resolution 59/25 (2004) that explicitly urge states and regional fishery management to use the pre cautionary principle. Moreover, the International Seabed Authority (ISA), established under the LOSC and 1994 Agreement, oversees seabed activities like deep-sea mining. Deep-sea mining is a legal minefield and the sovereignty to deep-sea mineral resources still makes it difficult for e.g. United States of America to sign and ratify the treaty (Allen 2001, DeMint; The Washington Times 2012).

Hydrothermal vents have become commercially interesting because of the mineral resources that become increasingly more important as terrestrial resources are depleted. This in turn leads to increased demand and prices of raw material. Nations with geothermal conditions and minerals in their seabed in their Exclusive Economic Zone (EEZ) find this particularly attractive. However, the consequences of mining are not obvious. Though, some effort to investigate consequences has been made on polymetallic nodule fields (Tiefsee-Umweltschultz and Thiel 2001). In parallel, the general interest in deep-sea minerals has generated a series of studies that aim to unravel the effects of deep-sea mining. Some find rather small effects (e.g. Radziejewska 2002), while others have seen a significant negative effects (e.g. Nath et al. 2012) and even indications of long-term effects have been reported (Borowski 2001). Deep-sea mining is at the brink of large-scale exploration and the media in

Norway announced already in 2013 the mineral resources at the AMOR as the new large-scale industrial goldmine. Even though the exploitation of the AMOR is relatively far away in time, the International Seabed Authority (ISA) will probably approve the first licenses to exploit mineral from the seabed in 2016. In order to handle the eminent activity at the seafloor one depends on focused scientific activity and an active community of scientists and advisors. The Centre for Geobiology (CGB) represents such a unit, and this work should continue in order to maintain the knowledge gathered. Presently one does not have knowledge of the vulnerability of these habitats and how they would respond to a possible mining situation. Recent and unpublished knowledge indicate that the deep-sea vents of the Arctic like Loki's Castle hosts an endemic and unique biodiversity. Future environmental and management advice must be based on knowledge that is still not at hand. The CGB will therefore play a pivotal role in gathering information and knowledge about the macro faunal benthic community.

4.3 Future directions

The future of deep-sea management holds many challenges. Scientific inter-collaborative initiatives in combination with an industrial dialogue will be instrumental to solve future scientific and environmental challenges. The CGB initiative is an example of a scientific inter-collaborative. The research at CGB combines applied science with more basic research questions. An example is the work with potential leakage of CO₂ from sub-seafloor storage sites where CGB has access to both the storage sites and CO₂-rich hydrothermal vents used as natural laboratories for CO₂ leakage.

Hydrothermal vents have become the new frontline of multidisciplinary studies of marine sciences, and geobiology has evolved from palaeontology towards e.g. applied studies of functional genes (e.g. thermophile prokaryotes, corrosion studies). The broad range of scientists gathered to describe the habitat from all angles including chemistry, geology and biology has led to a better understanding of the

interaction between geology and biology and created a foundation for scientists to continue their research.

However, game changing technological development has made it economically viable to utilize the resources, the society want to harvest this and some might even claim that we need to utilize the resources available. Isolated, this might not start a new ecological catastrophe, but we do not know the scale or the intensity of future exploration. We know, on the other hand, something about what we can lose (e.g. biodiversity and genetic resources). Inevitably humans will enter the realm of hydrothermal vents, and it is therefore of the uttermost importance that hydrothermal vents and ridge systems are implemented to future and existing management plans. International waters contribute to a challenging political landscape and collaboration where basic research and monitoring will be important pillars for sustainable management.

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