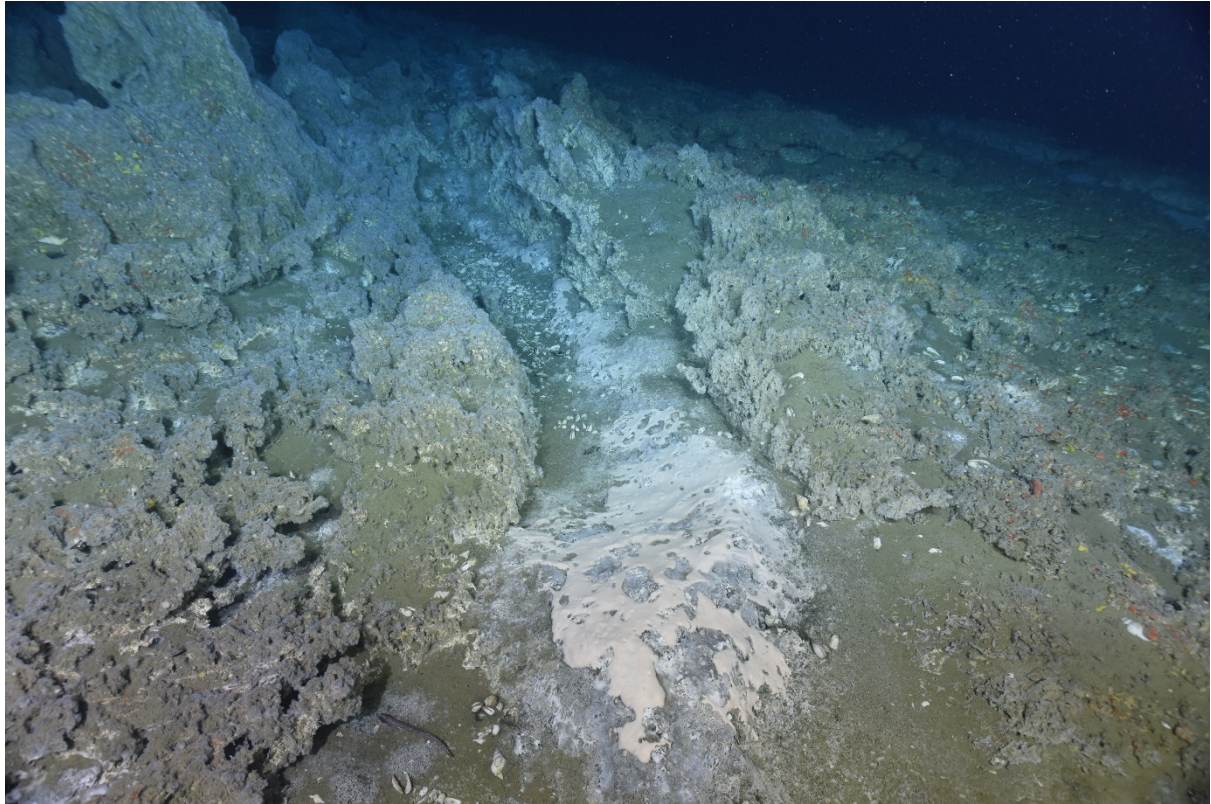


# Global and Regional Variations in Methane Seep Communities

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Microbial mats in between carbonate structures with red and yellow sponges on the carbonates

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# Abstract

The increasing focus on exploitation of the deep sea is raising considerable concerns about how these ecosystems will be impacted. There is increasing demands for natural resources to meet human population growth and declining resources on land, which are driving interest in deep sea resources. Deep sea organisms are often slow growing and long-lived, so destructive practices such as trawling and mining have the potential to cause considerable damage. Methane seeps are chemosynthetic ecosystems that rely on the microbial oxidation of methane to provide food for a diversity of fauna, and provide provisioning, regulating and cultural services to society. Despite these ecosystems being identified as vulnerable marine ecosystems by the Food and Agricultural Organisation of the United Nations (FAO), they have limited protection from anthropogenic disturbances.

My thesis addresses knowledge gaps on the global bioregional variations of methane seep communities and the regional effects of bottom trawling on methane seep ecosystems. I aimed to understand bioregional patterns of methane seep communities and the effects of trawling, to highlight the global diversity of methane seep ecosystems and provide insight for future management actions and decisions.

In chapter two, I conducted a global meta-analysis to examine the bioregional variation in methane seep meiofauna, macrofauna and associated benthic and pelagic communities. I found significant bioregional variation in methane seep communities where methane seeps clustered in the bioregions depicted by Costello et al. (2017). I also found that variations in methane seep communities correlate with changes in depth. My study highlights the importance of understanding methane seep bioregional variations in marine management. Understanding bioregional variations will ensure that there is representation of the diversity within methane seeps, in marine management to avoid biodiversity loss.

In chapter three I studied variations in the benthic and pelagic community at methane seeps on the Hikurangi Margin of New Zealand. I assessed how these variations correlated with depth, trawl intensity, and rugosity to understand the factors driving variations in methane seep communities. I found variation in methane

seep communities corresponded with variation in trawl intensity and depth. Increasing trawl intensity correlated with a decrease in the abundance of endemic methane seep taxa (*Lamellibrachia* tube worms, *Calyptogen*a clams and *Bathymodiolus* mussels). I also observed that in areas of high trawl intensity methane seep fauna were taking refuge around the carbonate structures at the methane seeps.

My results emphasise the need for management plans to be enacted to prevent anthropogenic disturbances to methane seeps. Management of methane seep ecosystems needs to ensure that the diversity of these ecosystems is represented within management plans, to avoid biodiversity loss in the deep sea. To do this management of methane seep ecosystems will need to take a bioregional approach and manage within bioregions at small scales so that the diversity of methane seep fauna is represented.



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# Table of Contents

|  |            |
|--|------------|
| <b>Abstract</b>  | <b>iii</b> |
| <b>Acknowledgements</b> .....  | <b>v</b>   |
| <b>Table of Contents</b> .....   | <b>vii</b> |
| <b>List of Figures</b> .....   | <b>xi</b>  |
| <b>List of Tables</b> .....  | <b>xv</b>  |
| <b>Chapter 1. Introduction</b> .....   | <b>1</b>   |
| 1.1 Ecosystem services .....   | 2          |
| 1.2 Threats to the deep sea .....  | 3          |
| 1.2.1 Trawling .....   | 3          |
| 1.2.2 Mining .....   | 4          |
| 1.2.2.1 Oil mining .....   | 5          |
| 1.2.2.2 Mineral mining .....   | 6          |
| 1.3 Deep-sea conservation .....  | 8          |
| 1.3.1 Ecologically and biologically significant areas .....                      | 11         |
| 1.3.2 Vulnerable marine ecosystems .....   | 11         |
| 1.4 Methane seeps.....   | 12         |
| 1.4.1 Methane seep ecosystem service.....  | 13         |
| 1.4.2 Methane seep protection .....  | 14         |
| 1.5 Aims and thesis structure .....  | 15         |
| <b>Chapter 2. Global Bioregional Variation in Methane Seep Communities</b> ..... | <b>16</b>  |
| 2.1 Introduction: .....  | 16         |
| 2.1.1 Biogeography in the deep sea .....   | 16         |
| 2.1.2 Methane seeps .....  | 17         |
| 2.2 Methods: .....   | 19         |
| 2.2.1 Data Collection:.....  | 19         |
| 2.2.2 Environmental data .....   | 20         |

|  |  |           |
|--|--|-----------|
| 2.2.3  | Statistical analysis:.....   | 27        |
| 2.2.3.1  | Bioregional variation in methane seep communities .....                  | 27        |
| 2.2.3.2  | Environmental variation in methane seep communities within<br>bioregions | 27        |
| 2.2.3.3  | Global environmental variation in methane seep communities ....          | 27        |
| 2.3  | Results .....  | 28        |
| 2.3.1  | Benthic and pelagic fauna.....   | 28        |
| 2.3.1.1  | Global environmental variation in methane seep communities ....          | 28        |
| 2.3.1.2  | Bioregional variation in methane seep communities .....                  | 28        |
| 2.3.1.3  | Environmental variation in methane seep communities within<br>bioregions | 29        |
| 2.3.2  | Macrofauna .....   | 31        |
| 2.3.2.1  | Global environmental variations in methane seep communities...           | 31        |
| 2.3.2.2  | Bioregional variation in methane seep communities .....                  | 32        |
| 2.3.2.3  | Environmental variation in methane seep communities within<br>bioregions | 32        |
| 2.3.3  | Meiofauna .....  | 35        |
| 2.3.3.1  | Global environmental variations in methane seep communities...           | 35        |
| 2.3.3.2  | Bioregional variation in methane seep communities .....                  | 35        |
| 2.3.3.3  | Environmental variation in methane seep communities within<br>bioregions | 35        |
| 2.4  | Discussion:.....   | 37        |
| 2.4.1  | Effects of bioregion on methane seep communities globally .....          | 38        |
| 2.4.2  | Environmental effects on methane seep communities .....                  | 38        |
| 2.4.3  | Small-scale community variability .....                                  | 40        |
| 2.5  | Conclusions.....   | 41        |
| <b>Chapter 3. Methane Seeps on the Hikurangi Margin: Comparisons of<br/>Communities and the Effects of Bottom Trawling .....</b> |  | <b>43</b> |

|   |           |
|---|-----------|
| 3.1 Introduction: .....                               | 43        |
| 3.1.1 Methane seep community diversity.....           | 43        |
| 3.1.2 Ecosystem services from methane seeps .....     | 45        |
| 3.1.3 Methane seep conservation .....                 | 46        |
| 3.2 Methods: .....                                    | 47        |
| 3.2.1 Study area.....                                 | 47        |
| 3.2.2 Video transects .....                           | 48        |
| 3.2.3 Environmental data .....                        | 49        |
| 3.2.4 Video analysis.....                             | 50        |
| 3.2.5 Statistical analysis.....                       | 51        |
| 3.3 Results .....                                     | 51        |
| 3.3.1 Site descriptions:.....                         | 51        |
| 3.3.1.1 Seep 1S .....                                 | 51        |
| 3.3.1.2 Seep 5S (Uruti) .....                         | 52        |
| 3.3.1.3 Seep 1N.....                                  | 53        |
| 3.3.1.4 Seep 3N.....                                  | 54        |
| 3.3.1.5 Seep site 2S (Glendhu) .....                  | 55        |
| 3.3.1.6 Seep site Kekerengu .....                     | 57        |
| 3.3.2 Environmental effects on the community .....    | 61        |
| 3.4 Discussion:.....                                  | 66        |
| 3.4.1 Community comparisons of the methane seeps..... | 66        |
| 3.4.2 Effects of environmental factors.....           | 67        |
| 3.4.3 Commercially important species .....            | 69        |
| <b>Chapter 4. Conclusions .....</b>                   | <b>72</b> |
| 4.1 Summary of findings .....                         | 72        |
| 4.2 Limitations .....                                 | 73        |
| 4.3 Future research.....                              | 75        |

|                   |   |           |
|-------------------|---|-----------|
| 4.4               | Management implications/ conclusion ..... | 76        |
| <b>References</b> |   | <b>77</b> |

# List of Figures

|  |    |
|--|----|
| Figure 1.1 Types of deep sea habitats with mineral resources. A) Ferromanganese nodules (polymetallic nodules) form abyssal plains. B) Active hydrothermal vent sulphides. C) Inactive hydrothermal vent sulphides. D) Cobalt rich crusts from seamounts. Figure taken from (Orcutt et al. 2020).....  | 7  |
| Figure 1.2 Global map showing the Exclusive Economic Zones (green) and the high seas (blue). This figure is from White C, Costello C (2014) Close the High Seas to Fishing? is licenced under CC by 4.0. ....  | 10 |
| Figure 1.3 Depiction of Regional Fisheries Management Organisations/ Arrangements (RFMO/As) that manage bottom fisheries and species other than tuna. This Figure was taken from Ban et al. (2014) Systematic Conservation Planning: A Better Recipe for Managing the High Seas for Biodiversity Conservation and Sustainable Use with permission.....   | 10 |
| Figure 2.1 Biogeographic regions delineated in Costello et al. (2017). Pink stars indicate methane seep sites from the current meta-analysis. Black lines indicate boundaries between bioregions. This work, Figure 2.1, is adapted from “Costello, M.J., Tsai, P., Wong, P.S. et al. Marine biogeographic realms and species endemism, Figure 2b. Nat Commun 8, 1057 (2017)” used under CC BY 4.0. Figure 2.1 is licensed under CC BY 4.0 by McKenzie Jones. .... | 20 |
| Figure 2.2 Non-metric MDS plot of presence/absence of the benthic and pelagic fauna methane seep communities. The label on the points is the seep code and can be found in Table 2.1. The seep code equates to the study the data was taken from (letter) and the methane seep number (number).....  | 30 |
| Figure 2.3 Bar graph of the relative abundance (percentage of the community) of benthic and pelagic fauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla.....   | 31 |
| Figure 2.4 Non-metric MDS plot of presence and absence macrofauna methane seep communities. The label on the points is the seep code and can be found in Table 2.2. The seep code equates to the study the data was taken from (letter) and the methane seep number (number). ....   | 34 |
| Figure 2.5 Bar graph of the relative abundance (percentage of the community) of macrofauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla. ....   | 34 |



|  |    |
|--|----|
| Figure 2.6 Non-metric MDS plot of presence and absence meiofauna methane seep communities. The data point label is the seep code and can be found in Table 2.3. The seep code equates to the study the data was taken from (letter) and the methane seep number (number). .....  | 36 |
| Figure 2.7 Bar graph of the relative abundance (percentage of the community) of meiofauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla. ....  | 37 |
| Figure 3.1 Map of the Hikurangi Margin methane seep sampling sites. Zoom in boxes of Northern and Southern sites. Starred points indicate methane seep sampling sites. Pink - Seep 3N, green - seep 1N, yellow - seep 5S (Uruti), orange- seep 2S (Glendhu), purple – seep 1S, blue – Kekerengu Bank. ....   | 48 |
| Figure 3.2 Trawl intensity at each site. Orange lines indicate trawls. Coloured flowers indicate the seep sites, pink - Seep 3N, green - seep 1N, yellow - seep 5S (Uruti), orange- seep 2S (Glendhu), purple – seep 1S, blue – Kekerengu Bank. Zoom in boxes show the 2km radius from the centre of the transect analysed indicated by the purple circle. Lines in the centre of the purple circles are the transect analysed. ....   | 50 |
| Figure 3.3 Pictures from seep site 1S. A) Tube worms in the cracks between carbonates and under carbonate ledges. Clam shells and clams on the sediment. B) Muddy sediment with Moridae (cod) on the bottom right. C) Muddy sediment with small carbonate pieces and clam shell hash. Reduced sediment patches and anemones on the sediment and carbonates. D) Zoom of <i>Munidopsis</i> squat lobster on a carbonate boulder. E) Small <i>Lamellibrachia</i> tube worm bush with <i>Bathymodiolus</i> mussels. <i>Munidopsis</i> squat lobster, <i>Calypptogena</i> clams and an eel also pictured. F) Small tube worm bed on the side of a carbonate, reduced sediment patches and <i>Munidopsis</i> on the carbonates. ....                                   | 52 |
| Figure 3.4 Pictures of seep site Uruti – 5S. A) Carbonates boulders with individual <i>Lamellibrachia</i> tube worms. Orange and yellow sponges on the carbonates and an eel poking out from the carbonates. B) A carbonate boulder with singular tube worms at the base and a starfish on the tube worms. C) Two carbonate boulders with clams and a clam bed/clamshell hash in the sediment between the boulders. D) Carbonate platforms with anemones on the top and singular flat tubeworms growing on the platform sides. Clamshells and clams in the gap between the carbonate platforms. E) Muddy sediment with small carbonate mounds. Singular flat tubeworms and an orange starfish. F) Carbonate platforms with singular flat tubeworms, clam shells, |    |

|  |    |
|--|----|
| and microbial mats within the gaps. Small patches of clam shells on the top of the carbonates. ....  | 53 |
| Figure 3.5 Pictures of seep site 1N. A) A small patch of clams and reduced sediments. B) A patch of clamshell hash, small microbial mats, and a shrimp. C) Buried carbonate with an Echinoid and small microbial mat. D) Two purple echinoids in muddy sediment. E) Buried carbonate and a stalked Crinoid in the middle. F) Muddy sediment and an anemone. ....   | 54 |
| Figure 3.6 Pictures from seep site 3N. A) <i>Sediment-covered carbonates with hermit crabs, Primnoidae and stylasterids</i> . B) <i>Muddy sediment with reduced patches and clam shells</i> . C) <i>Primnoidae in the sediment (Pink)</i> . D) <i>Glass sponge (white) in the sediment</i> . E) <i>Zoanthids (orange) with glass sponges on top (white) and glass sponges in the sediment</i> . F) <i>Two crinoids on buried carbonates</i> . ....   | 55 |
| Figure 3.7 Pictures from seep site Glendhu A) Carbonate platform covered in <i>Bathymodiolus</i> mussels. Thick microbial mats (white) and an eel in the bottom middle. Tube worms in the top right corner. B) Microbial mat in the middle of the mound surrounded by <i>Lamellibrachia</i> tube worms with barnacles on them and <i>Bathymodiolus</i> mussels. C) <i>Bathymodiolus</i> mussels in a hexagon shape on the sediment. D) Large bush of <i>Lamellibrachia</i> tube worms. E) <i>Bathymodiolus</i> mussels with microbial mats on them. F) <i>Lamellibrachia</i> tube worm bush with barnacles and <i>Munidopsis</i> squat lobsters. ....  | 56 |
| Figure 3.8 Pictures from Kekerengu seep site. A) Carbonate platforms with yellow and orange sponges and clams in the cracks between the carbonates. Singular lying down <i>Lamellibrachia</i> tube worms are also between the carbonates, and hermit crabs are on top of the carbonates. B) Hoki ( <i>Macruronus novaezelandiae</i> ). C) Laying down singular <i>Lamellibrachia</i> at the bases of carbonates and in the gaps between carbonates. D) Brasingida (orange) on the sides of a large carbonate block. Large bamboo corals, Stylasteridae (white) and Primnoidae on top of the carbonates. Moridae swimming next to the large carbonate. E) Oreo fish ( <i>Oreosomatidae</i> ), carbonate platforms and <i>Calyptogena</i> in the sediment. Orange and yellow sponges on the carbonate blocks. F) Orange and yellow sponges on the carbonates, <i>Calyptogena</i> clams in the gaps on the carbonates and a large anemone in the top middle of the carbonate. G) Thick Microbial mat in between the carbonates. H) Coral skeleton with Brasingida on it. .... | 58 |

Figure 3.9 Relative abundance (percentage of the community) of each phylum at the methane seeps sites. Depth is indicated by the seep name and a blue star indicates a seep with a trawl intensity >1. Colour depicts the phyla that were present – Seep endemic fauna have been listed to the lowest taxonomic ID and given hues of yellow.....63

Figure 3.10 Environmental factors at each methane seep. A) Trawl intensity (number of trawls in a 2km radius from the centre of the seep B) Depth (m). C) Rugosity. ....64

# List of Tables

|  |    |
|--|----|
| Table 2.1 Table of the methane seep data from the benthic and pelagic fauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep. ....                                   | 21 |
| Table 2.2 Table of the methane seep data from the macrofauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code used in this study, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep. ....                               | 24 |
| Table 2.3 Table of the methane seep data from the meiofauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code used in this study, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep. ....                                | 26 |
| Table 3.1 Table of methane seep sites and their corresponding environmental factors, depth (m), depth level (intermediate/deep), rugosity, trawl intensity. ....   | 49 |
| Table 3.2 Count of fauna per site. Fauna are Identified to the lowest taxonomic level (Fauna ID) and at the phylum level. Total number of fauna observed per site is indicated in the bottom row under 'total individuals'. ....   | 59 |
| Table 3.3 Table of results from the presence and absence DISTL-M sequential tests of methane seep communities and environmental factors. Environmental factors are depth (m), trawl intensity, depth level (intermediate, deep) and rugosity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column, the proportion of variation the environmental factor caused in the data is in the 'prop' column and cumulative portion of variation in the 'cumul.' Column. Significant results indicated by a "*" next to the p-value. .... | 65 |

Table 3.4 Table of results from the abundance DISTL-M sequential tests of methane seep communities and environmental factors. Environmental factors are depth (m), trawl intensity, depth level (intermediate, deep) and rugosity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column, the proportion of variation the environmental factor caused in the data is in the 'prop' column and cumulative portion of variation in the 'cumul.' Column. Significant results indicated by a "\*" next to the p-value.....65

Table 3.5 : DISTL-M marginal test results for the environmental factors depth (m), depth level (intermediate, deep) rugosity and trawl intensity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column and the proportion of variation the environmental factor caused in the data in the 'prop' column. Significant results indicated by a "\*" next to the p-value. ....65

# Chapter 1. Introduction

With the technological advances in the 21st century, humans have been able to explore deeper in the oceans, which has increased our knowledge of the deep sea (Danovaro et al. 2014). Increasing demands for natural resources and declining resources on land and in shallow waters, have led to increased interest in mining and trawling in the deep sea (Mengerink et al. 2014). The pace of exploitation in the deep-sea has raised concerns for the future of these essential ecosystems. This has led to the implementation of international obligations to protect and sustainably manage, so called Vulnerable Marine Ecosystems (VME), along with the formation of regional fisheries management organisations/ arrangements (RFMO/As) for management in the high seas (UNGA 2006; FAO. 2009; Haas et al. 2020).

The deep sea, the largest environment on Earth, encompasses 65% of the earth's surface (Sverdrup et al., 1942, as cited by Tyler, 2003) and is defined as any area in the ocean that is deeper than 200 m of water depth (Tyler 2003). Generally described as a homogeneous environment, the deep sea is characterised by low temperatures, high pressures and a lack of sunlight (Danovaro et al. 2014). Despite initially being thought of as devoid of life (Anderson and Rice 2006), the deep sea has been found to host a high diversity and abundance of life in a range of habitats and environments, including abyssal plains, deep ocean trenches, seamounts, continental slopes, submarine canyons, and chemosynthetic ecosystems (e.g. methane seeps and hydrothermal vents) (Glover and Smith 2003). Communities within these habitats are often slow growing, supporting organisms with long life spans and low reproductive rates. This makes these communities particularly susceptible to anthropogenic disturbances (Cordes et al. 2003; Roark et al. 2009). As well as hosting diverse life, the deep sea provides various ecosystem services (ES) that benefit society (Thurber et al. 2014). These benefits include food, pharmaceuticals, and non-market benefits such as regulating biogeochemical cycles and cultural/educational benefits (Armstrong et al. 2012; Levin et al. 2019).

## 1.1 Ecosystem services

With increasing interest in the deep sea for science, culture, tourism and exploitation, valuing the functions and services of the deep sea is a way to identify critical areas of importance (Armstrong et al. 2010, 2012). Understanding the value of the services that the deep sea provides also allows comparisons of causes and risks to be evaluated (Armstrong et al. 2010). Such work can highlight the potential services that may be lost due to anthropogenic disturbances (Turner et al. 2019). To understand the services the deep sea provides, deep-sea ecosystem functions and services have been split into four categories: provisioning, regulating, cultural and supporting services (Armstrong et al. 2012; Thurber et al. 2014; Le et al. 2017; Mejjad and Rovere 2021).

Provisioning services are those that provide something for humans, and are directly obtained from the deep sea, like fish, gas, minerals and pharmaceuticals (Armstrong et al. 2012; Mejjad and Rovere 2021). Deep sea fishing is a provisioning service that transfers energy directly from the deep sea to humans. Unfortunately, due to many deep-sea species' slow growth and long-life span, deep sea fishing can be destructive and severely impact benthic and seafloor-associated communities (Althaus et al. 2009; Pusceddu et al. 2014; Clark et al. 2016). Oil, gas and minerals are also provisioning services that can be found in the deep sea; however, these natural products have formed over millions of years and so are considered non-renewable resources within human timescales (Montserrat et al. 2019). In the deep sea, chemical compounds from organisms such as microorganisms, sponges and corals have the potential to be used medically. Scientists are currently studying these compounds for cancer, Alzheimer's, asthma, infections and bone grafting medication (Armstrong et al. 2010).

Regulating services come from natural cycles and processes in the deep sea. These services include gas and climate regulation, waste absorption and removal of pests (Armstrong et al. 2012; Le et al. 2017). The ocean is a significant contributor in climate regulation, with 90% of anthropogenic heat absorbed by the ocean (Levin et al. 2019). Carbon dioxide is also buried and sequestered in deep sea sediments (Levin et al. 2019). The deep sea releases approximately 85-300 Tg of



carbon annually; however, only approximately 2% of this gets released into the atmosphere due to microbial oxidation of methane (Armstrong et al. 2012). In addition, the biological pump is responsible for transferring organic material (nutrients) to the deep sea, and removing carbon dioxide from the atmosphere for primary production (Sabine et al. 2004).

Supporting services are the intermediate steps before regulating or provisioning services (Armstrong et al. 2012; Le et al. 2017; Mejjad and Rovere 2021). Examples of supporting services include chemosynthetic primary production which provides an alternate primary production source for deep sea species (Dubilier et al. 2008; Levin et al. 2016); provisioning of heterogeneous habitats provides habitats, nursery grounds and protection for fauna (Thurber et al. 2014); and nutrient cycling within ecosystems by organisms and microbes (Armstrong et al. 2012).

Cultural services are ecosystem services that spark inspiration for humans (Wakefield and Myers 2018). The deep sea has fascinated and sparked inspiration in humans for many years with deep sea tales in the literature dating back to 300 BC, deep sea science dating back to the 1800s, and now documentaries, shows and films are based on the deep sea (Thurber et al. 2014).

## 1.2 Threats to the deep sea

The long-lived and slow-growing nature of many deep-sea fauna means that anthropogenic activities disturb relatively undisturbed areas, which can have major impacts (Bergquist et al. 2000; Cordes et al. 2003; Roark et al. 2009). Anthropogenic disturbances such as trawling and mining for oil, gas and minerals can cause the extinction of endemic species and the destruction of benthic habitats (Gates and Jones 2012; Clark et al. 2016, 2019).

### 1.2.1 Trawling

The overexploitation of shallow water species in the 1960s and 1970s coincided with increasing interests and technologies for fishing in the deep sea (Roberts 2002). Of the anthropogenic disturbances, bottom trawling has been listed as one of the most important disturbances in the deep sea with habitats such as slope habitats,

methane seeps, hydrothermal vents and seamounts being identified as extremely vulnerable to trawling (MacDiarmid et al. 2012). Trawling has been described as one of the biggest threats to marine habitats due to its large area, non-selective nature and the destruction of benthic habitats (Jones 1992). In areas that are trawled, changes in the macrofauna, meiofauna, and megafauna benthic communities have been observed. In heavily trawled areas, reductions in the abundances of slow-growing suspension feeding fauna coincide with increases in scavenger and opportunistic species (Althaus et al. 2009; Maynou and Cartes 2012; Pusceddu et al. 2014). Reductions in species abundance, density, and diversity have also been observed due to the destruction of heterogeneous habitat and habitat-forming species and the smothering of benthic fauna from the resuspension of sediments (Clark and Rowden 2009). Not only has trawling been found to be destructive to the overall benthic community, but it was also found in the northwest Mediterranean Sea to decrease the turnover of organic carbon and the sequestration of methane (Pusceddu et al. 2014). Decreasing organic carbon turnover is likely due to constant disturbance and resuspension of the sediments, which reduces the food availability to the communities, potentially increasing the natural food limitations in the deep-sea (Pusceddu et al. 2014). Due to the nature of these ecosystems, recovery from disturbances is extremely slow (Althaus et al. 2009; Williams et al. 2010). In the Atlantic Ocean west of Scotland, it was found that eight years after the Darwin Mound was made a marine protected area (closed to fishing), there was little regeneration and no coral recolonisation (Huvenne et al. 2016).

### 1.2.2 Mining

Decreasing abundances of rare metals and minerals on land is increasing interest in mining the deep seafloor (Thurber et al. 2014). With the potential to become a multibillion-dollar industry, mining of the deep-sea is a prominent topic in marine management (Glover and Smith 2003). Mining of massive sulphides, cobalt-rich ferromanganese crusts, and polymetallic nodules is of particular interest (Mejjad and Rovere 2021). As well as mineral mining in the deep sea, offshore oil mining provides 33% of global oil production and 25% of natural gas production (Kark et al. 2015). Although advocates of deep sea mining proclaim that mining of the deep sea is more environmentally friendly than mining on land, the implications and

environmental impacts of deep-sea mining are poorly understood (Orcutt et al. 2020).

#### 1.2.2.1 Oil mining

In the 1960s, oil and gas exploitation moved from shallow waters to deeper offshore areas (Thurber et al. 2014). Deep-sea oil and gas mining occurs in the Arctic Ocean, Atlantic Ocean, Indian Ocean and Pacific Ocean, with ultra-deep mining of depths up to 3000 m in the Gulf of Mexico (Cordes et al. 2016). Deep sea drilling usually occurs in phases, the exploration phase, the production phase, and the well abandonment phase (Kark et al. 2015; Cordes et al. 2016). The initial phase of drilling is the exploration phase, where seismic surveys are used to identify potential oil/gas reservoirs, and exploration wells are drilled. Following the exploration phase is the production phase, where more wells are drilled, and hydrocarbon extraction occurs. The final phase of deep-sea mining is the abandonment phase, where wells are filled with cement and mining ceases (Kark et al. 2015). Unfortunately, the adverse impacts of mining occur through all phases of mining activity with noise and light pollution, chemical and toxin pollution, and destruction of habitats and sedimentation. Physical destruction of deep-sea habitats from sedimentation, installation of drilling equipment on the seafloor (pipelines anchors), and drilling can destroy habitats, especially for benthic sessile and filter-feeding communities (Vad et al. 2018). During the drilling process, drill cuttings, including rocks, mud and drill fluids, are released onto the seafloor or at the surface below drill rigs. This causes increases in sedimentation on the seafloor, smothers benthic communities, and depletes oxygen concentrations, reducing the density and diversity of these ecosystems (Trannum et al. 2010; Jones et al. 2012). At the Laggan oil field on the Faroe-Shetland Channel, Jones et al. (2012) observed severe smothering of the seafloor within 100 m of the drill site and evidence of drilling and sedimentation within 250 m of the drill site. They also observed that ten years after mining ceased, the communities surrounding the drill site had mostly recovered. However, rare species were absent, and drill cuttings were still apparent closer to the drill site. Similarly, Gates & Jones (2012) also noted that three years post-drilling at the Morvin Field on the continental slope of the Norwegian Sea, drill cuttings were still present, and there was a change in the community composition with decreased sessile species compared to the control sites. They also noticed increased concentrations of barium at the drill site, indicating

that the effects of the drill cutting (other than smothering) were still affecting fauna and habitat at the site.

As well as the negative impacts of the drilling itself, accidental spills and blowouts of oil have been reported to have catastrophic effects, not only at the site of the spill but also in surrounding ecosystems. On April 20th 2010, a blowout of the BP Deepwater Horizon Drilling rig in the northern Gulf of Mexico, released 3.19 million barrels of oil into the ocean (Beyer et al. 2016). From this oil spill, oil slicks covered >112,000 km<sup>2</sup> of the ocean surface, and a deep sea plume covered 930 km<sup>2</sup> of the deep sea (Fisher et al. 2016; Beyer et al. 2016). Montagna et al. (2013) observed that the most severe impacts on benthic communities were within 3 km of the drill zone. However, moderate effects of the spill were seen up to 17 km away from the drill zone. In the deep sea, the oil spill caused increased bacterial respiration, which decreased the oxygen concentration in the water column, and decreased densities and diversities of macrofauna, meiofauna, and megafauna benthic communities. No signs of recovery of the benthic communities were evident four years post-spill (Fisher et al. 2016). As well as significant impacts on the surrounding ecosystems, the BP oil spill also caused loss of jobs, decreased trade for the fisheries and tourism in affected coastal areas, killed wildlife, and affected 240 km of coastal shorelines (Levy & Gopalakrishnan, 2010).

#### 1.2.2.2 Mineral mining

Mining mineral resources in the high seas is regulated by the international seabed authority (ISA). Although the ISA has permitted no licences for exploitation mining, 29 exploratory licences have been issued to mine on 1.3 million km<sup>2</sup> of the seabed within the high seas (Orcutt et al. 2020). The resources that are targeted in mineral mining are massive sulphides on active and inactive hydrothermal vents, cobalt-rich ferromanganese crusts on sea mounts and polymetallic nodules on abyssal plains (Figure 1.1) (Gollner et al. 2017). Hydrothermal vents, seamounts and abyssal plains provide habitats for a diversity of fauna, some of which are rare and endemic to these habitats (Gollner et al. 2017). Mineral mining is a threat to deep-sea ecosystems due to the large amounts of sediment resuspended during the mining process, the destruction of habitats from mining equipment, the removal of hard substrate from the mine site, and the release of toxic chemicals into the water

column (Koschinsky et al. 2018; Weaver et al. 2018; Miller et al. 2021). The resuspension of sediments is a key issue with deep sea mining. It affects the immediate area surrounding the mine site and can disturb distant habitats due to the resuspension of sediments in the water column. For every tonne of manganese nodules mined from the seabed, approximately 2.5-5.5 tonnes of sediment will be resuspended (Sharma 2015). The resuspension of sediments is an issue for benthic filter-feeding communities, likely causing suffocation of benthic fauna. Mining equipment also destroys benthic habitats and removes hard substrates which causes the loss of habitats and will likely impede the recolonisation of the mine sites. Polymetallic nodules have an extremely slow growth rate of 1-6 mm per million years (Hein, 2004 as cited in Montserrat et al., 2019). The removal of the hard substrate, will cause community changes within the ecosystem and reduce the biodiversity and biomass of mined sites (Montserrat et al. 2019). In the tropical east Pacific, Miljutin et al. (2011) found that nematode communities had not recovered to their original state 26 years after one exploratory mining event for polymetallic nodules. Similarly, Simon-Lledó et al. (2019) found that 26 years after simulated polymetallic mining, mega benthic communities had changed significantly and had not recovered to their pre-mining state. These authors also concluded that the reductions in hard substrate had reduced the abundances in the communities.

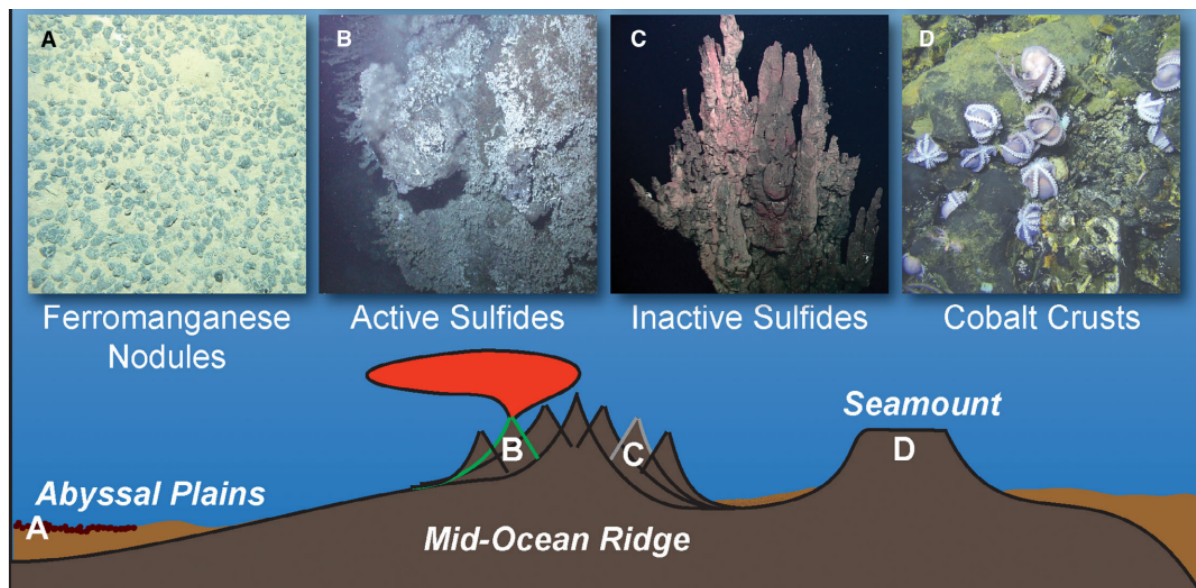


Figure 1.1 Types of deep sea habitats with mineral resources. A) Ferromanganese nodules (polymetallic nodules) form abyssal plains. B) Active hydrothermal vent sulphides. C) Inactive hydrothermal vent sulphides. D) Cobalt rich crusts from seamounts. This figure is from Orcutt et al. 2020 "[Impacts of deep-sea mining on microbial ecosystem services](#)" licenced under [CC by 4.0](#)

Anthropogenic disturbances can have disastrous effects on deep-sea communities where recovery rates are slow and the effects of disturbances can be potentially seen for decades. Due to the limited knowledge that we have about the ecosystems services that the deep sea provides, and the endemic/ rare species within the deep sea, management and conservation of these areas is imperative.

### 1.3 Deep-sea conservation

The deep sea occurs in areas of national jurisdiction/ exclusive economic zone (EEZs) and in areas beyond national jurisdiction where no nation has ownership. Under the United Nations General Assembly (UNGA), all states must protect and preserve the marine environment. In 2006, the UNGA resolution 61/105 called upon “states to take action immediately, individually, and through Regional Fisheries Management Organizations and Arrangements (RFMO/A), and consistent with the precautionary approach and ecosystem approach, to sustainably manage fish stocks and protect Vulnerable Marine Ecosystems (VME)” (Watling and Auster 2017). The CBD also requires that states, where possible and appropriate, should “provide a system of protected areas or area where special measures need to be taken to conserve biological diversity” (Hayashi 2004). Currently, international law uses Marine Protected Areas (MPAs) as the preferred method for marine conservation, with global targets to protect and conserve 30% of global oceans by 2030 (IUCN 2016). In 2018, MPAs covered 6.97% of the global ocean, with 16.03% of marine areas protected within national jurisdiction and only 1.18 % of the high seas protected (Gray 2018). Within an EEZ, the nation must conserve and manage the marine ecosystems within it. Due to this, a variety of techniques have been used for deep sea conservation. Within areas of national jurisdiction spatial management tools such as Special Areas of Conservation (SACs) are used in Europe to restrict anthropogenic activity and promote recovery of benthos and biodiversity (Rees et al. 2013). Ecologically and biologically significant areas (EBSAs) are used in Canada by Fisheries and Oceans Canada (DFO) to identify habitats that are ecologically or biologically significant. Benthic Protection Areas are used in New Zealand to prevent bottom trawling on VMEs (Howell et al. 2016).

The high seas cover over half of the earth's surface. They are the Areas Beyond National Jurisdiction (ABNJ) that have no clear ownership, and are open to extractive activities by all nations (Figure 1.2) (Ban et al. 2014; Haas et al. 2020). The high seas are also where a large portion of the deep sea exists and are potentially subject to overexploitation due to the limited management of the high seas (Davies et al. 2007). Until 1994, there were no regulations of fishing on the high seas unless it was enforced by the country that the fleet came from. In 1994, the United Nations Convention Law of the Sea (UNCLOS) provided an obligation to states to "cooperate in the conservation and management of living resources in the areas of the high seas... and shall negotiate with a view necessary for the conservation of the living resources concerned" (8 article 118 as cited by Haas et al., 2020). From this obligation, Regional Fisheries Management Organisations/arrangements (RFMO/As) were formed to manage fishing and the impacts of fishing in relevant geographic areas on the high seas (Figure 1.3). RFMO/As can legally implement fisheries actions on the high seas for member states of the RFMO (Haas et al. 2020). Although there has been a call for conservation management of the deep sea, in 2018, only nine MPAs covered 1.18 % of the high seas (Gray 2018). During this time, various management strategies were being formed for identifying and managing deep-sea ecosystems. The international seabed authority ISA was formed to regulate deep sea mining in the high seas (Lodge et al. 2014). The Vulnerable Marine Ecosystems (VME) Act was formed by the Food and Agricultural Organization of the United Nations (FAO), to manage adverse impacts from deep sea fisheries in the high seas (Van Dover et al. 2012). The Convention on Biodiversity (CBD) was formed to help identify and manage areas of Ecological or Biological significance in the deep sea and the high seas (Dunn et al. 2014).



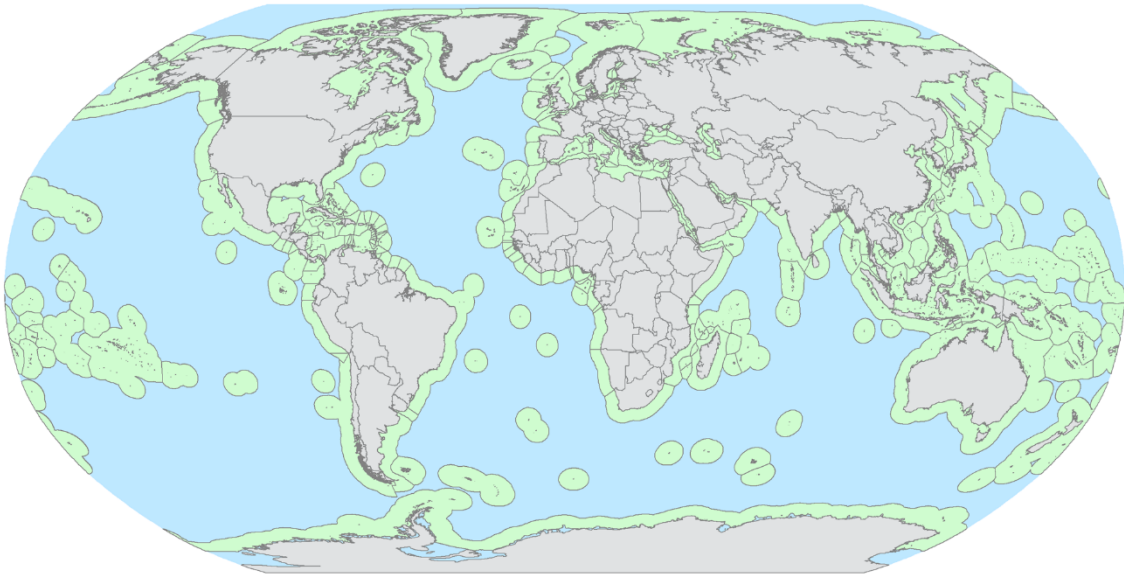


Figure 1.2 Global map showing the Exclusive Economic Zones (green) and the high seas (blue). This figure is from [White C, Costello C \(2014\) Close the High Seas to Fishing?](#) licenced under [CC by 4.0](#).

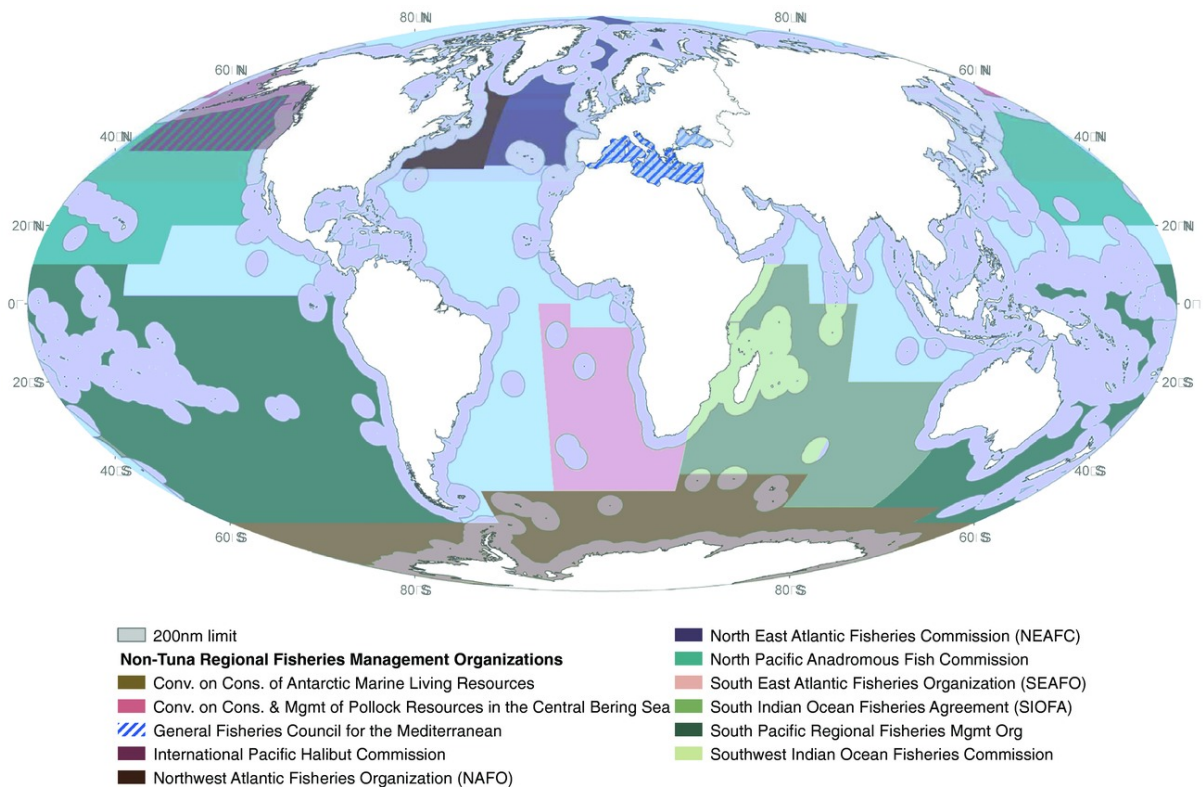


Figure 1.3 Depiction of Regional Fisheries Management Organisations/ Arrangements (RFMO/As) that manage bottom fisheries and species other than tuna. This Figure was taken from Ban et al. (2014) [Systematic Conservation Planning: A Better Recipe for Managing the High Seas for Biodiversity Conservation and Sustainable Use](#) with permission.

### 1.3.1 Ecologically and biologically significant areas

In 1992 the Convention on Biological Diversity (CBD) was formed following the UN Conference on Environment and Diversity. The CBD called upon states to “identify marine ecosystems exhibiting high levels of biodiversity and productivity and other critical habitat areas”, and to (...) “provide necessary limitations on use in these areas, through, inter alia, designation of protected areas” (UN 1992; Dunn et al. 2014). The CBD was formed to focus on conserving marine biodiversity in the deep sea and high seas using the precautionary approach (Dunn et al. 2014). In 2004 the CBD developed a set of criteria to identify Ecologically or Biologically Significant Areas (EBSAs) in the sea (Bax et al. 2016). The CBD developed a set of guidelines with seven criteria to identify habitats of importance and prioritise these areas for management and marine spatial planning (Clark et al. 2014; Bax et al. 2016). These criteria state that to be an EBSA, the area must have uniqueness or rarity; have particular importance for the life history stages of species; be important for threatened, endangered or declining species and/or habitats; be vulnerable, fragile, sensitive, or have slow recovery; be biologically productive; be natural; and be biologically diverse (Clark et al. 2014). In nine EBSA workshops held by CBD COP between 2011-2014, experts identified 203 EBSAs, with 31 of these EBSAs being within the high seas. Identifying EBSAs highlights areas highly susceptible to anthropogenic effects to inform area-based management to preserve these ecosystems (Bax et al. 2016). In 2010 a significant step forward in managing the high seas occurred in the north-east Atlantic, where six MPAs were established using EBSA to identify significant areas that require protection (O’Leary et al. 2012).

### 1.3.2 Vulnerable marine ecosystems

Following the acknowledgement of the impacts of deep sea fishing, in 2006 the UNGA called upon the Food and Agricultural Organisation of the United Nations (FAO) in resolution 61/105 to develop guidelines for RFMO/As to manage the impacts of deep-sea fisheries in the high seas (Ardrón et al. 2014; Watling and Auster 2017; Nations and Hosch 2020). The Vulnerable Marine Ecosystem (VME) guidelines were developed to identify ecosystems interpreted as rare or fragile. They aim to prevent significant adverse impacts on these ecosystems and promote the long-term sustainability of deep-sea fish stocks (Rice et al., 2014). The VME

guidelines use 5 criteria to identify a VME: Uniqueness or rarity, the functional significance of the habitat, fragility, life history traits that make a recovery difficult, and structural complexity of the habitat (FAO, 2009). VME indicator taxa were also outlined in the VME guidelines that can be used to identify a VME (Morato et al. 2018). Once a VME has been identified from fishing activity, the fishing activity must be managed and, in some cases, stopped in the area to protect the VME from significant adverse impacts (Rice et al. 2014; Morato et al. 2018). Since 2006, RFMO/As tools such as fishing closures and marine protected areas (MPAs) are used to reduce the impacts of bottom fishing (Thompson et al. 2016). Additionally, RFMO/As have implemented environmental impact assessments (EIAs) that must be completed prior to permitting bottom fishing to take place in new areas. EIAs are used to identify and manage VME's that may be within the area (Watling and Auster 2017).

Current ecosystems identified as VME's or EBSAs include hydrothermal vents, cold water coral reefs, seamounts, and methane seeps (FAO. 2009; Van Dover et al. 2012).

## 1.4 Methane seeps

Deep sea methane seeps are chemosynthetic ecosystems that rely on the microbial oxidation of methane, to provide nutrients for dense and diverse ecosystems (Levin et al. 2016). Occurring on passive and active continental margins, methane seeps have been found at a range of depths up to <7000 m (Fujikura et al. 1999). Often described as an oasis in the deep sea and a hotspot for biodiversity, methane seep ecosystems provide habitat and an alternate source of nutrition for diverse species, including endemic fauna, benthic and sessile fauna, and vagrant fauna (Demopoulos et al. 2010; Åström et al. 2018). Endemic fauna at methane seep sites includes species such as *Lamellibranchia* tube worms, vesicomyid clams and bathymodiolid mussels which are habitat-forming fauna that increase the heterogeneity at methane seep sites and further diversify the methane seep communities (Cordes et al. 2010; Zhao et al. 2020).

#### 1.4.1 Methane seep ecosystem service

As well as being a hotspot for biodiversity in the deep sea, methane seeps provide a suite of ecosystem provisioning, regulating and cultural services that benefit society (Armstrong et al. 2012; Niemann et al. 2013; Levin et al. 2016). Methane seeps provide regulating services through climate regulation and nutrient cycling (Levin et al. 2016). Deep sea sediments are a reservoir for methane, and the microbial oxidation of methane coupled with the reduction of sulphur increases the concentration of bicarbonate in the water column, causing carbonate to precipitate and methane to be sequestered into the benthic biomass (Boetius and Suess 2004; Panieri et al. 2017; Le et al. 2022). Not only does microbially-mediated carbon oxidation and sulphur reduction cause carbonate precipitation, but it also provides food for a diversity of microbial and faunal biomass and creates structural complexity, which increases the diversity of methane seep sites (Levin et al. 2016). Methane seep microbes also have the potential to be used for bioremediation tools for degrading oil spills (Mason et al. 2014; Scoma et al. 2017).

Methane seep provisioning services include: providing habitat, nutrition, aggregation points and nursery grounds for a variety of fauna. Commercially important species transfer chemosynthetic production directly to humans (Sellanes et al. 2008; Grupe et al. 2015; al. 2018; Seabrook et al. 2019). Commercially important species such as the long spine thorny head (*Sebastolobus altivelis*), rockfish, green halibut (*Reinhardtius hippoglossoides*), haddock (*Melanogrammus aeglefinus*), northern shrimp (*Pandalus borealis*), snow crab (*Chionoecetes opilio*), atlantic cod (*Gadus morhua*), crabs from the genus *Paralomis*, tanner crab (*Chionoecetes tanneri*), red crab (*Chaceon quinque-dens*) and patagonian toothfish (*Dissostichus eleginoides*), have been observed aggregating at methane seep sites (Sellanes et al. 2008; Niemann et al. 2013; Grupe et al. 2015; Sen et al. 2018; Seabrook et al. 2019; Turner et al. 2020). It is likely that the increased abundances of prey species, carbonate structures for habitat, and the potential for methane seeps to be breeding sites or nursery attract mobile species to methane seeps (Sellanes et al. 2008; Turner et al. 2020).

### 1.4.2 Methane seep protection

Despite the provisioning and regulating services that methane seeps provide, the management of these ecosystems is limited. The only protected area for methane seep ecosystems in the high seas is in the Mediterranean Basin and the Atlantic Ocean. In the Mediterranean Basin, trawling below 1000 m was banned in 2005, by the General Fisheries Council for the Mediterranean Basin (GFCM), to protect deep-sea habitats, including chemosynthetic ecosystems (De Juan and Lleonart 2010). The GFCM also uses Fisheries Restricted Areas (FRAs) to prevent the use of towed fishing gear on the Nile Delta, where there are high concentrations of methane seeps (De Juan and Lleonart 2010). In the Atlantic Ocean, the Hatton Rockall Basin was closed in 2015 to bottom fishing by the North East Atlantic Fisheries Commission (NEAFC) due to presence of methane seeps (Johnson et al. 2019).

Regionally within EEZ's, the Laurentian Fan cold seeps are recognised by Fisheries and Oceans Canada (DFO) as Ecologically and Biologically Significant Areas (similar to CBD EBSA) off the coast of Nova Scotia (Stortini 2015). Methane seeps are also recognised and protected from the impacts of gas and oil extraction in the Gulf of Mexico in waters from 400-3300 m by the Department of Interiors Bureau of Ocean Energy Management (BOEMRE) (Ardron et al. 2011). The European Commission has also listed methane-derived authigenic carbonates (MDAC) as habitats of community importance, and 27 marine protected areas have been designated for methane seeps in a network of special areas of conservation (SAC) (Noble-James et al. 2020; Judd et al. 2020). There are also potential methane seep ecosystems within the Parc Naturel De La Mer De Corail (Nature Park of the Coral Sea) surrounding New Caledonia. However, this nature park was established to protect coral reefs, not methane seeps (Ardron et al. 2011).

Methane seep ecosystems include long-lived and slow-growing fauna, so recovery from anthropogenic disturbances such as trawling and mining are likely to take a long time. Due to the limited protection for methane seep ecosystems globally, their vulnerable and important status and the ecosystem services they provide, management plans must be put in place to protect them from anthropogenic disturbances.

## 1.5 Aims and thesis structure

My thesis aims to explore biogeographic variations in methane seep communities globally, and variations within methane seep communities within the Hikurangi Margin of New Zealand. Through this work, I aim to highlight: i) the diversity of methane seep ecosystems between bioregions and within the New Zealand bioregion, ii) environmental variation in methane seep communities and iii) trawling impacts on methane seeps to address the importance of methane seep management globally and within New Zealand.

In chapter two, I investigate biogeographic patterns in methane seep communities using a global meta-analysis of data collected from the literature. I compare methane seep communities within and between biogeographic regions and against environmental data to provide insight into the drivers of community differences at methane seeps. I aim to highlight the diversity of methane seep communities to ensure that this diversity is represented in management. I hypothesise that bioregional variation in methane seep communities will occur and that depth will also cause variation in the communities.

In chapter three, I describe and compare six methane seep communities on the Hikurangi Margin of New Zealand with respect to the environmental characteristics depth, rugosity and trawl intensity. I aim to determine factors that may be influencing changes in the community compositions. I hypothesise that trawl intensity will correlate with reductions in the chemosynthetic communities at methane seeps.

# Chapter 2. Global Bioregional Variation in Methane Seep Communities

## 2.1 Introduction:

Biogeography is the study of large-scale (regional, national, global) patterns in species distributions (Whittaker et al. 2005, 2023). Understanding biogeographical patterns in marine ecosystems is important, as it can better inform management plans and ensure ecological representation when designating marine protected areas (Lourie and Vincent 2004). Representation, a key component in marine management, helps drive management actions towards a holistic approach. It aims to preserve all biodiversity in the system, not just areas that are perceived to be rare, important or 'beautiful' (Giakoumi et al. 2013; Zhao et al. 2020). Internationally, the Convention on Biodiversity (CBD) aims to establish representative networks of marine protected areas to protect 30% of the ocean by 2030 (Gissi et al. 2022). To ensure that ecological representation is considered in marine management, species distribution patterns and life history traits must be understood. The relationships between taxa and the environment, are essential and should be incorporated into area-based management (Álvarez-Noriega et al. 2020).

### 2.1.1 Biogeography in the deep sea

Biogeography relates species characteristics and traits to environmental variations to understand species distributions. It relates species characteristics such as, reproduction rate and life history, to environmental factors such as temperature, light availability, geology, topography, water availability and chemical availability (Myers and Giller 1988). Biogeographic theory was first applied to terrestrial ecosystems, where clear physical boundaries such as mountains, lakes, oceans and environmental conditions act as barriers to dispersal, preventing gene flow and creating genetically isolated communities (Ayari et al. 2019). Initial descriptions of biodiversity in the oceans proposed that species would have large ranges due to the vast nature and lack of perceived barriers in the sea. However, high levels of endemism and the presence of rare species in habitats and ecosystems indicate that barriers to gene flow do create isolated communities and biogeographic



provinces (Goldstien et al. 2006; Briggs and Bowen 2012; Freitas et al. 2019; Azovsky et al. 2020). In the deep sea, bathymetry (sea mounts, basins and continental margins), ocean water masses, oceanic fronts, deep sea currents, depth, hydrostatic pressure, food availability, pH, oxygen availability and distance can all be barriers to dispersal (Schauer et al. 2010; Rogers et al. 2012; Salazar et al. 2016; Lörz et al. 2021; Zhou et al. 2022). Of these barriers to dispersal, distance is often highlighted as a primary driver of biogeographic patterns (Lessios et al. 1998; Miller et al. 2010; Nakajima et al. 2014). Between Australia and New Zealand, biogeographic differences in seamount deep-sea coral communities have been primarily attributed to isolation by distance. However, even thousands of kilometres have not created completely isolated populations (Miller et al. 2010). For example, Miller et al. (2010) found that two species of coral on seamounts in Australia and New Zealand had recent and sustained gene flow. There were no signs of genetic differentiation between the populations despite the distance. Similarly, genetic studies of an echinoid (*Echinothrix diadema*) revealed extensive and recent gene flow across the Eastern Pacific Barrier (EPB). The EPB is deemed the “world’s most potent barrier” with 5400 km of deep water between the Line Islands in the central Pacific and the Clipperton Atoll in the eastern Pacific (Lessios et al. 1998). The authors concluded that this result does not preclude the EPB from being a significant barrier to dispersal, but that reproductive life history traits of species also influence the connectivity within sites and bioregional differences (Lessios et al. 1998; Miller et al. 2010).

### 2.1.2 Methane seeps

Methane seeps are chemosynthetic environments found worldwide on passive and active margins in depths up to 7,400 m (Fujikura et al. 1999; Levin 2005). Methane seep ecosystems host diverse communities supported by the microbial oxidation of methane and the reduction of sulphur that produces chemosynthetic primary production. Methane seeps have been discovered globally and can be identified by seep-characteristic fauna, including mytilid mussels, vesicomysid clams and siboglinid tube worms. The ubiquitous presence of seep characteristic fauna globally has led to hypotheses about the connectivity of methane seeps. The patchy and erratic nature of methane seep fluid flow and potential distances between methane seeps means

that the colonisation of new seeps and connectivity between seeps were initially considered to be limited (Levin et al. 2003). However, the presence of genetically similar characteristic seep fauna globally indicated that seeps may be highly connected (Miyazaki et al. 2010; Heijden et al. 2012; Teixeira et al. 2013). In chemosynthetic ecosystems such as methane seeps and hydrothermal vents, although separated by hundreds, if not thousands of kilometres, high levels of gene flow have been observed. High levels of connectivity in methane seep species Mytilid Mussels, Vesicomidae clams (*Abyssogena southwardae*) and two species of Alvinocarididae shrimp (*Alvinocaris markensis* and *Alvinocaris muricola*) and in the hydrothermal vent shrimp species *Rimicaris exoculate* have been observed (Miyazaki et al. 2010; Teixeira et al. 2012, 2013). Vesicomid clams have been found to have trans-Pacific distributions at both vents and seeps (Kojima et al. 2004). The initial hypothesis of methane seep connectivity suggested that other chemosynthetic systems such as vents, whalefalls and wood falls may act as stepping-stones for dispersal (Breusing et al. 2016). Other studies have alluded to delayed metamorphosis, metamorphosis triggered by environmental conditions or active guiding to suitable habitat may trigger colonisation of new seep areas by larvae (Teixeira et al., 2011).

Alternatively, high levels of regional endemism have led to the designations of biogeographic provinces. In the Japanese archipelago, 45% of the chemosynthetic sites have site endemic species and community similarities. This resulted in the designation of eight biogeographic provinces (Nakajima et al. 2014). In New Zealand the presence of ten species new to science and species-level differences in seep-associated fauna led to suggestions that New Zealand may also be a biogeographic province (Baco et al. 2010).

Understanding biogeographic differences in methane seep communities is essential to ensure that representation of the diversity in these ecosystems is met in management actions and protection. Methane seep ecosystems are currently threatened by anthropogenic disturbances such as bottom trawling, dredging and potential mining of gas hydrates and minerals (Baco et al. 2010; Ardron et al. 2011; Ramirez-Llodra et al. 2011; Bowden et al. 2013; Cordes et al. 2016). As well as containing rare and endemic species, methane seep communities are often slow

growing with low reproductive rates (Cordes et al. 2003; Roark et al. 2009). Therefore, recovery from disturbances may be unlikely or take a long time. Due to the slow-growing nature of many seep species, the high levels of endemism, and the ecosystem services that methane seeps provide, methane seeps have been recognised as vulnerable marine ecosystems by the United Nations General Assembly (UNGA). As a result obligated states and Regional Fisheries Management Organisations (RFMOs) need to protect and manage these vulnerable marine ecosystems (Ardron et al. 2014; Watling and Auster 2017; Nations and Hosch 2020).

In this chapter, I aim to understand bioregional patterns in methane seep communities to highlight the diversity of methane seeps and support future marine management. Specifically, I will investigate: i) bioregional changes in methane seep communities, ii) similarities (or dissimilarities) in methane seep communities within bioregions, and iii) determine environmental drivers of any differences in the methane seep communities.

## 2.2 Methods:

### 2.2.1 Data Collection:

Data for the meta-analysis were collected using the search engine Google Scholar. I searched for the keywords 'methane seeps', 'methane seep communities', 'methane seep depth', 'methane seep commercially important species', 'methane seep megafauna' and 'methane seep diversity'. An unstructured search was then completed by searching the references of relevant papers and retrieving additional published data as required. The data were collected from Supplementary materials and data tables from the literature. Data were determined adequate for the analysis if the data were count, density, or presence/absence data from an active methane seep. If multiple samples were taken per site, an average count of species per site was used so there was one set of data per site.

The data were converted to count data, except for presence/absence data. Density data were converted to count data by multiplying the density by the area. The data was split into two data sets: relative abundance (RA) data (containing the count data)

and presence/absence (PA) data (containing all the data). The abundance data was standardised to relative abundance by dividing the species abundance by the total abundance in the community and multiplying by 100. The data was further split into targeted fauna types to reduce the bias of comparing data sets of specific fauna types. The targeted fauna types were meiofauna (collected from within the sediment and smaller than 1 mm), infaunal macrofauna (visible to the naked eye and within the sediment) and benthic and pelagic fauna (any epifauna that is visible to the naked eye and pelagic fish/shark/Crustacea/echinoderms).

The location and name of the seeps were recorded to inform the delineation of the biogeographic regions. The locations of the seeps were then plotted in GIS (Figure 2.1) and categorised by the biogeographic regions determined by Costello et al. (2017) (Figure 2.1 and Table 2.1, 2.2, 2.3.).

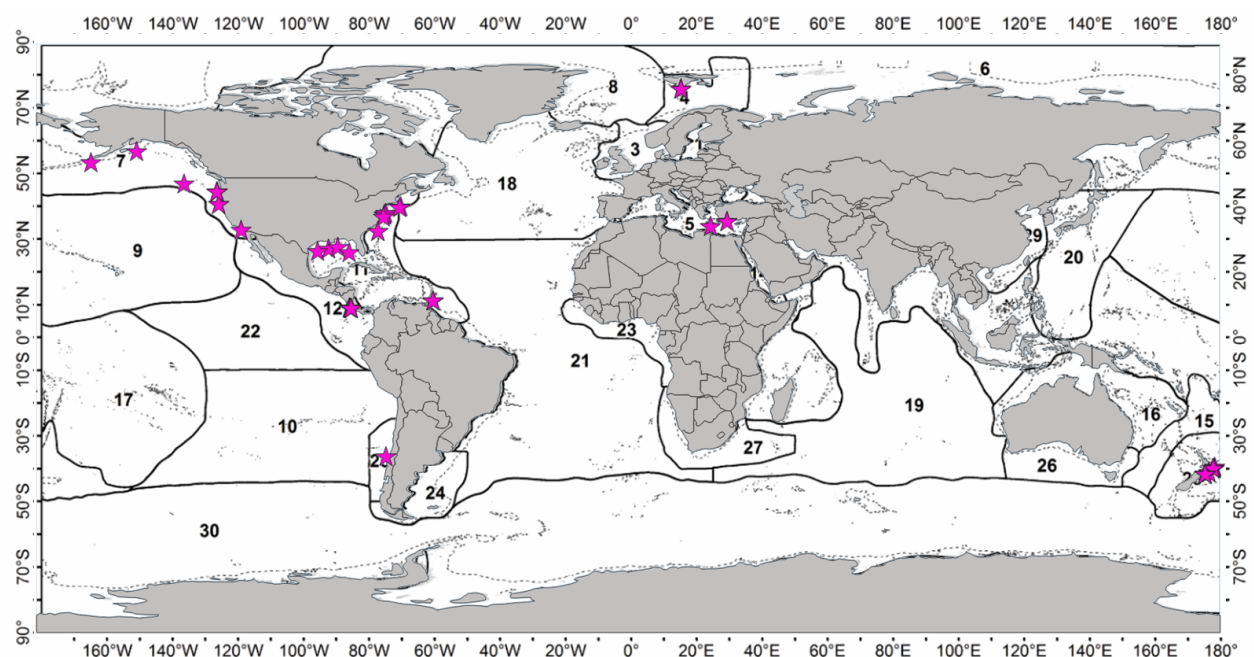


Figure 2.1 Biogeographic regions delineated in Costello et al. (2017). Pink stars indicate methane seep sites from the current meta-analysis. Black lines indicate boundaries between bioregions. This work, Figure 2.1, is adapted from “Costello, M.J., Tsai, P., Wong, P.S. et al. *Marine biogeographic realms and species endemism*, *Figure 2b. Nat Commun* 8, 1057 (2017)” used under [CC BY 4.0](#). Figure 2.1 is licensed under [CC BY 4.0](#) by McKenzie Jones.

## 2.2.2 Environmental data

Environmental factors at each seep were recorded to determine if environmental conditions correlated with any variation in the data (Table 2.1, 2.2, 2.3). The depth of

each site was recorded and converted into depth levels: shallow, intermediate, and deep (Table 2.1, 2.2, 2.3). The depth levels were determined following Turner et al. (2020) where depths <500 m were considered shallow, 501-1500 m were considered intermediate, and depths >1500 m were considered deep. The location of each seep (latitude and longitude) was also recorded, and then the distance between each pair of seep sites was calculated. To determine the distance between seeps, Google Earth was used to measure the shortest distance across the oceans between the seeps. The collection method was also considered an environmental factor, as different collection methods targeting different fauna may cause variations in the data (Table 2.1, 2.2, 2.3). The collection methods were: Cores (push core, box core, tube core); Video footage (recorded by a remote-operated vehicle (ROV) or deep towed imaging system (DTIS)); Cores and video (both video footage from ROV/DTIS and cores were used); Colonisation trays (analysed community compositions that had settled on colonisation trays that were put at methane seep habitats for seven months); Cores/bottom grab/video (using ROV manipulate grab, slurp and suck functions to bring up a piece of community to analyse in combination with ROV video footage and bottom cores); Cores and bottom grab (using cores and ROV grab, slurp, suck functions) and Video/Sled/ van veen grab/ multicore.

The data was collected from 17 papers for a total of 56 sites and 785 species observed at methane seep sites globally (Figure 2.1).

Table 2.1 Table of the methane seep data from the benthic and pelagic fauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep.

| Source           | Code | Seep Name     | Biogeographic region     | Depth (average) | Depth Level  | Collection Method | Targeted Fauna            |
|------------------|------|---------------|--------------------------|-----------------|--------------|-------------------|---------------------------|
| Amon et al. 2017 | A2   | Dome 1        | Caribbean/Gulf of Mexico | 1230.5          | Intermediate | Cores             | Benthic and Pelagic Fauna |
| Amon et al. 2017 | A3   | Mama d'leau   | Caribbean/Gulf of Mexico | 1269            | Intermediate | Cores             | Benthic and Pelagic Fauna |
| Amon et al. 2017 | A4   | La Diabliesse | Caribbean/Gulf of Mexico | 1607.5          | Deep         | Cores             | Benthic and Pelagic Fauna |

|                       |    |                         |                          |        |              |                                       |                           |
|-----------------------|----|-------------------------|--------------------------|--------|--------------|---------------------------------------|---------------------------|
| Van Dover et al. 2003 | G1 | Florida Escarpment      | Caribbean/Gulf of Mexico | 2155   | Deep         | Cores/bottom grab/video               | Benthic and Pelagic Fauna |
| Van Dover et al. 2003 | G2 | Barbados seep           | Caribbean/Gulf of Mexico | 2155   | Deep         | Cores/bottom grab/video               | Benthic and Pelagic Fauna |
| Sellanes et al. 2008  | H1 | Conception methane seep | Chile                    | 805    | Intermediate | Trawl                                 | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O1 | Rock Garden             | New Zealand              | 774    | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O2 | Builders Pencil         | New Zealand              | 792.5  | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O3 | Omakere Ridge           | New Zealand              | 1150.5 | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O4 | LM10                    | New Zealand              | 754.5  | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O5 | Hihi                    | New Zealand              | 786.5  | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O6 | Kereru                  | New Zealand              | 740    | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Baco et al. 2010      | O7 | North Tower             | New Zealand              | 1042.5 | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |

|                    |    |                   |                          |        |              |                                       |                           |
|--------------------|----|-------------------|--------------------------|--------|--------------|---------------------------------------|---------------------------|
| Baco et al. 2010   | O8 | South Tower       | New Zealand              | 1057.5 | Intermediate | Video, sled, van Veen grab, multicore | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q1 | TC21 GHP2         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q2 | TC18 GHP3         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q3 | TC25 GHP1         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q4 | TC25 GHP3         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q5 | ROV1 GHP5         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q6 | ROV2 GHP5         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Sen et al. 2018    | Q7 | ROV3 GHP5         | Norwegian Sea            | 380    | Shallow      | Video and cores                       | Benthic and Pelagic Fauna |
| Amon et al. 2017   | A1 | Dome 2            | Caribbean/Gulf of Mexico | 1034   | Intermediate | Cores                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B1 | Pick Up Sticks    | Caribbean/Gulf of Mexico | 420    | Shallow      | Video                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B2 | Baltimore         | Caribbean/Gulf of Mexico | 385    | Shallow      | Video                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B3 | Shallop west      | Caribbean/Gulf of Mexico | 350    | Shallow      | Video                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B4 | Chincoteague east | Caribbean/Gulf of Mexico | 1035   | Intermediate | Video                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B5 | Norfolk west      | Caribbean/Gulf of Mexico | 1565   | Deep         | Video                                 | Benthic and Pelagic Fauna |
| Turner et al. 2020 | B6 | Veatch            | Caribbean/Gulf of Mexico | 1415   | Intermediate | Video                                 | Benthic and Pelagic Fauna |

Table 2.2 Table of the methane seep data from the macrofauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code used in this study, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep.

| Source                  | Code | Seep Name            | Biogeographic region     | Depth (average) | Depth Level  | Collection Method | Targeted Fauna |
|-------------------------|------|----------------------|--------------------------|-----------------|--------------|-------------------|----------------|
| Levin et al. 2003       | D1   | Eel River            | North Pacific            | 520             | Intermediate | Cores             | Macrofauna     |
| Grupe et al. 2015       | E1   | Del Mar methane seep | North Pacific            | 1030            | Intermediate | Video and cores   | Macrofauna     |
| Demopoulos et al. 2010  | F1   | Green Canyon         | Caribbean/Gulf of Mexico | 1425            | Intermediate | Cores             | Macrofauna     |
| Demopoulos et al. 2010  | F2   | Atwater Valley       | Caribbean/Gulf of Mexico | 2391            | Deep         | Cores             | Macrofauna     |
| Demopoulos et al. 2010  | F3   | Alaminos Canyon      | Caribbean/Gulf of Mexico | 2230            | Deep         | Cores             | Macrofauna     |
| Levin and Mendoza, 2007 | J1   | Florida Escarpment   | Caribbean/Gulf of Mexico | 3271            | Deep         | Cores             | Macrofauna     |
| Levin and Mendoza 2007  | J2   | Kodiak seep          | North Pacific            | 4425            | Deep         | Cores             | Macrofauna     |
| Levin and Mendoza 2007  | J3   | Unimak seep          | North Pacific            | 3275            | Deep         | Cores             | Macrofauna     |
| Levin et al. 2015       | L1   | Mound 12             | Gulf of California       | 994             | Intermediate | Cores             | Macrofauna     |
| Levin et al. 2015       | L2   | Mound 11             | Gulf of California       | 1016            | Intermediate | Cores             | Macrofauna     |
| Levin et al. 2015       | L3   | Mound Quepos         | Gulf of California       | 1216            | Intermediate | Cores             | Macrofauna     |



|                     |    |                                    |                    |         |              |                    |            |
|---------------------|----|------------------------------------|--------------------|---------|--------------|--------------------|------------|
| Levin et al. 2015   | L4 | Jaco Wall                          | Gulf of California | 1656    | Deep         | Cores              | Macrofauna |
| Levin et al. 2006   | M1 | Californian methane seep Eel River | North Pacific      | 525     | Intermediate | Colonisation trays | Macrofauna |
| Levin et al. 2010   | N1 | CA seep                            | North Pacific      | 512     | Intermediate | Cores              | Macrofauna |
| Levin et al. 2010   | N2 | OR seep                            | North Pacific      | 770     | Intermediate | Cores              | Macrofauna |
| Ashford et al. 2021 | P1 | Mound 12                           | Gulf of California | 990     | Intermediate | Cores              | Macrofauna |
| Ashford et al. 2021 | P2 | Jaco Scar                          | Gulf of California | 1817    | Deep         | Cores              | Macrofauna |
| Ashford et al. 2021 | P3 | Quepos Land Slide                  | Gulf of California | 397     | Shallow      | Cores              | Macrofauna |
| Ashford et al. 2021 | P4 | Parrita Seep                       | Gulf of California | 1462    | Intermediate | Cores              | Macrofauna |
| Ashford et al. 2021 | P5 | Mound 11                           | Gulf of California | 1007    | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R1 | Bears Paw                          | New Zealand        | 1102.5  | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R2 | Kaka                               | New Zealand        | 1169    | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R3 | Lm-3                               | New Zealand        | 665     | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R4 | LM-9                               | New Zealand        | 1143    | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R5 | North Tower                        | New Zealand        | 1052.00 | Intermediate | Cores              | Macrofauna |
| Thurber et al. 2013 | R6 | South Tower                        | New Zealand        | 1043    | Intermediate | Cores              | Macrofauna |

|                     |    |        |             |      |              |       |            |
|---------------------|----|--------|-------------|------|--------------|-------|------------|
| Thurber et al. 2013 | R7 | Takahe | New Zealand | 1060 | Intermediate | Cores | Macrofauna |
|---------------------|----|--------|-------------|------|--------------|-------|------------|

Table 2.3 Table of the methane seep data from the meiofauna that was used in the meta-analysis. The table also includes the literature the data was collected from (source), the seep code used in this study, the seep name, biogeographic region, depth average, depth level, collection method used to collect the data and type of fauna targeted. The seep code is the code used in this study to identify the seep. The letter indicates the source of the data, and the number is the number of the seep.

| Source                 | Code | Seep Name           | Biogeographic region | Depth (average) | Depth Level  | Collection Method     | Targeted Fauna |
|------------------------|------|---------------------|----------------------|-----------------|--------------|-----------------------|----------------|
| Olu-Le Roy et al. 2004 | C1   | Napoli              | Mediterranean        | 2000            | Deep         | Cores and bottom grab | Meiofauna      |
| Olu-Le Roy et al. 2004 | C2   | Milano              | Mediterranean        | 2000            | Deep         | Cores and bottom grab | Meiofauna      |
| Olu-Le Roy et al. 2004 | C3   | Amsterdam           | Mediterranean        | 1850            | Deep         | Cores and bottom grab | Meiofauna      |
| Olu-Le Roy et al. 2004 | C4   | Kazan               | Mediterranean        | 1850            | Deep         | Cores and bottom grab | Meiofauna      |
| Olu-Le Roy et al. 2004 | C5   | Faulted Ridge       | Mediterranean        | 1850            | Deep         | Cores and bottom grab | Meiofauna      |
| Guilini et al. 2012    | K1   | Hydrate Ridge north | North Pacific        | 601             | Intermediate | Cores and bottom grab | Meiofauna      |
| Guilini et al. 2012    | K2   | Hydrate Ridge south | North Pacific        | 773             | Intermediate | Cores and bottom grab | Meiofauna      |
| Guilini et al. 2012    | K3   | Hydrate Ridge east  | North Pacific        | 880             | Intermediate | Cores and bottom grab | Meiofauna      |
| Rosli et al. 2016      | S1   | South Tower         | New Zealand          | 1053            | Intermediate | Cores                 | Meiofauna      |
| Rosli et al. 2016      | S2   | North Tower         | New Zealand          | 1052            | Intermediate | Cores                 | Meiofauna      |

### 2.2.3 Statistical analysis:

The data was analysed with PRIMER (V7) with the PERMANOVA add-on. The data was analysed at the lowest taxonomic ID (LTID), family, order, class, and phyla levels.

For the presence/absence (PA) and relative abundance (RA) data, the data was split by targeted fauna and analysed. The PA data had no outliers in the Non-metric Multidimensional scaling plots (NMDS), so no transformations were necessary. For the RA data the benthic and pelagic fauna data was not transformed as there were no outliers in the NMDS. The macrofauna data was square-root-transformed and the meiofauna data was  $\log(x+1)$  transformed to remove funnelling and outliers.

#### 2.2.3.1 Bioregional variation in methane seep communities

The PA and RA data was analysed using permutational analysis of variance (PERMANOVA) tests to determine if bioregion was correlated with any significant differences in community structure. The data was then analysed using a similarity percentage analysis (SIMPER) to compare the dissimilarities between bioregions and the species that were attributed to these differences.

#### 2.2.3.2 Environmental variation in methane seep communities within bioregions

The data was then analysed using a similarity percentage analysis (SIMPER) to compare the similarities of methane seeps within bioregions. The PA data was split into the bioregions. Within the bioregions methane seep community variation was compared to environmental factors depth, depth level, latitude, longitude, and collection method, using a DISTL-M with an AIC and stepwise model to understand correlations of community variation with environmental variation. The RA data was not analysed in the DISTL-M due to small sample sizes and lack of variation in the environmental factors.

#### 2.2.3.3 Global environmental variation in methane seep communities

The RA and PA data split by fauna type were analysed using a DISTL-M with an AIC and stepwise model with the factors: depth (average), depth (level), latitude, longitude, and collection method. A RELATE test was also used to compare the

Bray-Curtis resemblance matrix of the PA and RA data and distance matrix to determine if distance was correlated with differences in methane seep communities.

## 2.3 Results

### 2.3.1 Benthic and pelagic fauna

#### 2.3.1.1 Global environmental variation in methane seep communities

Collection method and depth explained 40-60% of the variation in methane seep benthic and pelagic fauna communities (Supplementary material Table 1). At the LTID for the RA data, collection method and depth average explained 54% of the variation in methane seep communities (Supplementary material Table 1). In the PA data at the LTID, collection method, depth level and depth average explained 75% of the variation in methane seep communities (Supplementary material Table 1). The distance between methane seeps correlated with variation in methane seep communities in the PA and RA data at all taxonomic levels (LTID, PA  $R^{OS}=0.395$ ,  $P\approx 0.1$ , RA  $R^{OS}=0.665$ ,  $P\approx 0.1$ ) (Supplementary material Table 2).

#### 2.3.1.2 Bioregional variation in methane seep communities

Bioregion significantly correlated with changes in the benthic and pelagic fauna communities ( $P\leq 0.02$ , for all taxonomic levels, Supplementary material Table 3). The methane seeps in the New Zealand, Chile and the Norwegian Sea bioregions individually clustered, indicating that bioregion correlates with changes in the community composition (Figure 2.2). Within the RA data, the Caribbean/Gulf of Mexico bioregion and the Norwegian Sea bioregion were significantly different (Supplementary material Table 4).

For the PA data, the Chilean bioregion was not significantly different from the New Zealand or Caribbean/Gulf of Mexico bioregions ( $P>0.05$ , Supplementary material Table 5). All other bioregions were significantly different from each other ( $P<0.05$ , Supplementary material Table 5). The dissimilarity was  $>65\%$  between the bioregions at the LTID, family, order and class levels (Supplementary material Table 6). For the PA data at the LTID, the dissimilarity between the Caribbean/Gulf of Mexico bioregion and the Norwegian Sea bioregion was 99%, with Actiniaria,

Ophiuroidea, *Actinia (uticina)*, *Chaceon quinquedens* and *Cancer borealis* contributing to 90% of the differences in the communities.

#### 2.3.1.3 Environmental variation in methane seep communities within bioregions

Within the New Zealand bioregion, there was 27-50% similarity in the community composition of the seeps at the LTID, family and order levels. There was 62-82% similarity in the methane seep communities at the class and phyla levels (Supplementary material Table 7). Of the environmental factors at the LTID, depth significantly correlated with changes in the community composition at the methane seeps within the New Zealand bioregion, and accounted for 24% ( $F=1.98$ ,  $P=0.024$ ) of the variation in species present (Supplementary material Table 8).

Within the Caribbean/Gulf of Mexico bioregion, there was low similarity (>30%) at all taxonomic levels (Supplementary material Table 7). No species contributed more than 5% to the similarity at LTID in the RA data. In the PA data the similarity in the Caribbean/Gulf of Mexico bioregion was low, >40% at the LTID, Family, Order and Class levels but was 63% at the phylum level (Supplementary material Table 7). There was large variability in the methane seep communities within the Caribbean/Gulf of Mexico bioregion, with different phyla being the most prominent in the communities at different methane seeps (Figure 2.3). Collection method, depth average and depth level correlated with 85% of the variation in methane seep communities at the LTID in the PA data in the Caribbean/ Gulf of Mexico bioregion (Supplementary material Table 8) ( $F>2.81$ ,  $P<0.039$ ).

Within the Norwegian Sea bioregion, the similarity between seeps was between 49 and 64% across all taxonomic levels (Supplementary material Table 7). The Norwegian Sea methane seep communities are composed of similar species with similar abundances within the communities (Figure 2.3). No environmental factors significantly correlated with changes in methane seep communities at the LTID ( $P<0.05$ ) (Supplementary material Table 8).

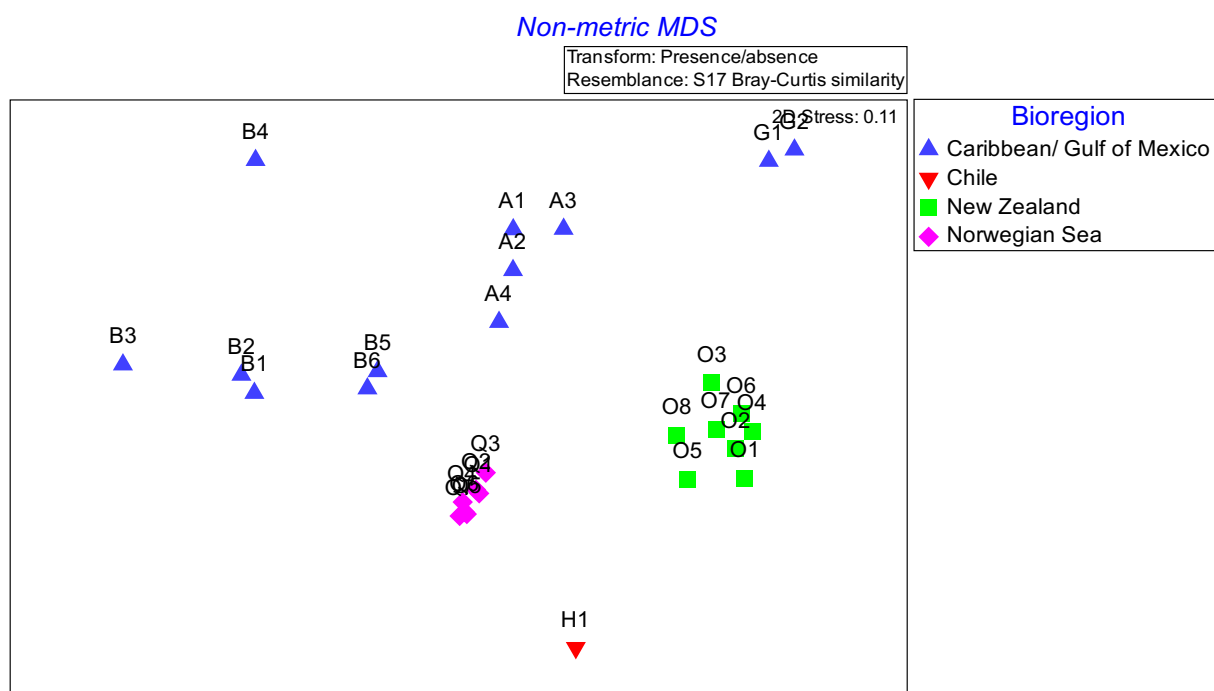


Figure 2.2 Non-metric MDS plot of presence/absence of the benthic and pelagic fauna methane seep communities. The label on the points is the seep code and can be found in Table 2.1. The seep code equates to the study the data was taken from (letter) and the methane seep number (number).

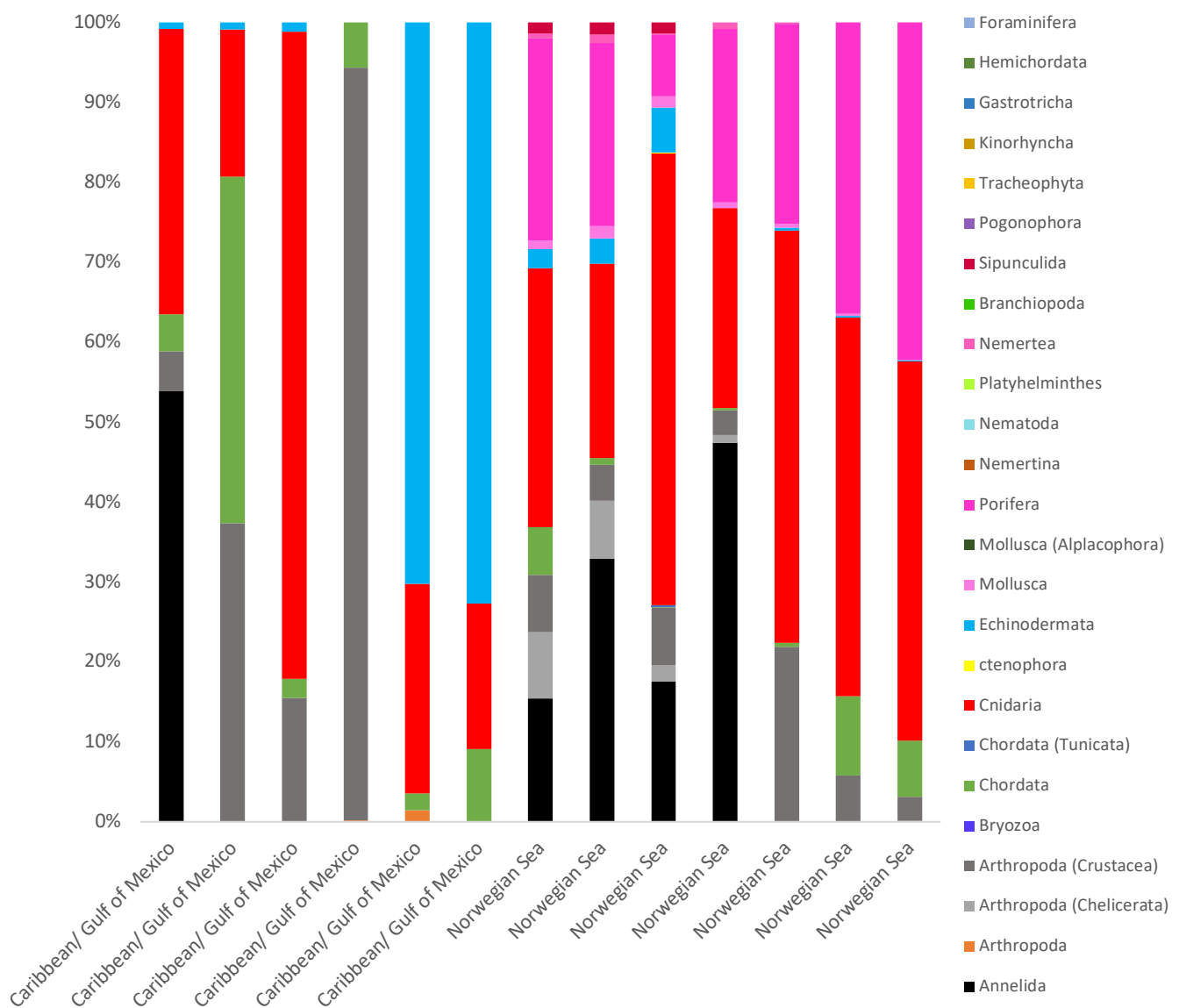


Figure 2.3 Bar graph of the relative abundance (percentage of the community) of benthic and pelagic fauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla.

## 2.3.2 Macrofauna

### 2.3.2.1 Global environmental variations in methane seep communities

Longitude, latitude, depth, and collection method explained 35-60% of the global variation in methane seep macrofauna communities (Supplementary material Table 9). At the LTID longitude, latitude, depth average and collection method cumulatively explained 52% for the PA data and 54% for the RA data of the variation in methane seep macrofauna communities (PA  $F > 1.99$ ,  $P < 0.002$ , RA  $F > 2.35$ ,  $P < 0.001$ ) (Supplementary material Table 9). Distance to neighbouring seeps also correlated

with variation in methane seep macrofauna communities (LTID PA  $R^{OS}=0.337$ ,  $P\approx 0.1$ , RA  $R^{OS}=0.359$ ,  $P\approx 0.2$ ) (Supplementary material Table 10).

#### 2.3.2.2 Bioregional variation in methane seep communities

Bioregion significantly correlated with changes in the macrofauna community composition of methane seeps (Supplementary material Table 11). The methane seep communities clustered into bioregions (Figure 2.4). For the PA data, all macrofauna bioregions were significantly different from each other (Supplementary material Table 13). For the RA data, the Caribbean/Gulf of Mexico bioregion was not significantly different from the North Pacific or Gulf of California bioregion ( $P \geq 0.05$ ) (Supplementary material Table 12). In the North Pacific and Caribbean/Gulf of Mexico bioregions, there were relatively similar species in the phyla Annelida, Mollusca and Crustacea within the methane seep communities (Figure 2.5). The methane seep macrofauna community compositions in the North Pacific and the Gulf of California bioregions were significantly different ( $F>1.75$ ,  $P<0.008$ , Supplementary material Table 9). The North Pacific bioregion and the Gulf of California cluster within the Pacific Ocean and share a boundary; however, the North Pacific appears to have a higher abundance of molluscs within the methane seep communities (Fig 2.5). The PA and the RA data sets showed that all bioregions were highly dissimilar at the LTID (dissimilarity  $>80\%$ ), with dissimilarity between bioregions decreasing at higher taxonomic levels (e.g., dissimilarities at the phyla level were  $<40\%$ ) (Supplementary material Table 14).

#### 2.3.2.3 Environmental variation in methane seep communities within bioregions

Within the North Pacific bioregion, the similarities in the methane seep community composition and species presence ranged from 27-55% at the LTID, family and order levels and increased to 62-80% at the class and phylum levels (Supplementary material Table 15). The North Pacific bioregions were composed of similar species at the phyla level. However, the relative abundance within the seep communities varied (Figure 2.5). Depth accounted for 43% ( $F=3.89$ ,  $P=0.005$ ) of the variation in the methane seep community composition in the North Pacific bioregion at the LTID (Supplementary material Table 16).



In the Gulf of California bioregion, methane seep communities had between 20-48% similarity at the LTID, family, order and class levels. At the phyla level, there was 58-63% similarity in the Gulf of California seep bioregion (Supplementary material Table 15). At the phyla level, methane seeps in the Gulf of California were dominated by annelids, crustaceans and molluscs, although the relative abundances of these fauna varied depending on the seep (Figure 2.5). No environmental factors significantly correlated with changes in methane seep communities at the LTID ( $P < 0.05$ ) (Supplementary material Table 16).

The New Zealand bioregion had the highest within bioregion similarity of the methane seep macrofauna data, which ranged from 54-83% similarity (Supplementary material Table 15), driven by similar relative abundances of annelids and crustaceans (Figure 2.5). No environmental factors significantly correlated with changes in methane seep communities at the LTID ( $P < 0.05$ ) (Supplementary material Table 16).

Within the Caribbean/Gulf of Mexico bioregion, the average similarity in methane seep communities was between 36-54% for the LTID, family, order and class levels (Supplementary material Table 15). At the phyla levels, the average similarity within the Caribbean/Gulf of Mexico bioregion was 75% (Supplementary material Table 15). None of the factors significantly correlated with differences in methane seep species presence at the LTID ( $P > 0.05$ ) (Supplementary material Table 16).

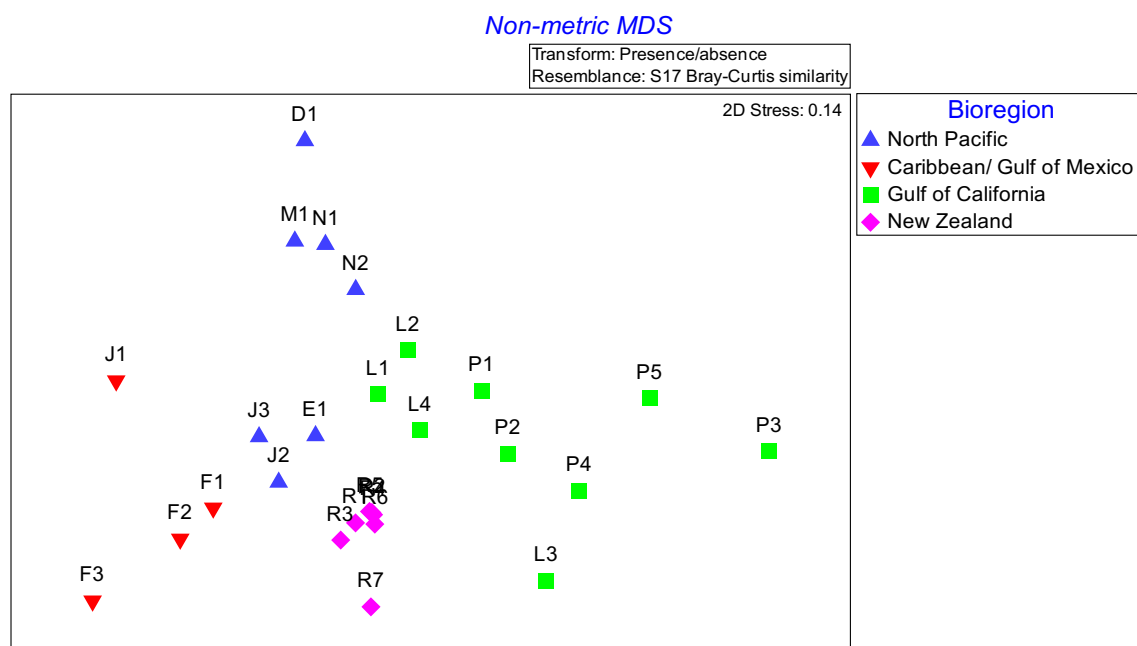


Figure 2.4 Non-metric MDS plot of presence and absence macrofauna methane seep communities. The label on the points is the seep code and can be found in Table 2.2. The seep code equates to the study the data was taken from (letter) and the methane seep number (number).

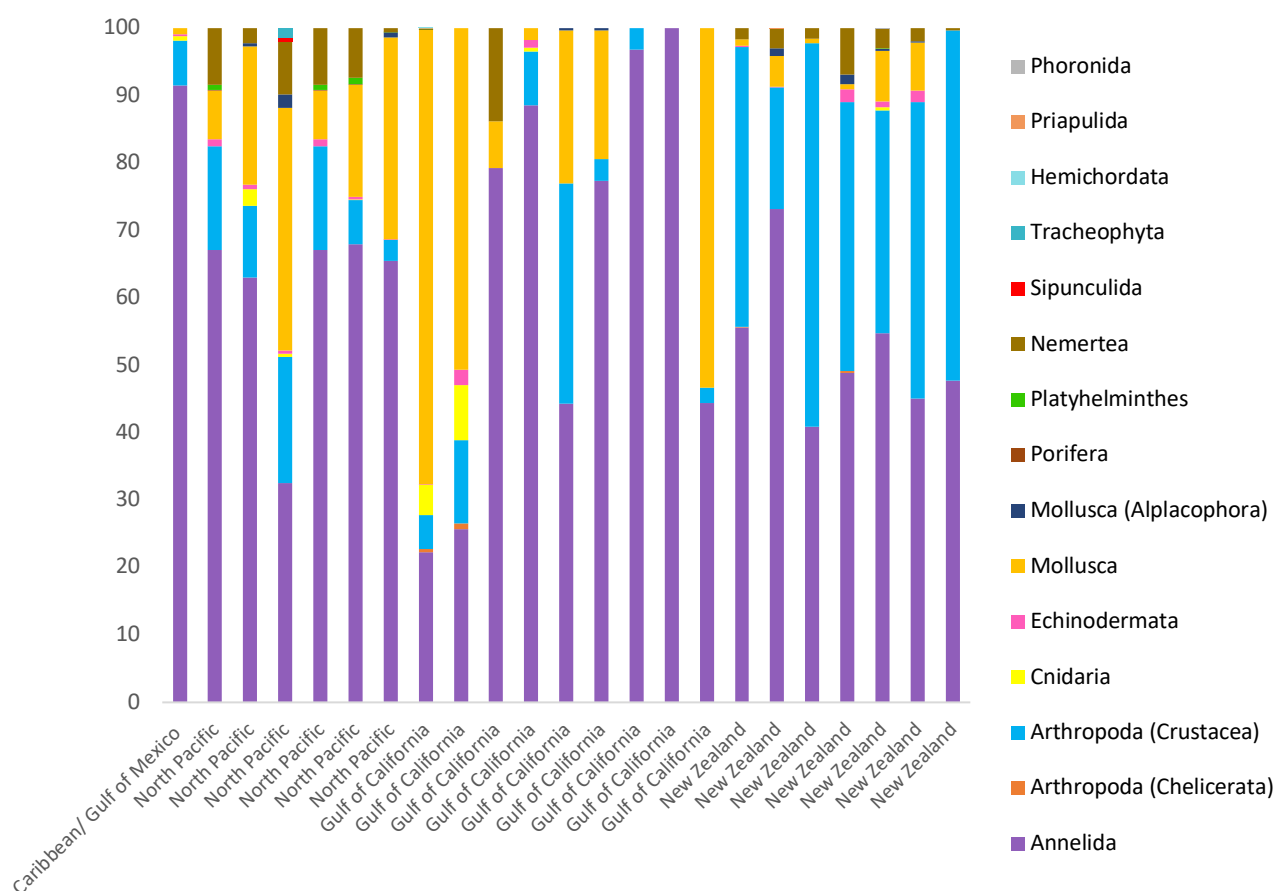


Figure 2.5 Bar graph of the relative abundance (percentage of the community) of macrofauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla.

### 2.3.3 Meiofauna

#### 2.3.3.1 Global environmental variations in methane seep communities

Depth, collection method and latitude explained 84-96% of the variation in methane seep meiofauna communities (Supplementary material Table 17). At the LTID, for the PA data, depth level and collection method cumulatively explained 85% of the variation in methane seep meiofauna communities ( $F > 5.54$ ,  $P < 0.005$  Supplementary material Table 11). For the RA data at the LTID, latitude and collection method cumulatively explained 96% of the variation in methane seep meiofauna communities ( $F > 2$ ,  $P < 0.027$  Supplementary material Table 17).

There was a relationship between methane seep community similarity and distance to neighbouring seeps observed (LTID PA  $R^{OS} = 0.843$ ,  $P \approx 0.1$ , RA  $R^{OS} = 0.802$ ,  $P \approx 3.7$ ) (Supplementary material Table 18).

#### 2.3.3.2 Bioregional variation in methane seep communities

Bioregion significantly correlated with differences in the meiofauna community composition ( $P \leq 0.05$ , Supplementary material Table 19, and Table 20). The methane seep community data clustered into bioregions (Figure 2.6). The New Zealand bioregion has an increased abundance of Crustaceans, Molluscs and Kinorhyncha compared to the North Pacific (Figure 2.7). For the RA and PA data, there was a relatively low dissimilarity between the North Pacific and New Zealand bioregions at all taxonomic levels (dissimilarity  $< 55\%$ ) (Supplementary material Table 21), with high abundances of Nematodes, Annelids and Arthropods present in both bioregions (Figure 2.7, Supplementary material Table 21). In the PA data, the Mediterranean is highly dissimilar from the North Pacific and New Zealand (dissimilarity  $> 90\%$  at the LTID) (Supplementary material Table 21).

#### 2.3.3.3 Environmental variation in methane seep communities within bioregions

Within the North Pacific bioregion, the similarity between methane seeps was between 71-95% at all taxonomic levels (Supplementary material Table 22). No environmental factors correlated with changes in the methane seeps communities at the LTID ( $P > 0.05$ ) (Supplementary material Table 23).

Within the New Zealand bioregion, there was 80-100% similarity between methane seep community relative abundances (Supplementary material Table 22). The effects of environmental factors on community composition were not tested due to the small sample size (N=2).

The similarity between methane seep communities within the Mediterranean bioregion was 58-67% (Supplementary material Table 22). No environmental factors correlated with variation in the Mediterranean methane seep communities at the LTID ( $P>0.05$ ) (Supplementary material Table 23).

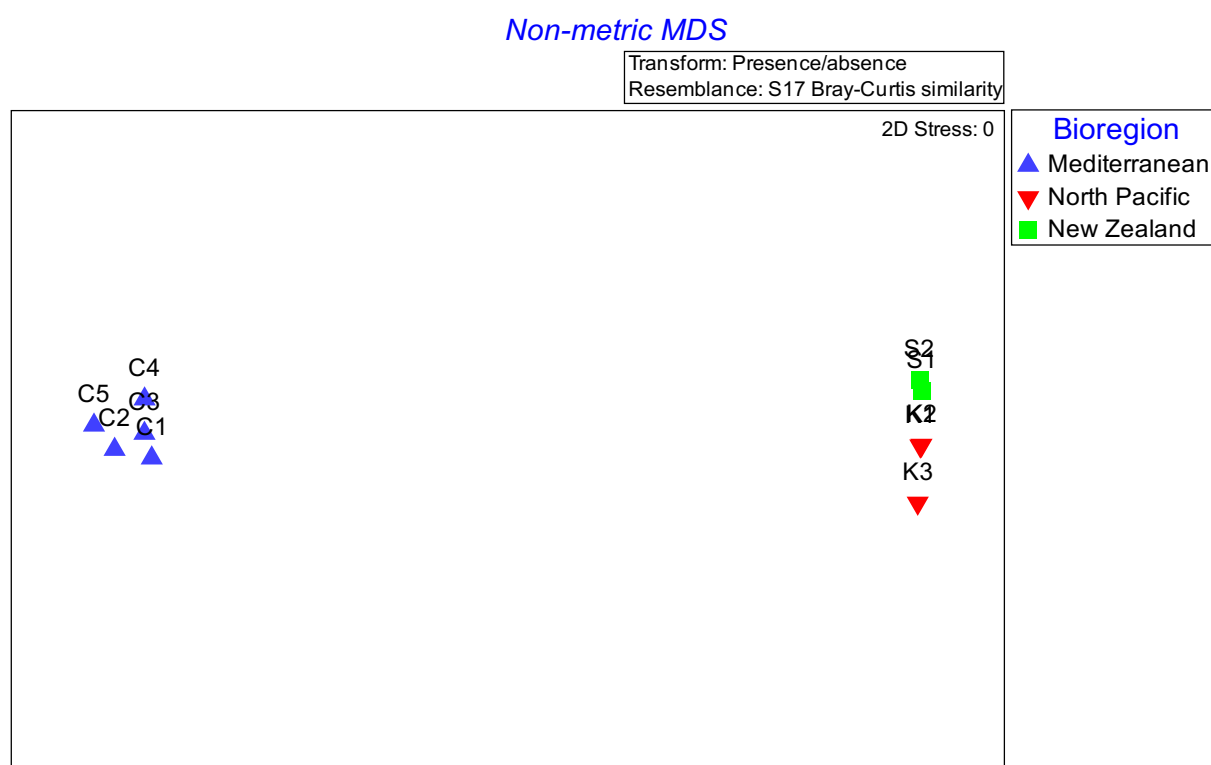


Figure 2.6 Non-metric MDS plot of presence and absence meiofauna methane seep communities. The data point label is the seep code and can be found in Table 2.3. The seep code equates to the study the data was taken from (letter) and the methane seep number (number).

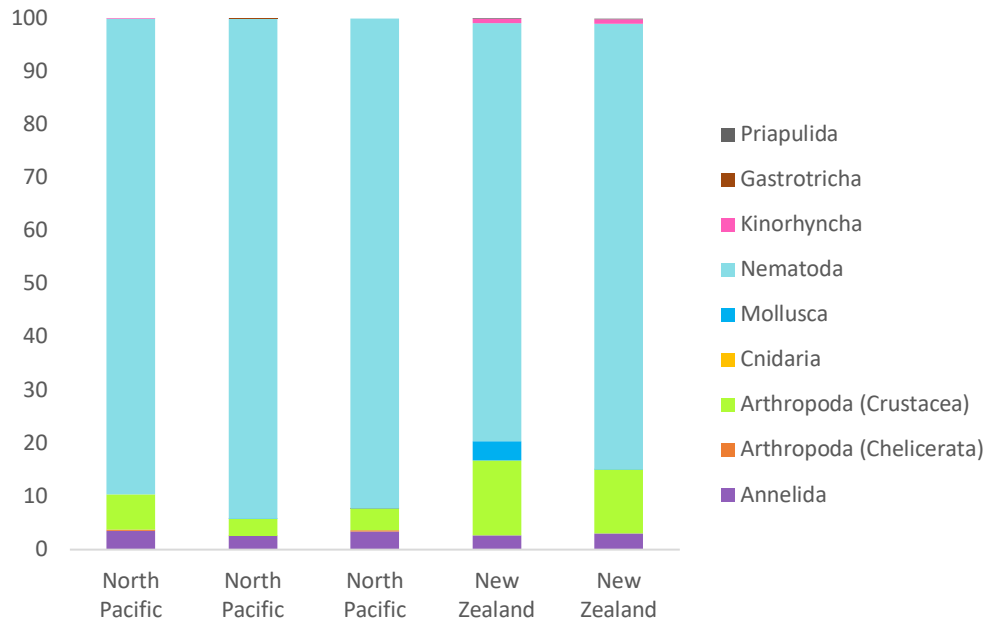


Figure 2.7 Bar graph of the relative abundance (percentage of the community) of meiofauna phyla at methane seeps. The bioregion is indicated on the x axis and the colour of the bars indicates the phyla.

## 2.4 Discussion:

In this chapter I aimed to identify if methane seeps follow global bioregional patterns. I also assessed environmental factors that cause variation in methane communities globally and within bioregions. The bioregions established by Costello et al. (2017) correlated with significant variation in methane seep communities highlighting the global diversity and heterogeneity of these environments. Globally, collection method and depth correlated with large amounts of variation in the methane seep community compositions. Within bioregions, methane seep community similarities ranged from highly dissimilar (>30% similarity) in the Caribbean/Gulf of Mexico bioregion (benthic and pelagic fauna), to highly similar (95-100% similarity) in the New Zealand and North Pacific bioregions (meiofauna). Within bioregions, depth correlated with changes in community composition. However, for some bioregions, such as the North Pacific (meiofauna), Mediterranean (meiofauna), Gulf of California (macrofauna), Caribbean/Gulf of Mexico (macrofauna) and the Norwegian Sea (benthic and pelagic fauna), no environmental factors correlated with changes in methane seep communities. The unbalanced design, small sample sizes and lack of variation in environmental factors within bioregions likely reduced the statistical power of the results and potentially obscured some environmentally driven patterns.

Small sample sizes also meant that natural variation in communities reduced the power of potential variation due to environmental factors.

#### 2.4.1 Effects of bioregion on methane seep communities globally

This is the first global-scale analysis of bioregional differences in methane seep communities. Similar to what has been suggested for the deep sea abyssal and bathyal zones and ecosystems such as hydrothermal vents (Van Dover et al. 2002; Watling et al. 2013), I have shown bioregional variation in methane seep community compositions. In my study, there was low similarity in the methane seep communities within the bioregions Caribbean/Gulf of Mexico (11%) and Norwegian Sea (49%), for the benthic and pelagic fauna communities and the North Pacific (27.92%) and the Gulf of California (20.30%) for the macrofauna communities. This suggests that the bioregions depicted by Costello et al. (2017) do not adequately reflect bioregional variations in methane seep communities. In the Japanese Archipelago, Nakajima et al. (2014) found that due to the presence of site endemic species and community dissimilarity in chemosynthetic ecosystems, the Japanese Archipelago (bioregion 20) was best represented by eight biogeographic regions. Similarly, in Southern Australia (bioregion 26), differences within deep-sea benthic communities caused the delineation of seven biogeographic regions (Tanner et al. 2018). This highlights the need for further research to understand global bioregional variations in methane seep communities, and the possible delineation of biogeographic regions specifically for methane seeps.

#### 2.4.2 Environmental effects on methane seep communities

Collection method and depth correlated with variation in the methane seep communities for all fauna types. Collection methods accounting for large amounts of variation in the data highlights the need for a standardised method of analysis. Due to the challenges in understanding deep-sea environments, studies on methane seeps often occur in one area, leading to patchy data and data localised in areas where deep-sea research is available. In future studies, implementation of standard collection practices while at sea, as well as an increased effort to uniformly sample and study global seep systems, will be essential to better understand global variability of methane seep communities.

There is a common understanding in the literature that methane seep communities are highly structured by depth, with some studies suggesting that depth is a stronger driver of methane seep community compositions than distance (Olu et al. 2010; Cordes et al. 2016). Generally, shallow methane seep (>400 m) communities include a high abundance of non-endemic background fauna, and deep methane seep communities encompass a high abundance of endemic fauna (Sahling et al. 2003). At deep methane seeps, the diversity generally decreases (Sibuet and Olu 1998) and at intermediate depths high levels of diversity are attributed to the presence of fauna from both the shallow and deeper communities (Olu et al. 2010). Knowledge of variation in methane seep communities with depth is often based on studies of benthic fauna (Sahling et al. 2003; Nakajima et al. 2014; Quattrini et al. 2015; Rybakova et al. 2022). In my study, meiofauna and macrofauna community variations also correlated with changes in depth. Methane seep meiofauna variations with depth are not well studied in the literature. However, deep sea meiofauna community variation with depth has been observed, where meiofauna abundance decreases with depth (Vanhove et al. 1995; Rex et al. 2006; Rosli et al. 2018). In methane seep macrofauna communities, observations of increasing species richness with depth have been reported in the Bering Sea (Rybakova et al. 2022). It is not overly surprising that macrofauna and meiofauna communities varied with depth as the literature shows that variations in these communities can be linked to increased heterogeneity of the site, often caused by endemic benthic megafauna which are structured by depth (Levin and Mendoza 2007; Cordes et al. 2010). Disentangling the exact factors that cause community changes with depth is challenging as changes in depth also correlate with change in other environmental factors. These factors include photosynthetic productivity/food availability, pressure, temperature, and light availability (Sahling et al. 2003; Carney 2005; Harris and Whiteway 2009; Olu et al. 2010; Nakajima et al. 2014; Levin et al. 2016).

In this study I used latitude, longitude, depth, and collection method as my environmental factors to compare to methane seep community variation. The environmental factors that influence methane seep community compositions and bioregional patterns are not limited to these factors, as methane seeps can also be influenced by other factors such as: deep-water currents, topography, oxygen

minimum zones, temperature, and seawater chemistry (Van Dover et al. 2002; Dambach et al. 2016; Puerta et al. 2020). These factors can cause variations in the connectivity between sites and can cause environmental variations in global bioregional patterns of methane (Van Dover et al. 2002; Carney 2005; Nakajima et al. 2014; Dambach et al. 2016; Puerta et al. 2020)

### 2.4.3 Small-scale community variability

My results showed large variation in the similarities of the methane seep communities within the bioregions. The methane seep community similarity within bioregions ranged from highly similar in the North Pacific and New Zealand meiofauna communities (95-100% similarity), to highly dissimilar in the Caribbean/Gulf of Mexico benthic and pelagic fauna communities (>30% similarity). My results highlight the diversity of communities at methane seeps, which are likely attributable to the dynamic nature of methane seeps. One explanation for the variation seen in methane seep communities within bioregions is due to the successional stage or age of the seep. Methane seep fluid flow is not an infinite source, so flux rates can be highly dynamic and change with time. Due to the variation in methane seep fluid flow, methane seep communities often follow successional patterns in community compositions and can vary depending on the age of the seep and the fluid flow rate at the seep (Bowden et al. 2013; Levin et al. 2015). This means that potential variation in the fluid flow or age of the methane seeps within a bioregion could cause variation in the communities present, and decrease the methane seep community similarity within a bioregion.

The presence of foundation fauna that create biogenic habitats at methane seeps could also be driving variation in the methane seep community composition. Foundation fauna create heterogeneous habitats for species to colonise and alter the chemical composition of the sediment and pore water surrounding the biogenic habitat (Levin et al. 2010; Cordes et al. 2010; Rybakova et al. 2022). Foundation fauna also occur in areas of differing sulphide concentrations, which can alter the communities occupying the biogenic habitats (Levin et al. 2003; Grupe et al. 2015). Typically, microbial mats are found in areas with the highest sulphide concentrations, with the communities occupying microbial mats having high densities but low species



diversity (Levin et al. 2003; Sahling et al. 2003; Bowden et al. 2013). Sulphide concentrations are also high within clam beds at methane seeps. However, highly diverse communities in clam beds are attributed to bioturbation from the clams, which reduces surface sediment sulphide concentrations (Sahling et al. 2002; Rybakova et al. 2022). Low sulphide concentrations have been reported in mussel beds, and consequently, increased abundances of background species have been observed occupying these biogenic habitats (Sahling et al. 2002; Menot et al. 2010; Guillon et al. 2017). In addition, tubeworm aggregations have diverse communities of surface deposit feeders due to their ability to access sulphate deep within the sediment through their root-like systems (Menot et al. 2010). Successional changes in community composition also occur within biogenic habitats. Often endemic species occupy younger habitats and background fauna colonise older and more complex biogenic habitats (Bergquist et al. 2003). Thus, communities present within methane seeps will depend on the foundation fauna and the successional stage of the communities within the biogenic habitats.

## 2.5 Conclusions

Methane seep ecosystems are currently threatened by anthropogenic disturbances such as bottom trawling, dredging and potential mining of gas hydrates and minerals. Methane seep communities are highly susceptible to disturbance due to their slow-growing nature, low reproductive rates, and rare and endemic species.

Understanding biogeographic differences in methane seep communities is essential to ensure representation of the diversity in methane seep ecosystems is included in management and protection. In my study, global bioregional patterns in methane seep ecosystems were identified. Globally and within bioregions, variation in methane seep community composition were driven by depth. I have shown that methane seep communities vary globally and correlate with global bioregional patterns. This highlights the importance of taking a bioregional approach in the management of methane seeps.

Methane seeps were only discovered in 1983 and since then, have been reported globally. However, a lack of standard sampling techniques limits global comparisons of methane seep communities. Future research should be driven by international

collaborations that follow standard collection methods to aid our knowledge of global patterns in methane seep communities.

# Chapter 3. Methane Seeps on the Hikurangi Margin: Comparisons of Communities and the Effects of Bottom Trawling

## 3.1 Introduction:

Deep sea methane seeps have been described as biodiversity hotspots in food-limited deep sea environments (Sahling et al. 2003; Åström et al. 2018). The dense and diverse communities observed at these sites are supported by chemosynthetic primary production (e.g. microbial oxidation of methane), which provides an alternative source of production to surrounding communities. Methane seeps were first discovered in the Gulf of Mexico in 1983 (Paull et al. 1984) and have continued to be described on continental margins globally, across a range of depths from <15m to >7,400m (Sibuet and Olu 1998; Levin 2005; Rybakova et al. 2022)

### 3.1.1 Methane seep community diversity

Methane seeps across the globe have host diverse and rare communities, with some authors suggesting that methane seep sites are island-like habitats (Bergquist et al. 2003; Van Gaever et al. 2009; Rybakova et al. 2022). Most of the deep seafloor is relatively homogeneous, muddy sediment that provides limited substrate for colonisation for sessile and filter-feeding fauna (Vanreusel et al. 2010).

Contrastingly, methane seep ecosystems include carbonate structures such as cobbles, boulders, flats, and pavements that sessile species can colonise and inhabit. In addition, endemic fauna (e.g. *Bathymodiulus* mussels, *Lamellibrachia* tube worms, and *Calypptogena* clams) create biogenic habitats by increasing the complexity of the ecosystem, and altering the chemistry of the water and sediment through processes such as bioturbation (Rybakova et al. 2022). Within these biogenic habitats chemosynthetic foundation fauna can host a diversity of heterotrophic invertebrates and background fauna (Cordes et al. 2010; Zhao et al. 2020). As well as sessile fauna, methane seeps also provide habitats, and in some cases food, for various mobile predator species such as fish, crabs and sharks (Grupe et al. 2015; Sen et al. 2018; Seabrook et al. 2019). The high complexity and

abundance of prey species at methane seeps, have been shown to attract mobile predator species to these ecosystems (Grupe et al. 2015).

Methane seep communities also follow strong successional patterns dependent on fluid flux rates (Bergquist et al. 2003; Cordes et al. 2005). Methane seeps are dynamic ecosystems where fluid flow is not continuous, so the communities often reflect the seep's age and the fluid flow rate, among other environmental factors. As a seep develops, the community composition will follow successional stages. Generally, when fluid flow is initiated, and fluid flux rates are at their highest: i) microbial mats of thiotrophic and methanotrophic bacteria will be predominant at the seep site; ii) *Calyptogena* clams colonise the seep, and carbonate precipitation begins; iii) Carbonates continue to be precipitated, and *Lamellibrachia* tube worms colonise the seep, followed by *Bathymodiolus* mussels; and iv) As the carbonate continues to precipitate, it can decrease or redirect methane fluid flow, reducing the abundance of chemosynthetic fauna and leading to the colonisation of the seep site by background fauna (Bowden et al. 2013). Following the cessation of fluid flow, carbonate structures and shells from chemosynthetic species will persist and be further colonised by background fauna, where they will continue to support communities even after fluid flow has stopped (Levin et al. 2015).

Depth has also been shown to be an important factor driving variation in methane seep community composition (Olu et al. 2010; Nakajima et al. 2014; Turner et al. 2020). This is attributed to the factors that co-vary with depth, such as surface production, temperature, light availability, pressure, oxygen availability, predation, sediment texture and bottom water currents (Sahling et al. 2003; Olu et al. 2010; Turner et al. 2020). Generally, at shallow methane seeps (<400 m), the communities lack chemosynthetic species and are more representative of non-seep background habitats (Sahling et al. 2003). At deep-sea methane seeps >400 m, the communities are dominated by chemosynthetic species (Sahling et al. 2003). At shallow methane seeps, the community often represents background assemblages as nutrient availability is not limited, so background species can outcompete chemosynthetic species (Sahling et al. 2003; Sen et al. 2018). In the deep sea, photosynthetic primary production availability is limited. Chemosynthetic primary production from methane seeps removes nutrient limitations and provides an alternative source of

nutrition not only to endemic seep fauna but also a variety of background species (Åström et al. 2018; Seabrook et al. 2019; Turner et al. 2020). The influence/benefits of chemosynthetic primary production in the deep sea have a vast influence. Symbiont-bearing species, *Calyptogena* clams and feather duster worms have been observed up to 150 m and 300 m away from methane seep ecosystems respectively (Wagner et al. 2013; Goffredi et al. 2020).

### 3.1.2 Ecosystem services from methane seeps

As well as providing a habitat for a diversity of fauna, methane seeps provide ecosystem services to society. Methane seeps provide many provisioning (providing goods from the ocean), regulating (regulating habitats and ecosystems), supporting (causing other ecosystem services to happen) and cultural (non-material services) services (Armstrong et al. 2012; Thurber et al. 2014). Methane seep microbial communities are major sinks for carbon in the ocean. The anaerobic oxidation of methane and precipitation of carbonates removes methane from the ocean and puts it into the benthic biomass (Levin et al. 2016). The anaerobic oxidation of methane is thought to remove up to 90% of the methane released through the seafloor sediments, reducing the release of methane into the atmosphere to around 2% of the global flux (Armstrong et al. 2012). Methane seep ecosystems also provide habitat and aggregation points for some commercially important species (Grupe et al. 2015; Sen et al. 2018; Seabrook et al. 2019; Turner et al. 2020). Observations and evidence of the ingestion of chemosynthetic material by tanner crabs (*Chionoecetes tanneri*), red crab (*Chaceon quinque-dens*) and the increased abundances of commercially important species relative to surrounding habitats at methane seeps, indicate that these habitats may provide refuge, nutrition and nursery grounds for some commercially important species (Grupe et al. 2015; Sen et al. 2018; Seabrook et al. 2019; Turner et al. 2020).

Methane seep ecosystems are threatened by human activities, such as bottom trawling, dredging, and mining for gas hydrates and minerals, as well as climate change (Amon et al. 2017). The impact of these activities on methane seep ecosystems and the services they provide remains poorly understood. The destruction of methane seep habitats from anthropogenic sources will likely have a

catastrophic effect, due to the slow-growing nature of the environment (Hove and Moreau 2007). Deep sea ecosystems experience little natural disturbance, which leads to low resilience of the system to anthropogenic disturbances (Armstrong et al. 2012). Evidence of the negative impacts of bottom trawling has been observed on the Hikurangi Margin of New Zealand. Observations of coral and *Calypptogena* shell debris were observed alongside trawl marks. Chemosynthetic fauna was also observed taking refuge around the base of carbonates and in the cracks between carbonate structures (Baco et al. 2010).

### 3.1.3 Methane seep conservation

Globally, there is limited management of methane seep ecosystems. Due to the vulnerability of methane seep ecosystems and anthropogenic threats they face, the international conventions United Nations General Assembly (UNGA) and the Convention on Biodiversity (CBD), have described methane seep ecosystems as Vulnerable Marine Ecosystems (VMEs). They have also been described as Ecologically or Biologically Significant Areas (EBSAs) to highlight the importance of methane seep ecosystems (FAO., 2009; CBD., 2008 as referenced by Rice et al., 2014). Within the EBSA and VME concepts, management of these ecosystems aims to ensure that representation of the biodiversity within these ecosystems is managed to avoid biodiversity loss (Gleason et al. 2006).

Within the New Zealand Exclusive Economic Zone (EEZ), methane seeps are recognised as sensitive environments. This is due to the adverse impacts of anthropogenic disturbances and the expected slow recovery rates (10-20 years) of methane seep ecosystems (Makgill et al. 2012). This means that if a methane seep or sensitive environment is encountered during an environmental impact assessment (EIA), “measures are taken to avoid, mitigate, or remedy the adverse effects of the activity on any sensitive environment encountered” (Makgill et al. 2012). Although methane seeps are recognised as sensitive environments within the New Zealand EEZ, there are no designated protected/managed areas for methane seeps. In order to manage and conserve methane seep ecosystems, it is important to understand the diversity of the communities within them. This is so that representation of the

species within these ecosystems can be managed to prevent potential extinction events.

In this study, I describe six methane seep ecosystems on the Hikurangi Margin of New Zealand, across a range of depths and bottom trawling impact. I compare the communities at these methane seeps to environmental characteristics such as depth, rugosity, and trawl intensity to determine the factors influencing the methane seep ecosystems. I hypothesise that trawl intensity and depth will drive variations in methane seep community composition across the Hikurangi Margin.

## 3.2 Methods:

### 3.2.1 Study area

Video surveys of the methane seep communities were conducted at six active methane seep sites on the Hikurangi Margin of New Zealand (Figure 3.1). These sites were selected based on the presence of bubble hydro-acoustic flares observed by Watson et al. (2020). The Hikurangi Margin is at the southern end of the Tongan-Kermadec-Hikurangi subduction zone, where the Pacific plate subducts under the Indo-Australian plate on the east coast of the North Island of New Zealand (Greinert et al. 2010; Clark et al. 2019). Methane seeps were first discovered on the Hikurangi Margin by fishermen who observed anomalies in shipboard acoustics that were interpreted to be fish. These anomalies were likely to be methane bubbles as they also recovered chemosynthetic clams and carbonates from trawls (Greinert et al. 2010). Following these observations, methane seeps were first investigated in 1996 (Lewis and Marshall 1996). The Hikurangi margin encompasses a variety of methane seep sites, with over 30 methane seeps being described by Greinert et al. (2010) and sites still being discovered to this day (Greinert et al. 2010)

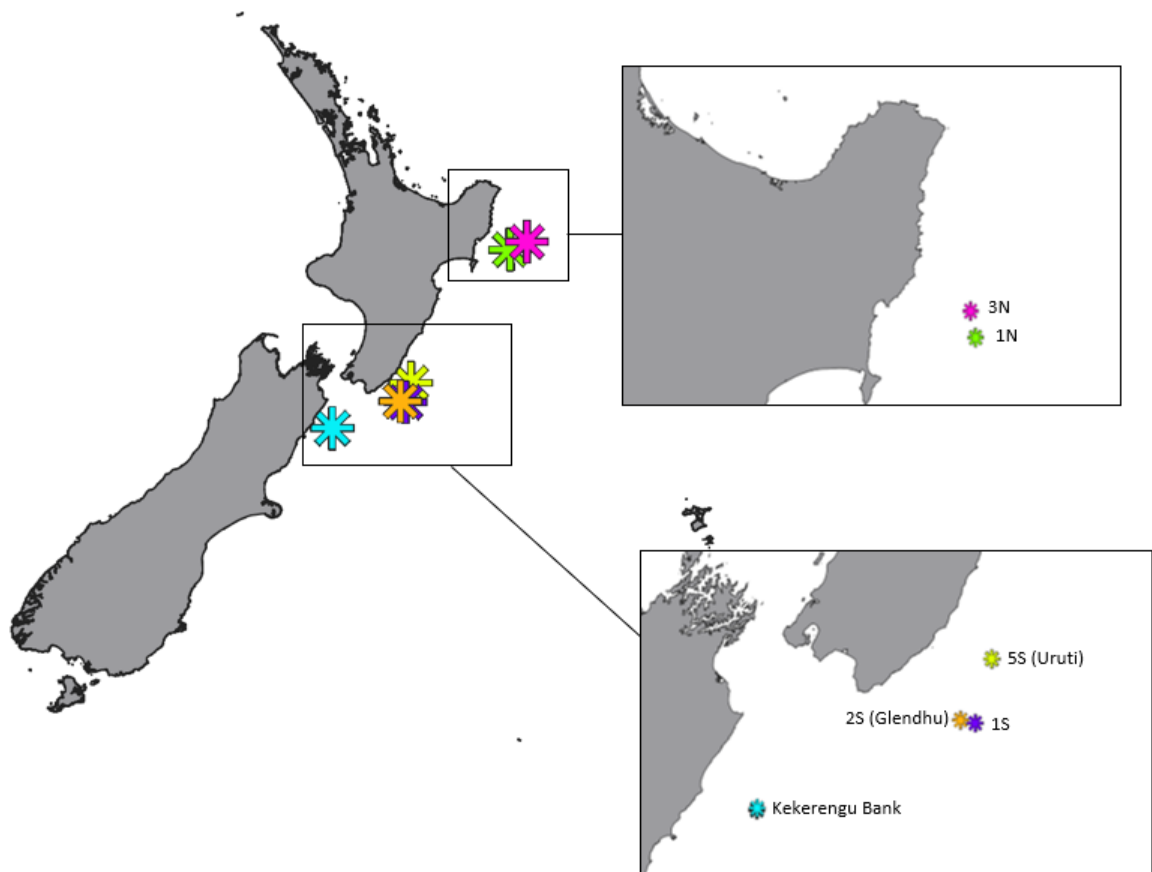


Figure 3.1 Map of the Hikurangi Margin methane seep sampling sites. Zoom in boxes of Northern and Southern sites. Starred points indicate methane seep sampling sites. Pink - Seep 3N, green - seep 1N, yellow - seep 5S (Uruti), orange- seep 2S (Glendhu), purple – seep 1S, blue – Kekerengu Bank.

### 3.2.2 Video transects

Video surveys were conducted using the Remotely Operated Vehicle (ROV) Jason deployed from R/V Roger Revelle in January/February of 2019 (RR1901) and ROV ROPOS deployed from R/V Tangaroa in February/March of 2021 (TAN2102). Video data from RR1901 was used for seep 1N, 3N, 1S and 5S (Uruti) and video from TAN2102 was used for seep 2S (Glendhu) and Kekerengu Bank. Continuous video was recorded from ROV JASON and ROPOS, with dive duration ranging from 6 - 44 hours. The cameras on ROV JASON and ROPOS were angled vertically downwards.

Data on methane seep communities were obtained by analysing two hours of video per sampling site. To be suitable for analysis, the video had to be focused on the seafloor; have a clear view without any obstructions (e.g., scientific equipment or sediment plumes); be close enough to the seafloor that small fauna of approximately



3-5 cm (e.g. *Munidopsis* squat lobsters) were able to be identified; and the ROV had to be in transit and covering unsurveyed seafloor for approximately 1.3 km.

### 3.2.3 Environmental data

Environmental data including depth (the average depth at the site), trawl intensity and rugosity were recorded for each sampling site. Depth was recorded from the ship log and was then converted to depth levels following Turner et al. (2020). Depths <500 m were considered shallow; 501-1500 m were considered intermediate; and depths >1500 m were considered deep. Rugosity was determined using QGIS and derived from bathymetry data gridded at 20-25 m resolution. Data on bottom contact trawling from the 1989-1990 fishing year until the 2020-2021 fishing year were sourced from Fisheries New Zealand. Trawling intensity was determined by calculating the sum of all the trawl lines intersecting a 2 km radius around the midpoint of the methane seep sample transects, following the methods in Bowden et al. (2016). The 2 km radius exceeds the area of the individual transects; however, this was to account for the broader trawling footprint around the seep as well as potentially unreported activity and disparities between GPS systems.

Table 3.1 Table of methane seep sites and their corresponding environmental factors, depth (m), depth level (intermediate/deep), rugosity, trawl intensity.

| Methane seep | Depth (m) | Depth level  | Rugosity | Trawl intensity |
|--------------|-----------|--------------|----------|-----------------|
| 1S           | 2402      | Deep         | 17.57837 | 0               |
| 5S (Uruti)   | 1225      | Intermediate | 3.286533 | 1               |
| 1N           | 1475      | Intermediate | 28.39396 | 24              |
| 3N           | 954       | Intermediate | 7.764438 | 44              |
| 2S (Glendhu) | 1989      | Deep         | 6.365156 | 0               |
| Kekerengu    | 840       | Intermediate | 6.015632 | 16              |

### 3.2.4 Video analysis

The video was analysed using the Ocean Floor Observation Protocol (OFOP) 3.3.8a software to record habitat characteristics and visible benthic and pelagic fauna. The species were identified to the lowest taxonomic level, which ranged from species to phylum level. Methane seep characteristic fauna were generally recorded to a finer taxonomic resolution, and non-chemosynthetic species were recorded to a coarser taxonomic resolution (i.e. sea urchins were recorded as echinoid). The data were then compiled into a single data set that recorded the number of each species present per site.

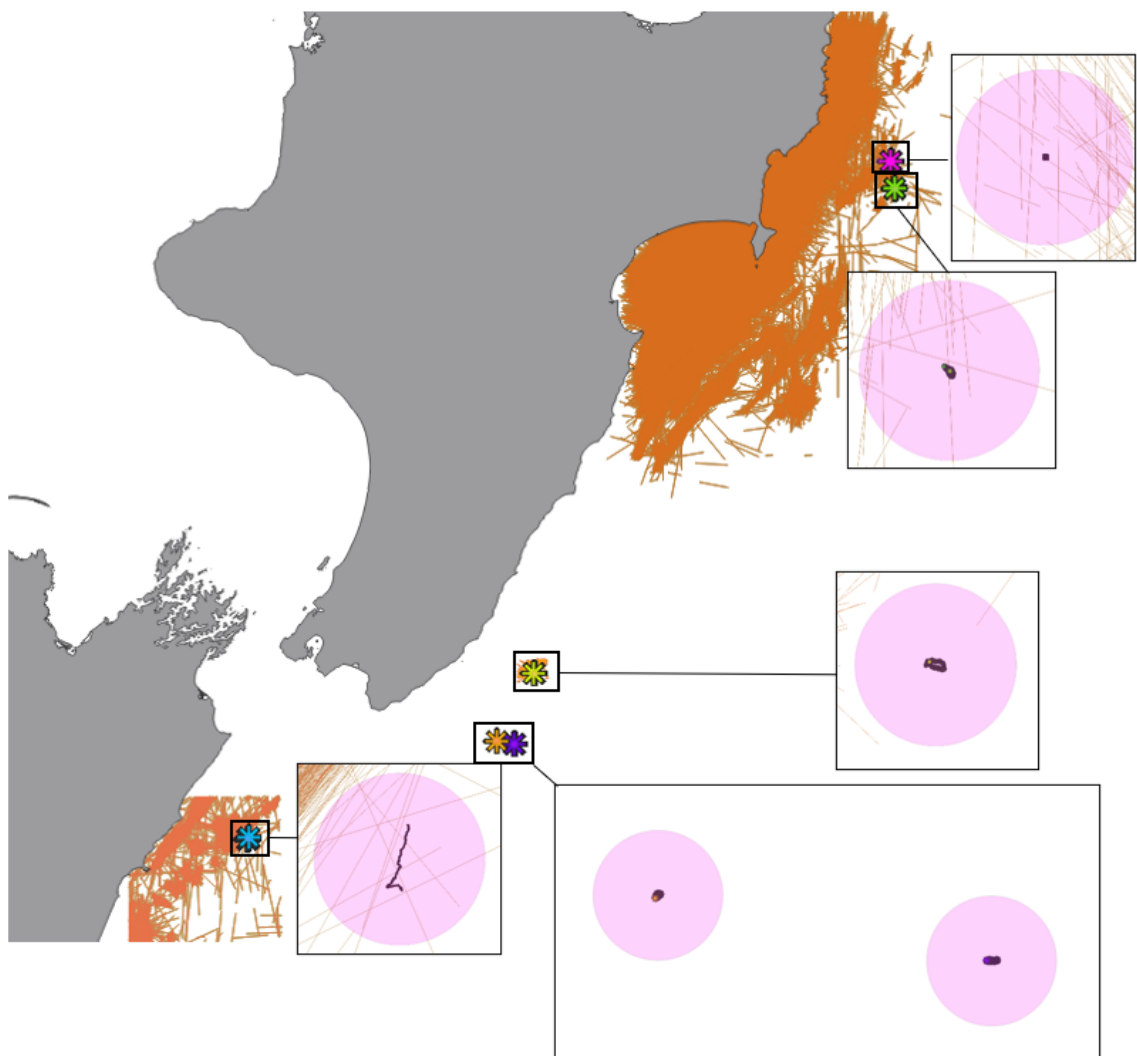


Figure 3.2 Trawl intensity at each site. Orange lines indicate trawls. Coloured flowers indicate the seep sites, pink - Seep 3N, green - seep 1N, yellow - seep 5S (Uruti), orange- seep 2S (Glendhu), purple – seep 1S, blue – Kekerengu Bank. Zoom in boxes show the 2km radius from the centre of the transect analysed indicated by the purple circle. Lines in the centre of the purple circles are the transect analysed.

### 3.2.5 Statistical analysis

The PRIMER v7 software with the PERMANOVA + add-on was used to analyse the community composition at each methane seep habitat. The data was run using presence/absence data and abundance data to account for the fact that colonial organisms were counted as individuals i.e. 1 coral or 1 sponge.

The abundance data were square-root-transformed to reduce the influence of abundant taxa.

Community similarities between methane seep sites were compared using a similarity percentage (SIMPER) test. The relative influence of environmental factors depth, depth level, rugosity, and trawl intensity on methane seep community compositions were explored using a distance-based linear model (DISTLM) test with an AIC stepwise model.

## 3.3 Results

### 3.3.1 Site descriptions:

#### 3.3.1.1 Seep 1S

Seep site 1S was on average 2402m deep and the bottom characteristics were complex muddy sediments. Seep site 1S had the lowest species diversity of all the surveyed sites (21 species) and high abundances of hydroids. Towards the end of the transect, typical seep characteristics were observed around a pockmark with blocks of carbonate, reduced dark sediments, scattered clam shells, microbial mats, tube worms, *Lamellibrachia columna* bushes, high abundances of *Munidopsis* sp. squat lobsters and a small patch of *Calyptogena* sp. clams. Around the seep site, an increased abundance of anemones was observed. The non-seep areas of seep site 1S were generally dominated by hydroids, isopods, swimming holothurians (*Enypniastes eximia*), asteroids, Pennatulacea, pycnogonids and shrimp.

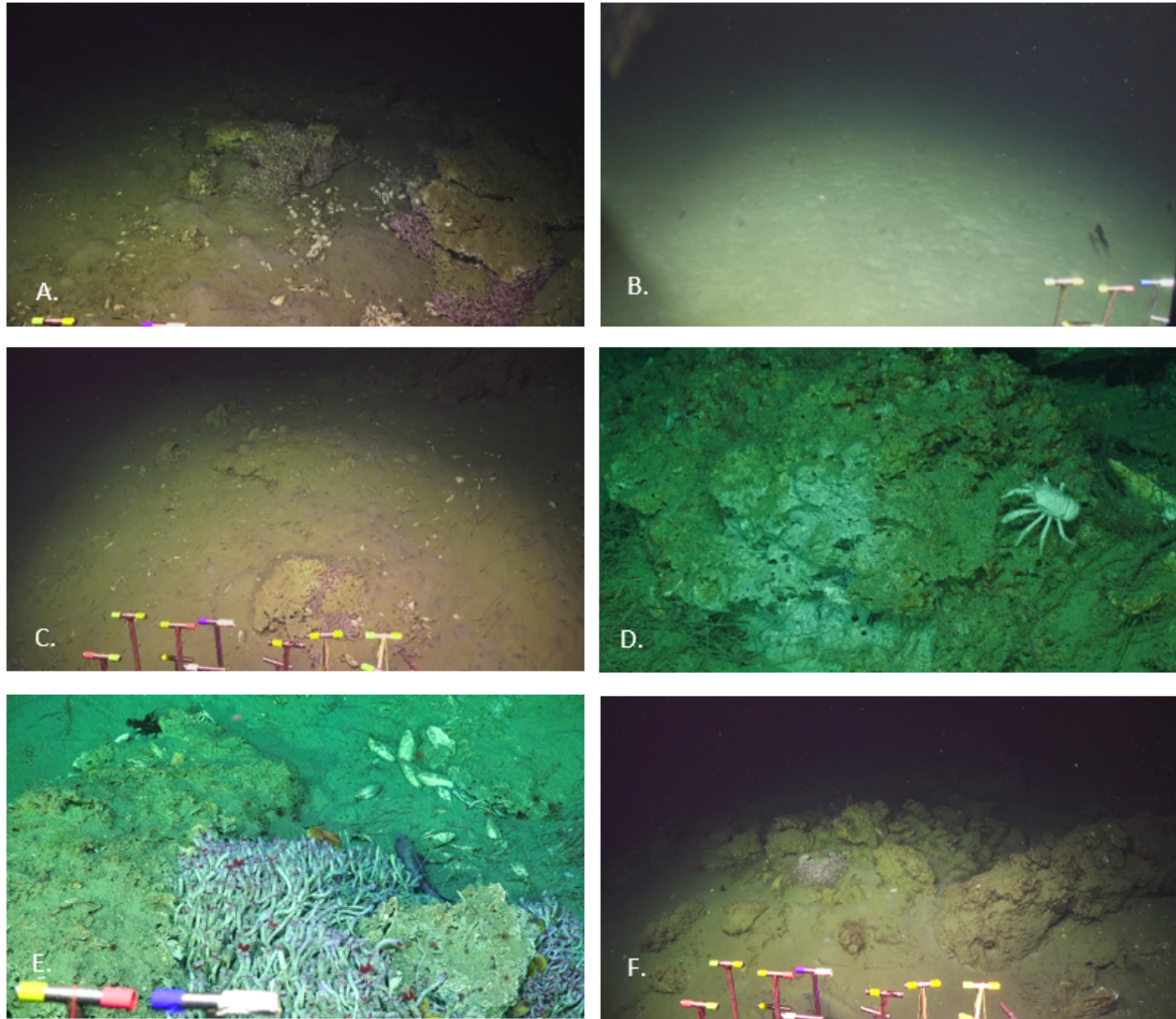


Figure 3.3 Pictures from seep site 1S. A) Tube worms in the cracks between carbonates and under carbonate ledges. Clam shells and clams on the sediment. B) Muddy sediment with Moridae (cod) on the bottom right. C) Muddy sediment with small carbonate pieces and clam shell hash. Reduced sediment patches and anemones on the sediment and carbonates. D) Zoom of *Munidopsis* squat lobster on a carbonate boulder. E) Small *Lamellibrachia* tube worm bush with *Bathymodiolus* mussels. *Munidopsis* squat lobster, *Calymene* clams and an eel also pictured. F) Small tube worm bed on the side of a carbonate, reduced sediment patches and *Munidopsis* on the carbonates.

#### 3.3.1.2 Seep 5S (Uruti)

Seep site Uruti had an average depth of 1225m and the bottom characteristics were predominantly muddy sediment with high abundances of white and purple echinoids, large asteroids, anemones, and holothurians. At the characteristic seep sites there were small microbial mats, increased abundances of broken clamshells and individual tube worms. Complex carbonate boulders were often covered in sediments at the characteristic seep sites. The tube worms at seep site Uruti often appeared flat on the sediment and were observed at the bases and under ledges of carbonates. Similarly, microbial mats were observed in the cracks of the carbonates.



This site had a relatively high abundance of rattails (*Coelorinchus spp.*), eels and ghost sharks (*Hydrolagus sp.*), with cods (*Moridae spp.*) also observed.

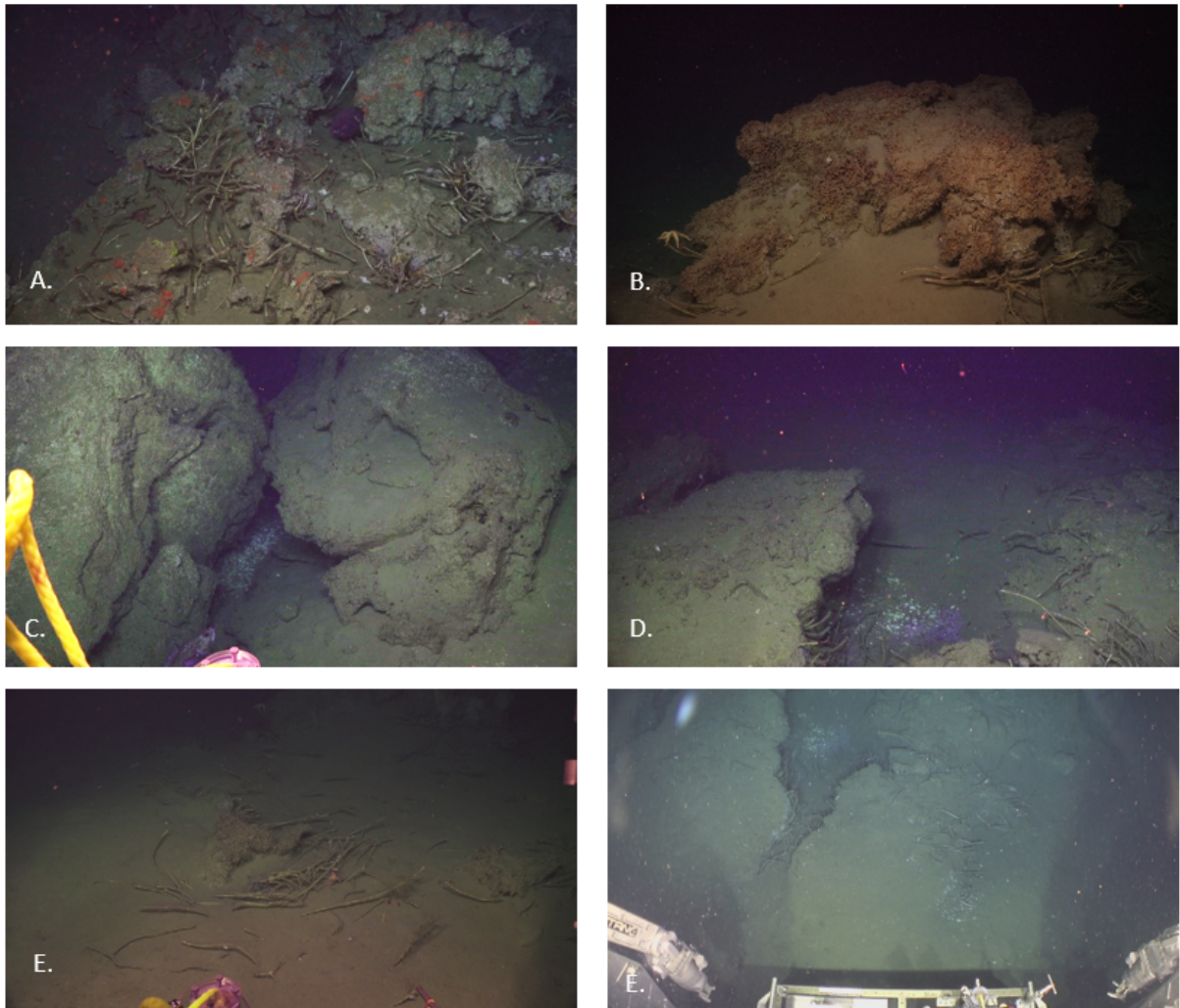


Figure 3.4 Pictures of seep site Uruti – 5S. A) Carbonates boulders with individual *Lamellibrachia* tube worms. Orange and yellow sponges on the carbonates and an eel poking out from the carbonates. B) A carbonate boulder with singular tube worms at the base and a starfish on the tube worms. C) Two carbonate boulders with clams and a clam bed/clamshell hash in the sediment between the boulders. D) Carbonate platforms with anemones on the top and singular flat tubeworms growing on the platform sides. Clamshells and clams in the gap between the carbonate platforms. E) Muddy sediment with small carbonate mounds. Singular flat tubeworms and an orange starfish. F) Carbonate platforms with singular flat tubeworms, clam shells, and microbial mats within the gaps. Small patches of clam shells on the top of the carbonates.

### 3.3.1.3 Seep 1N

Seep site 1N had an average depth of 1475m and the bottom characteristics were predominantly muddy sediment with flats of buried carbonate rocks, with limited characteristic seep sites observed. There was a high abundance of sea pens, shrimp, holothurians, large purple echinoids and ophiuroids (stalked and star crinoids). Cup corals were also present. Site 1N was the only seep site that Scleractinia corals were observed at. At seep site 1N small microbial mats, a small

patch of clam shells, and flats of carbonate rock that appeared to be buried in sediments were observed.



Figure 3.5 Pictures of seep site 1N. A) A small patch of clams and reduced sediments. B) A patch of clamshell hash, small microbial mats, and a shrimp. C) Buried carbonate with an Echinoid and small microbial mat. D) Two purple echinoids in muddy sediment. E) Buried carbonate and a stalked Crinoid in the middle. F) Muddy sediment and an anemone.

#### 3.3.1.4 Seep 3N

Seep site 3N had an average depth of 954m and was characterised by muddy sediments and minimal characteristic seep areas. Despite the lack of chemosynthetic habitats, this site had a high diversity of species (30 species). Within the muddy sediment of seep 3N white demosponges, glass sponges (Hexactinellida), zoanthids, sea pens, hydroids, primnoids, stylasterids, shrimp, and anemones were abundant. Site 3N also had six fauna identified as site endemic fauna. Seep characteristic areas within seep 3N consisted of small patches of reduced sediment, a relatively large patch of clamshells and buried carbonate rubble

and pavements. The carbonate rubble and pavements hosted diverse assemblages of Primnoidae, Pennatulaceae, asteroids, hydroids, brisingids and hermit crabs. At this site, squid, a stingray, a blobfish (*Psychrolutes microporos*), slick heads (Alepocephalidae), Pycnogonids and Crinoids were also observed.



Figure 3.6 Pictures from seep site 3N. A) Sediment-covered carbonates with hermit crabs, Primnoidae and stylasterids. B) Muddy sediment with reduced patches and clam shells. C) Primnoidae in the sediment (Pink). D) Glass sponge (white) in the sediment. E) Zoanthids (orange) with glass sponges on top (white) and glass sponges in the sediment. F) Two crinoids on buried carbonates.

#### 3.3.1.5 Seep site 2S (Glendhu)

Glendhu had an average water depth of 1989m and a high abundance of chemosynthetic fauna, with a relatively low overall species diversity (22 species observed). Glendhu has a dense community of *Lamellibrachia* tube worms, barnacles, *Bathymodiulus* mussels and *Calymene* clams. *Munidopsis* squat lobsters, asteroids, tube worms, eels, lanternfish (Myctophidae), holothurians, shrimp and anemones were also observed at Glendhu. Glendhu was the only seep site



where lantern fish were observed. Chemosynthetic habitats at Glendhu were generally characterised by large carbonate pavements and boulders, extensive mussel and clam beds, expansive tube worm bushes, microbial mats, and exposed methane hydrates. Pockmarks and areas of active bubbling were also observed in the Glendhu video transects. In some areas of Glendhu seep site, smaller invertebrates and benthic fauna could not be identified because the camera's height above the seafloor was too high.

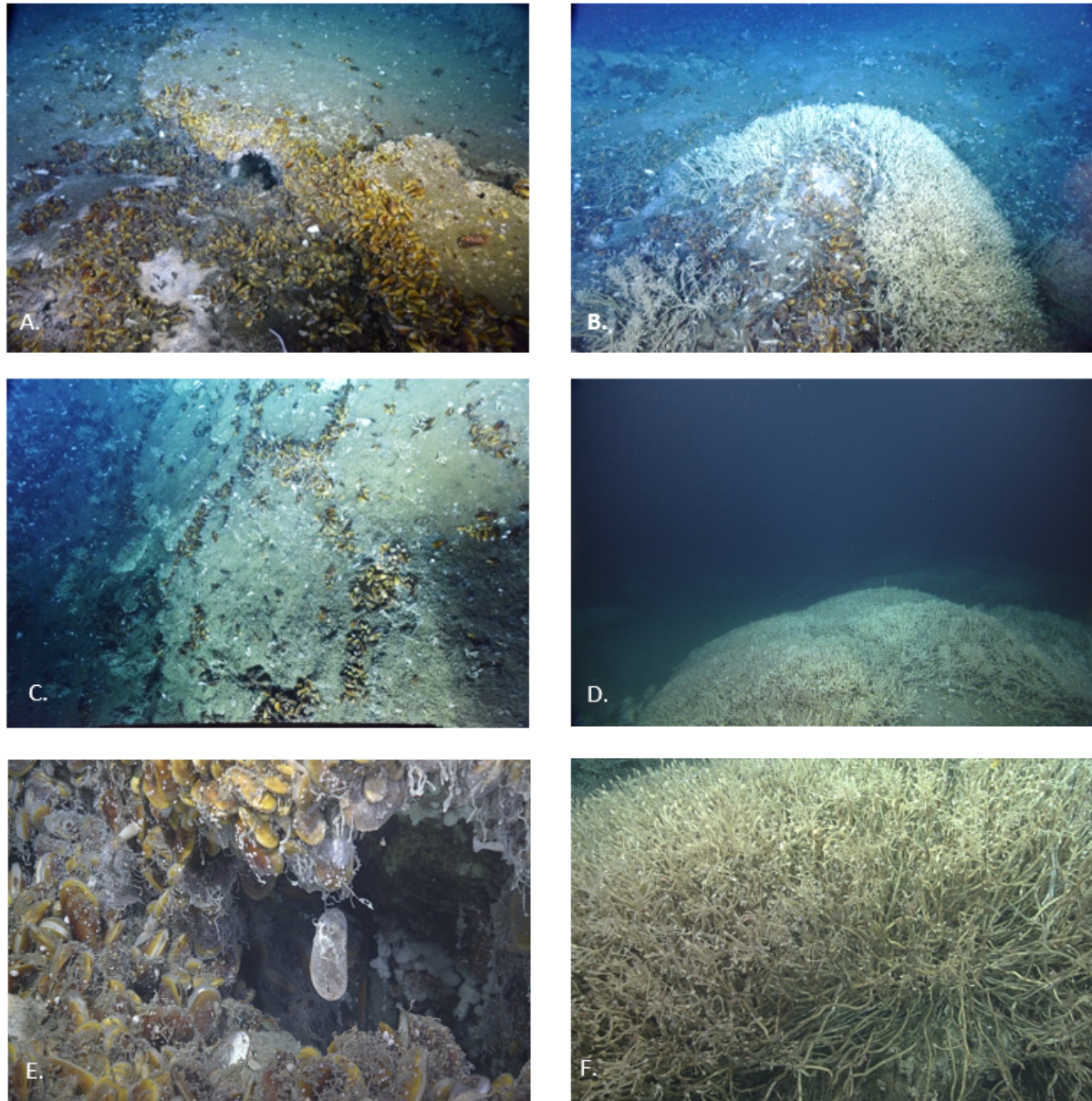


Figure 3.7 Pictures from seep site Glendhu A) Carbonate platform covered in *Bathymodiulus* mussels. Thick microbial mats (white) and an eel in the bottom middle. Tube worms in the top right corner. B) Microbial mat in the middle of the mound surrounded by *Lamellibrachia* tube worms with barnacles on them and *Bathymodiulus* mussels. C) *Bathymodiulus* mussels in a hexagon shape on the sediment. D) Large bush of *Lamellibrachia* tube worms. E) *Bathymodiulus* mussels with microbial mats on them. F) *Lamellibrachia* tube worm bush with barnacles and *Munidopsis* squat lobsters.



#### 3.3.1.6 Seep site Kekerengu

Kekerengu seep site had an average depth of 840m with a high diversity of species and a high abundance of fish (including fisheries species) but a low abundance of characteristic seep fauna. There were ten fauna that were 'site endemic' fauna and were only identified at Kekerengu. In characteristic seep areas, there were small patches of microbial mats, patches of clamshell hash and singular tube worms. When tubeworms and microbial mats were observed, they appeared to be flat on the sediment and in cracks of carbonates/ under carbonate ledges. Although there were low abundances of characteristic seep fauna, the Kekerengu seep site comprised carbonate boulders, flats, rubbles, or gravel. On carbonates, there were high abundances of stylasterids, Primnoidae, and demosponges. Hermit crabs, hydroids, anemones, shrimp, rattails (*Coelorinchus spp.*), brisingids, black corals (*Antipatharia*), bubble gum corals (*Alcyonacea*) and bamboo corals (*Alcyonacea*) were also observed at Kekerengu. Asteroids were also commonly seen in the sediment and Brisingids on the carbonates. At Kekerengu, most of the sessile species observed were on large carbonate structures and not in the sediment. Large dead coral skeletons were observed in the sediment and recorded in the un-analysed sections of the video (Figure 8h). There was also a significantly increased diversity and density of fish at this site, with eels, rattails (*Coelorinchus*) and ghost sharks (*Hydrolagus sp*) most prominent. Hoki (*Macruronus novaezelandiae*), oreo (*Oreosomatidae*), *Trachyscorpia*, orange roughy (*Hoplostethus atlanticus*), coffin fish (*Chaunacidae*) and cucumber fish (*Paraulopus sp*) were also observed at Kekerengu.

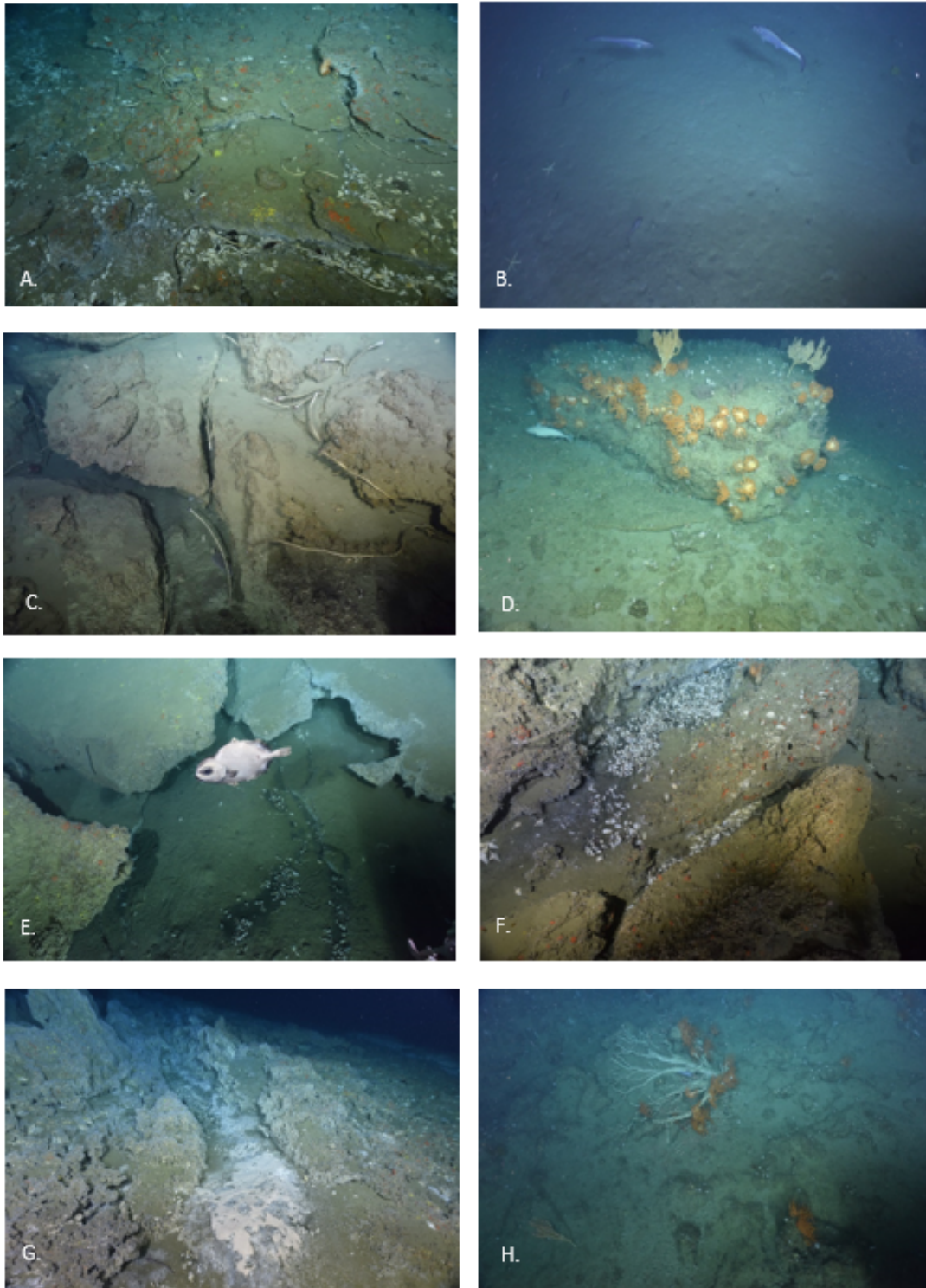


Figure 3.8 Pictures from Kekerengu seep site. A) Carbonate platforms with yellow and orange sponges and clams in the cracks between the carbonates. Singular lying down *Lamellibrachia* tube worms are also between the carbonates, and hermit crabs are on top of the carbonates. B) Hoki (*Macruronus novaezealandiae*). C) Laying down singular *Lamellibrachia* at the bases of carbonates and in the gaps between carbonates. D) Brisingida (orange) on the sides of a large carbonate block. Large bamboo corals, Stylasteridae (white) and Primnoidae on top of the carbonates. Moridae swimming next to the large carbonate. E) Oreosoma fish (*Oreosomatidae*), carbonate platforms and *Calyptogena* in the sediment. Orange and yellow sponges on the carbonate blocks. F) Orange and yellow sponges on the carbonates, *Calyptogena* clams in the gaps on the carbonates and a large anemone in the top middle of the carbonate. G) Thick Microbial mat in between the carbonates. H) Coral skeleton with Brisingida on it.

Table 3.2 Count of fauna per site. Fauna are Identified to the lowest taxonomic level (Fauna ID) and at the phylum level. Total number of fauna observed per site is indicated in the bottom row under 'total individuals'.

| Phyla      | Fauna ID                    | 1S | 5S | 1N  | 3N | Glendhu | Kekerengu |
|------------|-----------------------------|----|----|-----|----|---------|-----------|
| Annelida   | Annelid                     | 1  |    |     |    |         |           |
| Arthropoda | Barnacles                   | 50 |    |     |    | 29270   |           |
| Arthropoda | Crustacean (shrimp)         | 22 | 14 | 166 | 12 | 301     | 119       |
| Arthropoda | Crustacean (crab)           |    |    |     | 3  |         | 1         |
| Arthropoda | Hermit crab                 |    | 25 | 10  | 72 |         | 143       |
| Arthropoda | Isopoda                     | 34 |    |     | 8  | 8       |           |
| Arthropoda | Pycnogonid                  | 1  |    | 6   |    |         |           |
| Chordata   | <i>Trachysoropia</i> (fish) |    |    |     |    |         | 4         |
| Chordata   | Ascidian                    |    |    | 6   | 4  | 1       | 4         |
| Chordata   | Blob fish                   |    |    |     | 1  |         |           |
| Chordata   | Bony fish                   |    | 3  | 1   |    |         | 3         |
| Chordata   | Coffin fish                 |    |    |     |    |         | 1         |
| Chordata   | Cucumber fish               |    |    |     |    |         | 13        |
| Chordata   | Eels                        | 9  | 26 | 20  | 15 | 19      | 83        |
| Chordata   | Ghost shark                 |    | 8  |     |    |         | 26        |
| Chordata   | Hoki                        |    |    |     |    |         | 11        |
| Chordata   | Lantern fish (Myctophidae)  |    |    |     |    | 3       |           |
| Chordata   | Moridae (cods)              | 8  | 3  | 1   |    | 1       | 29        |
| Chordata   | Orange roughy               |    |    |     |    |         | 1         |
| Chordata   | Oreo fish                   |    |    |     |    |         | 2         |

|               |                              |     |     |     |     |     |      |
|---------------|------------------------------|-----|-----|-----|-----|-----|------|
| Chordata      | Rattails                     | 2   | 79  | 36  | 14  | 4   | 382  |
| Chordata      | Skate                        | 1   | 1   |     |     | 1   | 2    |
| Chordata      | Salp                         |     |     |     |     |     |      |
| Chordata      | Stingray                     | 1   |     | 1   | 2   | 2   |      |
| Chordata      | Slick head (fish)            |     |     |     | 2   |     |      |
| Cnidaria      | Primnoidae                   |     |     |     | 120 |     | 612  |
| Cnidaria      | Scleractinia                 |     |     | 2   |     |     |      |
| Cnidaria      | Stylasteridae                |     |     |     | 29  |     | 3308 |
| Cnidaria      | Alcyonacea                   |     |     |     | 1   |     |      |
| Cnidaria      | Anemones                     | 154 | 165 | 40  | 10  | 267 | 210  |
| Cnidaria      | Antipatharia                 |     |     |     |     |     | 43   |
| Cnidaria      | Hydroids                     | 922 | 9   | 61  | 494 | 27  | 88   |
| Cnidaria      | Bamboo coral<br>(Alcyonacea) |     |     |     |     |     | 4    |
| Cnidaria      | Keratoisididae               |     |     |     | 1   |     |      |
| Cnidaria      | Paragorgidae                 |     |     |     |     |     | 7    |
| Cnidaria      | Pennatulacea                 | 21  | 6   | 137 | 69  |     | 27   |
| Cnidaria      | Zoanthidea                   |     | 2   |     | 190 |     |      |
| Echinodermata | Asteroid                     | 15  | 75  | 15  | 4   | 40  | 160  |
| Echinodermata | Brisingida                   |     | 36  | 2   | 4   |     | 671  |
| Echinodermata | Cidaroida                    |     |     |     | 1   |     |      |
| Echinodermata | Crionoid                     |     |     | 21  | 10  |     | 9    |
| Echinodermata | Echinoid                     |     | 432 | 73  | 2   |     | 35   |
| Echinodermata | <i>Enypniastes eximia</i>    | 14  | 1   | 11  | 2   | 9   |      |

|               |   |      |      |     |      |        |      |
|---------------|---|------|------|-----|------|--------|------|
| Echinodermata | Holothurian                             |      | 61   | 89  | 2    | 8      | 11   |
| Echinodermata | Ophiuroid                               | 1    |      | 60  | 3    | 2      | 2    |
| Mollusc       | Squid                                   |      |      |     | 2    |        | 1    |
| Mollusc       | Octopus                                 |      | 2    | 1   |      | 1      | 3    |
| Porifera      | Sponges<br>(Hexactinellida)             |      |      | 1   | 18   |        |      |
| Porifera      | Porifera<br>(Demospongiae)              |      | 4    |     | 58   |        | 426  |
| Seep endemic  | <i>Bathymodiolus</i> sp.                | 120  |      |     |      | 26622  | 51   |
| Seep endemic  | <i>Calymene</i> sp.                     | 114  | 100  |     |      | 15130  |      |
| Seep endemic  | <i>Lamellibrachia</i><br><i>columna</i> | 1311 | 646  |     |      | 34563  | 49   |
| Seep endemic  | <i>Munidopsis</i> sp.                   | 76   |      | 1   |      | 1352   |      |
| Seep endemic  | Tube worms                              | 1591 | 100  |     |      | 1178   | 6    |
|               | Total individuals                       | 4468 | 1798 | 761 | 1153 | 108809 | 6547 |

### 3.3.2 Environmental effects on the community

In the presence and absence data, depth and trawl intensity were included in the model and accounted for 74% of the variation seen in the community ( $F > 3.77$ ,  $P < 0.39$ ) (Table 3.3).

None of the environmental factors significantly correlated with changes in the methane seep community composition in the sequential model ( $F \leq 2.2221$ ,  $P \geq 0.079$ ; Table 3.4).

Depth level was significantly correlated with 35% of the variation in community composition (Marginal test,  $F = 2.2221$ ,  $P = 0.049$ ) (Table 3.5). Intermediate and deep categories were almost 80% (77.99%) different, with abundances

of *Bathymodiolus* sp, *Calyptogena* sp, *Munidopsis* sp. *Lamellibrachia columna* and barnacles more than 10x higher at deep seeps. Higher abundances of Echinoderms, Chordates, Poriferans and Cnidarians were observed in the intermediate seeps relative to the deep seeps (Figure 3.9). The intermediate depth methane seeps had 37% similarity in the seep communities with Hydroids, anemones, rattails (*Coelorinchus spp.*), shrimp, sea pens, hermit crabs, eels, echinoids and asteroids all contributing over 5% to the community similarity. At the deep methane seeps, there was 30.46% similarity in the communities. In the deep methane seep sites (which also coincided with non-trawled seeps), chemosynthetic species *Lamellibrachia*, *Bathymodiolus*, *Calyptogena* and 'other' tube worms were responsible for 62% of the community similarities, and anemones attributed a further 10% to this.

Trawl intensity was very close to significant in the abundance data (Marginal tests,  $F=1.91$ ,  $P=0.0570$ , Table 3.5). Methane seeps with a trawl intensity  $\leq 1$  have similar communities, and methane seeps with a trawl intensity  $>1$  have similar communities. As trawl intensity increases, the abundances of Cnidarians and Poriferans also increase at the methane seep sites (Figure 3.9). Seeps with high trawl intensity also have very low abundances of chemosynthetic fauna especially *Lamellibrachia* tube worms (Figure 3.9).

Rugosity did not correlate with variations in methane seep community compositions.

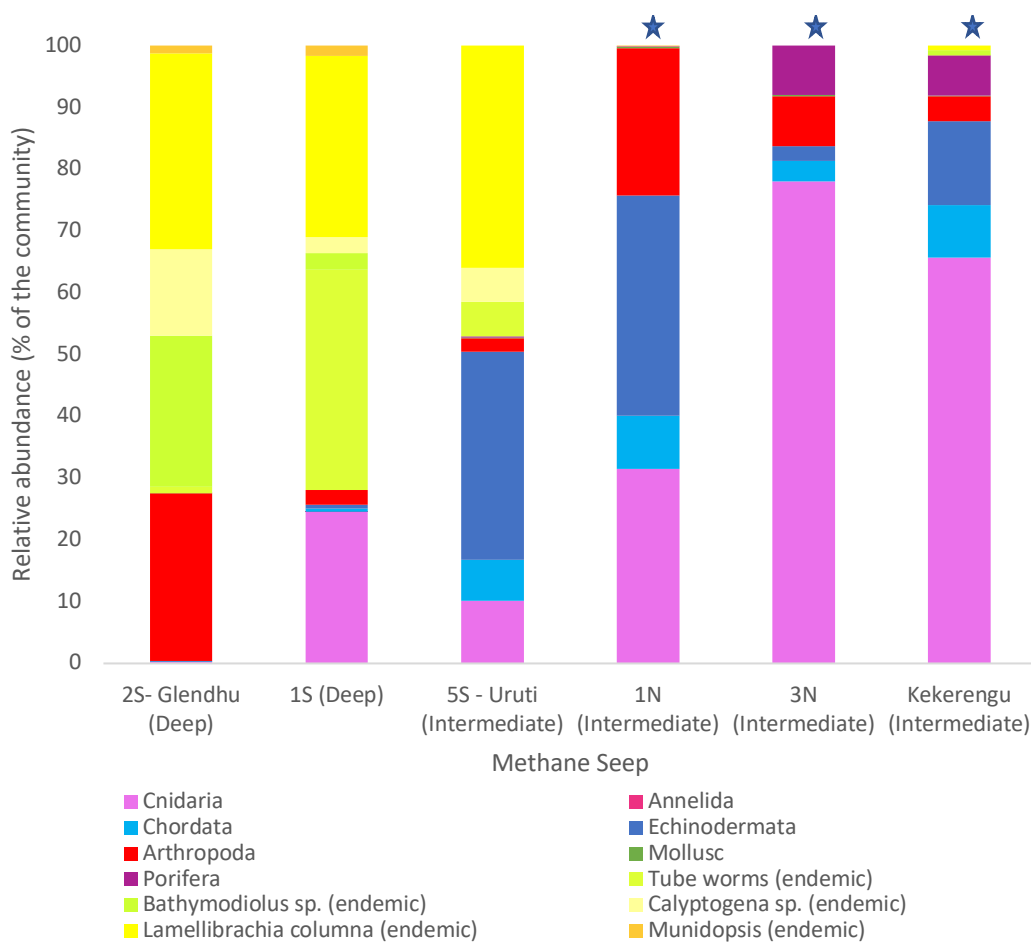


Figure 3.9 Relative abundance (percentage of the community) of each phylum at the methane seeps sites. Depth is indicated by the seep name and a blue star indicates a seep with a trawl intensity >1. Colour depicts the phyla that were present – Seep endemic fauna have been listed to the lowest taxonomic ID and given hues of yellow.

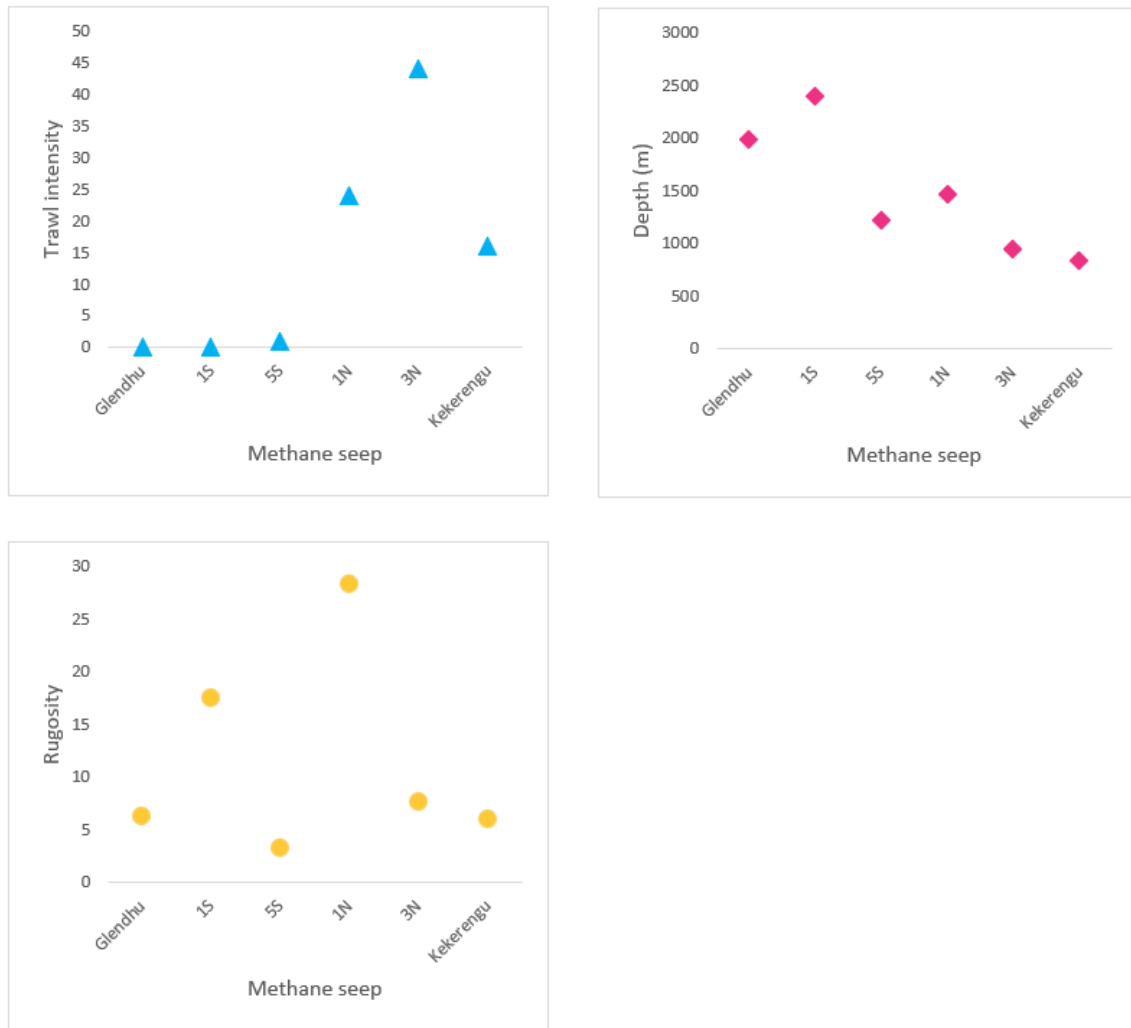


Figure 3.10 Environmental factors at each methane seep. A) Trawl intensity (number of trawls in a 2km radius from the centre of the seep B) Depth (m). C) Rugosity.



Table 3.3 Table of results from the presence and absence DISTL-M sequential tests of methane seep communities and environmental factors. Environmental factors are depth (m), trawl intensity, depth level (intermediate, deep) and rugosity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column, the proportion of variation the environmental factor caused in the data is in the 'prop' column and cumulative portion of variation in the 'cumul.' Column. Significant results indicated by a "\*" next to the p-value.

| Environmental factor | AIC    | SS(trace) | Pseudo-F | P      | Prop.    | Cumul.  |
|----------------------|--------|-----------|----------|--------|----------|---------|
| Depth                | 39.928 | 2257      | 3.7745   | 0.006* | 0.48549  | 0.48549 |
| Trawl Intensity      | 37.627 | 1223.9    | 3.1439   | 0.039* | 0.26328  | 0.74877 |
| Depth Level          | 36.588 | 464.13    | 1.319    | 0.36   | 0.099839 | 0.84861 |
| Rugosity             | 34.787 | 330.28    | 0.8843   | 0.542  | 0.071046 | 0.91966 |

Table 3.4 Table of results from the abundance DISTL-M sequential tests of methane seep communities and environmental factors. Environmental factors are depth (m), trawl intensity, depth level (intermediate, deep) and rugosity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column, the proportion of variation the environmental factor caused in the data is in the 'prop' column and cumulative portion of variation in the 'cumul.' Column. Significant results indicated by a "\*" next to the p-value

| Environmental factor | AIC    | SS(trace) | Pseudo-F | P     | Prop.   | Cumul.  |
|----------------------|--------|-----------|----------|-------|---------|---------|
| Depth Level          | 47.496 | 4690.4    | 2.2221   | 0.079 | 0.35713 | 0.35713 |
| Trawl Intensity      | 47.16  | 2722.6    | 1.4278   | 0.181 | 0.2073  | 0.56443 |
| Depth                | 46.051 | 2313.4    | 1.3579   | 0.348 | 0.17614 | 0.74057 |
| Rugosity             | 45.265 | 1266      | 0.5912   | 0.632 | 0.09639 | 0.83696 |

Table 3.5 : DISTL-M marginal test results for the environmental factors depth (m), depth level (intermediate, deep) rugosity and trawl intensity. F-value is indicated in the 'pseudo-F' column, p-value in the 'P' column and the proportion of variation the environmental factor caused in the data in the 'prop' column. Significant results indicated by a "\*" next to the p-value.

| Environmental factor | SS(trace) | Pseudo-F | P      | Prop.   | res.df | regr.df |
|----------------------|-----------|----------|--------|---------|--------|---------|
| Depth                | 3837.5    | 1.6512   | 0.089  | 0.29219 | 4      | 2       |
| Depth Level          | 4690.4    | 2.2221   | 0.049* | 0.35713 | 4      | 2       |
| Rugosity             | 1515.3    | 0.52171  | 0.928  | 0.11538 | 4      | 2       |
| Trawl Intensity      | 4253      | 1.9157   | 0.057* | 0.32383 | 4      | 2       |

### 3.4 Discussion:

In this chapter I aimed to characterise and compare six methane seep sites on the Hikurangi Margin of New Zealand. I also explored the correlation between variations in methane seep communities and environmental variations. From my results, six different methane seep communities were observed. Across all six sites, 56 different fauna groups from the phyla Cnidaria, Annelida, Chordata, Echinodermata, Arthropoda, Mollusca, and Porifera were identified. Of the 56 different fauna observed, seventeen were identified at only one site (site endemic), with ten of these site endemic fauna at Kekerengu, six at 3N, one at Glendhu and one at 1N. At all seep sites, evidence of methane seepage was determined based on the presence of methane-derived carbonate, clam shells, microbial mats and reduced sediments. Endemic methane seep fauna were observed at all methane seep sites. However, there were decreased abundances of endemic fauna, especially *Lamellibrachia columna* at all seep sites where the trawl intensities were greater than 1. At seep sites 1N and 3N, only small patches of clams were observed. Within trawled zones, endemic fauna was observed under ledges and in cracks of carbonate instead of in the open sediment. At Kekerengu and Uruti seep sites, tube worms were observed as singular tube worms, often lying flat in the sediment, unlike the expansive bushes observed at Glendhu. Based on my results, depth level and trawl intensity correlate with changes in the community composition in both the presence and absence and the abundance data. In low trawl intensity seep sites Uruti, 1S, and Glendhu, endemic species compose  $\geq 40\%$  of the community composition; at the high trawl intensity seep sites 1N, 3N and Kekerengu, endemic species contribute  $\leq 5\%$  to the relative abundance of fauna in these communities.

#### 3.4.1 Community comparisons of the methane seeps

At Kekerengu, 3N and 1N, there were low abundances of endemic methane seep fauna observed. However, carbonate structures indicate active seeping occurred at the methane seep sites for hundreds of years. Descriptions of methane seeps on the Hikurangi Margin with complex carbonate structures and reduced abundances of endemic fauna have also been reported by Greinert et al. (2010) and Jones et al. (2010). They concluded that it was likely that the Hikurangi Margin methane seeps are in a later successional stage due to reduced or ceased methane fluid flow. At

sites 3N and 1N, particularly, the clam patches observed appeared to have high abundances of broken clamshells and dead clams, which could indicate a decrease or cessation of methane seep fluid flux (Bowden et al. 2013). Although it is plausible that methane seep communities at Kekerengu, 3N and 1N could represent late successional stages due to decreased methane seep fluid flux, this seems unlikely as these methane seep sites were selected as ‘active’ seep sites and have been observed actively bubbling on acoustic sonars and visually in ROV videos (Watson et al. 2020)

### 3.4.2 Effects of environmental factors

In my study, depth and trawl intensity correlated with changes in the methane seep community and methane seep diversity. The literature consistently states that methane seep communities are strongly structured by depth (Sahling et al. 2003; Olu et al. 2010; Nakajima et al. 2014; Turner et al. 2020). This study showed an 80% difference in the community compositions at intermediate and deep methane seeps. Deep methane seep communities comprised endemic methane seep fauna, Arthropods, and Cnidarians, while intermediate methane seep communities comprised Echinoderms, Cnidarians, Arthropods, Poriferans and Chordates. These variations in the Hikurangi Margin methane seep communities follow the general patterns described in the literature, where the abundances of endemic fauna observed increased with depth, and the abundances of background species decrease with depth (Sahling et al. 2003; Nakajima et al. 2014; Quattrini et al. 2015; Rybakova et al. 2022). Although the variations in methane seep community compositions I observed correlated with variation in depth, depth also correlated with changes in trawl intensity, where intermediate depth seeps were trawled, and deep seeps were not. This means that variations in methane seep communities could be due to variations in trawl intensity, depth, or both.

In my study, trawl intensity correlated with changes in the methane seep communities. At the high trawl intensity methane seep sites Kekerengu, 3N and 1N (trawl intensities between 16 and 44) there were low percentages of endemic species observed, especially *Lamellibrachia* tube worms. This indicates a potential correlation of trawl intensity decreasing the abundance of endemic species in

methane seep communities. Many endemic species are slow growing species that are susceptible to disturbances such as trawling, which could prohibit their recovery or development in highly disturbed areas (Hove and Moreau 2007). In my study it was observed that methane seep sites that have had as few as 16 trawls across them in the last 30 years have reduced abundances of endemic fauna in their communities. Tubeworms, and microbial mats, were also observed as singular tube worms lying flat on the surface and occurring at the bases of carbonates or in cracks of carbonates at highly trawled seeps. Baco et al. (2010) also noted that characteristic methane seep species, tube worms, clams and mussels appeared to be concentrated in carbonate cracks. These observations occurred alongside observations of trawl gear, trawl marks, clam shells and coral debris at the seep. Bottom trawls are known to remove and smother benthic fauna by dragging large trawl nets weighing 8-10 tonnes across the seafloor (de Groot 1984). Endemic fauna within the cracks of carbonates and around the bottom of carbonates would likely avoid trawl gear as nets will not have access to these areas and likely avoid these areas to avoid loss of trawl gear. The reduction in chemosynthetic species could also be linked to trawl intensity disturbing methane seep flow and pushing the sulphate methane transition zone deeper into the sediment. Trawling disturbs not only the slow growing chemosynthetic communities, but also the surface sediments at methane seeps. In shallow water systems, trawling alters denitrification pathways (removal of bioavailable nitrogen from nitrogen deficient waters) (Ferguson et al. 2020). Trawling removed burrows and pits in the sediments and homogenised the sediment, which changed the concentrations of ammonium, dissolved organic nitrogen, dissolved oxygen, and nitrate fluxes in the sediment. This reduces denitrification by up to 50% (Ferguson et al. 2020). Disturbance of surface sediments could potentially redistribute methane seep pathways or alter the sulphate-methane transition zone, which could affect the presences of chemosynthetic communities, whose abundance is determined by sulphide flux from this zone (Van Dover et al. 2003; Fischer et al. 2012).

In contrast, fauna that are highly susceptible to trawling, such as sea pens, bamboo corals and hydro corals, were present in high densities at Kekerengu seep (Bax and Cairns, 2014; Clark et al., 2016). These species were observed in high densities on and around large carbonate structures, which may reduce their susceptibility to trawl

impacts. These areas may not be subject to direct bottom trawling due to entanglement risks on carbonate structures. A large coral skeleton was also observed in the sediment at Kekerengu seeps site.

In this study, rugosity was not correlated with changes in the methane seep communities; however, it has been observed in earlier studies that increased habitat complexity increased the diversity of the community (Cordes et al., 2010; Åström et al., 2018). The resolution of the rugosity data was too coarse for the detection of small changes in the rugosity of the seafloor, such as what would have been expected from endemic fauna increasing the heterogeneity of the site. Even though rugosity was not significantly correlated with changes in methane seep communities, observations of high densities of fauna such as squat lobsters, shrimp, mussels, ascidians, and barnacles within tube worm bushes could indicate increased habitat heterogeneity which increases the abundances/ diversity of the site.

### 3.4.3 Commercially important species

At the Hikurangi Margin methane seeps, commercially important species managed in the New Zealand Quota Management System (QMS) were observed within the seep habitats. These were Ghost sharks (*Hydrolagus sp.*), Hoki (*Macruronus novaezelandiae*), Orange Roughy (*Hoplostethus atlanticus*), Oreo fish (Oreosomatidae) and squid (Fisheries New Zealand 2020). The ROV survey likely under-sampled the commercially important species at methane seeps due to the disturbances by the ROV. Light pollution and noise pollution from the ROV could potentially reduce the observed abundance of mobile species, or they were not observed as they were too high by the water column to be observed by the downwards facing camera. Observations of commercially important species at methane seeps globally have been reported in the literature. Observations of long spine thorny heads (*Sebastolobus altivelis*), rockfish, green halibut (*Reinhardtius hippoglossoides*), haddock (*Melanogrammus aeglefinus*), northern shrimp (*Pandalus borealis*), snow crab (*Chionoecetes opilio*), atlantic cod (*Gadus morhua*), crabs from the *Paralomis* spp., tanner crabs (*Chionoecetes tanneri*), red crab (*Chaceon quinque-dens*) and Patagonian toothfish (*Dissostichus eleginoides*) have been reported at methane seep ecosystems (Sellanes et al. 2008; Grupe 2014; Nakajima

et al. 2014; Sen et al. 2018; Seabrook et al. 2019; Turner et al. 2020). Aggregations of commercially important species at methane seeps are likely due to the increased abundances of prey species, potential shelter or protection provided by the carbonates (Sen et al. 2018). Seeps have also been reported as potential breeding grounds for species such as blobfish (*Psychrolutes sio*) and red crabs (Sellanes et al. 2008; Turner et al. 2020). Observations of tanner crabs and red crabs foraging and feeding on bacterial mats and mussel shells and stable isotope analyses have confirmed the ingestion of chemosynthetic production in these species (Seabrook et al. 2019; Turner et al. 2020).

Methane seep ecosystems occur on continental margins in areas of high economic interest where trawling occurs, and sediments with oil and gas-rich fluids exist (Clark et al. 2016; Cordes et al. 2016). Deep-sea trawling and potential mining of deep-sea methane seeps have been highlighted as a significant threat to methane seep communities due to the long-lived, slow-growing nature of methane seep ecosystems (Bowden et al. 2013). Methane seeps are important ecosystems that host a diversity of fauna and provide regulating, provisioning, and cultural services to society. Although methane seeps are high-value ecosystems, New Zealand currently has limited protection for methane seeps, recognising seeps as sensitive environments but having no spatial protection from anthropogenic disturbances (Makgill et al. 2012).

Six New Zealand methane seeps have been described and analysed to compare and contrast community compositions. Across the six methane seep sites, all sites had carbonate structures and varying abundances of seep endemic species. At seep sites with high trawling intensities, there were reductions in the abundances of endemic seep fauna, and endemic seep fauna took refuge in cracks between carbonates or at the bases of carbonates. Variation in depths (intermediate or deep) was also correlated with changes in community compositions at the methane seeps; however, depth level also correlated with trawling activity. This study supported the hypothesis that trawling intensity and depth drive changes in methane seep communities. However, direct observations of the effects of trawling on the communities suggested that these variations were driven more by trawl intensity than depth. Several commercially important species were also identified at the seep

sites, which could suggest that chemosynthetic production influences humans directly. Due to the vulnerability of methane seeps to anthropogenic effects and the lack of management of methane seep sites within New Zealand, area-based management plans that represent the diversity of methane seep ecosystems should be a top priority for marine management.

## Chapter 4. Conclusions

### 4.1 Summary of findings

In this study I aimed to explore the diversity of methane seep communities both globally and within New Zealand. I investigated global bioregional patterns in methane seep communities and compared variations in methane seep communities to variations in environmental factors depth, latitude, longitude, and collection methods. I also investigated methane seep community variations on the Hikurangi Margin of New Zealand, and compared them to the environmental factors depth, trawl intensity and rugosity, to understand the factors that may be driving the variations in these communities.

In Chapter two, I found global bioregional variation in methane seep communities, underpinned by local patterns of diversity and endemism. Variation in global methane seep communities correlated with water depth and collection methods while local variation in methane seep communities varied with depth. Within the bioregions I found that methane seep community similarity was variable; some methane seep communities had very similar communities and others were dissimilar. High variability within bioregions may indicate that further partitioning of the bioregions outlined by Costello et al. (2017) is required, to better represent methane seep communities. Further research into the bioregional patterns of methane seeps using a larger sample size and a better global representation is required, to further understand the environmental factors that drive bioregional variations in methane seep communities. To do this a standardised sampling method for methane seep community analysis needs to be agreed upon and used by researchers so that data can be compared more easily. Further investigations into methane seep bioregional patterns and community composition would also be beneficial for the development of effective management plans, that values the global variability within methane seep communities.



In Chapter three, I explored variation within methane seep communities on the Hikurangi Margin of New Zealand. I studied six methane seep sites across the Hikurangi Margin and correlated variation in these communities with depth and trawl intensity. Within trawled areas of the Hikurangi Margin, there were reductions in the abundance of seep endemic species, with highly trawled sites having few to no endemic seep taxa. At trawled methane seeps, the seep endemic genera *Lamellibrachia*, *Calyptogena* and *Bathymodiolus* were observed taking refuge in the gaps between carbonates, under carbonate ledges or on the sides of carbonate ledges. Observations of chemosynthetic species taking refuge around carbonate structures and reductions in chemosynthetic species abundances at trawled sites indicated that bottom trawling may be decreasing the presence of endemic species at methane seep sites. Reductions in endemic fauna at trawled sites may also indicate that trawling is affecting methane seep fluid flux pathways by homogenising the sediment. Further investigations into the relationship between trawl intensity and reductions in chemosynthetic fauna in methane seep communities is required. This will allow us to understand the implications of trawling on methane seeps and understand how anthropogenic disturbance is affecting methane seep communities.

## 4.2 Limitations

The metanalysis in chapter two was limited by the data not being collected and recorded in the same way, and the small sample sizes that reduced the power of the statistical tests within the analysis. Unfortunately, due to the unbalanced design and small sample sizes, natural variation in the data may have obscured the expected environmental drivers of variation. There were also large amounts of variation that were observed in the data due to the source of the data. In the NMDS plots, although the data would generally cluster by bioregion, the data would almost always cluster by the data source even if the collection methods were the same. This highlights the need for standardised sampling practices to be used when surveying methane seep communities, so that global analysis of variation can occur. In the meta-analysis there were also some bioregions that only had methane seep data from one scientific paper within them, e.g., Chile and the Norwegian Sea Bioregions. This likely decreased the variation within the bioregions that only had one paper as it was seen in the NMDs plots that seeps often clustered in the papers that the data was

collected from. The meta-analysis was also limited by the fact that it is not a true global analysis, as there was no representation of methane seeps in the Indian Ocean or the Western Pacific Ocean near Asia. This was because for data to be included in the meta-analysis I had to have access to the data and the information about it. For some of the papers that I came across the community abundances/compositions for the individual seep sites were not included in the text or in supplementary material, so I was unable to include them in the meta-analysis. Another limitation of the meta-analysis is that because the community data were collected by different people, the fauna were identified to different taxonomic levels. This means that community similarities may be more or less similar depending on the taxonomic level that the fauna was recorded to. E.g if a Brasingid was recorded as Brasinigida by one person and Asteroidea by another person these would count as two different taxa at the lowest taxonomic identification and cause more variation in the communities than there actually is.

In Chapter three, analysing video that was designed to be for exploration rather than analysis meant that the camera view was not constant, and likely resulted in the misidentifications of some species, and an underrepresentation of the abundance of species that were present. At times the ROV ascended and changed the height of the camera above the benthos, which obscured the view of the benthic fauna and made identification of small species difficult. The camera on the ROV also went in and out of focus and changed angle and perspective which made correct identifications and abundance estimations difficult, particularly for smaller fauna. The abundances of commercially important species and pelagic fauna are also likely underrepresented, due to species in the water column not being in view of the downwards facing camera. The ROV's also had bright lights on them and made loud noises which likely disturbed/scared some mobile species away from the view of the camera. The study in chapter three was also limited by colonial organisms such as corals being counted as individuals. Another limitation of chapter three is that non-seep fauna were identified to a higher taxonomic resolution which likely reduced some of the community variability in the methane seep sites. Fauna such as sea urchins were recorded generally as echinoids, however, different species of echinoids were seen at different sites. E.g. small white echinoids at seep site Uruti

and the large purple echinoids at site 1N would further increase the variation at the sites if they were identified to a finer taxonomic level.

Additionally, due to the constraints of the study only one transect of each seep site was analysed for chapter three. This meant that the different habitats within seep sites may be underrepresented or overrepresented, and variations in the habitat (e.g., habitats that were 'characteristic' seep sites) could have been missed.

The fact that there was no 'control' non-trawled site at the intermediate depth is also a limitation of this study. Without controls it can be hard to disentangle whether the community variations observed are due to depth or trawl intensity. However, seep site Uruti had a trawl intensity of one and so acted as somewhat of a control site. Having a control non-trawled site at the intermediate depth and a trawled site in the deep depths (although it is too deep to trawl) would have made the relationship between trawl intensity and variations in the methane seep communities clearer.

### 4.3 Future research

Further research on global methane seep community composition using standardised sampling techniques, and including methane seep communities from the Western Pacific Ocean around Asia and the Indian Ocean, is required to truly depict global bioregional variations in methane seep communities. This would also require analysing the macrofauna, meiofauna and benthic and pelagic fauna communities within each bioregion. It is crucial to understand bioregional variation in methane seep communities for marine management, to ensure that the biodiversity within these ecosystems is represented within marine spatial planning.

Continuing research on the effects of bottom trawling on methane seep ecosystems, specifically looking into the effect of bottom trawls on endemic methane seep communities is required to understand the relationship between trawling and methane seep endemic species. Additionally, understanding how bottom trawling effects the sulphate-methane transition zone and the concentrations of sulphate and methane within the sediment and water column will also provide further insights into these relationships. This will also provide insights into the disturbances that bottom trawling is having on methane seep ecosystems, from carbon and nutrient cycling to ecological structure and function. Understanding the disturbances that bottom

trawling is causing on methane seep ecosystems is imperative to ensure that management of methane seep ecosystems protects the biodiversity within these ecosystems and the ecosystem services that they provide.

#### 4.4 Management implications/ conclusion

The results of my thesis illustrate that methane seep communities can vary drastically and show bioregional patterns in community composition. Bioregional differences in methane seep communities indicate that management for methane seeps cannot be uniform and one size fits all but must be tailored to the specific area and bioregions. In this thesis I showed that trawl intensity correlates with changes in the community compositions, and reductions in endemic fauna abundance in methane seep communities. This is an important relationship to understand and explore as to my knowledge this is the first report of this relationship in the literature. This study has shown that the limited amount of protection for methane seeps globally is unlikely to represent the diversity of the fauna within these ecosystems and prevent biodiversity loss. Ensuring that representation is achieved in management within bioregions and globally is also imperative as trawling is correlated with reductions in the endemic species present. This may also affect the productivity of the site and the ecosystem services it provides. These negative correlations with trawling emphasise the need for increased management plans and actions for methane seep communities globally and within bioregions to conserve the biodiversity within them.

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