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ECOLOGICAL STUDIES OF AN ABYSSAL NODULE PROVINCE TO INFORM THE MANAGEMENT OF DEEP-SEA MINING

by

KIRSTY ALEXANDRA MCQUAID

A thesis submitted to the University of Plymouth

In partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Biological and Marine Sciences

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Acknowledgments

It is traditional, but also hugely important, that I say a big, big thank you:

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Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee. Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Relevant scientific and International Seabed Authority seminars, conferences and meetings were regularly attended, at which work was often presented, research cruises were undertaken, and work is currently underway to prepare scientific papers from Chapters 2-4.

Presentations and conferences attended:

2018 **Oral presentation:** 15th Deep-Sea Biology Symposium, Monterey, USA, "Top down, Broad scale Habitat Classification as a Tool to Support MSP".

2017 **Oral presentation:** Challenger Society Deep Sea Special Interest Group, Oban, UK, "Habitat Classification to Support Spatial Management of Deep-Sea Mining".

Oral presentation: Plymouth Marine Science and Education Foundation Conference (PlyMSEF), Plymouth, UK, "Habitat Classification to Support Spatial Management of Deep-Sea Mining".

Oral presentation: University of Plymouth Marine and Coastal Policy Research Group, Plymouth, UK, "Habitat Classification to Support Spatial Management of Deep-Sea Mining".

2016 **Oral presentation:** International Seabed Authority 22nd Annual Session, Kingston, Jamaica, "Marine Spatial Planning Associated with Deep-Sea Mining".

Several internal University of Plymouth Marine Biology and Ecology Research Centre presentations were also given.

Research cruises during PhD:

1. RRS James Cook, cruise reference JC136; Wyville Thomson Ridge, Darwin Mounds, Rosemary Bank & Anton Dohrn Seamount; June 2016, 3 weeks
2. RV Kilo Moana, cruise reference KM1808; Clarion-Clipperton Fracture Zone - APEIs 1, 4 & 7; May 2018, 5 weeks

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Abstract

Ecological studies of an abyssal nodule province to inform the management of deep-sea mining

Kirsty A. McQuaid

The abyssal environment remains one of the most poorly studied parts of the planet. While predominantly an environment dominated by soft sediments, some abyssal regions are known to harbour potato-sized, rock-like formations known as polymetallic nodules. These nodule provinces are now the subject of exploration by nations and their nominated contractors keen to develop a new deep-sea mining industry. This new industry has the potential for large-scale environmental impacts, but at present these impacts are difficult to predict, and therefore mitigate, as a result of the lack of ecological data and scientific understanding of these areas.

The studies carried out in this thesis aimed to contribute to our understanding of the epibenthic megafauna communities in areas targeted for mining, and to support the environmental management of mining activities through informing recommendations on environmental survey design and spatial planning. Imagery from nine transects of 800 m^2 in the central Clarion Clipperton Fracture Zone (CCZ) were analysed to describe the epibenthic megafaunal communities at both regional (>1 000 km) and local (2 km) scales. The relationship between biological data derived from image analysis and modelled environmental data was examined to determine potential drivers of community composition and diversity, and rarefaction and extrapolation curves were used to assess levels of sampling required to establish baseline faunal assessments. Finally, clustering algorithms were used to classify broad-scale, modelled environmental data into different habitat types, to assess the effectiveness of the existing protected area network in the CCZ.

Megafauna morphotypes most vulnerable to mining, including rare, nodule-specific, suspension feeding, and sessile organisms, formed a large proportion of the CCZ epibenthic megafauna. Several dominant morphotypes were homogenous over large scales, but there was high turnover of rare morphotypes at regional and local scales. In addition, broad-scale bathymetric position index was identified as an important driver of both megafauna and metazoan diversity at regional scales. To characterise the community at 99% sample coverage, sampling units of ~2 800 - 4 600 m^2, or 780 - 960 individuals, were required, with 26 - 27 x 800 m^2 replicate transects. This sampling effort was much greater than is generally used in the deep sea. Finally, a top-down, broad-scale habitat classification of the CCZ identified 46 habitat types and revealed that many of these were underrepresented in the current protected area network, with several occurring almost exclusively in mining areas.

The body of research contained in this thesis suggests that 1) those morphotypes most vulnerable to mining form a substantial proportion of the megafauna communities in the CCZ, 2) greater sampling effort is required to fully characterise baseline environmental conditions of the CCZ, and 3) the current protected area network established in the CCZ does not adequately capture the range of habitats present. This thesis advocates for the use of Regional Environmental Assessment to address some of the pressing issues preventing progress in environmental management of deep-sea mining.

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Chapter 1.

General introduction

1.1 Introduction

The deep sea, defined as any part of the ocean deeper than 200 m, covers over 60% of the earth's surface and carries out many vital functions, including providing supporting, provisioning, regulating and cultural services (Thurber *et al.*, 2014). Abyssal plains, generally considered those areas between the foot of the continental slope and 6 500m depth (Harris *et al.*, 2014) are the largest deep-sea habitat (Ramirez-Llodra *et al.*, 2010), and consist of vast, relatively flat expanses of soft sediment, with topographic features such as abyssal hills and seamounts (Harris *et al.*, 2014). The faunal and microbial communities associated with abyssal plains support ecosystem services through a range of activities. These activities include, for example, respiration and bioturbation, which are ecological functions underpinning supporting ecosystem services like nutrient regeneration and biogeochemical cycling (e.g. Sweetman *et al.*, 2019). These services can, in turn, support provisioning services like fisheries through increased surface productivity (Thurber *et al.*, 2014). In addition, abyssal habitats may provide provisioning services through bioprospecting (Skropeta, 2008) and mineral extraction (Hein *et al.*, 2013), and biodiversity has been shown to support ecosystem functioning and efficiency in the deep sea (Danovaro *et al.*, 2008). Finally, abyssal habitats may provide cultural services, which are non-material benefits to humans, including educational opportunities and inspiration for art (Le, Levin & Carson, 2016; Thurber *et al.*, 2014). Although some of these functions and associated processes may occur at low rates, when scaled up to the large areas of abyssal seafloor, they can contribute significantly to ecosystem services like energy flow and carbon sequestration on a global scale (Thurber *et al.*, 2014).

Despite the importance of the deep sea, it is one of the most understudied environments on our planet (Ramirez-Llodra *et al.*, 2011; Thistle, 2003), as a result of both its remoteness, making it difficult and expensive to reach, as well as the immense size of the area (Glover & Smith, 2003). Human activities such as oil and gas extraction, fishing, waste disposal, and seabed mining occur in the deep sea, and, together with climate change, have been shown to negatively impact this environment (Ramirez-Llodra *et al.*, 2011). With advancements in technology, human activities are moving into deeper and more remote parts of the ocean, and there is increasing evidence of impact to deep-sea ecosystems (Pham *et al.*, 2014; Ramirez-Llodra *et al.*, 2011).

With a growing global population, rapid industrialization, and a move towards green technologies, the demand for metals that are used in the manufacturing of consumer electronics such as cell phones, laptops and batteries, as well as renewable energy technology, is rising (Glover *et al.*, 2018; Hein *et al.*, 2013; Petersen *et al.*, 2016; Valsangkar, 2003). As a result, over the past 50 years there has been great interest in developing a deep-sea mining industry to recover metals such as copper, nickel, cobalt, manganese, molybdenum, gold, silver, bismuth, platinum, and rare earth elements from the seafloor (Valsangkar, 2003).

Deep-sea mining is targeting three main mineral resources: polymetallic nodules, ferromanganese crusts and polymetallic sulphides, and includes a major focus on Areas Beyond National Jurisdiction (ABNJ), in other words areas beyond the national borders of any nation (Hein *et al.*, 2013). Phosphorites, found in shallower waters on continental margins, are also being considered; however, these resources occur primarily within the territory of coastal nations (Thiel, 2003). In international waters, mining for polymetallic nodules on abyssal plains, in particular, has seen the greatest progress in terms of exploration, evaluation of environmental consequences, development of technology, economic analysis, and development of a legal and regulatory framework (Markussen, 1994).

Polymetallic nodule mining has the potential to impact marine biodiversity in abyssal environments (Jones *et al.*, 2017; Thiel, 2001), and the scale at which mining activities are proposed is large (Amos & Roels, 1977; Borowski, 2001; Glover & Smith, 2003), comparable in extent only to bottom trawling (Halpern *et al.*, 2008). There have been calls from the global science and conservation communities, as well as at the state level, for mining to be managed in a responsible way, so that regional biodiversity, and associated ecosystem processes, functions and services, within targeted mining areas is conserved (Mengerink *et al.*, 2014; Van Dover, 2011; Wedding *et al.*, 2015). Scientific research is essential to this endeavour, and should inform decision-making for the management of mining activities.

This thesis aims to contribute to our understanding of the epibenthic megafauna communities in abyssal areas targeted for nodule mining, and to support the environmental management of mining activities through recommendations on sampling design and spatial planning. This introductory chapter reviews what is currently known about polymetallic-nodule mining, with a focus on ABNJ, from the history and status of activities, to the formation and distribution of nodule deposits, mining technology and operations, fauna associated with nodule fields, potential environmental impacts, and the regulatory framework governing mining for nodules in ABNJ. It concludes with a general outline of the thesis and descriptions of each chapter.

1.2 History and status of activities

Polymetallic nodules, potato-sized mineral deposits, were first recovered from the seafloor during the British *HMS Challenger* expedition in 1873 (Murray & Renard, 1891), and for nearly a century were considered to be of only scientific value, with little or no economic importance (Glover & Smith, 2003). During the 1960s, however, interest in polymetallic nodules grew substantially as they were identified as a potentially important source of commercially valuable metals such as nickel, copper and cobalt (Mero, 1965). The 1960s and '70s saw high global economic growth, and by this time it had been established that vast expanses of the deep seafloor are covered with polymetallic nodules (Markussen, 1994; Nath & Sharma, 2000).

The '60s and '70s were characterised by intensive exploratory activities and technological development for deep-sea mining (Glasby, 2000; Markussen, 1994; Valsangkar, 2003). Several international consortia and private companies invested hundreds of millions of dollars into developing mining and processing technology (Markussen, 1994), and testing various prototype mining systems (Glasby, 2000; Valsangkar, 2003). The United States of America (USA), France, Germany and Russia were all involved in early exploratory activities, and made significant advances in the development of mining equipment (Glasby, 2000; Valsangkar, 2003). Exploration activities included surveys to explore the distribution, grades and mineralogy of nodules, sedimentology of associated substrates and environmental conditions (Valsangkar, 2003). Japan and India commenced activity in 1981, with China and South Korea following suit at the end of the decade (Markussen, 1994; Valsangkar, 2003).

Progress in exploratory activities slowed markedly in the 1980s. This resulted from unfavourable market conditions for metals, strong dissatisfaction of some nations with several of the regulations proposed for deep-sea mining, and a feeling that deep-sea mining was going to be less economically feasible than initially believed (Glasby, 2000; Markussen, 1994; Valsangkar, 2003). Private investment in exploratory work and technology development declined, and was replaced instead by funding from government bodies, who were more interested in long-term prospects and supply considerations (Markussen, 1994).

Commodity prices increased again greatly in the first decade of the 2000s, driven by major growth in developing countries, particularly China (Chen, 2010; Erten & Ocampo, 2012). With this came revived interest and investment in deep-sea mining (Jaeckel, Ardron & Gjerde, 2016). Although this boom then slowed, as decelerating global economic growth reduced demand pressures and the metals market declined (Erten & Ocampo, 2012; Matsumoto, 2015), recent years have seen an increase in some metal prices (e.g. Hamilton, 2017) and efforts are being made to push forward with the exploitation of seabed minerals. Regulations for nodule exploitation in ABNJ are currently under development (ISA, 2019b) and technological challenges associated with mining are being overcome (e.g. Kyodo, 2017; Petersen *et al.*, 2016; Tejonmayam, 2017; Woody, 2017), supported by initiatives such as the European Commission's recent multimillion-euro projects, Blue Mining and Blue Nodules, which address technological difficulties associated with mining activities (BlueNodules, 2017).

As of September 2019, 17 exploration contracts for polymetallic nodule mining in ABNJ (each ~75,000 km^2) have been awarded by the International Seabed Authority (ISA), the intergovernmental body tasked with regulating seabed mining in ABNJ. Of these, 16 are for a large abyssal area in the central Pacific, called the Clarion-Clipperton Fracture Zone (CCZ) (Figure 1.1), while the remaining one is for an area in the Central Indian Basin (CIB)

(Petersen *et al.*, 2016). Exploration contracts in the CCZ are held by a combination of government and private contractors, including from several Pacific Island states and the United Kingdom (UK), and in the CIB by the Government of India (ISA, 2019c). With each exploration contract granted to a developed state, a reserved area of equal estimated commercial value is set aside for mining activities by the ISA for developing states. Some of the existing exploration contracts are thus for reserved areas that have been claimed by developing states, e.g. China. Although six of the 17 exploration contracts expired in 2016, no applications for commercial *exploitation* of mineral resources in ABNJ have been made to date, as exploitation regulations are still being developed, and contractors have instead applied for extensions to their exploration contracts (ISA, 2019c).

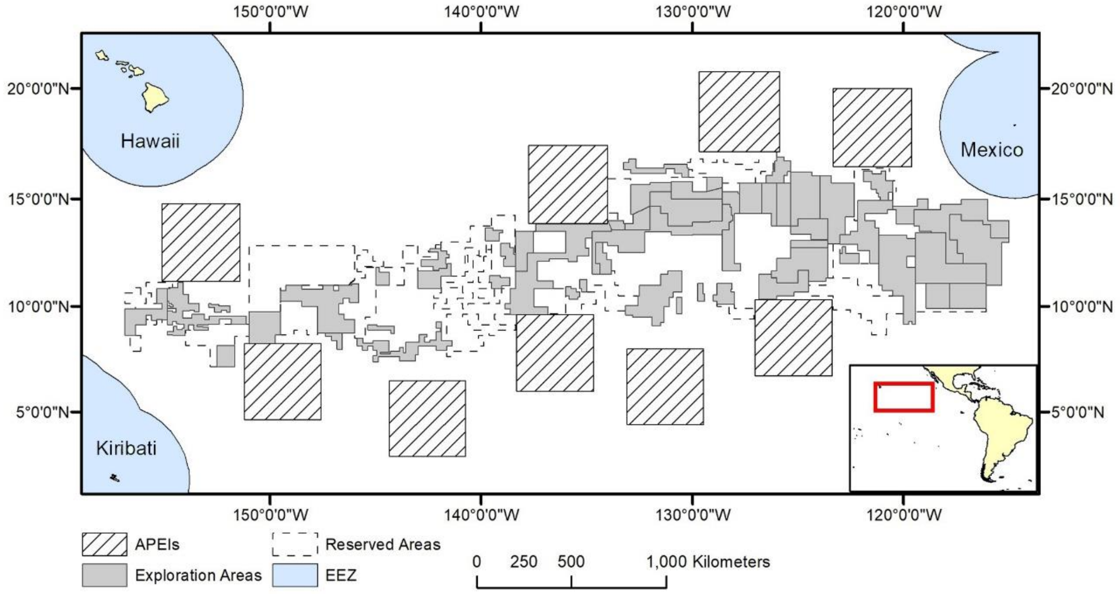

Figure 1.1 Polymetallic nodule exploration areas in the CCZ, showing reserved areas and Areas of Particular Environmental Interest (APEIs). Contractors from developed states are obliged to set aside reserved areas that are of equal commercial value to their exploration claim, for exploitation by developing states or the ISA. APEIs are protected areas that are closed to mining. This figure does not show the exploration area recently awarded to China Minmetals Corporation in May 2017.

1.3 Nodule formation and distribution

1.3.1 Formation

Polymetallic nodules, also known as manganese nodules or ferromanganese nodules, are composed of metals such as manganese, nickel, cobalt and copper (Figure 1.2). Nodules can form through a hydrogenous process, when metals precipitate out of ambient seawater, or a diagenetic process, when metals precipitate out of the sediment at the sediment-water interface (Halbach *et al.*, 1981; McMurtry, 2001). The metals attach to a nucleus, for example a shark's tooth, plankton shell, rock fragment or even an older fragment of nodule (Glover & Smith, 2003), and the formation process determines the concentrations of different metals (Halbach *et al.*, 1981; Hein *et al.*, 2013). Nodules contain up to fifty metallic and non-metallic elements, the most commercially important of which are nickel, cobalt, copper, manganese, and zinc (Markussen, 1994; McMurtry, 2001). They also contain some rare earth elements (REE), including the light REEs Cerium and Neodymium, and heavy REEs such as Yttrium (Hein & Koschinsky, 2014; McMurtry, 2001; Spickermann, 2012).

Figure 1.2 A polymetallic nodule, also called a manganese nodule or ferromanganese nodule. Image credit: K. McQuaid.

Nodules range in size from microscopic to greater than 10 cm in diameter (Valsangkar & Khadge, 1989), with the most common size between 4-6 cm diameter (Valsangkar, 2003). To form, they require high concentrations of metals in either seawater or sediments, low sedimentation rates (<20 mm per thousand years) and relatively stable seabed conditions (Morgan, Odunton & Jones, 1999; Petersen *et al.*, 2016). Nodules grow extremely slowly, at rates of 1-100 mm every million years (Hein & Koschinsky, 2014; McMurtry, 2001), and growth rates vary depending on the formation process and various environmental parameters, including the supply of organic and inorganic matter, rates of sedimentation, the composition and flow of bottom water, and bioturbation (Petersen *et al.*, 2016). Most nodules form through a combination of hydrogenous and diagenetic processes (Banakar, 1990). In the Pacific, abyssal nodules are estimated to grow at a rate of 1-2 mm per million years (McMurtry, 2001), and approximately 1-3 mm per million years in the Indian Ocean (Banakar, 1990).

1.3.2 Distribution

Polymetallic nodules are found in all of the major ocean basins, typically on sediment-covered abyssal plains at depths of 3 000 - 6 500 m (Hein & Koschinsky, 2014; Morgan, Odunton & Jones, 1999; Valsangkar, 2003) (Figure 1.3). They can also be found in some freshwater lakes (Morgan, Odunton & Jones, 1999). Unlike most terrestrial mineral deposits, nodules lie on or near the seafloor surface, and may be partially or completely buried by sediment (Hein *et al.*, 2013). The abundance of nodules varies with bottom topography (Valsangkar, 2003), with the highest abundances occurring on abyssal plain areas with a gentle slope (Morgan, Odunton & Jones, 1999; Petersen *et al.*, 2016).

Estimates of the global area that is considered favourable for polymetallic nodules have changed over time, but range between approximately 38 million km^2 (Petersen *et al.*, 2016) and 46 million km^2 (Glover & Smith, 2003), located primarily (81%) in ABNJ (Petersen *et al.*, 2016).

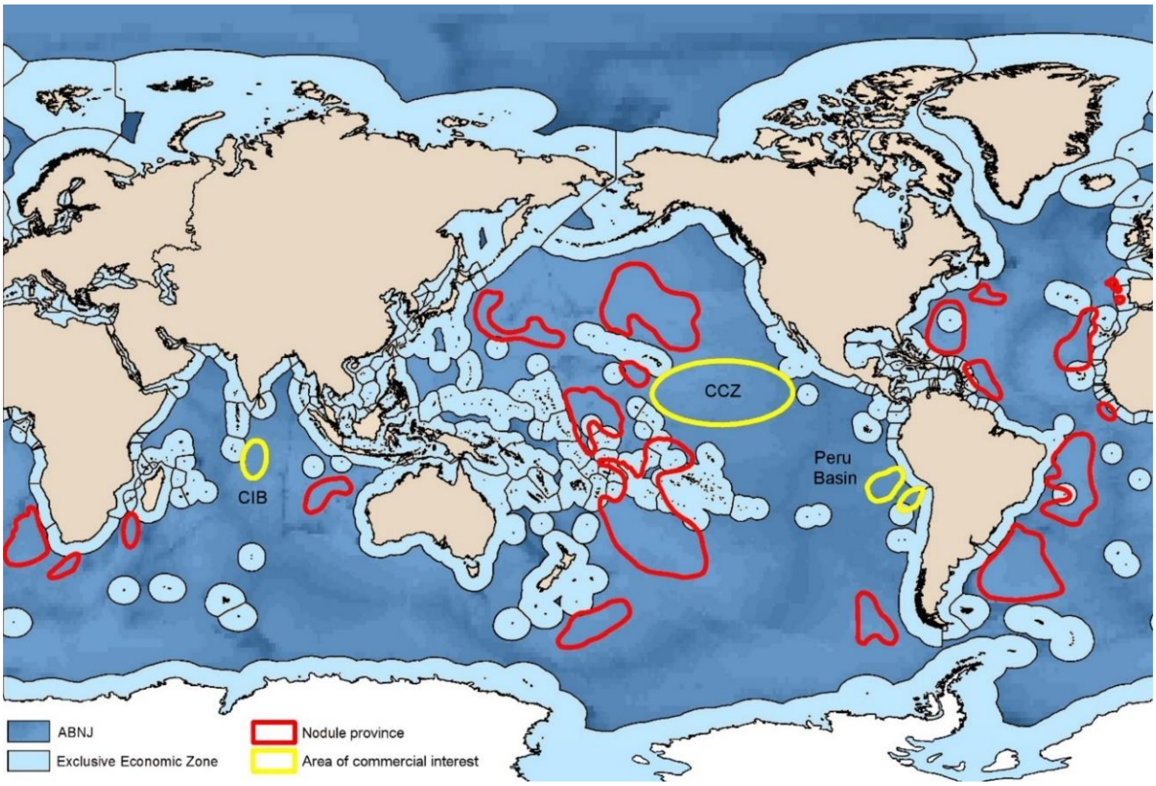

Figure 1.3 Map showing the global distribution of nodule provinces in all major ocean basins (in red). Prospective areas of commercial interest in ABNJ are in yellow. Nodules may be found in other locations in smaller patches with sparse coverage. Reprinted and modified from Hein *et al.* (2013), with permission from Elsevier.

The greatest concentrations of nodules are found in two areas in the Pacific Ocean, the CCZ and the Peru Basin, and in the CIB, in the Indian Ocean (Hein & Koschinsky, 2014). The CCZ is located in the eastern equatorial Pacific Ocean, extending from the west coast of Mexico to Hawaii, between the Clarion and Clipperton fracture zones. This area is approximately 6 million km^2 and contains the greatest global concentration of high grade nodules, with abundances of 10-15 kg.m^2 (Thiel *et al.*, 1998). The commercial value of nodule deposits is dependent on the grade of the ore and the abundance of nodules, and mining is considered financially viable when nodules reach at least 10 kg.m^2, with an average nickel and copper content > 2.25% (Markussen, 1994). In the CCZ, the highest abundances of nodules are located in the central and northern areas, and mining contracts in this region cover over 1 million km^2 (Petersen *et al.*, 2016).

Areas of interest for polymetallic nodule mining have also been identified in the national waters of several Pacific island states, for example the Cook Islands (Hein *et al.*, 2015; SPC, 2013). Although nodules are known to occur in the Atlantic and polar regions, there is very little known about their distribution in these areas (Hein & Koschinsky, 2014).

1.4 Mining technology and operations

Although polymetallic nodule mining occurs at great depths, it is, in other respects, much less of a technical challenge than mining of sulphides found at hydrothermal vents, or ferromanganese crusts on seamounts (Hein & Koschinsky, 2014). This is because nodules are not attached to the seabed, and the mining process essentially involves collecting and lifting nodules off the seafloor (Hein & Koschinsky, 2014; Schrope, 2013).

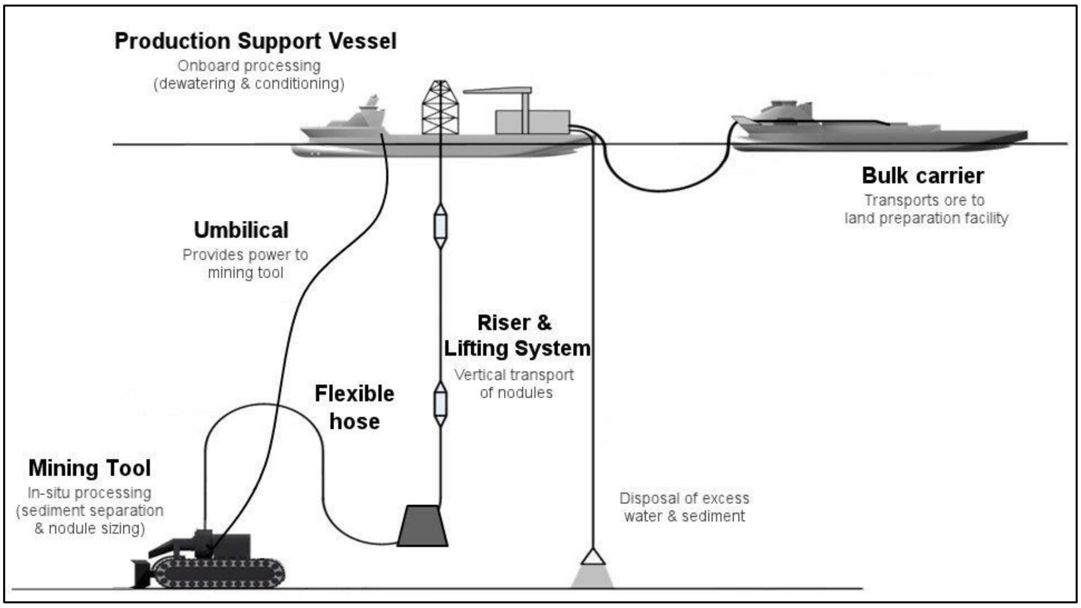

Figure 1.4 Schematic showing proposed hydraulic system for polymetallic nodule mining. This system includes a mining tool at the seafloor, a riser and lifting system to transport nodules to the surface, a production support vessel, and a bulk carrier to transport nodules to land. Reprinted and modified with permission from Laurens de Jonge (Blue Nodules, 2017).

Several different prototype mining systems have been proposed and tested since the 1970s (Glasby, 2000; Valsangkar, 2003). The current most popular system proposed is a hydraulic system, which consists of four basic components: a mining tool at the seafloor, a vertical transport system with hydraulic pumps called a riser and lifting system (which connect the mining tool with the production support vessel at the surface), a production support vessel, and an ore preparation facility on land (Oebius *et al.*, 2001; Thiel, 2001; Verlaan, 2015) (Figure 1.4).

In simple terms, the methods for extracting nodules in this way involve: collecting nodules off the seafloor; sizing, breaking larger nodules into a slurry, and discharging excess sediment collected (in-situ processing); pumping nodules and slurry by means of a vertical transport system from the seafloor to a production support vessel at the surface; separating nodules from excess seawater and sediment (discharged back to seafloor), and conditioning (on-board processing); and transporting nodules to land for metallurgical processing (BlueNodules, 2017; ISA, 2011; Markussen, 1994; Motorship, 2017; Oebius *et al.*, 2001; Schrope, 2013; Thiel, 2001; Verlaan, 2015).

It is expected that the mining tool will work across the seabed in swathes approximately 6 m wide (Oebius *et al.*, 2001), collecting nodules along parallel tracks with strips of unmined area (0.1-10 km) in between (Jones *et al.*, 2017; Thiel *et al.*, 2001). These may be small strips of commercially valuable nodules or larger areas of low-value sediment that occur between mined patches of high-value nodules (Jones *et al.*, 2017; Thiel *et al.*, 2001). According to Madureira *et al.* (2016), approximately 8 500 km^2 of seabed, mined over 20 years, would be required per contractor for mining to be commercially viable. This represents ~11% of a 75,000 km^2 exploration claim area. On-land processing of the nodules to extract targeted metals requires the development of new green technology, and is expected to be the most cost-intensive step of the deep-sea mining process (Hein & Koschinsky, 2014).

While commercial mining has not yet commenced, there have been intense exploratory activities over the past 5 decades, particularly in the CCZ, in efforts to locate high quality, dense nodule fields (Valsangkar, 2003). These surveys have included multibeam bathymetric mapping with side-scan sonar, high resolution bathymetry mapping with autonomous vehicles, seismic surveys, magnetic and gravity surveys, grab and core sampling, dredging, and visualising the seafloor using remotely operated vehicles (Hein & Koschinsky, 2014). In addition, biological sampling and environmental surveys have been undertaken to establish environmental baselines and assess potential environmental impacts of seabed mining (Hein & Koschinsky, 2014).

Polymetallic nodule mining comes with a suite of technical challenges that are primarily associated with working on the seabed at great depths, and dealing with the extreme physical and chemical conditions of this environment (Valsangkar, 2003). The principal challenges include operating at very low temperatures and high pressures, operating on soft seabed surfaces, minimising collection of sediments with nodules, minimising turbidity generation, ensuring long-term reliability and maintaining equipment (Verlaan, 2015). Although technological issues associated with mining in the deep sea are being overcome, it is unlikely that this technology will become available within the very near future (~5 years) (Petersen *et al.*, 2016). This is also affected by the absence of environmental standards and thresholds against which the impacts of mining operations will be measured. In order to develop these standards and thresholds, a better understanding of the impacts of mining (discussed below) is required, as well as clear definitions of what levels of harm will be deemed acceptable (Levin *et al.*, 2016). Once determined, these should then inform technology development, so that, where possible, harmful effects of mining may be reduced or mitigated.

1.5 Fauna of nodule provinces

The deep sea was once considered a depauperate and homogenous environment. However, biological sampling in the 1960s revealed a plethora of life, with heterogeneous environments and diverse faunal communities (Hessler & Sanders, 1967). Abyssal plains, stretching from the foot of the continental slope to 6 500 m deep (Harris *et al.*, 2014), are the largest habitat on earth, covering over 75% of the seafloor (Ramirez-Llodra *et al.*, 2010). The fauna of abyssal plains occur at lower densities than in shallower waters, but are highly diverse, with many rare species (Ebbe *et al.*, 2010). Nodule provinces are one of the most heterogeneous abyssal environments due to the presence of hard substrate, creating a unique habitat in an otherwise soft sediment environment (Ebbe *et al.*, 2010).

Despite being the largest ecosystem on Earth, abyssal plains are poorly explored, with many taxa undescribed, and many new species discovered with every research cruise (Glover *et al.*, 2016a; Smith, Drazen & Mincks, 2006). Scientific research in the deep sea often lags behind, and is dependent upon, industry-related exploratory work (O'Leary *et al.*, 2012), and the current limited knowledge of biological communities associated with nodule provinces is an example of this. While the discovery of polymetallic nodules as a potentially important economic resource in the 1960s was followed by a suite of geological studies (e.g. Barnes & Dymond, 1967; Chester & Hughes, 1967; Ku & Broecker, 1967; Mero, 1965; Somayajulu, 1967), examination of the associated biota only really began around 1975 (Amos *et al.*, 1975; Amos, Roels & Paul, 1976), and we are still trying to characterise the baseline biological communities of nodule provinces, with new records of species, and indeed new species, frequently observed (e.g. Amon *et al.*, 2016; Amon *et al.*, 2017; Dahlgren *et al.*, 2016; Glover *et al.*, 2016b; Grischenko, Gordon & Melnik, 2018; Lim *et al.*, 2017; Miljutin & Miljutina, 2009; Wiklund *et al.*, 2017).

Although there is still much to understand about the benthic fauna and microbial communities, there is evidence that nodule provinces host a diverse range of life, from sessile and encrusting fauna on the nodules themselves (or nearby seamounts), to sediment-dwelling infauna, mobile scavengers and distinct bacterial communities (e.g. Amon *et al.*, 2016; Gooday *et al.*, 2017b; Shulse *et al.*, 2016).

The dominant epibenthic megafauna in nodule fields, which are the focus of this thesis, include highly motile taxa, such as ophiuroids, holothurians and crustaceans, and less motile fauna, such as asteroids, anemones and giant Foraminifera (Amon *et al.*, 2016; Glover *et al.*, 2016b; Gooday *et al.*, 2017b; Morgan *et al.*, 1993; Morgan, Odunton & Jones, 1999; Vanreusel *et al.*, 2016). The term megafauna is used here (and throughout the thesis) to refer to those organisms greater than 10 mm in size (as per Durden *et al.*, 2015; Howell, Davies & Narayanaswamy, 2010; Simon-Lledó *et al.*, 2019b). In the CCZ, sessile megafauna are dominated by cnidarians (mainly alcyonaceans and actiniarians) and Porifera, while the mobile megafauna are predominantly echinoderms, including holothurians, ophiuroids, echinoids and asteroids (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Vanreusel *et al.*, 2016). High abundances of giant foraminiferan xenophyophores have also been observed, with numbers often far exceeding those of the metazoan community (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b). In addition, there are many rare and unknown species (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b).

The megafauna form an important component of abyssal communities in terms of biomass (Haedrich & Rowe, 1977), and although these organisms occur at low densities when compared to shallower waters (Rex *et al.*, 2006), there is a high diversity of megafauna observed in abyssal environments (Smith *et al.*, 2008a). Benthic megafauna in these systems support regulating ecosystem services such as carbon cycling and mineralisation through the consumption of phytodetritus deposited on the seafloor and bioturbation activity (Bett *et al.*, 2001; Smith *et al.*, 2008a), and may support provisioning services associated with bioprospecting (Le, Levin & Carson, 2016). In addition, megafauna can be useful indicators of change, responding to both natural and anthropogenically induced fluctuations in conditions (e.g. Bluhm, Schriever & Thiel, 1995; Durden, Bett & Ruhl, 2020).

Other benthic fauna associated with nodule fields include macrofauna (infauna and epifauna), dominated by polychaetes, tanaid crustaceans, isopods and bivalves (De Smet *et al.*, 2017; Larsen, 2011; Morgan, Odunton & Jones, 1999; Sharma *et al.*, 1997), and meiofauna, consisting predominantly of nematodes, copepods and Foraminifera (Bussau, Schriever & Thiel, 1995; Miljutin & Miljutina, 2009; Min *et al.*, 2018; Morgan, Odunton & Jones, 1999; Pape *et al.*, 2017; Sharma *et al.*, 1997). Nodules themselves provide a habitat for epifaunal species that are dependent on hard substrate, such as sponges, corals, crinoids, bryozoans and Foraminifera (Amon *et al.*, 2016; Dahlgren *et al.*, 2016; Dugolinsky, Margolis & Dudley 1977; Foell & Pawson, 1986; Gooday, Goineau & Voltski, 2015; Gooday *et al.*, 2017b; Grischenko, Gordon & Melnik, 2018; Mullineaux, 1987; Vanreusel *et al.*, 2016; Veillette *et al.*, 2007), and a range of fauna is found in the crevices of individual nodules, including nematodes and copepods (Bussau, Schriever &

Thiel, 1995; Thiel *et al.*, 1993) (Figure 1.5). Highly diverse bacterial and microbial communities have also been observed in nodule provinces (Shulse *et al.*, 2016).

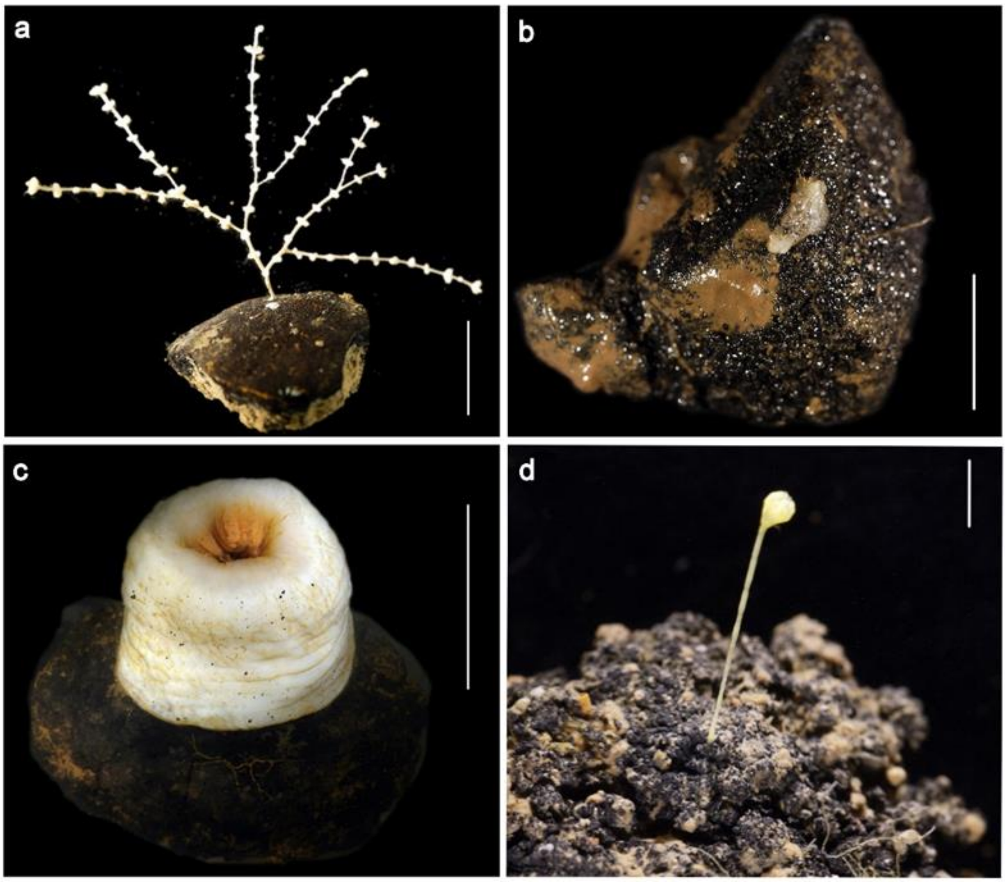

Figure 1.5 Examples of sessile epifauna found attached to polymetallic nodules during the Abyssal Baseline (Abyssline) research cruise in the eastern CCZ. (a) Encrusting octocoral, *Abyssoprimnoa gemina*. (b) New species of sponge (small white object) currently under description, possibly a new genus related to Hemiasteralla. (c) Large anemone, belonging to the Hormathiidae family. (d) Crinoid echinoderm. Scale bars (a) 2cm, (b) 1cm, (c) 5cm, (d) 3mm. Image credits: (a) and (c) Glover, Dahlgren & Wiklund 2016, (b) Glover, Dahlgren & Wiklund, (d) Glover, Dahlgren & Wiklund 2015, with permission to reproduce granted by A. Glover.

There is evidence that the habitat heterogeneity associated with nodule provinces supports a higher density and/or diversity of macro- and megafauna than soft-sediment environments with fewer or no nodules (Amon *et al.*, 2016; De Smet *et al.*, 2017; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016). Higher densities of both sessile and mobile epibenthic megafauna have been recorded in nodule-rich areas, compared to those with few or no nodules, and a positive relationship has been observed between nodule cover and megafauna abundance (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016). In the CCZ, Vanreusel *et al.* (2016) found that alcyonaceans and antipatharians were virtually absent from areas with no nodules, and densities of ophiuroids and echinoids were also far lower.

The link between increasing nodule abundance and megafauna diversity is less clear, with one study observing no change in diversity across a nodule cover gradient of 1-20% (Simon-Lledó *et al.*, 2019c), and another recording higher estimated total species richness in areas of low (<15%) nodule cover compared to areas of medium cover (15-50%) (Amon *et al.*, 2016). A significant positive correlation has been observed between macrofauna diversity and nodule abundance, although this was attributed mostly to environmental factors (such as the flux of particulate organic carbon to the seafloor), rather than habitat heterogeneity (De Smet *et al.*, 2017). Amon *et al.* (2016) argue that there may be a threshold after which increasing nodule cover and volume may reduce species richness by decreasing habitat availability for soft-sediment fauna.

In areas where there are not significant differences in nodule abundance, meio-, macro- and mega-fauna abundance, diversity and community composition have been shown to be relatively homogenous over scales of 1 to 100s km (Amon *et al.*, 2016; De Smet *et al.*, 2017; Pape *et al.*, 2017). Pape *et al.* (2017) suggest that limited spatial variability in meiofauna distribution in nodule provinces may point to broad distribution ranges and high connectivity, although further molecular work is required to support this. Studies on sediment-dwelling macrofauna and a newly-described encrusting sponge, *Plenaster craigi*, suggest that these organisms show genetic connectivity over scales of ~1 000 - 3 000 km (Glover *et al.*, 2002; Janssen *et al.*, 2015; Taboada *et al.*, 2018). The connectivity of megafauna populations in the CCZ is still unknown, although these organisms are expected to have large geographic ranges (Smith, Drazen & Mincks, 2006). Amon *et al.* (2016) hypothesise that at the scale of individual nodules, tens of metres (as nodule cover varies) and at regional scales, megafauna communities will be highly heterogeneous.

Despite almost 40 years of research in the CCZ and over 130 research cruises (Glover *et al.*, 2016a), our knowledge of the fauna associated with nodule fields remains remarkably patchy. The majority of species are undescribed, and for this reason there is almost no information on the biogeographic ranges of species, or the general pattern of species ranges that may be useful to understand potential extinction risks (Glover *et al.*, 2016a). The principal reason for this data gap is that although many samples have been taken, no contractors, regulators or governments have funded the taxonomic work that is required. Modern DNA taxonomy methods may provide an opportunity for this work to be done in the future, and on a large and rapid scale, but they still require careful specimen collection and combined comparative morphology and molecular approaches (Glover *et al.*, 2016a). The creation of a library of quality taxonomic information linked to DNA sequences could also enable future high-throughput metabarcoding approaches for the monitoring of CCZ faunal biodiversity (e.g. Sinniger *et al.*, 2016).

1.6 Potential impacts of nodule mining

Owing to concern over the potentially harmful effects of deep-sea mining on the environment, a number of benthic impact experiment studies have been undertaken since the 1970s to assess the effects of nodule mining on biological communities associated with nodule provinces (Table 1.1a).

These studies assessed baseline environmental conditions and faunal communities, simulated mining operations through seafloor disturbance and sediment discharge, and monitored recolonization and recovery over various time scales. Several key problems in predicting benthic community responses to nodule mining were brought to light through these experiments, most importantly a lack of baseline knowledge on the taxonomy, biology, population dynamics, rates of dispersal, biogeography, food-web dynamics, successional patterns, and life history of deep-sea organisms in nodule areas (Glover & Smith, 2003; Jumars, 1981). A recent meta-analysis by Jones *et al.* (2017) provides an in-depth overview of the experiments and biological responses observed.

More recent projects have shifted focus to establishing environmental baselines for important nodule provinces (specifically the CCZ), and gaining a better understanding of the functioning of these ecosystems (Table 1.1b). This represents an effort to address some of the key factors that are limiting the assessment of mining impacts.

Table 1.1 Studies carried out since the 1970s to assess potential impacts of polymetallic nodule mining on benthic faunal communities and establish environmental baselines for nodule provinces.

Study name	**Location**	**Reference examples**
(a)		
Deepsea Ventures Inc. (DVI) experimental dredging	CCZ	Amos, 1975; Amos *et al.*, 1972
Disturbance and Recolonisation experiment (DISCOL)	Peru Basin	Borowski & Thiel, 1998; Schriever *et al.*, 1997; Thiel *et al.*, 2001
Deep Ocean Mining Environmental Study (DOMES)	CCZ	Bischoff, Heath & Leinen, 1979; Bischoff & Piper, 1979; Burns, 1980
Indian Deep-sea Experiment (INDEX)	Indian Ocean Basin	Ingole *et al.*, 1999; Ingole *et al.*, 2001; Rodrigues, Sharma & Nath, 2001; Sharma *et al.*, 2001
Inter Ocean Metal Joint Organization Benthic Impact Experiment (IOM-BIE)	CCZ	Kotlinski & Tkatchenko, 1997; Radziejewska, 2002
Ocean Minerals Company (OMCO) experimental dredging	CCZ	Khripounoff *et al.*, 2006; Miljutina *et al.*, 2011; Morgan *et al.*, 1993
National Oceanic and Atmospheric Administration Benthic Impact Experiment (NOAA-BIE)	CCZ	Trueblood & Ozturgut, 1997

Japan Deep-Sea Impact Experiment (JET 1 & JET 2)	CCZ	Fukushima, 1995; Fukushima *et al.*, 2002; Fukushima, Shirayama & Kuboki, 2000; Kaneko, Maejima & Teishima, 1997; Kaneko, Ogawa & Fukushima, 1995; Yamazaki, Kuboki & Uehara, 2001
(b)		
Kaplan Project	CCZ	Smith *et al.*, 2008c
Nodinaut project	CCZ	Larsen, 2011; Miljutin & Miljutina, 2009
European Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) project	CCZ	Purser *et al.*, 2016; Vanreusel *et al.*, 2016
European Managing Impacts of Deep-Sea Resource Exploitation (MIDAS) project	CCZ	MIDAS, 2016b
Abyssal Baseline (Abyssline) project	CCZ	Amon *et al.*, 2016; Glover *et al.*, 2016b; Gooday, Goineau & Voltski, 2015

The following section briefly describes the three main potential impacts of nodule mining that have been identified through simulated mining disturbance experiments. This summary is focused on marine biodiversity, particularly the benthos, and does not consider impacts on the pelagic environment (see Christiansen, Denda & Christiansen, 2019), other users of the sea or sites of archaeological or heritage importance.

1.6.1 Loss of nodule habitat

The most severe impact of nodule mining is associated with the physical removal of nodules from the seafloor (Burns, 1980; Sharma *et al.*, 2001; Thiel *et al.*, 1993). Nodules have been shown to support a diverse range of fauna, with many encrusting and epifaunal species that are not found in surrounding soft sediments (e.g. Amon *et al.*, 2016; Bussau, Schriever & Thiel, 1995; Dugolinsky, Margolis & Dudley 1977; Gooday, Goineau & Voltski, 2015; Grischenko, Gordon & Melnik, 2018; Mullineaux, 1987; Thiel *et al.*, 1993; Vanreusel *et al.*, 2016; Veillette *et al.*, 2007). Removal of nodules reduces habitat heterogeneity by taking away the only hard substrate present on much of the abyssal seafloor. Experiments simulating mining disturbance have shown a complete loss of nodule-associated fauna after nodules were removed or buried (Bluhm, 2001; Burns, 1980; Foell, Thiel & Schriever, 1990). This particularly affects sessile organisms that are attached to the hard surfaces of nodules, and are unable to live in surrounding soft sediment (Jumars, 1981; Mullineaux, 1987; Vanreusel *et al.*, 2016).

Removal of nodules could result in the permanent loss of fauna that depend on this habitat for attachment, as the extremely slow growth rates of these minerals means recovery will be in the order of decades to millions of years (Glover & Smith, 2003; Jumars, 1981; Vanreusel *et al.*, 2016). Various experimental disturbance tracks that have been revisited show no signs of recovery of nodule epifauna after seven (Bluhm, 2001), and even 37 (Vanreusel *et al.*, 2016) years. The benthic fauna within disturbance tracks have changed from mixed assemblages (i.e. including nodule epifauna), to communities dominated by soft-bottom fauna (Bluhm, 2001; Schriever *et al.*, 1997; Vanreusel *et al.*, 2016).

This loss of habitat could also affect organisms higher up the food-chain that are dependent on nodule fauna (Jumars, 1981). In a recent study in the Pacific, deep-sea octopods were observed brooding egg clutches on stalks of dead sponge attached to nodules (Purser *et al.*, 2016). This study provides some evidence that loss of nodule habitat could have knock-on effects extending up the food chain.

1.6.2 Physical disturbance to seafloor

Physical disturbance to the seafloor is a natural phenomenon in both shallow (Dernie, Kaiser & Warwick, 2003; Harris, 2012) and deep waters (Aller, 1997; Kukert & Smith, 1992); however, the degree of disturbance expected from commercial mining is far greater than any natural occurrence (Bluhm, 2001). The current proposed mining operations will disturb the top 10-20 cm of the seafloor sediment layer, and fauna in the direct vicinity of mining tracks will be crushed by the mining tools (Glover & Smith, 2003; Markussen, 1994; Valsangkar, 2003). This impact was observed in one of the very first benthic impact experiments (DOMES), carried out in 1978, where the authors noted that "the [disturbance] track is a zone of almost total destruction of benthic biota" (Burns, 1980, p. 441). This was also recorded in later studies, where faunal abundance was observed to decrease greatly inside experimental disturbance tracks immediately after disturbance (Foell, Thiel & Schriever, 1990; Ingole *et al.*, 1999; Ingole *et al.*, 2001; Raghukumar *et al.*, 2001; Rodrigues, Sharma & Nath, 2001; Schriever *et al.*, 1997; Sharma *et al.*, 2001).

With time, sediment-dwelling fauna have shown some recovery in experimental disturbance tracks. This is usually characterised by repopulation of mobile and scavenging species (Bluhm, 2001; Borowski & Thiel, 1998; Foell, Thiel & Schriever, 1990; Schriever *et al.*, 1997; Vanreusel *et al.*, 2016). Some faunal communities in disturbed areas have shown total recovery in terms of abundance (Borowski, 2001; Schriever *et al.*, 1997); however, this has not always been the case (Bluhm, 2001; Vanreusel *et al.*, 2016). Even if communities do recover in abundance, this may take several years (e.g. 7 years recorded by Borowski (2001)), and, more importantly, recovery of abundance does not necessarily indicate the complete recovery of a community (Schriever *et al.*, 1997). During the DISCOL experiment, although overall faunal abundance within experimental disturbance tracks appeared to recover, the diversity of the Polychaeta, the most abundant taxon in all samples and all expeditions, was significantly lower than in undisturbed control sites three years after disturbance (Borowski & Thiel, 1998; Schriever *et al.*, 1997). This decreased diversity was still recorded, although less intensely, after seven years (Borowski, 2001). Similarly, 26 years after experimental mining by OMCO, Miljutina *et al.* (2011) observed significantly lower nematode density, biomass and diversity in the mining tracks compared to undisturbed control sites.

The top layer of sediment on the seafloor is generally accepted as the most faunally rich (Jumars, 1981), and a greater impact is expected on animals in seafloor surface sediments, than on those living deeper in the sediment (Schriever *et al.*, 1997). Some studies have recorded a change in the vertical distribution of fauna within the sediment following experimental disturbance, with the highest concentrations of fauna found at greater sediment depth than before, and this change was still present after several years (Borowski & Thiel, 1998; Ingole *et al.*, 1999; Radziejewska, 2002).

Another impact observed following physical disturbance by experimental mining is the surfacing of deep sediments with physicochemical properties that differ from pre-disturbance surface sediments (Khripounoff *et al.*, 2006; Koschinsky *et al.*, 2001). There does not seem to be consensus on whether subsurface sediments that are exposed through disturbance will be rich in organic nutrients (Ingole *et al.*, 1999; Koschinsky *et al.*, 2001; Raghukumar *et al.*, 2001) or poor (Glover & Smith, 2003; Oebius *et al.*, 2001). Some studies have attributed increases in faunal density or biological activity in disturbance tracks to increased nutrient availability through the surfacing of deeper sediments, suggesting that disturbance could have some 'beneficial' effects (Khripounoff *et al.*, 2006; Raghukumar *et al.*, 2001). Whether disturbance temporarily increases or decreases organic nutrient availability, it is thought that surface sediment properties will not return to a pre-disturbance state for some time. When one of the

OMCO disturbance tracks was visited after 26 years, Khripounoff *et al.* (2006) observed that the sediment properties still had not recovered.

1.6.3 Resuspension and redeposition of sediment

Potentially the most far-reaching impact of nodule mining is the resuspension and redeposition of sediment during mining operations. As the mining tool collects nodules, it will remove the top layer of sediment, which it will then sift out and discharge back to the seafloor (Burns, 1980; Valsangkar, 2003). Some of this resuspended sediment will fall directly back to the seabed in the immediate area, with high redeposition expected up to 100 m away (Yamazaki & Kajitani, 1999), while some will be carried further afield by bottom currents and eddies (Aleynik *et al.*, 2017; Burns, 1980; Sharma *et al.*, 2001; Valsangkar, 2003). Similarly, excess sediment and seawater that is removed during on-board processing of nodules on the production support vessel will be discharged back into the ocean near the seafloor (BlueNodules, 2017; Motorship, 2017), leading to sediment redeposition in localised areas. During the INDEX benthic impact experiment, Sharma *et al.* (2001) observed that the concentration of suspended particles in the area of disturbance increased by 300% during disturbance.

Sediment that is redeposited on the seafloor may negatively affect benthic fauna through smothering and burial (Bluhm, 2001; Morgan, Odunton & Jones, 1999; Sharma *et al.*, 2001; Thiel, 2001). Redeposited sediment can clog the feeding apparatus of organisms, alter burrowing ability and affect food supply (Jumars, 1981; Radziejewska, 2002; Trueblood & Ozturgut, 1997). These impacts may then affect the reproductive success, recruitment, interspecific relationships and ultimately biodiversity of faunal communities (Radziejewska, 2002). Redeposition of disturbed sediment has also been shown to change the physicochemical conditions of the seafloor both in and out of disturbance tracks, and this is expected to impact the recolonization process (Koschinsky *et al.*, 2001; Radziejewska, 2002; Sharma *et al.*, 2001).

Experiments have shown that redeposition of sediment can lead to a decrease in faunal abundance, and that these impacts may last from several months to years (Bluhm, 2001; Fukushima, Shirayama & Kuboki, 2000; Kaneko, Maejima & Teishima, 1997; Kaneko, Ogawa & Fukushima, 1995; Raghukumar *et al.*, 2001; Rodrigues, Sharma & Nath, 2001; Trueblood & Ozturgut, 1997; Yamazaki & Kajitani, 1999). During the DISCOL experiment, several organisms were found to be coated in sediment 6 months after disturbance (Foell, Thiel & Schriever, 1990). Some experiments have observed recovery of certain taxa, particularly highly mobile and scavenging fauna like ophiuroids, holothurians, asteroids, crustaceans and fish, or whole faunal communities (although several compared their findings with pre-disturbance or baseline study levels rather than undisturbed control sites) (Bluhm, 2001; Fukushima, Shirayama & Kuboki, 2000; Kaneko, Maejima & Teishima, 1997; Radziejewska, 2002; Schriever *et al.*, 1997), while others recorded little effect of resedimentation on overall species diversity (Trueblood & Ozturgut, 1997). Radziejewska (2002) and Rodrigues, Sharma and Nath (2001) both recorded increases in abundance of certain taxa that could be in response to high organic carbon load of redeposited sediment.

Taxa show different levels of response to increased turbidity and sedimentation rates, due to their differing abilities to cope with these impacts (Fukushima, Shirayama & Kuboki, 2000; Jumars, 1981; Rodrigues, Sharma & Nath, 2001). Organisms that are able to swim away from the area of sedimentation, such as scavenging fish, shrimps or larger amphipods, may be able to avoid areas of high redeposition and turbidity (Jumars, 1981). Suspension feeders and sessile organisms are therefore likely to be the worst affected by sediment plumes (e.g. byrozoans, Grischenko, Gordon & Melnik, 2018). When the DISCOL site was revisited 26 years after experimental mining, the abundance of suspension feeders was significantly reduced in disturbed areas (Simon-Lledó *et al.*, 2019a). What is more, Kersten, Smith and Vetter (2017) found that benthic invertebrate larvae in the CCZ were most abundant in the benthic boundary layer, and suggest that any sediment plume could thus affect the dispersal and recruitment abilities of these organisms. Vertical distribution in the sediment will also play a role in determining how fauna are affected by sedimentation (Kaneko, Maejima & Teishima, 1997).

The severity of resuspension and redeposition impacts will be determined by the migration and settlement of the sediment plume, which depends on factors such as the strength of bottom currents and the type of particle assemblages (Sharma *et al.*, 2001), as well as the extent and duration of mining activity (Burns, 1980). The highest degree of redeposition is expected to occur in the direct vicinity of mining tracks (Sharma *et al.*, 2001) and up to 100 m away (Yamazaki & Kajitani, 1999), but the sediment plume may be carried further afield by bottom currents and eddies (Aleynik *et al.*, 2017; Burns, 1980; MIDAS, 2016b; Sharma *et al.*, 2001; Valsangkar, 2003). Model simulations of benthic sediment plumes suggest that sediment redeposition could exceed 1 mm for more than 10 km from the disturbance site (MIDAS, 2016b), and both benthic plumes and those resulting from on-board processing discharge could affect greater areas, in the region of 100 km (MIDAS, 2016a; Rolinskia, Segschneiderb & Sundermann, 2001). It is thus anticipated that the impacts of sediment plumes will extend beyond the direct area of mining disturbance, although the severity of these impacts is unknown.

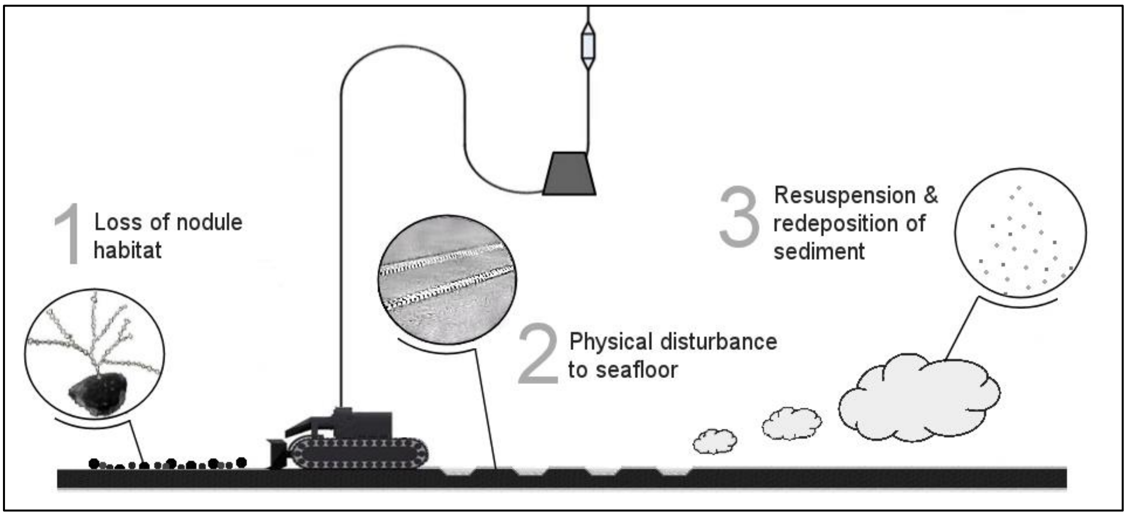

Figure 1.6 The three main potential impacts of polymetallic nodule mining. Mining concept based on Blue Nodules (BlueNodules, 2017).

These three principal impacts of nodule mining (Figure 1.6) are expected to affect ecosystem services associated with nodule provinces, although the services provided and the impact of mining on these services are not currently understood (Le, Levin & Carson, 2016).

1.6.4 Lessons from other industries

Although there are many differences between polymetallic nodule mining and shallow-water extractive industries or deep-sea bottom trawling, it is possible to draw lessons from these industries as they involve similar activities. Both shallow-water aggregate extraction and diamond mining involve the removal of substrate from the seafloor, i.e. direct physical disturbance to the seafloor, and the discharge and subsequent redeposition of sediment (De Groot, 1996; Hitchcock & Bell, 2004; Newell, Seiderer & Hitchcock, 1998; Newell *et al.*, 2004; Penney *et al.*, 2007). Mining for phosphorites has not yet commenced, but is expected to have similar impacts to diamond mining (Currie, 2013). Finally, deep-sea bottom trawling can create plough marks (i.e. disturbance tracks) on the seabed (Krost *et al.*, 1990) and suspend seafloor sediments (Churchill, 1989).

Many studies have been undertaken to investigate the environmental impacts of aggregate extraction, with fewer focussing on diamond mining impacts. The findings include a loss of species richness, abundance and biomass due to physical disturbance, burial and smothering, and changes in physicochemical properties of seabed sediments (e.g Boyd & Rees, 2003; Cooper *et al.*, 2011; De Groot, 1979; Desprez, 2000; Desprez, Pearce & Le Bot, 2010; Kenny & Rees, 1996; Newell, Seiderer & Hitchcock, 1998; Newell *et al.*, 2004; Penney *et al.*, 2007; Poiner & Kennedy, 1984; Sarda *et al.*, 2000; Savage, Field & Warwick, 2001; Waye-Barker *et al.*, 2015). There have also been records of significant increases in abundance and species richness of certain taxa in adjacent benthic areas, due to enrichment and the creation of favourable conditions (Poiner & Kennedy, 1984; Sarda *et al.*, 2000). Recovery and recolonisation of disturbed areas has been recorded, with recovery rates ranging from months to years depending on substrate type and life history strategy (e.g. Boyd *et al.*, 2005; Desprez, 2000; Newell, Seiderer & Hitchcock, 1998; Sarda *et al.*, 2000; Van Dalfsen *et al.*, 2000; Waye-Barker *et al.*, 2015). Recovery from nodule mining is expected to take longer than this, as biological processes in the deep sea, and thus recolonisation and recovery, are slower (Grassle, 1977), and nodules take millions of years to form (Reyss, Marchig & Ku, 1982).

Demersal fishing occurs over vast areas of the ocean (Halpern *et al.*, 2008), and studies on the environmental impacts of bottom trawling have shown high levels of disturbance to benthic invertebrates, with decreases in faunal abundance, biomass, species richness and diversity recorded (e.g. Bradstock & Gordon, 1983; Collie, Escanero & Valentine, 1997; Hiddink *et al.*, 2006; Hinz, Prieto & Kaiser, 2009; Hutchings, 1990). Most of these studies have focused on coastal waters and continental shelf areas, with fewer in deeper waters (Clark *et al.*, 2016a). Observations include fauna directly within the path of trawl doors being crushed or dislodged, with larger, sessile organisms, such as corals and sponges, particularly vulnerable to this (Althaus *et al.*, 2009; Clark *et al.*, 2016a; Durán Muñoz *et al.*, 2012; Hall-Spencer, Allain & Fossa, 2002; Koslow *et al.*, 2001). The resuspension of sediment has been shown to increase turbidity in the water column, changing biogeochemical properties of the system and smothering and burying fauna (Duplisea *et al.*, 2001; Jones, 1992; Kaiser *et al.*, 2002; Pilskaln, Churchill & Mayer, 1998; Riemann & Hoffmann, 1991; Stevens, 1987). Many of the benthic organisms affected by bottom trawling play a role in creating habitat complexity and heterogeneity (e.g. coral reefs), and removal of, or damage to, these structures through trawling can change the composition of associated communities (Auster *et al.*, 1996; Kaiser *et al.*, 2002; Levin *et al.*, 2010; Watling & Norse, 1998). In addition, trawling has been shown to affect predator-prey interactions, and may have impacts higher up the food-chain (Auster *et al.*, 1996; Kaiser *et al.*, 2002). Areas with low natural disturbance (like the deep sea) show the least resilience to trawling impacts (Hiddink *et al.*, 2006; Watling & Norse, 1998), and many of the fauna affected by deep-sea trawling are slow growing and long-lived, with expected recovery times of decades to centuries (Clark *et al.*, 2016a; Jones, 1992; Watling & Norse, 1998).

As Jones *et al.* (2017) conclude, it is difficult to quantify the potential impacts of mining from simulated disturbance experiments, as variation in the severity of disturbance, sampling equipment and effort, and other methodological aspects prohibits the generalisation of results. In addition, the scale of proposed commercial mining is so much greater than that of the studies. No natural disturbance of the scale or intensity proposed for polymetallic nodule mining occurs in the deep sea, and it is thus inaccurate to compare or extrapolate results from community responses to natural deep-sea disturbances (Jumars, 1981). The cumulative impacts of multiple mining activities over extended spatial and temporal scales are also unknown. In spite of this uncertainty, mining is expected to have large-scale, long-term impacts on deep-sea ecosystems, because of the scale of proposed activities and the inherently slow growth rates of both nodules and deep-sea organisms (Glover & Smith, 2003; Jones *et al.*, 2017; Thiel, 2001). Observations from existing industries with similar impacts suggest that reductions in faunal abundance, biomass and diversity will occur, and this supports findings from small-scale benthic impact experiments.

1.7 Legal framework, regulations and management

1.7.1 Overarching legal framework

The 1982 United Nations Convention on the Law of the Sea (UNCLOS)[1] is an international agreement that outlines the rights and responsibilities of member states with regard to the ocean. It was adopted in 1982, came into force on 16 November 1994, and has been ratified by 167 countries (excluding the United States of America) and the European Union. UNCLOS provides the international legal framework for deep-sea mining in ABNJ through Part XI, as modified and interpreted by the 1994 Implementation Agreement[2]. It declares all areas of the ocean beyond national borders, and all resources in these areas, to be the “common heritage of mankind” (UNGA, 1970). This means that all mineral resources in ABNJ, also called the Area, belong to humankind as a whole (article 136), and any financial or other economic benefits arising from seabed mining should be shared equally, through an appropriate mechanism and on a non-discriminatory basis

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[1] United Nations Convention on the Law of the Sea. Adopted 10 December 1982, entered into force 16 November 1994. Montego Bay, Jamaica.

[2] 1994 Agreement Relating to the Implementation of Part XI of the United Nations Convention on the Law of the Sea of 10 December 1982. Adopted 28 July 1994, entered into force 28 July 1996. New York, USA.

(article 140). Exactly how this principle will be operationalised once commercial mining begins remains a problematic area that requires further consideration (Jaeckel, Ardron & Gjerde, 2016).

The ISA was established in 1994 through UNCLOS and the 1994 Implementation Agreement to administer mineral resources in ABNJ (article 156). It acts on behalf of humankind as a whole, and oversees all activities for mineral prospecting, exploration and exploitation in areas beyond the national borders of coastal states. States or persons thus have to claim rights to mineral resources in ABNJ through the ISA. The ISA is also responsible for adopting regulations to ensure effective protection of the marine environment from harmful effects that may arise from mining activities (article 145), and member states are obliged to take actions to comply with this (article 192). This includes preventing, reducing and controlling pollution, protecting and conserving the natural resources of the Area, and preventing damage to the flora and fauna of the marine environment.

1.7.2 Regulations for mineral prospecting, exploration and exploitation

Since its inception, the ISA has developed regulations for the prospecting and exploration of polymetallic nodules, polymetallic sulphides, and ferromanganese crusts, collectively called the Mining Code (ISA, 2000; ISA, 2010a; ISA, 2012). Each of these sets of regulations contains guidelines on what is required in an application for exploration of the mineral resource in question. Under an exploration contract, contractors are obliged to, *inter alia*, establish environmental baselines, carry out monitoring to determine any impacts of mining-related activities, and develop contingency plans in the case of environmental emergencies. In addition, contractors are also required to designate impact and preservation reference zones (IRZs and PRZs) within their exploration contract area, to facilitate monitoring and ensure preservation of marine biodiversity (discussed below).

In 2013, the ISA initiated the process of developing a regulatory framework for the exploitation of seabed mineral resources, and in March 2015 the Legal and Technical Commission of the ISA released a draft regulatory framework (ISA, 2015b). This has been revised and developed into a set of draft exploitation regulations through several workshops and stakeholder comment periods, with the latest working draft released in March 2019 (ISA, 2019b). These regulations include a section on protection and preservation of the marine environment, with articles on general obligations of contractors, the development of environmental standards, pollution control, and the preparation of Environmental Impact Statements (a report coming from an Environmental Impact Assessment) and Environmental Management and Monitoring Plans. At this stage, an over-arching framework for all mineral exploitation is being developed, with the development of resource-specific rules, regulations and procedures to follow at a later stage. It is expected that this will initially be focused on polymetallic nodules.

The development of these exploitation regulations is an urgent requirement, as several initial exploration contracts granted by the ISA in 2001 were scheduled to expire in 2016. In these cases, contractors have applied for extensions to existing exploration contracts, rather than submitting plans of work for exploitation (e.g. ISA, 2017c; ISA, 2017a). Because deep-sea mining has not yet started, stakeholders are able to provide input into the formation of exploitation regulations, including those relating to the protection of the marine environment, before exploitation activities begin (Petersen *et al.*, 2016). Key challenges to this process include incorporating transparency (Ardron, 2014), the precautionary approach (Wedding *et al.*, 2015) and adaptive management (Jaeckel, 2016) into the environmental regulations, developing mechanisms to facilitate sharing of benefits arising from seabed mining (Jaeckel, Ardron & Gjerde, 2016), establishing environmental standards and thresholds to guide mining activities (Levin *et al.*, 2016), and developing an overarching environmental management strategy for deep-sea mining in the Area (ISA, 2017b; Jaeckel, 2015).

1.7.3 Regional environmental management plans

In addition to ISA regulations for mineral prospecting, exploration and exploitation, a regional Environmental Management Plan (EMP) has been developed for nodule mining in the CCZ (ISA, 2011), and work is currently underway to develop a strategic EMP for hydrothermal vent mining activities in the mid-Atlantic (Dunn *et al.*, 2018; ISA, 2015c). The CCZ EMP is a spatial management plan that strives to support mining exploitation, while preserving marine biodiversity in the CCZ region (Lodge *et al.*, 2014). It is based on the guiding principles of the common heritage of mankind; the precautionary approach; the protection and preservation of the marine environment; prior environmental impact assessment; conservation and sustainable use of biodiversity; and transparency, and drew largely from the outputs of a collaborative scientific project called the Kaplan Project (Smith *et al.*, 2008c).

The CCZ EMP includes the designation of nine Areas of Particular Environmental Interest (APEIs), which are areas excluded from mining and intended to protect regional biodiversity and ecosystem functioning (Figure 1.1). These areas were selected using widely applied design principles for marine protected area networks, such as representativeness and size, and considered, amongst others, spatial variation across the CCZ, driven by a strong productivity gradient from east to west, species ranges, and gene flow (Wedding *et al.*, 2013). Each of the APEIs has a core area of 200 x 200 km, with an additional 100 km buffer to protect this area from mining activities occurring directly adjacent to the APEI (ISA, 2011) (Figure 1.7).

The total area of the network is 1.44 million km^2, compared to approximately 1.25 km^2 million covered by current exploration contracts (including the maximum size after relinquishments of the recently awarded contract to China, May 2017), and 6 million km^2 of the total size of the CCZ management area (Madureira *et al.*, 2016; Petersen *et al.*, 2016). Although there is currently less area under contract for exploration than area protected, the APEI network falls short of one of the design criteria: protecting 30-50% of the total management area (currently 24%) (ISA, 2011). In addition, because the APEI network was designed and implemented after many of the exploration contracts were already in place, it was restricted by honouring existing claim areas, and the APEIs are thus located in the periphery of the central CCZ, in areas of lower commercial interest and lower estimated nodule abundance (ISA, 2010b). It is also unclear whether the network represents the full range of habitats in the CCZ, particularly areas of high nodule abundance.

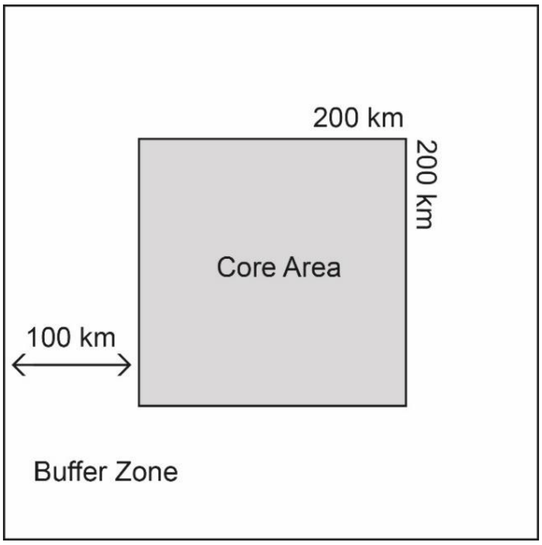

Figure 1.7 Schematic showing the configuration of Areas of Particular Environmental Interest (APEI). APEIs are a spatial management measure aimed at protecting regional biodiversity and ecosystem functioning. They consist of a core area of 200 x 200 km, surrounded by a 100 km buffer. Figure not drawn to scale.

Other spatial management measures identified in the EMP include the designation of IRZs and PRZs by contractors, as per the exploration regulations, to facilitate monitoring of mining impacts and ensure preservation of biodiversity. The former should be located within the mined area of a claim, while the latter should be located outside of the mined area of a claim, but should still contain nodules (ISA, 2011) (Figure 1.8). PRZs are intended to provide a control for monitoring and to support recolonisation of exploited claim areas. Although no formal guidelines have been established for designating reference zones, work has been carried out to develop recommendations on IRZ and PRZ design and monitoring criteria (ISA, 2018; Jones *et al.*, 2018).

The ISA has committed to reviewing the CCZ EMP every 5-10 years, providing an opportunity to update and improve it. The first review was carried out in July 2016 and found that additional APEIs may be required in the CCZ to ensure adequate protection of regional biodiversity (ISA, 2016). It also highlighted the need for guidelines on establishing IRZs and PRZs, and a working group of expert scientific consultants.

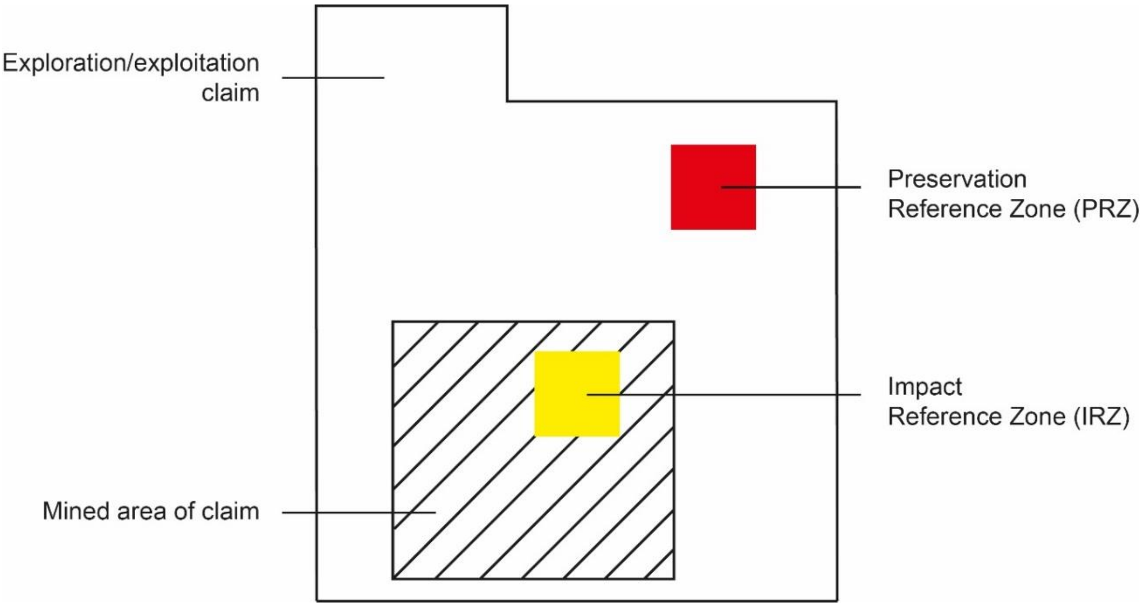

Figure 1.8 Schematic showing the configuration of impact reference zones (IRZ) and preservation reference zones (PRZ) within an exploration contract area. Figure not drawn to scale.

1.8 Thesis aims and outline

Although commercial deep-sea mining has not yet become a reality, a number of applications have been made in national waters of coastal states (e.g. in New Zealand, Government of New Zealand, 2015; and Namibia, Shihepo, 2016), and regulations for exploitation in ABNJ are due for completion in 2020 (IISD, 2018). There is a need for strong environmental regulations before mining activities commence; however, little is still known about the taxonomy, ecology, geographic ranges, connectivity and functional roles of deep-sea fauna in areas targeted for mining, as well as the scale of potential environmental impacts of mining (Jones *et al.*, 2017). There is therefore a great need for scientific research that bridges the gap between policy demands and available science. Fundamental baseline information on the fauna of nodule provinces is crucial to support conservation and environmental management of mining activities, as is Marine Spatial Planning (MSP) (Lodge *et al.*, 2014; Wedding *et al.*, 2015).

The overall aims of this thesis were to improve our understanding of the epibenthic megafauna communities associated with abyssal nodule provinces, and to support environmental management of deep-sea mining through recommendations on sampling and spatial planning, including through assessment of the current protected area network. Specific aims of each study are presented at the end of the introduction to every chapter.

The studies in this thesis are focussed on the abyssal nodule province of the eastern equatorial Pacific, called the CCZ. Using historical imagery of the seafloor from the central CCZ, Chapter 2 investigates patterns in the composition, life history characteristics and diversity of the epibenthic megafauna communities at both a regional and local scale, and investigates potential environmental drivers of these patterns. Good baseline knowledge on community ecology is fundamental to our ability to predict potential impacts of deep-sea mining on abyssal ecosystems, and thus support effective environmental management and conservation planning.

Chapter 3 then uses the same data to examine the sampling effort required to capture megafauna diversity at different levels of completeness. Guidelines on appropriate sampling units and numbers of replicates in the deep sea are sparse, and there is a crucial need to make sure we are using appropriate sampling units to capture the diversity of benthic communities. This chapter informs discussions around minimum sampling requirements for baseline studies, monitoring and EIAs in the deep sea, and specifically relating to polymetallic nodule mining.

Finally, Chapter 4 presents a classification tool to support spatial planning in deep-sea areas that are very large, with poor biological data. This chapter produces a top-down, broad-scale habitat classification and map of the CCZ using environmental surrogates. Biological data from the previous two chapters is used to ground truth the classification, which is then used to test the benthic habitat representativity of the current network of protected areas in the CCZ.

In each case the implications of the findings for environmental management of deep-sea activities, and particularly nodule mining, are discussed and areas of future research identified. The final chapter, Chapter 5, synthesises the results of each chapter and discusses how the findings advance both our understanding of the ecology of epibenthic megafauna in abyssal nodule provinces and environmental management of nodule mining. It also identifies general limitations of the studies and potential areas of future research.

Chapter 2.

Epibenthic megafauna community ecology of abyssal nodule fields in the CCZ

2.1 Introduction

2.1.1 Abyssal ecology

Abyssal plains are the largest benthic habitat on earth, covering 75% of the seafloor (Ramirez-Llodra *et al.*, 2010). Harris *et al.* (2014) defined the abyssal zone as those areas of the seafloor below the foot of the continental slope, and above 6 500 m deep. These plains consist of vast, relatively flat expanses of soft sediment, with topographic features such as abyssal hills and mountains (or seamounts) (Harris *et al.*, 2014).

Despite early misconceptions that the deep sea was a desolate and depauperate environment, abyssal plains are now known to host diverse faunal communities (Hessler & Sanders, 1967). Although at abyssal depths meiofauna and bacteria tend to dominate in terms of biomass and abundance (Hessler & Jumars, 1974; Hessler & Sanders, 1967; Rex *et al.*, 2006), diverse megafaunal communities are also found (Smith *et al.*, 2008a). Megafauna in this environment are thought to have broad geographic ranges (Smith, Drazen & Mincks, 2006) and be generally well connected over distances of 100s to 1 000s of kilometres, except where they cross an ecologically important depth band (Taylor & Roterman, 2017); however, information is woefully lacking. Abyssal plains remain relatively poorly explored, with many taxa undescribed, and many new species discovered with every research cruise (Glover *et al.*, 2016a; Smith, Drazen & Mincks, 2006). The most common metazoan species found encrusting polymetallic nodules in the Pacific, a demosponge, was only recently described (Lim *et al.*, 2017), as well as new species of annelids (Wiklund *et al.*, 2019), molluscs (Wiklund *et al.*, 2017) and xenophyophores (Gooday *et al.*, 2020; Gooday *et al.*, 2017c), to name just a few examples.

Abyssal plains have long been considered to host fairly stable environmental conditions. However, while some of the abiotic features are relatively stable, such as temperature, salinity, and dissolved oxygen, others are dynamic, episodic or seasonal, such as bottom currents and food availability (Tyler, 1988). Those parameters that vary have the potential to influence megafauna species distributions, and studies on the drivers of abyssal benthic megafaunal distributions have demonstrated a number of parameters play a role.

Food availability is arguably the most important environmental variable determining the distribution of organisms in the abyssal deep sea (Cartes & Sarda, 1993; Gooday, 2002; Levin *et al.*, 2001; Ruhl & Smith, 2004; Smith *et al.*, 1997; Smith *et al.*, 2008a; Wei *et al.*, 2011; Woolley *et al.*, 2016). Apart from at chemosynthetic habitats, like hydrothermal vents and cold seeps, or organic food falls, deep-sea communities are entirely dependent on sinking particles from surface waters for food (Gooday *et al.*, 1990; Smith & Baco, 2003; Turner, 1973; Van Dover, 2000). Particulate Organic Carbon (POC) flux to the seafloor in abyssal environments is generally low, and abyssal plains are thus considered food limited (Smith *et al.*, 2008a). Variations in POC have been shown to structure communities in abyssal plain areas both spatial and temporally, with local highs in megafauna abundance, biomass and diversity associated with high POC (e.g. Kuhnz *et al.*, 2014; Smith *et al.*, 1997; Smith *et al.*, 2008a; Thurston, Rice & Bett, 1998).

Depth and topography also play an important role in determining species distribution in the deep sea, with species showing preference for certain depth ranges and topographic conditions (Hecker, 1990; Rice, Thurston & New, 1990; Rowe & Menzies, 1969). The abundance and biomass of fauna is known to decrease with depth, with higher standing stock of fauna recorded in bathyal than abyssal areas (Rex *et al.*, 2006). Although there was once thought to be a unimodal relationship between diversity and depth, with peaks in diversity recorded at intermediate depths around 2 000 m (Rex, 1981), we now know that this relationship is not universal, and that diversity may in fact increase, decrease or show no relationship with depth (Stuart & Rex, 2009). This is reflected in high abyssal diversity recorded in the Pacific (Glover *et al.*, 2002; Smith *et al.*, 2008a). Depth itself is not an environmental driver of species distributions but serves as a proxy for a number of environmental parameters that vary with depth (Howell, 2010).

Topography also serves as a proxy for other important environmental drivers of species distributions. Slope of the seafloor, for example, affects local hydrodynamics by directing benthic currents, and current velocity is expected to be strong where there is a steep slope (Genin *et al.*, 1986). This influences the flow of food and impacts suspensions feeders particularly (Gage & Tyler, 1996). Biological responses to topographic heterogeneity have been observed at abyssal plains in the North Atlantic (Durden *et al.*, 2015; Morris *et al.*, 2016; Stefanoudis, Bett & Gooday, 2016) and central Pacific (Leitner *et al.*, 2017; Simon-Lledó *et al.*, 2019b) and are suggested to be linked to topographically-enhanced local hydrodynamics (Thistle, Ertman & Fauchald, 1991), sediment composition (Durden *et al.*, 2015) and food availability (Morris *et al.*, 2016).

Finally, substrate type is important in determining species distributions in the deep sea (e.g. Buhl-Mortensen *et al.*, 2010; Howell, 2010). As in shallow waters, presence of hard substrate introduces habitat heterogeneity to soft sediment environments (MacDonald *et al.*, 2010) and provides substrate for attachment (Meyer *et al.*, 2016; Van Dover, Berg & Turner, 1988). The presence and extent of hard substrate is therefore expected to influence the composition of benthic communities (Smith & Demopoulos, 2003; Young, 2009). Hard substrate habitats supporting benthic communities in abyssal environments are diverse (Young, 2009) and include seamounts (Clark *et al.*, 2010), canyons (De Leo *et al.*, 2010), hydrothermal vents (Lutz & Kennish, 1993), whale (Smith & Demopoulos, 2003) and wood falls (Turner, 1973), drop-stones (MacDonald *et al.*, 2010; Meyer *et al.*, 2016), and polymetallic nodules (Mullineaux, 1987).

2.1.2 The CCZ abyssal plain

The Clarion-Clipperton Fracture Zone (CCZ) is a large abyssal plain area in the eastern central Pacific that is being targeted for polymetallic nodule mining. The CCZ is a highly heterogeneous environment, with variations in nodule size and abundance (ISA, 2010b), a large-scale and strong productivity gradient from east to west and north to south (Smith *et al.*, 1997), increasing depth from east to west (Pushcharovsky, 2006), and topographic features like abyssal hills, seamounts, troughs and ridges (Klitgord & Mammerickx, 1982). It has been suggested that these varying environmental conditions have led to greater diversity of CCZ benthic communities when compared to other abyssal plain areas (Amon *et al.*, 2016; Glover *et al.*, 2002; Gooday *et al.*, 2017b; Janssen *et al.*, 2015; Kamenskaya, Melnik & Gooday, 2013; Simon-Lledó *et al.*, 2019b; Tilot *et al.*, 2018).

In recent years, research cruises to the CCZ have focussed on establishing environmental baselines, to gain a better understanding of the environmental conditions of the area prior to any commercial-scale mining activities. The academic research community are still trying to characterise the baseline biological communities of nodule provinces, with new records of species, and indeed new species, frequently observed (e.g. Amon *et al.*, 2016; Amon *et al.*, 2017; Dahlgren *et al.*, 2016; Glover *et al.*, 2016b; Lim *et al.*, 2017; Miljutin & Miljutina, 2009; Wiklund *et al.*, 2017).

Although there is still much to understand about the benthic fauna, there is evidence that nodule provinces host a diverse range of life, from sessile and encrusting fauna on the nodules themselves, to sediment-dwelling infauna, mobile scavengers and distinct bacterial communities (e.g. Amon *et al.*, 2016; Amon *et al.*, 2017; Gooday *et al.*, 2017b; Shulse *et al.*, 2016). The dominant epibenthic megafauna in nodule fields includes highly motile taxa, such as ophiuroids, holothurians, echinoids, asteroids and crustaceans, and sessile fauna, such as actiniarians, alcyonaceans, sponges and giant monothalamous Foraminifera called xenophyophores (Amon *et al.*, 2016; Glover *et al.*, 2016b; Gooday *et al.*, 2017b; Kamenskaya, Melnik & Gooday, 2013; Morgan *et al.*, 1993; Morgan, Odunton & Jones, 1999; Simon-Lledó *et al.*, 2019b; Tilot *et al.*, 2018; Vanreusel *et al.*, 2016). Nodules themselves also provide a habitat for epifaunal species that are dependent on hard substrate, such as sponges, corals, bryozoans, crinoids, and Foraminifera (Amon *et al.*, 2016; Dahlgren *et al.*, 2016; Dugolinsky, Margolis & Dudley 1977; Foell & Pawson, 1986; Gooday, Goineau & Voltski, 2015; Gooday *et al.*, 2017b; Grischenko, Gordon & Melnik, 2018; Mullineaux, 1987; Vanreusel *et al.*, 2016; Veillette *et al.*, 2007).

There is evidence that the habitat heterogeneity introduced by nodules supports both a higher density and diversity of megafauna than soft-sediment environments with no nodules (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016). In addition, there appears to be a positive relationship between megafauna and nodule abundance, with more sessile and mobile epibenthic megafauna recorded in nodule-rich areas, compared to areas with low nodule cover (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016). Simon-Lledó *et al.* (2019c) found that an initial increase in nodule cover from 1% - 3% was most important, resulting in a doubling of metazoan and xenophyophore standing stock.

The relationship between increasing nodule cover and megafauna diversity is less clear. Simon-Lledó *et al.* (2019c) observed that biodiversity did not vary along a nodule cover gradient of 1 - 20%, but that the composition of the megafauna community changed continuously. Amon *et al.* (2016), on the other hand, reported greater accumulation of species and higher estimated total species richness in areas of low (<15%) nodule cover compared to areas of medium cover (15-50%). Vanreusel *et al.* (2016) suggest that the same conditions that create a favourable environment for high abundances of commercially valuable nodules (i.e. low sedimentation rates and moderate bottom currents at elevated parts of the abyssal seafloor) may also be important in supporting local biodiversity, and that areas of high nodule abundance will therefore be important for conserving abyssal biodiversity. Amon *et al.* (2016) argue that there may be a threshold after which increasing nodule cover and volume may reduce species richness by decreasing habitat availability for soft-sediment fauna.

In areas where there are not significant differences in nodule abundance, megafauna density, diversity and community composition in the CCZ have been shown to be relatively homogenous over scales of 1 to 100s km (Amon *et al.*, 2016). The connectivity of megafauna populations in the CCZ is still unknown, although these organisms are expected to have large geographic ranges (Smith, Drazen & Mincks, 2006). Amon *et al.* (2016) hypothesise that at the scale of individual nodules, tens of metres (as nodule cover varies) and at regional scales, megafauna communities will be highly heterogeneous.

2.1.3 Environmental management

Nodule mining will impact benthic megafaunal communities of the CCZ. The three main environmental impacts of nodule mining relate to the removal of the nodules themselves, physical disturbance to the seafloor and the resuspension and redeposition of sediment (Burns, 1980), as reviewed in Chapter 1. All of these impacts are expected to negatively affect fauna associated with nodule fields, although the extent of these impacts is still unknown (Jones *et al.*, 2017) and will depend on the life histories of different taxa.

The removal of nodules from the seafloor will decrease habitat availability for sessile organisms that depend on hard substrate for attachment and are unable to live in surrounding soft sediment environments (Burns, 1980; Vanreusel *et al.*, 2016). This impact may result in the permanent loss of nodule-specific fauna, and could affect organisms higher up the food-chain (Jumars, 1981; Purser *et al.*, 2016). The production of a sediment plume will have potentially the most far-reaching impact, and is expected to be particularly detrimental for sessile, suspension feeding organisms (Grischenko, Gordon & Melnik, 2018; Jumars, 1981). Sediment that is redeposited to the seafloor will smother and bury fauna, clogging the feeding apparatus of organisms, and may lead to reduced reproductive success, recruitment, interspecific relationships and ultimately diversity (Bluhm, 2001; Radziejewska, 2002). This will affect not only those organisms in the direct vicinity of mining activities, but also further afield as the plume is carried by eddies and currents (Aleynik *et al.*, 2017; Sharma *et al.*, 2001).

Before mining activities begin on a commercial scale, the International Seabed Authority (ISA), the intergovernmental body tasked with regulating seabed mining in Areas Beyond National Jurisdiction (ABNJ), is developing exploitation regulations to ensure that the natural environment, and particularly the fauna, are protected from any potential harmful effects of these activities (ISA, 2019b), as is required under the 1982 United Nations Convention on the Law of the Sea (UNCLOS) (article 145).

Safeguards that have been put in place to protect the marine environment from exploration and exploitation activities include a Regional Environmental Management Plan (REMP) for the CCZ, and through this the establishment of a network of nine marine protected areas, called Areas of Particular Environmental Interest (APEI) (ISA, 2011).

Further ways to protect the marine environment from mining activities (e.g. operational standards and guidelines) are being explored through the development of the exploitation regulations (e.g. ISA, 2017b; ISA, 2019d). However, one of the current fundamental problems with trying to manage seabed mining from an environmental perspective, is that there are still large knowledge gaps relating to the fauna in areas being targeted (Glover *et al.*, 2016a), and thus also the scale of potential impacts of mining. The CCZ, as this thesis's case study, is an area of approximately 6 million km^2, and while there have been many research and commercial cruises to the CCZ, there remain large gaps in our understanding of the ecology of the area.

2.1.4 Aims

Good baseline knowledge on community ecology is fundamental to our ability to predict potential impacts of deep-sea mining on abyssal ecosystems and thus support effective environmental management and conservation planning.

The aims of this study were to:

1) Describe the composition and life history traits of epibenthic megafauna communities in the CCZ, at regional (> 1 000 km) and local (2 km) scales, in order to improve baseline ecological knowledge of abyssal nodule provinces and predict potential impacts of mining activities;

2) Quantify diversity of the epibenthic megafauna communities in the CCZ at

regional (> 1 000 km) and local (2 km) scales and place in the context of current knowledge of abyssal and CCZ diversity; and

3) Identify environmental parameters that may be driving community composition and diversity of abyssal megafauna across the CCZ to support future development of modelling approaches.

2.2 Methods

2.2.1 Study site

The CCZ is a large area that spans approximately 6 million km^2 between the Clarion and Clipperton fracture zones in the eastern central Pacific. It is an abyssal plain area, with topographic features including abyssal hills, seamounts, troughs and ridges, and hosts the highest global concentration of high-grade polymetallic nodules (Thiel *et al.*, 1998).

There are strong environmental gradients across the CCZ, in both an east-west and north-south direction. POC, depth and sediment characteristics vary across the region, resulting in differences in both nodule size and abundance as well as the composition of faunal communities (ISA, 2010b; Smith *et al.*, 1997; Wedding *et al.*, 2013).

Sampling sites for this study were concentrated in the central CCZ, between 11.6° N 136.3° W and 13.7° N 126.3° W (Figure 2.1). The transects were located in flat abyssal plain areas, with a range of environmental conditions (see Table 2.2).

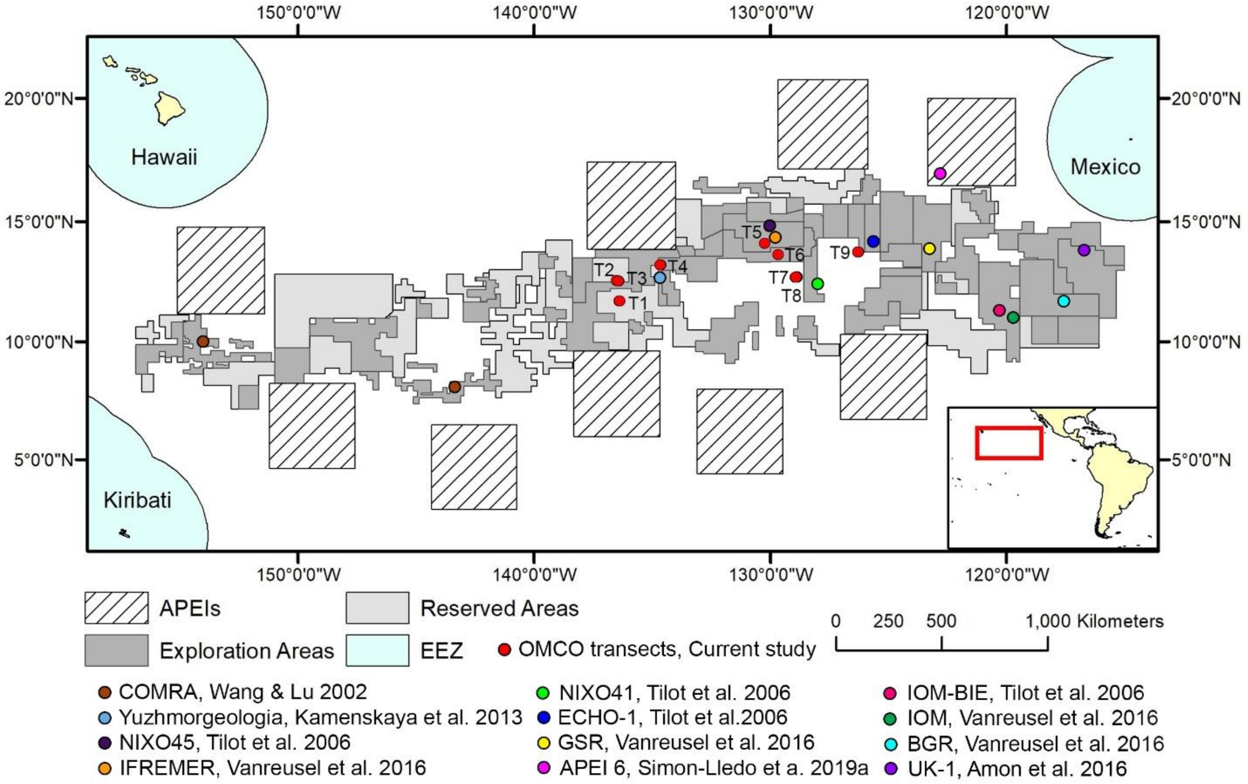

Figure 2.1 Location of image transects from this study and others within the CCZ management area. Land masses in cream, surrounded by Exclusive Economic Zones (EEZs) in pale blue. APEI = Area of Particular Environmental Interest. Transects from the current study are numbered sequentially from west to east. The minimum distance between transects is 2.5 km (between transects T7 and T8), with a maximum distance of 1 150 km (between transects T1 and T9).

2.2.2 Image analysis

2.2.2.1 OMCO data set

The data set used in this study was a collection of archived images of the seabed that were taken by Lockheed Martin and the Ocean Minerals Company (OMCO) consortium during exploration activities in the CCZ from 1978-1981, as part of a commercial effort to characterise the polymetallic nodule deposits of the region. Images were taken using a Benthos 377 deep-sea towed camera system with 35 mm film, and the whole data set includes approximately 79 transects. The film was scanned into digital format by Woods Hole Oceanographic Institute (WHOI), and resulted in a dataset of 143 967 images. A subset of these images was then geolocated by UK Seabed Resources Ltd (UKSRL) where information was available in archived cruise reports, environmental baseline reporting to U.S National Oceanographic and Atmospheric Agency (NOAA) and camera run logs. Some of the original (film) images from the OMCO data set were examined in a preliminary analysis by (Morgan *et al.*, 1993) prior to scanning, and their findings are referred to briefly in this study.

2.2.2.2 Transect selection

It is well known that manual image annotation is an extremely time-consuming process, and represents a bottleneck in image data processing at present (Piechaud *et al.*, 2019). Although there was a large data set available for analysis, time spent annotating was a limiting factor as image analysis commenced at a late stage in the PhD due to unforeseen circumstances. This restricted the number of transects and images that could be annotated in this study. However, efforts were made to ensure that the number and size of transects analysed were sufficient (see below for size).

Several transects contained only a few images, and these were dismissed from the data set. From the remaining subset, transects were then selected for analysis based on a number of visual criteria, including the quality of the images within each transect, suitable altitude range, and the amount of sediment plume (generated by impact of the camera system on the seafloor) visible.

Once these criteria were satisfied, transects were selected in order to achieve a stratified design with replication between habitat classes modelled in Chapter 4. Chapter 4 carries out a habitat classification in the CCZ to predict and model habitat types across the region. A key step in the classification process is to ground-truth modelled habitat types using biological data, to assess whether they reflect variation in faunal communities and are thus biologically meaningful. Transects were therefore selected to ensure a replicated, stratified design for validating the habitat classification of Chapter 4, taking into account time restrictions.

The locations of selected transects are shown in Figure 2.1, with a minimum distance between transects of 2.5 km (between transects T7 and T8), and a maximum distance of 1 150 km (between transects T1 and T9).

2.2.2.3 Image selection

Once the final transects had been selected, several steps were undertaken to standardised the quality and quantity of imagery in each transect. Blurred and overlapping images were removed, and those remaining were then filtered to within an altitude range of 2 - 4.6 m, with no sediment plume visible within the frame. In some cases, this did not allow for enough images and so the altitude range was increased to 1.8 - 5.5 m, with <5% sediment plume visible. Of this subset, images were then randomly selected (where possible) until a standardised sample area of 800 m^2 of seabed was reached.

The sampling unit size of 800 m^2 was chosen based on previous studies considering species accumulation curves and stabilisation of ecological parameter estimates in the deep sea. Foster, Foggo and Howell (In prep.) show that species accumulation curves begin to approach the asymptote after 100 images (equivalent to 800 m^2 of seabed) in their study of megafauna at 1 200 m depth in the North Atlantic. Simon-Lledó *et al.* (2019b), studying the megafauna of the CCZ, examined the effect of sampling unit size (either transect area in km^2 or number of individuals) on the accuracy and precision of several ecological parameters (including faunal density and several biodiversity indices) and found that “increases in precision were modest for most parameters with sampling unit sizes…700-900 m^2”. The effect on accuracy was more variable, but estimations of faunal density, and Shannon's and Simpson's biodiversity indices were shown to stabilise at 700 - 1 000 m^2 and 400 - 600 m^2 respectively. The sampling unit size used in the current study drew from, and was developed in parallel with, the studies of Drs Foster and Simon-Lledó.

The field of view ranged in images from 1.4 m^2 to 18.5 m^2, and this therefore resulted in a different number of images per transect (range 71 - 122, Table 2.1). The length of transect from which images were selected was also standardised as far as possible (ranging from 1.5 - 2.5 km), to reduce the likelihood of testing different types of diversity. Metadata, including the altitude, compass heading and time stamp, were manually extracted from each image as part of this study.

Table 2.1 Summarised metadata for transects analysed in this study. The centre point of each transect is indicated by latitude and longitude (in decimal degrees).

Transect	Centre latitude (⁰)	Centre longitude (⁰)	Water depth (m)	Transect length (km)	Images analysed (n)	Total area imaged (m^2)	Mean area per image ± st dev (m^2)	Habitat class
T1	11.688	-136.399	4920-4921	1.46	110	800.3	7.3 ±3.49	11
T2	12.536	-136.515	5019-5041	1.47	122	800.1	6.6 ±2.59	9
T3	12.520	-136.438	4984-5001	1.37	105	799.2	7.6 ±2.88	13
T4	13.193	-134.660	4796-4811	1.94	99	801.7	8.1 ±2.55	13
T5	14.085	-130.244	4906-4928	1.91	71	799.8	11.3 ±4.54	13
T6	13.620	-129.672	4839-4859	1.92	74	801.5	10.8 ±5.18	9
T7	12.677	-128.948	4800-4802	2.36	113	800.7	7.1 ±2.95	11
T8	12.686	-128.895	4787-4792	2.26	104	799.4	7.7 ±3.16	9
T9	13.723	-126.290	4607-4645	1.51	97	799.4	8.2 ±3.26	11
				TOTAL	895	7 201.1		

2.2.2.4 Image annotation

Images were annotated using the freely-available online software, BIIGLE 2.0 (Langenkämper *et al.*, 2017). Fauna greater than 10 mm were identified to the lowest possible taxonomic ranking and assigned a morphotype label, following the approach of

Howell, Davies and Narayanaswamy (2010). This approach allows for standardisation of annotations across images taken at different altitudes.

A megafauna morphospecies catalogue developed by Simon-Lledó *et al.* (2019b) for the CCZ region was used together with relevant literature to support this work, and images across all transects were annotated in a random order (Durden *et al.*, 2016). Morphotypes were assigned a feeding strategy based on what is known in the literature about similar organisms (e.g. Iken *et al.*, 2001), and individuals attached to a nodule were assigned a “nodule-attached” label.

Following image annotation, quality assessment was carried out by Dr Erik Simon-Lledó to ensure consistency in identification across studies.

2.2.3 Community ecology analyses

Previous studies have found that including xenophyophores in analyses of community data can substantially affect the outcomes (Simon-Lledó *et al.*, 2019b), and several reasons for analysing them separately have been suggested, including difficulty in determining whether individuals are living (Hughes & Gooday, 2004) and body size mismatch due to the very small size of xenophyophore protoplasm volume (Levin & Gooday, 1992). In this study, therefore, patterns in the metazoan community were examined separately, in addition to those in the megafauna community as a whole (metazoans and xenophyophores).

2.2.3.1 Community composition

To examine patterns in the composition of epibenthic megafauna and metazoan communities of the CCZ, the density of individuals per metre squared (m^2) was calculated. Patterns in community composition were explored at both a regional scale

(>1 000 km), using pooled megafauna/metazoan data across all transects, and at a local scale (2 km), by comparing transects.

In addition, hierarchical agglomerative clustering with group average linkage was applied to Bray-Curtis similarity matrices of multivariate log transformed megafauna and square-root transformed metazoan community composition data in the statistical software Plymouth Routines In Multivariate Ecological Research (PRIMER) v.6 (Clarke & Gorley, 2006). Similarity Profile (SIMPROF) permutational tests were carried out to determine whether clustering was statistically significant (Clarke, Somerfield & Gorley, 2008).

2.2.3.2 Life history characteristics

To examine patterns in the life history characteristics of the epibenthic megafauna and metazoan communities, the proportion of individuals with different feeding strategies, mobility (sessile or mobile) and substrate attachment (nodule-associated or free-living) was calculated at both a regional (all transects pooled) and local scale (comparing transects). For substrate attachment, those sessile organisms observed in contact with a nodule were treated as nodule-associated.

There are many different feeding mechanisms proposed for xenophyophores, although information around all of these is still unclear (Goldstein & Corliss, 1994). It was hypothesised that the two most abundant groups of xenophyophores observed in this study were suspension feeders, based on their morphological similarity to other species with this feeding strategy (e.g. *Psammina limbata*, Gooday *et al.*, 2017b; *Reticulammina* sp., Levin & Thomas, 1988; *Syringammina* sp., Robinson *et al.*, 2011). These two morphotypes were upstanding (plate-like) and hemispheric (reticulate) forms, and occupied elevated habitats (either by attaching to nodules or by nature of their upright morphology) that provided access to the near-bottom water layer (Levin & Thomas, 1988). There is some uncertainty as to whether reticulate forms are suspension feeding or particle trapping (Levin & Thomas, 1988), but for the purposes of this study they were treated as suspension feeders as in the context of potential mining impacts it was assumed that these feeding strategies will be equally vulnerable to the effects of a sediment plume.

There was no information available on the potential feeding strategy of the tubular xenophyophores, which mostly resembled *Aschemonella monile* (Gooday *et al.*, 2017a), and this group, together with a general group of xenophyophores, occurred at far lower densities than the plate-like and reticulate xenophyophores. They were thus included in calculations as suspension feeders, accepting that this may not be accurate.

2.2.3.3 Diversity

Several biodiversity indices were calculated for megafauna and metazoan data: Chao 1 and Chao 2 estimates of morphotype richness; and the first three Hill's numbers, species richness (here referred to as morphotype richness, MR), the exponential of Shannon's entropy index (H') and the inverse of Simpson's concentration index (1/D).

Chao and Hill's biodiversity estimates were calculated in R x64 3.5.1 using the 'vegan' and 'iNEXT' (iNterpolation/EXTrapolation) packages (Hsieh, Ma & Chao, 2016; Oksanen *et al.*, 2018). Individual- and sample-based rarefaction and extrapolation (R/E) curves were fitted to the reference (observed) sample size, double the reference sample size and asymptote, with 95% confidence intervals obtained through bootstrapping of 1 000 replications. Extrapolations of morphotype richness beyond double the reference sample may be unreliable due to prediction bias (Chao *et al.*, 2014), and these estimates were therefore treated with caution.

Patterns in diversity were examined at a regional scale, using the nine 800 m^2 transects as the sampling units, and at a local scale, using the images of each transect as the sampling unit. For local comparisons, non-overlapping confidence intervals were interpreted as a significant difference in diversity among transects, as per Cumming, Fidler and Vaux (2007).

2.2.4 Environmental links

In order to test whether there was a significant relationship between modelled environmental variables and community composition and diversity of epibenthic megafauna and metazoans in the CCZ, regression analyses were carried out in PRIMER and R. Linear regression modelling has several assumptions relating to the relationship between explanatory and response variables, and the distribution and variance of model residuals (Quinn & Keough, 2002), and these assumptions were not met with the current data set, potentially due to the low sample size of nine transects. A non-parametric distance-based linear regression approach was thus used, called distance-based linear modelling (DistLM). DistLM still quantifies the relationship between response and explanatory variables, but is permutational and does not have the strict assumptions of linear regression (Anderson, Gorley & Clarke, 2008).

2.2.4.1 Environmental variable selection

The first step in the process was to identify relevant environmental variables that may be important in driving species distributions in the CCZ. Although no direct measures of environmental variables were available with the transect imagery, some broadscale modelled data were available. Previous studies have suggested that topography, POC, and nodule abundance all play a role in determining species distributions in abyssal environments, and specifically in the CCZ (e.g. Leitner *et al.*, 2017; Simon-Lledó *et al.*, 2019b; Simon-Lledó *et al.*, 2019c; Smith *et al.*, 1997).

2.2.4.1.1 Topography

Bathymetry and derived variables representing different topographic conditions were used as possible explanatory variables in this study. Derivatives of bathymetry that were used were broad-scale bathymetric position index (BBPI) and fine-scale bathymetric position index (FBPI). Slope was also considered an important topographic variable to include, but showed little variation among transects and was therefore excluded from the study.

Bathymetric position index (modified from topographic position index) is a second-order derivative of bathymetry, and gives the relative elevation of a point in relation to the overall landscape (Lundblad *et al.*, 2006). Positive values indicate features rising above the surrounding terrain (e.g. peaks and crests), while negative values indicate depressions such as valleys and troughs. Areas with constant slope or flat areas are represented by near-zero values. BPI is a scale dependent phenomenon that can be calculated on both a broad- (BBPI) and fine-scale (FBPI) (Figure 2.2) (Weiss, 2001). BPI acts as a surrogate for various environmental factors that affect species distribution, such as exposure to current, current speed and sedimentation, without confounding the effects of other variables (e.g. temperature and salinity) (Evans, Peckett & Howell, 2015).

Topographic variables were derived from the General Bathymetric Chart of the Oceans (GEBCO) bathymetry layer. All variables were generated in ArcMap 10.4 using the Benthic Terrain Modeler extension (Wright *et al.*, 2005). BPI was derived at both broad and fine scale, to capture topographic features at different scales in the CCZ. BBPI was derived with an inner radius of 1 and an outer radius of 100, with a scale factor of 100 km. This broad scale layer identified large geomorphological units, such as abyssal plains, steps and troughs. This choice of scale drew from the US Federal Geographic Data Committee’s Coastal and Marine Ecological Classification Standard (CMECS) (FGDC, 2012). FBPI was derived with an inner radius of 1 and an outer radius of 10, with a scale factor of 10 km. This finer scale layer identified smaller megahabitats or features on the scale of kilometres to tens of kilometres, as defined in Greene *et al.* (1999). These features included seamounts, abyssal hills, canyons, plateaux, large banks and terraces.

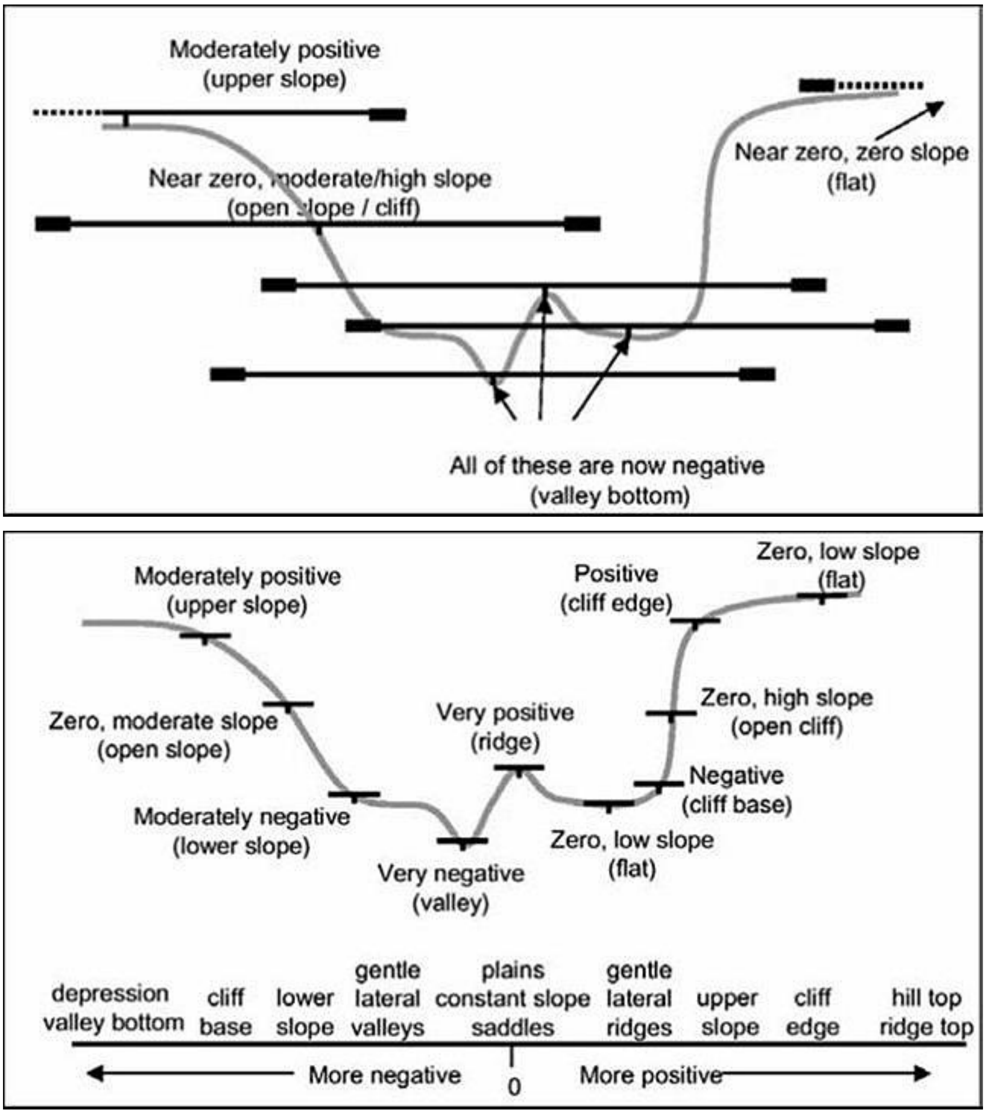

Figure 2.2 Identifying different topographic features using Bathymetric Position Index at a broad (top) and fine (bottom) scale. Figures from Weiss (2001).

2.2.4.1.2 Particulate Organic Carbon

In the CCZ there is a strong gradient in POC, decreasing from east to west, and from south to north (Lutz *et al.*, 2007; Pennington *et al.*, 2006; Smith *et al.*, 1997). Equatorial upwelling results in high productivity and moderate to high POC flux to the abyssal seafloor in south-eastern parts of the CCZ. To the north, however, the North Pacific Gyre leads to oligotrophic conditions and very low POC flux. This gradient in POC across the CCZ has been linked to differences in faunal communities (e.g Smith *et al.*, 1997; Smith *et al.*, 2008a; Vanreusel *et al.*, 2016; Veillette *et al.*, 2007), and flux of POC to the seafloor was thus identified as a key driver of faunal communities in the CCZ.

Estimates for POC in the CCZ were obtained from a global model produced by Lutz *et al.* (2007). Lutz *et al.* (2007) modelled flux of POC to the seafloor based on water depth and seasonal variability in remote-sensed net primary productivity between 19 August 1997 and 24 June 2004.

2.2.4.1.3 Substrate

Although there is no global map of seabed substrate, in the CCZ polymetallic nodules provide most of the hard substrate in an otherwise soft sediment environment (Ebbe *et al.*, 2010) and nodule abundance thus gives a general idea of substrate across the region. Modelled estimates of nodule abundance across the CCZ region were obtained from *ISA Technical Study No. 6: A Geological Model of Polymetallic Nodule Deposits in the Clarion Clipperton Fracture Zone* (ISA, 2010b). This model was developed by a group of technical experts in response to a need identified by the ISA for better resource assessments across the CCZ. The model used both publically available and proprietary data.

While this list of predictor variables is not exhaustive of all variables driving community composition and diversity in the CCZ, these were thought to be some of the most important ones that would have the strongest influence on biological communities in this area, and that showed some variation between transects (Table 2.2).

Table 2.2 Environmental characteristics of each transect, as a mean value.

Transect	Depth (m)	BBPI	FBPI	POC (g Corg m^{-2} y^{-1})	Nodule abundance (kg m^{-2})
T1	4921	56	5	1.5909	6.90
T2	5034	-113	-10	1.4938	5.18
T3	4992	-69	23	1.4697	8.77
T4	4807	9	-29	1.5124	24.87
T5	4915	40	21	1.4325	9.25
T6	4846	13	42	1.6202	3.97
T7	4800	16	2	1.6637	6.30
T8	4790	23	12	1.6801	4.96
T9	4627	-2	43	1.5914	6.82

2.2.4.2 DistLM

Multivariate community composition data were log transformed for megafauna data to account for high abundances of xenophyophores, and square-root transformed for metazoan data to account for high abundances of common morphotypes. Bray-Curtis similarity matrices were then produced for multivariate megafauna and metazoan community composition data, and matrices based on Euclidean distances were produced for univariate megafauna and metazoan diversity indices (morphotype richness, Shannon diversity and Simpson diversity). Environmental variables were visualised using draftsman’s plots, transformed to approximate normality (where applicable) and normalised. Correlations between transformed environmental variables were also assessed using the Spearman correlation coefficient.

DistLM was run separately on multivariate community composition and univariate diversity data against all environmental variables using stepwise selection and the Akaike information criterion for small sample sizes (AICc, Akaike, 1978), with 999 permutations. Significant models were visualised using distance-based redundancy analysis (dbRDA). All analyses were carried out in PRIMER v.6.

2.2.5 Altitude tests

In order to test whether there were significant differences in altitude between transects, and whether this had affected annotations, several tests were carried out. Data were visualised using plots and tested for normality using Shapiro-Wilk, Lilliefors and Pearson's chi-squared tests. The distribution of residuals was not normal ($p<0.05$ in all cases), and a Kruskal-Wallis test was therefore used to test for significant differences in altitude between transects. Kruskal-Wallis is a non-parametric test with no assumptions about normality, and is appropriate for use with unequal sample sizes. Post-hoc pairwise comparisons were carried out using Wilcoxon signed-rank tests to identify where the significant differences occurred.

In addition, annotations were pooled into groups based on altitude (differing by one metre above the seafloor) and a Kruskal-Wallis test carried out to test for significant differences in the number of annotations at different altitudes. This was followed by Wilcoxon post-hoc tests. All analyses were carried out in R x64 3.5.1.

2.3 Results

2.3.1 Habitat description

During the analysis of images for megafauna data, some qualitative observations were made of the habitats encountered (Figure 2.3). Nodule abundance within and between transects ranged greatly, from no nodules visible with an image, to nearly complete cover of the seafloor. Based on observations, nodule abundance was predominantly medium to high. Nodule size also appeared to vary (as seen in Figure 2.3b and c). In addition to nodules, other hard substrates encountered were cobbles, some boulders (although not common), and whale bones. On several occasions trace fossils called *Paleodictyon nodosum* were also observed. These are a regular hexagonal network of tunnels or holes in the seafloor, and it is currently unclear if these trace fossils are a biogenic structure (like a burrow), or an organism (such as a sponge or a xenophyophore) (Durden *et al.*, 2017b). Finally, the topography of the study area was primarily flat, with some gently sloping areas observed.

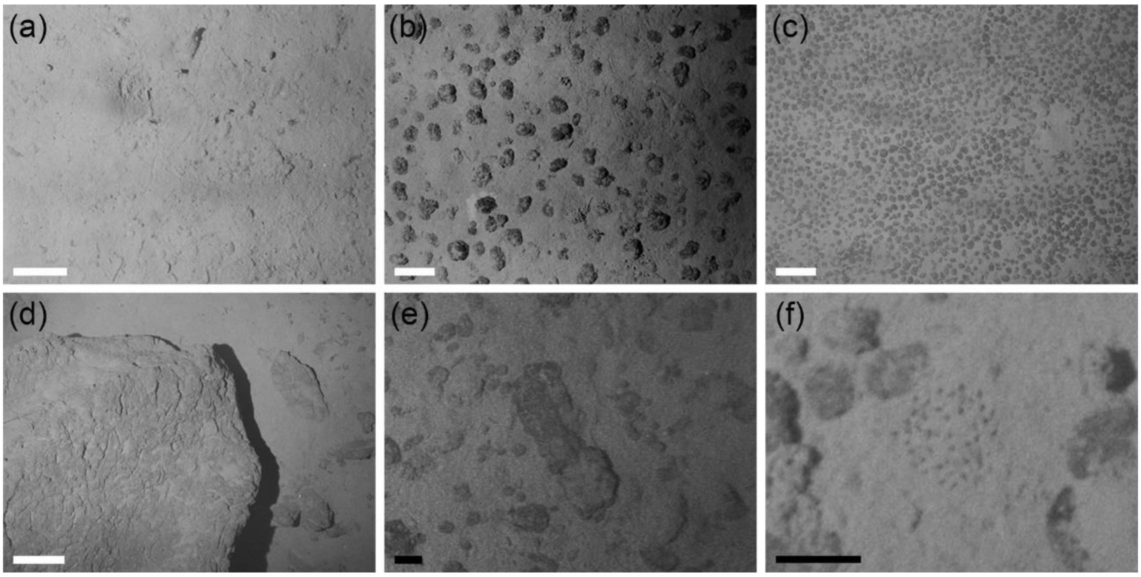

Figure 2.3 Nodule cover in the study area ranged from (a) no nodules, to (b) medium nodule cover and (c) high nodule cover. Also observed in the transects were (d) large boulders, (e) whale bones and (f) *Paleodictyon nodosum* fossils. White scale bar = 20cm, black scale bar = 5cm.

2.3.2 Epibenthic megafauna communities in the CCZ

2.3.2.1 Regional community composition

A total of 7 201.98 m^2 of seabed was observed in this study, comprising 895 images and 32 120 megafauna individuals (30 464 protists and 1 656 metazoans). In total 90 morphotypes were identified, belonging to nine phyla: eight metazoan – Annelida, Cnidaria, Bryozoa, Chordata, Arthropoda, Echinodermata, Mollusca and Porifera; and one protistan – Foraminifera (Figure 2.4, Table A.1, Table A.2). As with other studies using image sampling, poor image quality and resolution, difficulty with identifying organisms from images and the presence of cryptic species mean that this is likely an underestimate of the true number of species in the area. There were also many organisms that could not be identified.

The most morphotype-rich phylum was the Echinodermata, with 35 morphotypes, 21 of which were holothurians (Table 2.3). This group also consisted of several morphotypes of Asteroidea, Crinoidea, Echinoidea, and Ophiuroidea. The next phylum with the highest number of morphotypes was Cnidaria, with 29. This group had a number of different Actiniaria and Antipatharia morphotypes, with fewer Pennatulacea morphotypes and just 1-2 morphotypes each of Alcyonacea, Hydrozoa, Ceriantharia and Coralliomorpharia. Although there are several alcyonacean species or morphotypes in the CCZ (e.g. Amon *et al.*, 2016; Dahlgren *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Tilot *et al.*, 2018; Vanreusel *et al.*, 2016), this group was impossible to differentiate further given the image quality and resolution.

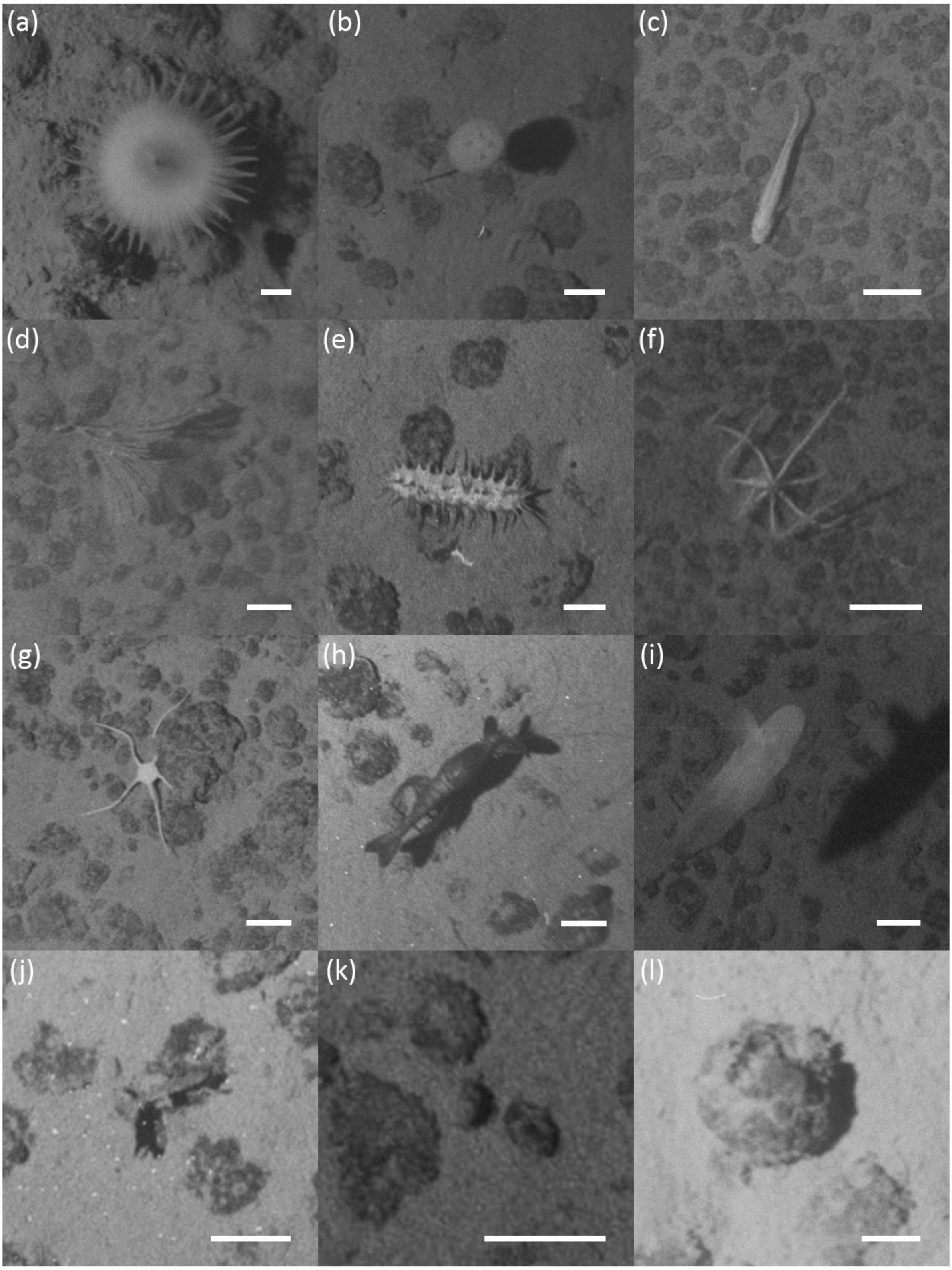

Figure 2.4 Examples of megafauna identified in image analyses from the central CCZ. (a) Actiniaria morphotype, (b) *Hyalonema* morphotype, (c) Ophidiidae morphotype, (d) Antipatharia morphotype, (e) Holothuroidea morphotype, (f) *Freyella* morphotype, (g) Ophiuroidea morphotype, (h) *Cerataspis* morphotype, (i) *Grimpoteuthis* morphotype, (j) plate-like xenophyophore, (k) reticulate xenophyophore, (l) tubular xenophyophore. Scale bars represent 5 cm.

There were six Chordata morphotypes, divided amongst Actinopterygii and Ascidiacea, and the remaining six phyla all contained four or less morphotypes. All Annelida were grouped under a generic morphotype label due to difficulty with identification, and Bryozoa, Arthropoda and sponges all comprised of four morphotypes. Sponges were extremely difficult to identify to lower taxonomic groupings due to image quality and resolution, and this group was thus split into a generic Porifera group, Demospongiae, Hexactinellida and *Hyalonema*. There were three Mollusca morphotypes identified, and, finally, the xenophyophores were divided into four groups: a generic xenophyophore group and plate-like, reticulate and tubular morphotypes. Although this is a gross underrepresentation of the diverse xenophyophore community found within the CCZ (Gooday *et al.*, 2017b), xenophyophore species are difficult to distinguish, even with collected specimens, and generic groupings were thus used.

The mean megafauna density across all transects was 4.46 ind.m^{-2} (s.e. = 0.4), with a mean metazoan density of 0.23 ind.m^{-2} (s.e. = 0.03). By far the most numerically abundant group was the protistan xenophyophores, which made up 95% of the total megafauna abundance observed. Plate-like and reticular morphotypes were found in an order of magnitude greater density than morphotypes in all other phyla. The next most abundant group was the Cnidaria (Table 2.3), which was numerically dominated by actiniarians and alcyonaceans. This was followed by echinoderms, principally echinoids, ophiuroids and holothurians, and sponges. Annelids, arthropods, bryozoans, chordates, and molluscs were all found in extremely low densities, with fewer than 10 individuals observed in each phylum across all nine transects.

Table 2.3 Megafauna morphotype richness and average density. Data pooled across all transects.

Phylum	**Morphotype richness**	**Av density (ind.m^{-2} ± s.e.)**
Annelida	1	0.014 (0.003)
Arthropoda	4	0.0007 (0.0003)
Bryozoa	4	0.001 (0.0005)
Chordata	6	0.001 (0.0005)
Cnidaria	29	0.10 (0.02)
Echinodermata	35	0.06 (0.02)
Mollusca	3	0.0008 (0.0003)
Porifera	4	0.04 (0.01)
Total (metazoans)	**86**	**0.23 (0.03)**
Xenophyophores	4	4.23 (0.43)
Total (megafauna)	**90**	**4.46 (0.40)**

Of the 90 morphotypes observed, 68 (76%) had fewer than 10 individuals recorded, and 25 (28%) were observed on only one occasion (Figure 2.5). These rare morphotypes were mainly arthropods, bryozoans, chordates and molluscs, although many cnidarian and echinoderm morphotypes were also observed on less than ten occasions, e.g. several actiniarian, antipatharian, sea pen, hydrozoan, asteroid, crinoid, echinoid and holothurian morphotypes. Excluding xenophyophores, just four phyla accounted for 98% of the metazoan abundance: Annelida, Cnidaria (mainly Actiniaria and Alyconacea), Echinodermata (mainly Echinoidea, Holothuroidea and Ophiuroidea), and Porifera.

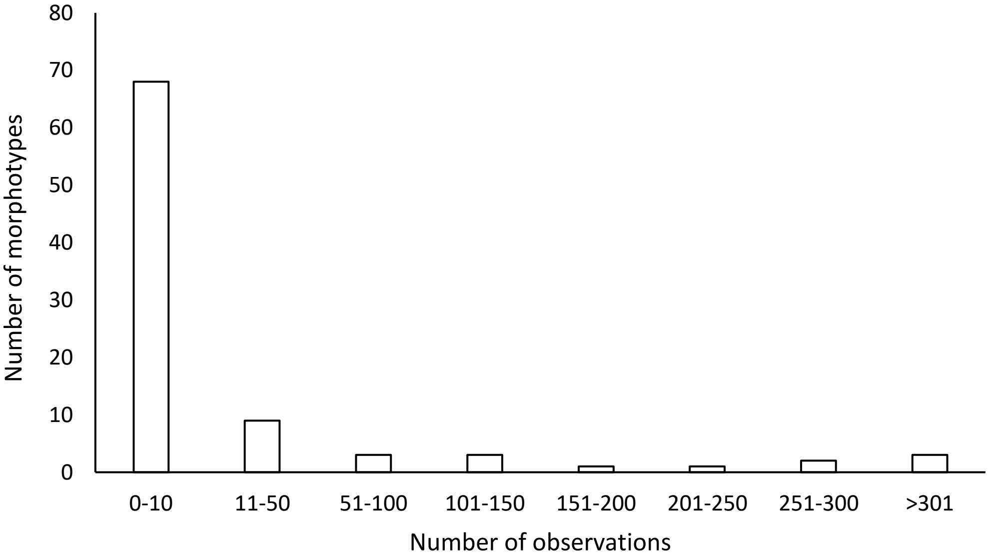

Figure 2.5 Frequency of morphotype observations in the CCZ, with data pooled across all transects. The OMCO megafauna communities observed were dominated in terms of number of individuals by a few, very abundant morphotypes, with many rare morphotypes observed less than 10 times across all transects.

2.3.2.2 Local community composition

All transects were fairly similar in terms of community composition (Bray-Curtis similarity ranged from 67.1 – 93.9%, Table A.4). Transects T5 and T6 were the most similar, followed by T4 and T9 and T2 and T4. Transects T1 and T3 were the most different. When metazoan density data was analysed separately from xenophyophores, Bray-Curtis similarity indices were lower (Table A.5). Transects T7 and T9 were the most similar, while transects T1 and T9 were the most different. This was followed closely by T5 and T9.

There was some variation in the density of megafauna recorded in different transects (Figure 2.6, Figure 2.10a, Table 2.5). Transects T2 and T9 both had over double the megafauna density of T6 and T5. When metazoans were considered alone, these differences became more pronounced. This was driven by overall lower densities in T1 and T5, but particularly fewer Actiniarians, Alcyoneans, Holothurians and sponges. Interestingly, transect T9 had extremely high numbers of echinoids compared to all other transects, and a high number of ophiuroids (Figure 2.6, Table A.1). Transect T2, on the other hand, had very few ophiuroids but much higher numbers of Actiniarians and Alyconaceans than all other transects.

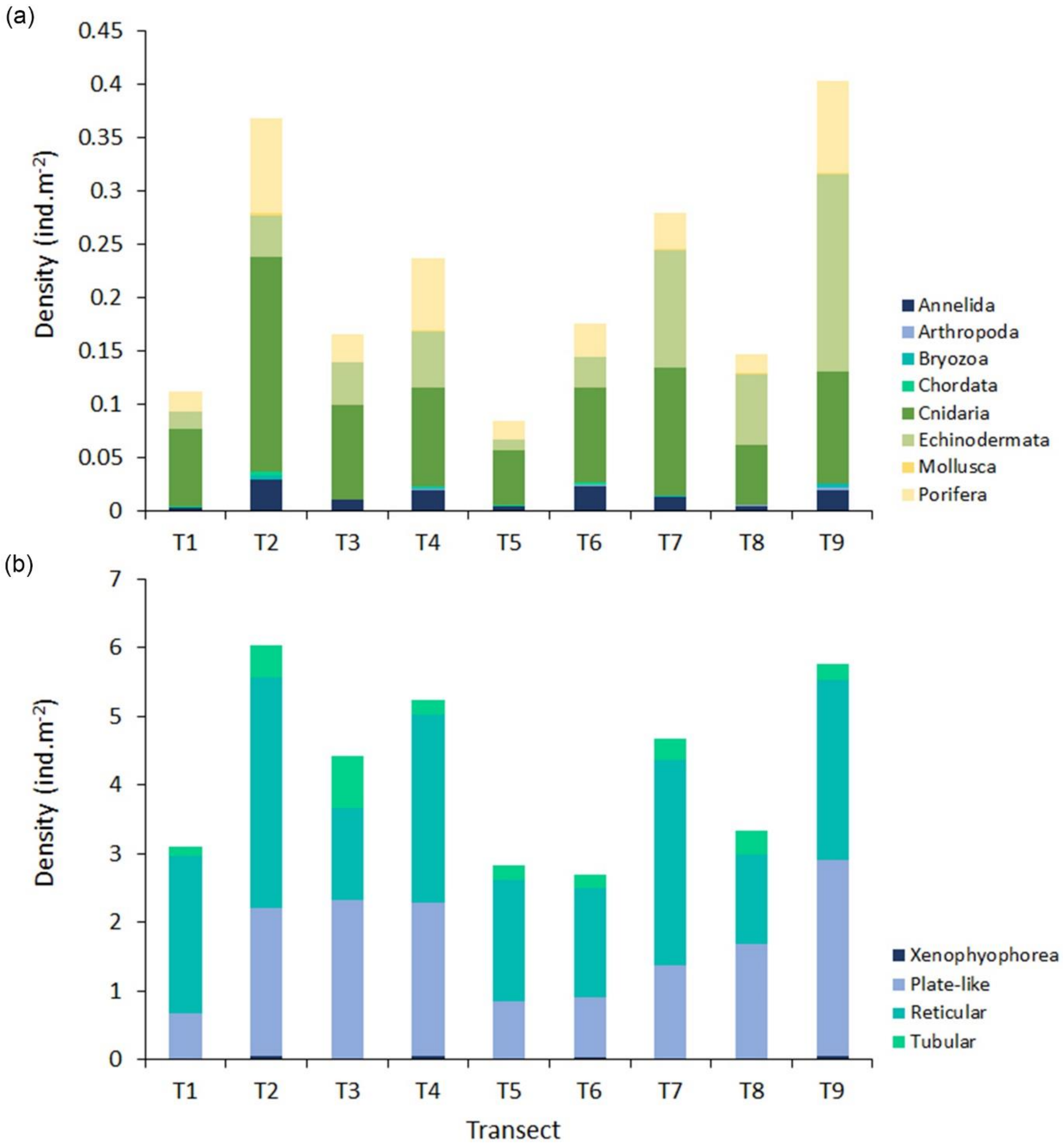

Figure 2.6 Density of (a) metazoan phyla and (b) xenophyophore morphotypes, showing variation in overall densities and taxonomic composition among transects.

Cluster analysis identified two significant clusters (Table A.10). Megafauna data clustered into one group consisting of transect T9, and another group containing all other transects. Metazoan data clustered into two, more even groups (Figure 2.7). No further significant clusters were identified within either data set, and transects in close geographic proximity did not cluster together. The clusters produced for metazoan data were broadly separated by depth, although this was not formally tested (Figure 2.7).

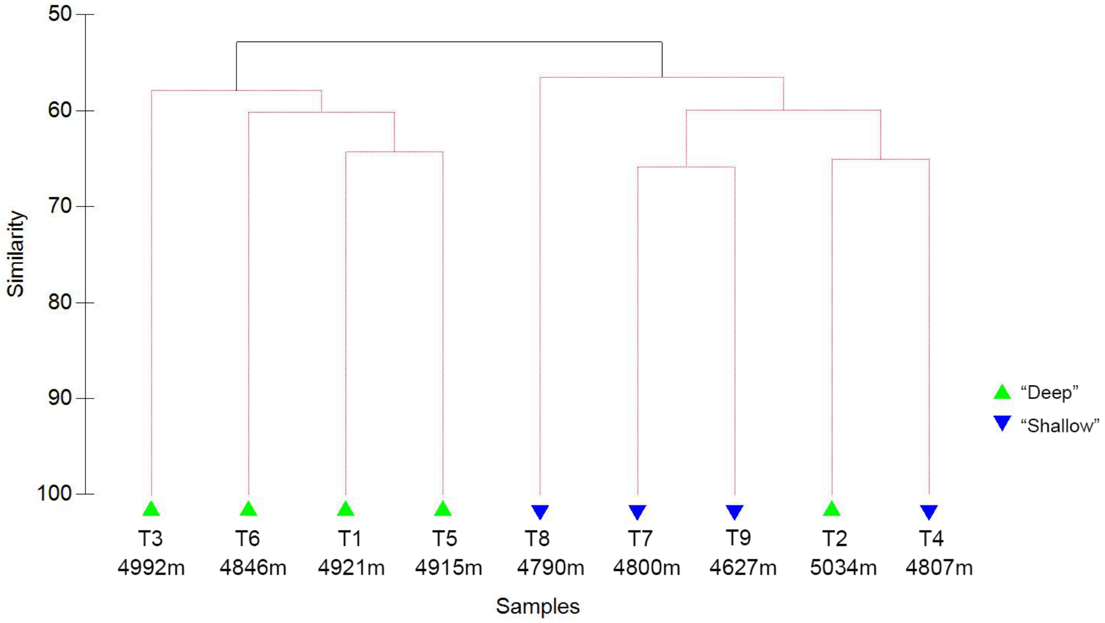

Figure 2.7 Hierarchical agglomerative clustering with group average linkage identified two significant clusters of square-root transformed metazoan community composition data. Solid black lines indicate significant clustering; red dashed lines indicate clustering that could not be significantly differentiated.

2.3.2.3 Regional life history characteristics

In terms of feeding strategies, 51% of the megafauna morphotypes (across all transects) were suspension feeders, with 29% of the morphotypes deposit feeders, and a smaller proportion (20%) predators and scavengers. In terms of abundance, 72% of the metazoans observed were suspension feeders (mainly cnidarians and sponges), with 25% deposit feeders and the remainder predators and scavengers (Table 2.4, Figure 2.8a). When xenophyophores were included, the CCZ megafauna consisted of > 98% suspension feeders in terms of abundance, due to the highly dominant nature of xenophyophores.

The number of megafauna and metazoan morphotypes that were mobile and sessile was approximately equal, with roughly 50% of the morphotypes in both communities belonging to each functional group. When taking into consideration the abundance of fauna, however, a different picture was seen (Table 2.4, Figure 2.8b). Over 98% of megafauna individuals were sessile, driven by the dominance of sessile xenophyophore morphotypes. In the metazoan community, on the other hand, 66% of individuals recorded were sessile.

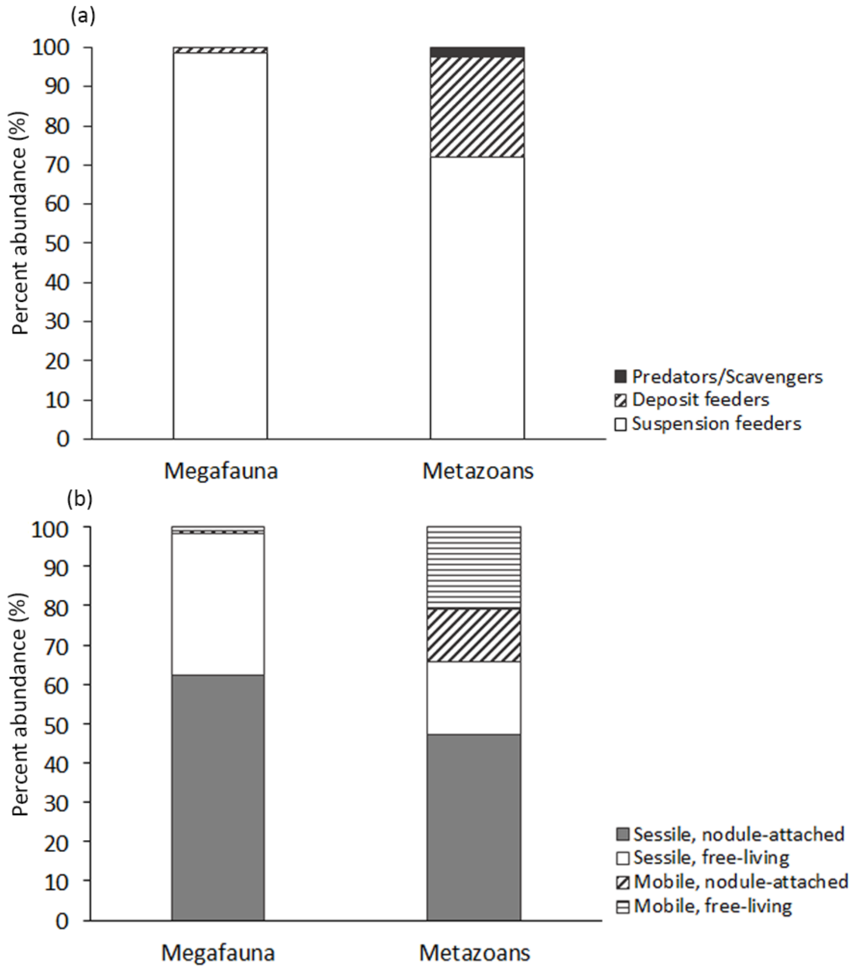

Figure 2.8 Percent abundance (%) of megafauna and metazoan individuals with different (a) feeding strategies, and (b) mobility and substrate attachment.

When comparing the total number of organisms observed attached to nodules, and those that were free-living, 63% of all megafauna observations were attached to nodules (Table 2.4). This figure was similar for metazoans when analysed separately, with 61% attached to nodules. A third of all morphotypes (30 morphotypes) had >70% of observations attached to nodules, although half of these were singletons.

Of the suspension feeding metazoans, 73% of individuals were found attached to nodules, with 48% of suspension feeding morphotypes having >70% of observations attached to nodules (although again, nearly half of these were singletons). Of the sessile metazoan fauna, 72% were attached to nodules, with the remaining 28% observed on the seafloor. The free-living sessile metazoans (34%) included some actinians, alcyonaceans, antipatharians and sponges. Interestingly, 40% of mobile metazoans were associated with nodules, including several holothurians, ophiuroids and asteroids, although this could be a result of high nodule abundance in images rather than preferential habitat choice or association. Of the 98% of megafauna that were sessile, 63% were attached to nodules. This included many xenophyophores as well as actinians, alcyonaceans, antipatharians, bryozoans, crinoids, sea pens and sponges. The 37% of sessile megafauna found free-living on the seafloor were mainly reticulate xenophyophore morphotypes (Figure 2.9).

Table 2.4 Life history characteristics of megafauna and metazoans observed in the CCZ, for all transects pooled at the regional level. Values given are percent abundance.

Life history characteristics	Megafauna	Metazoans
Suspension feeder (%)	98.6	72.1
Deposit feeder (%)	1.3	25.4
Predator/scavenger (%)	0.1	2.5
Sessile (%)	98.2	65.6
Mobile (%)	1.8	34.4
Nodule-attached (%)	62.9	60.8

Figure 2.9 One of the most abundant morphotypes observed was a reticulate xenophyophore, similar to species of the genus *Syringammina*. This morphotype was treated as a single group, but is more likely to include several different species. (a) Reticulate xenophyophores observed in the central CCZ during this study, (b) and (c) *Syringammina fragilissima* fields on the UK Atlantic margin. Image credits: (b) and (c) courtesy of the NERC funded Deep Links Project - Plymouth University, Oxford University, JNCC, BGS.

2.3.2.4 Local life history characteristics

There were some differences observed in the proportion of different feeding strategies amongst transects (Figure 2.10b, Table 2.5). For megafauna data, all transects were dominated (>96%) by suspension feeders, as a result of high densities of xenophyophores. The metazoan community also comprised a high proportion of suspension feeders, greater that 50% in all cases and as high as 90% suspension feeders in T5, which was dominated by actiniarians (Figure 2.6). Transect T7 had the highest proportion of deposit feeders at 30%, driven by a large number of ophiuroids, compared to the lowest record of only 4% in T5. Transect T9, with the lowest proportion of suspension feeders, had the highest number of predator and scavenger records (mainly annelids and decapods) at 24%. In T2, however, only 3% of metazoans recorded were predators or scavengers.

There was also some variation in the mobility of megafauna and metazoan communities observed (Figure 2.10c, Table 2.5). The megafauna community was dominated by sessile individuals, which formed >96% of the community in all transects, again as a result of the extremely high densities of sessile xenophyophores. The proportion of sessile and mobile individuals was more variable when looking at the metazoan community. Here, transect T9 had the lowest proportion of sessile individuals with approximately half of the individuals observed being mobile. This was driven by high densities of ophiuroids, holothurians and urchins. Transect T5 had the highest proportion of sessile individuals (84%), driven by a large proportion of actiniarians and overall low densities of all other taxa.

Finally, in terms of substrate attachment, transect T2 had the highest proportion of megafauna individuals attached to nodules at 77%, compared to only 49% of individuals in T1 (Figure 2.10d, Table 2.5). Transect T5 also had a high number of individuals attached, with 69%. When metazoans were analysed separately, the range of values was fairly similar (40% - 78%), but the rank order was different. Transect T8 had the lowest level of attachment with just 40% of individuals on nodules, and T2 still had the greatest level of attached fauna. This was likely driven by large differences in the number of sessile actiniarians, alcyonaceans and sponges in T2 and T8 (Table A.1).

Table 2.5 Summarised megafauna and metazoan data per transect. Abundance is the total number of organisms > 1 cm recorded. SF = suspension feeder, DF = deposit feeder, P = predator, SC = scavenger; NA = Nodule-attached. Life history data are given as percent of total abundance at each transect.

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Megafauna									
Abundance	2 571	5 150	3 663	4 396	2 323	2 305	3 971	2 783	4 958
Density (ind.m^{-2})	3.21	6.44	4.58	5.48	2.90	2.88	4.96	3.48	6.20
No. morphotypes	29	44	42	36	21	30	33	35	49
SF (%)	99.38	99.32	99.10	98.95	99.70	98.96	97.66	98.13	96.85
DF (%)	0.39	0.47	0.57	0.34	0.13	0.56	1.81	1.08	1.47
P/SC (%)	0.23	0.21	0.33	0.71	0.17	0.48	0.53	0.79	1.67
Sessile (%)	99.34	98.85	98.88	98.59	99.53	98.13	97.38	97.99	96.45
Mobile (%)	0.66	1.15	1.12	1.41	0.47	1.87	2.62	2.01	3.55
NA (%)	48.74	77.16	54.44	63.38	68.83	66.07	59.51	52.86	65.35
Metazoans									
Abundance	92	316	137	196	69	145	235	121	345
Density (ind.m^{-2})	0.11	0.39	0.17	0.24	0.09	0.18	0.29	0.15	0.43
No. morphotypes	25	40	38	32	17	26	29	31	45
SF (%)	82.61	88.92	75.91	76.53	89.86	83.45	60.43	57.02	54.78
DF (%)	10.87	7.59	15.33	7.65	4.35	8.97	30.64	24.79	21.16
P/SC (%)	6.52	3.48	8.76	15.82	5.80	7.59	8.94	18.18	24.06
Sessile (%)	81.52	81.33	70.07	68.37	84.06	70.34	55.74	53.72	48.99
Mobile (%)	18.48	18.67	29.93	31.63	15.94	29.66	44.26	46.28	51.01
NA (%)	46.74	77.85	49.64	65.31	71.01	64.14	53.19	40.50	59.71

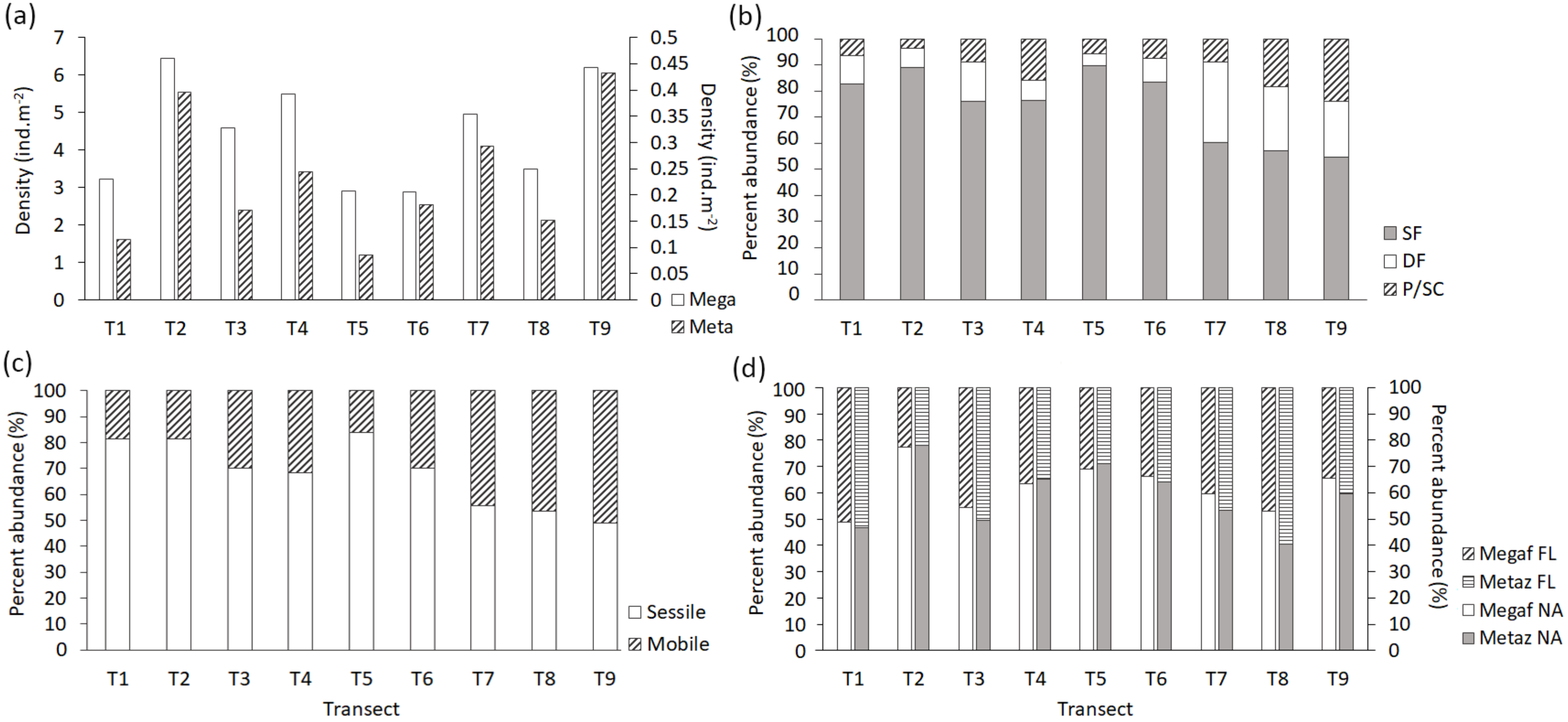

Figure 2.10 Density and life history characteristics of megafauna and metazoan communities by transect, showing (a) density of individuals, (b) feeding strategies, (c) mobility and (d) substrate attachment. Megafauna data are not shown in panels (b) and (c) as this community was dominated by suspension feeders and sessile individuals due to the highly abundant nature of xenophyophores, with very little variation amongst transects. SF = suspension feeder, DF = deposit feeder, P = predator, SC = scavenger, NA = nodule-attached, FL = free-living.

2.3.2.5 Regional diversity

At a regional level, megafauna and metazoan diversity estimates followed similar patterns, although metazoan diversity estimates were lower than megafauna estimates for all but individual-based Shannon and Simpson diversity estimates (Table A.3, Figure A.1, Figure A.2). Observed megafauna morphotype richness was 90, with asymptotic estimates ranging from 114 – 134, depending on data type (individual- or sample-based). Metazoan morphotype richness was 86, with asymptotic estimates from 110 – 130.

For both megafauna and metazoan data, estimates of morphotype richness at double the reference sample and asymptote were greater for individual-based data than for sample-based curves. Individual-based asymptotic morphotype richness and Chao 1 estimates were nearly 1.5 times observed values, while sample-based asymptotic estimates were only slightly greater than observed values. Asymptotic estimates of morphotype richness should, however, be treated with caution as they may be subject to large prediction bias.

Observed megafauna Shannon and Simpson diversity for individual-based data were much lower than metazoan estimates. Increases in Shannon diversity and particularly Simpson diversity at double the reference sample size and asymptote were small for both individual- and sample-based data, implying that these estimates were near asymptote for the given sample.

2.3.2.6 Local diversity

At the transect, or local, level morphotype richness estimates were higher for megafauna for both individual- and sample-based data (Table A.6), while Shannon and Simpson diversity estimates were higher for metazoans (Table A.7). This is a result of the dominant nature of the xenophyophores and the differing sensitivity to rare species of each diversity index (Soetaert & Heip, 1990). It is important to note here that estimates of morphotype richness beyond double the reference sample size should be treated with caution, as they may be unreliable due to large prediction bias.

While the rank order of transects changed at times depending on whether individual- or sample-based data was used, and the reference sample, double the reference sample or asymptote, there were some general patterns observed in the data. For the megafauna, several transects had consistently high diversity estimates (T2, T3, T8 and T9, Figure 2.11). Although the order of highest diversity estimate changed amongst these transects, they were consistently at the top and often had significantly higher estimates than other transects. There were also two transects with consistently low diversity estimates (T5 and T1).

Slightly different patterns were observed for the metazoan community (Figure 2.12). Like the megafauna data, transects T3 and T8 consistently had high diversity estimates, with significantly greater estimates for all diversity measures than most other transects, and T5 generally had low diversity estimates. Unlike the megafauna data, however, T1 had some of the highest diversity measures, while T2 had some of the lowest. Another key difference was T9, which had fairly middle of the range to low Shannon and Simpson diversity estimates, where for megafauna data this transect consistently had some of the highest diversity estimates.

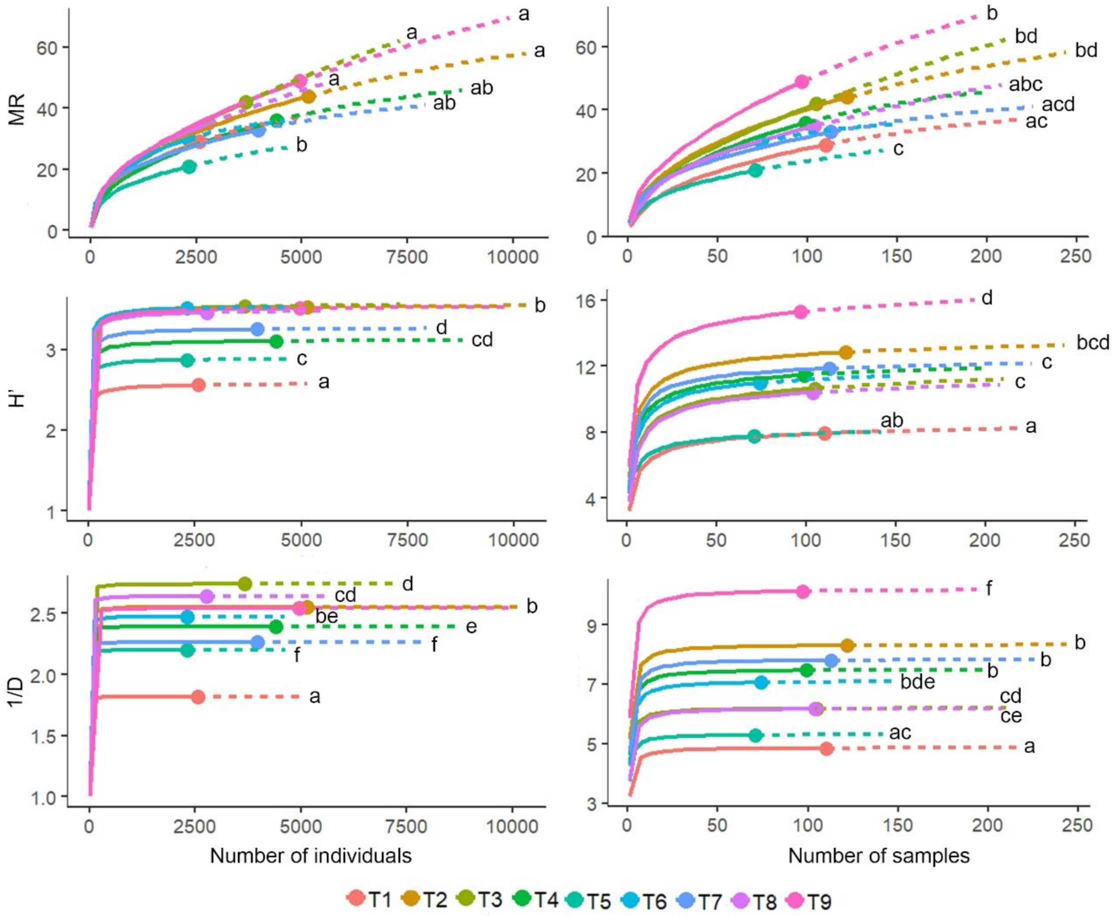

Figure 2.11 Rarefaction and extrapolation curves of diversity estimates for sample- and individual-based megafauna data, comparing transects T1-T9 in the CCZ. Morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (individual or image). Solid lines indicate rarefaction, dashed lines indicate extrapolation, and solid points indicate observed values. Significant differences identified through non-overlapping confidence intervals (removed for clarity) are denoted with letters a-f.

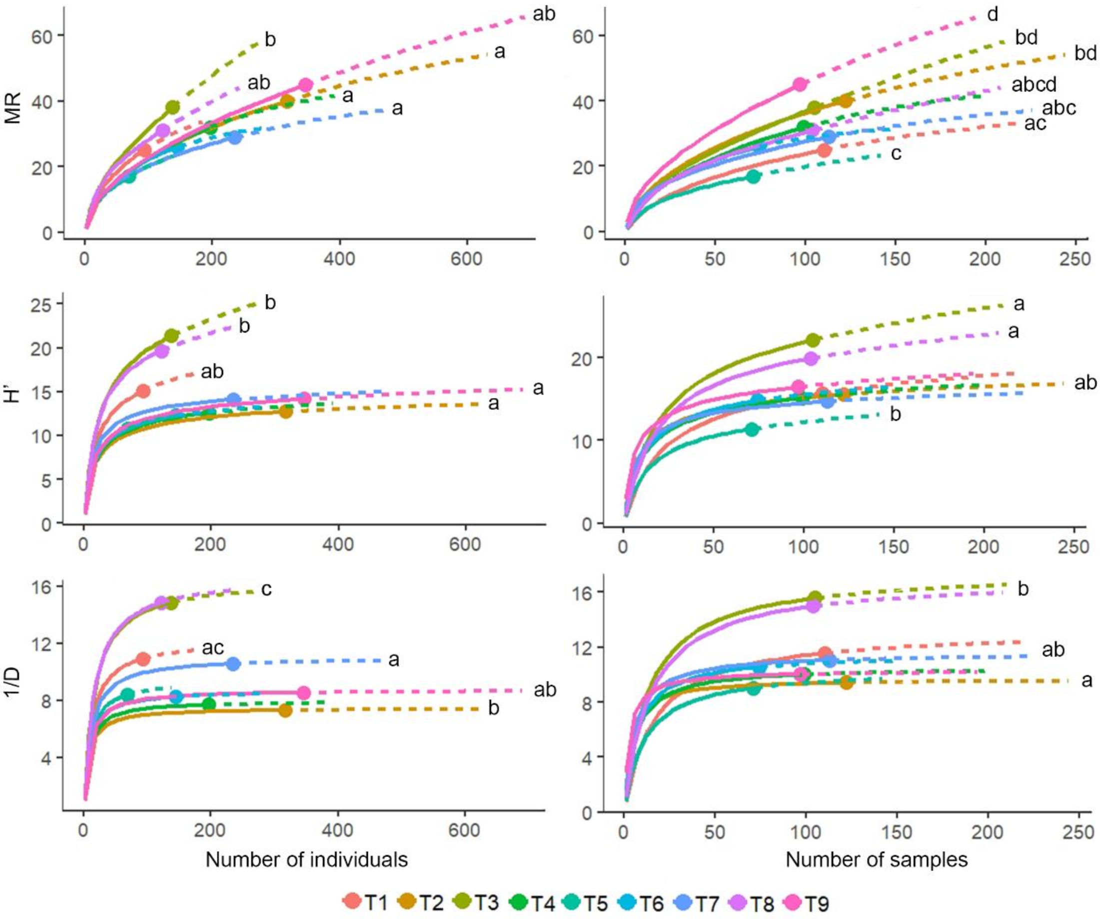

Figure 2.12 Rarefaction and extrapolation curves of diversity estimates for sample- and individual-based metazoan data, comparing transects T1-T9 in the CCZ. Morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (individual or image). Solid lines indicate rarefaction, dashed lines indicate extrapolation, and solid points indicate observed values. Significant differences identified through non-overlapping confidence intervals (removed for clarity) are denoted with letters a-d.

2.3.3 Environmental links

The results of DistLM identified several environmental variables as explaining a significant proportion of the variation in megafauna and metazoan community composition and diversity (Figure 2.13, Table 2.6). Depth explained 21% of the variation in megafauna community composition, while BBPI explained 56% of the variation in megafauna morphotype richness ($p<0.05$). Given BBPI, depth was close to significant in explaining a further 21% of variation in megafauna morphotype richness ($p=0.059$). BBPI also explained 61% of the variation in megafauna Shannon diversity, and together with POC explained a further 18% of the variation, to account for a total of 79% of the total variation ($p<0.05$ in most cases). BBPI also explained 60% of the variation in megafauna Simpson diversity ($p<0.05$).

No environmental variables were significant in explaining variation in metazoan community composition, although depth was close to significant in explaining 19% ($p=0.077$). BBPI explained 56% of the variation in metazoan morphotype richness ($p<0.05$), and, given BBPI, depth explained a further 21%, to explain a total of 77% of the variation ($p=0.06$).

There were no strong correlations between environmental variables (Table A.11).

Table 2.6 DistLM outputs, identifying environmental variables explaining a significant proportion of megafauna and metazoan community composition and diversity. MR = morphotype richness, H’ = Shannon diversity, 1/D = Simpson diversity.

Measure	**Environmental variable**	**Pseudo -F**	**Proportion of variation explained**	**p**
Megafauna				
Community composition	Depth	1.8166	0.20604	0.041
MR	BBPI	8.8527	0.55844	0.018
	+ Depth	5.6845	0.21482	0.059
H’	BBPI	10.794	0.60661	0.011
	+ POC	5.2908	0.18434	0.058
1/D	BBPI	10.409	0.59792	0.011
Metazoans				
Community composition	Depth	1.6327	0.18913	0.077
MR	BBPI	8.8527	0.55844	0.023
	+ Depth	5.6845	0.21482	0.06

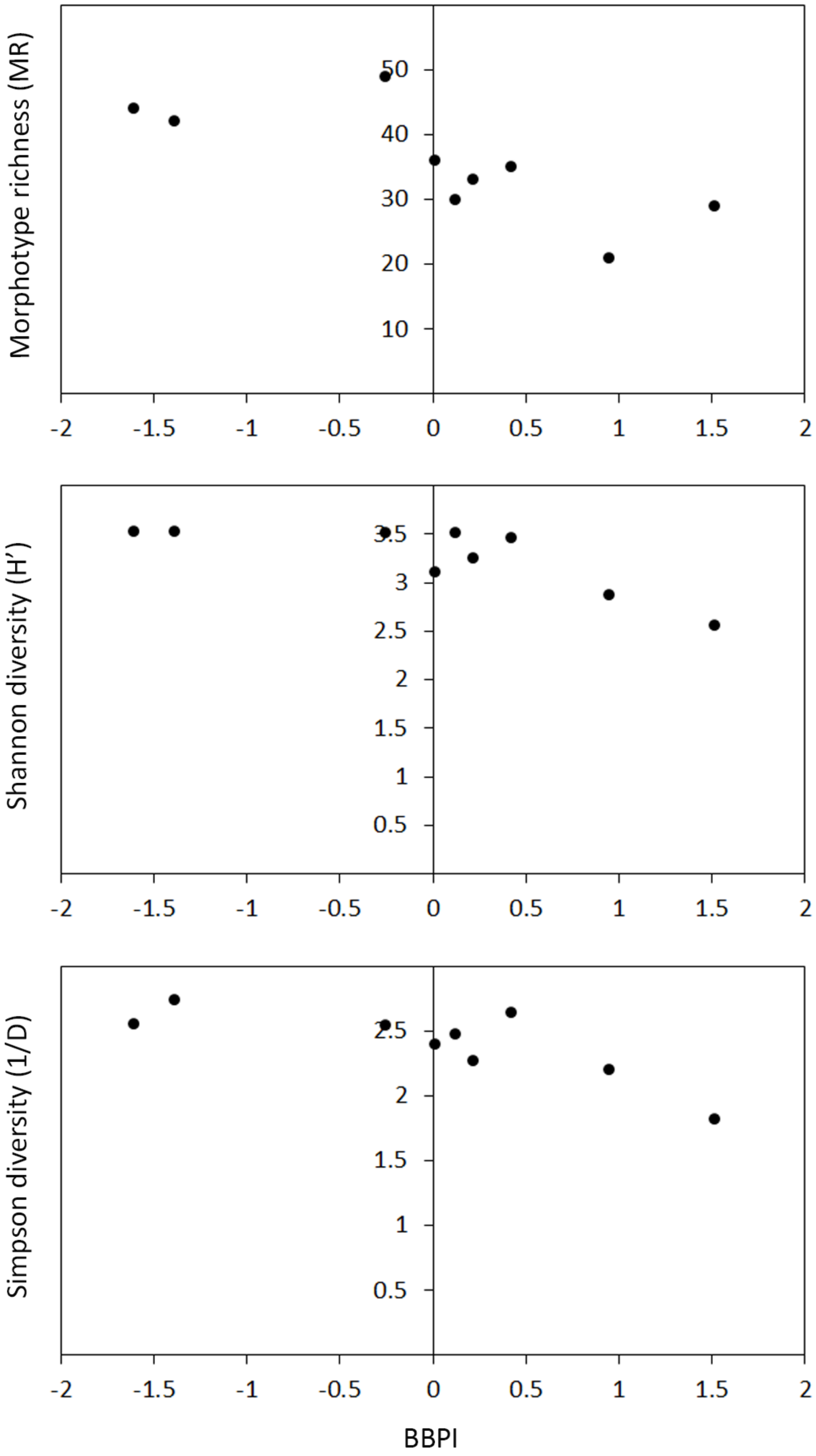

Figure 2.13 Relationship between megafauna morphotype richness, Shannon diversity and Simpson diversity and transformed, normalised broad-scale bathymetric position index (BBPI).

2.3.4 Altitude tests

The transects in this study were found to have significantly different altitudes (Kruskal-Wallis chi-squared = 90.623, df = 8, p<0.05) (Figure 2.14). In addition, there was a significant difference in the number of megafauna and metazoan annotations made in images at different altitudes, with significantly fewer individuals recorded per metre squared in images with higher altitude (Kruskal-Wallis chi-squared = 226.73, 75.69, df = 4, p<0.05) (Figure 2.15). However, transects with similar altitudes and thus similar densities of annotations did not cluster together (Figure 2.7).

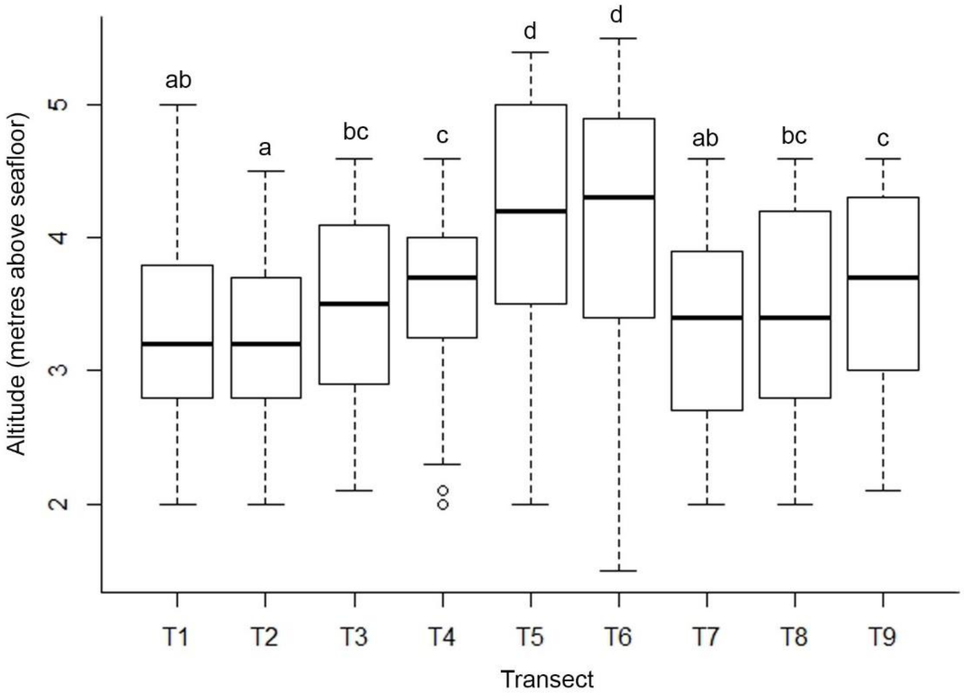

Figure 2.14 The average altitude per transect. Letters denote significant differences (p<0.05).

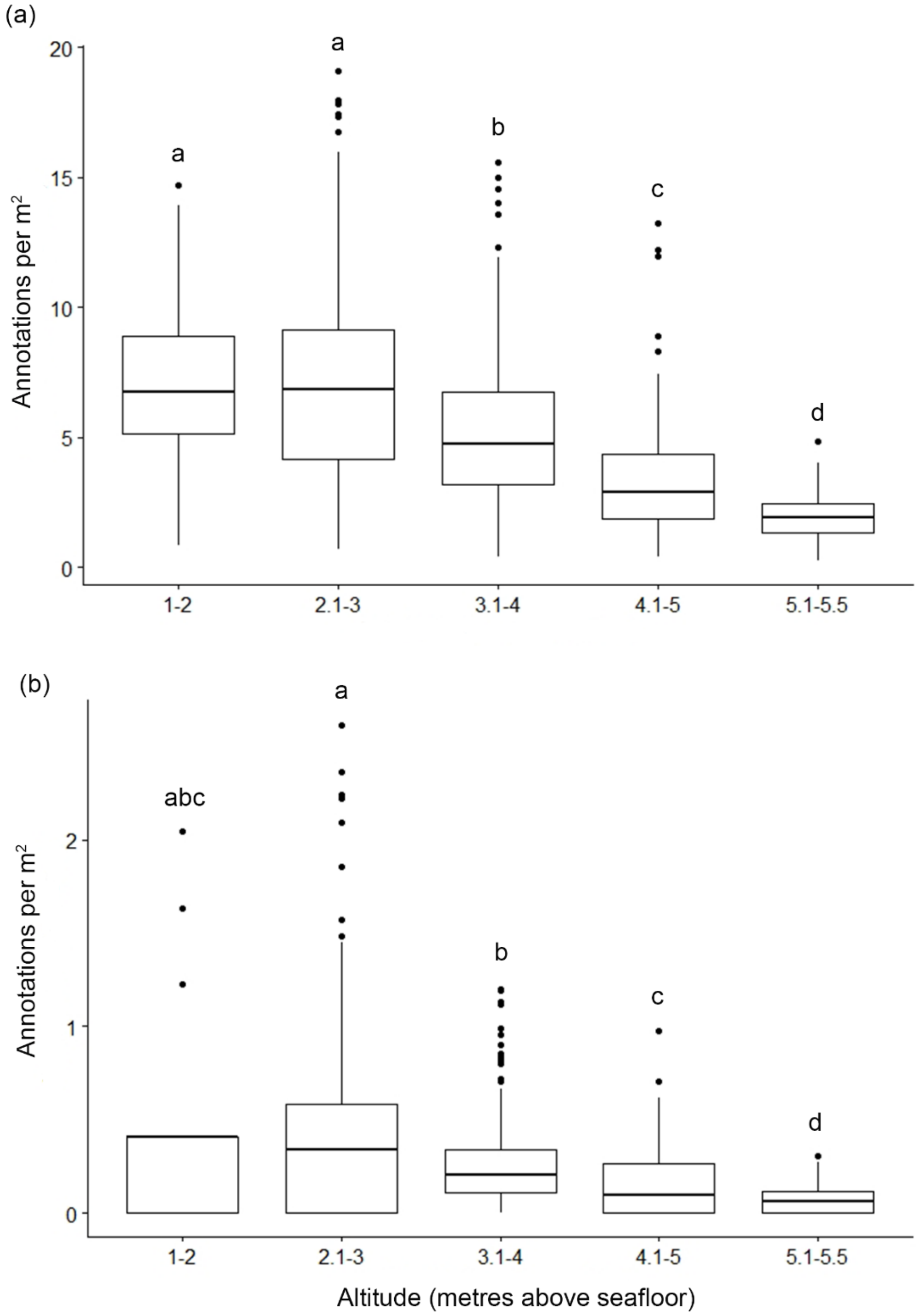

Figure 2.15 The number of annotations per m^2 at different altitudes above the seafloor for (a) megafauna and (b) metazoan data. Letters denote significant differences ($p<0.05$).

2.4 Discussion

The aims of this study were to increase baseline ecological knowledge of the epibenthic megafauna communities associated with nodule provinces, and to identify any potential environmental drivers of patterns in megafauna distribution. As previous studies have found that including xenophyophores in analyses of community data can substantially affect the outcomes (Simon-Lledó *et al.*, 2019b), patterns in the metazoan community were examined separately, in addition to those in the megafauna community as a whole (metazoans and xenophyophores).

In this discussion, key findings in the community composition, life history characteristics and diversity of the megafauna and metazoan communities at both a regional and local scale are highlighted, and observations are compared to other CCZ and nodule-free abyssal plain sites. The importance of various environmental variables in explaining megafauna and metazoan community composition and diversity are then discussed.

2.4.1 Epibenthic megafauna communities in the CCZ

2.4.1.1 Regional community composition

The regional megafauna community observed here had a core group of a few, widespread, dominant morphotypes distributed over 1 000 km across the CCZ. This was consistent with current understanding of abyssal megafauna distributions, which are thought to be broad (Smith, Drazen & Mincks, 2006), but was based on morphological types and needs molecular work to allow species-level identification and genetic connectivity studies (Gollner *et al.*, 2017). There were many rare morphotypes found in very low densities, and often observed in only one location. This pattern is highly characteristic of deep-sea communities, and has been previously observed in the CCZ megafauna (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Tilot, 2006; Vanreusel *et al.*, 2016).

By far the most dominant group was the protist xenophyophores, which occurred at several orders of magnitude greater density than any metazoan morphotype. Dominance of benthic megafauna communities by xenophyophores has been observed in the CCZ (e.g. Amon *et al.*, 2016; Kamenskaya, Melnik & Gooday, 2013; Simon-Lledó *et al.*, 2019b); however, the xenophyophore densities observed here were exceptionally high in comparison to other studies. An average of 4.23 ind.m^{-2} (± 0.4) was observed in this study, which is far higher than the averages recorded in various sites across the central to eastern CCZ: 0.16 ind.m^{-2} (Kamenskaya, Melnik & Gooday, 2013, although this study saw a maximum of 12 ind.m^{-2}), 0.65 ind.m^{-2} (Amon *et al.*, 2016, no range given) and 2.22 ind.m^{-2} (Simon-Lledó *et al.*, 2019b, with a range of 1.54 - 2.99 ind.m^{-2}) . As the data were quality controlled using multiple observers and photographs were at lower resolution than these other studies, the high densities of xenophyophores observed here likely represent a real difference.

The metazoan community consisted predominantly of echinoderm and cnidarian morphotypes, and these phyla were also the most numerically abundant. There were several anthozoan orders with very low densities and few records (Antipatharia, Ceriantharia, Coralimorpharia and Pennatulacea), as well as all hydrozoans, bryozoans, chordates, arthropods and molluscs, and two echinoderm classes (Asteroidea and Crinoidea). These observations were consistent with what was found by (Morgan *et al.*, 1993) as well as other studies in the central, eastern and north-eastern CCZ (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Tilot *et al.*, 2018; Vanreusel *et al.*, 2016). Most of the morphotypes observed were similar to or the same as those recorded by (Amon *et* *al.*, 2016) and Simon-Lledó *et al.* (2019b), as documented in the latter's megafauna morphospecies catalogue for the CCZ region. However, there were several new morphotypes observed in the current study, which have since been added to the catalogue. These were all rare morphotypes (observed on less than ten occasions) and included a tunicate, two actiniarians, an antipatharian, a hydroid, three pennatulaceans, an asteroid, an echinoid and several holothurians (examples in Figure 2.16).

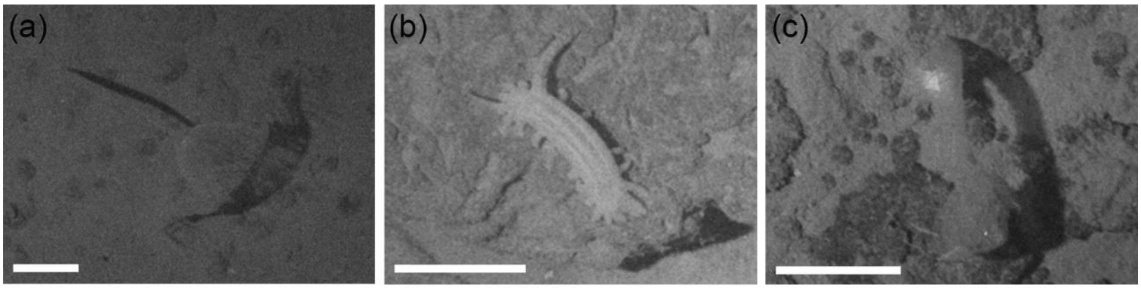

Figure 2.16 Examples of new morphotypes observed in this study: (a) a hydroid, (b) a *Scotoplanes* holothurian, and (c) a tunicate. White scale bars = 10cm.

2.4.1.2 Local community composition

At the local level, differences in metazoan density between transects was driven primarily by the density of echinoderms, cnidarians and sponges. Two transects, in particular, had much higher densities than others, with one further to the east dominated by echinoderms (mainly echinoids and ophiuroids), and the other further to the west dominated by cnidarians (mainly actiniarians and alcyonaceans). One transect in the very west had no records of ophiuroids. Unlike other studies, where alcyoncean corals dominated Cnidaria abundance (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Vanreusel *et al.*, 2016), actiniarians were more important in this study, with alcyonaceans only showing greater density in one transect.

Two transects in the centre of this study site had particularly low metazoan densities, and it is possible that this could be a result of lower detection rates due to a higher average altitude. Tests showed that a significantly lower number of annotations were made in images with higher altitude, although transects with similar average altitudes did not cluster together. Transects were selected based on a stratified design to validate the habitat classification in Chapter 4 (see section 2.2.2.2), and unfortunately no transects with lower altitude were available for analysis.

There was very little overlap of the many (76%) rare morphotypes between sites. This would suggest that there are many species that occur at very low densities across the CCZ or have very limited geographic ranges, although species accumulation curves also showed that sampling at each site was incomplete. Two transects that were separated by just 7.5 km in the west both contained an actiniarian morphotype that was not observed elsewhere. This would suggest a range size of less than 95 km (the distance to the next nearest transect), although of course incomplete sampling at sites could also have played a role, and this is based on morphological types not molecular studies. In addition, many holothurian morphotypes were observed in only one transect. This means that over a seabed area of ~7 200 m^2, these morphotypes were observed on only one occasion.

When transects were clustered, there was no grouping by geographic proximity, suggesting that there is high turnover of rare species even at very small scales. Two transects in the east (T7 and T8) that were separated by just 2.5 km shared 24 of the same morphotypes, and had 20 morphotypes that were contained in only one or the other transect. In addition, transect T7 contained double the number of actiniarians and nearly four times the number of ophiuroids as T8, although image quality in T8 was poor.

Some morphotypes, including an asteroid, a sea pen, an antipatharian, a holothurian and two mollusc morphotypes, were observed in one or more transects to the west, and then only seen again in a very easterly transect. This would suggest that these organisms have fairly large geographic ranges, but occur at extremely low densities. The genetic connectivity between these organisms is unknown, and the wider population-level consequences of removing even a few individuals of these low density species from mining areas are unknown.

Other morphotypes were found only in transects in the east, or only in those to the west. One Pennatulacea and two hydrozoan morphotypes were observed only in the west, while another three Pennatulacea morphotypes, a holothurian (*Psychronaetes* sp.) and a stalked crinoid were observed only in eastern transects. This suggests perhaps a preference for certain environmental conditions linked to longitude, but also potentially limited geographic ranges as a result of this. Rare species with small geographic extents would be more vulnerable to the potential impacts of mining than abundant, widespread species. Extinction risks could be high if, for example, entire populations of rare species were located within exploration contract areas in the central CCZ.

The impact on ecosystem functioning of losing rare species is not well understood (Jain *et al.*, 2014; Lyons *et al.*, 2005), and a precautionary approach to conservation would thus involve protecting rare species, as well as those that are more common (Lyons *et al.*, 2005; Tekalign *et al.*, 2017). This approach presents a conundrum for managing any activities in the deep sea, but particularly mining, because the geographic ranges of rare species in nodule dominated abyssal plains are not known (Paterson *et al.*, 2014), nor are the functions of these species and the services they provide (Le, Levin & Carson, 2016). In addition, there is little information available on population connectivity and larval dispersal of organisms in the CCZ (Kersten, Smith & Vetter, 2017; Paterson *et al.*, 2014). Further work in these areas is required in order to better understand and predict the extent of the potential impacts of seabed mining.

2.4.1.3 Life history characteristics

At the regional level, 51% of all morphotypes (metazoans and xenophyophores) were suspension feeders, and in terms of abundance, 72% of all metazoans recorded were suspension feeders (mainly cnidarians and sponges). Although the proportion of metazoan suspension feeders varied at the local level (between transects), they were always found in high (>50%) abundance. Suspension feeding organisms are thought to be particularly at risk from the resuspension and redeposition of sediment associated with mining activities, with plumes expected to bury and smother these organisms, clogging their feeding apparatus (Jumars, 1981; Radziejewska, 2002; Sharma *et al.*, 2001). As resuspended sediment is carried away from the area of mining by bottom currents and eddies, the effects of plumes are expected to be far reaching (Aleynik *et al.*, 2017; Bluhm, 2001), and our results suggest that a substantial proportion of the benthic metazoan community could be affected by this. The ability of organisms to resist and survive these conditions, as well as the wider ecosystem-level impact that loss of affected populations could have, are currently unknown (Gollner *et al.*, 2017), and are crucial to understand to support the development of thresholds and standards for mining (Levin *et al.*, 2016).

There was also a high proportion (>50%) of sessile metazoans in all transects, except T9, which was furthest east and had a high abundance of mobile ophiuroids and echinoids. This proliferation of sessile fauna was linked to the abundance of suspension feeding actiniarians and sponges. Transects further to the west were dominated by sessile metazoans, with up to 84% sessile. This has serious implications for the potential scale of impacts from mining. It is clear that everything in the direct path of mining equipment will be destroyed and it is highly unlikely that even mobile taxa like ophiuroids, echinoids, and holothurians will be able to move fast enough to escape. But the high proportion of sessile individuals observed means that the vast majority of megafauna individuals will also be unable to move to escape unfavourable conditions brought about by a sediment plume further afield. What is more, this has implications for the recovery of affected ecosystems. Mobile fauna may be able to move back into the area, but sessile fauna will not, and will have to rely on larval dispersal to recolonise over generations. It is important to note that this applies only to sediment-dwelling sessile fauna. Recovery times for hard substrate obligates would be on the scale of millions of years, due to the extremely slow growth rates of nodules (Gollner *et al.*, 2017). Some mobile fauna may also be reliant on sessile fauna, as is the case with xenophyophores (Levin, 1991) (discussed below). The connection between sessile life forms and suspension feeding is also highly problematic for mining impacts.

Finally, over half of the individuals (both megafauna and metazoan) observed in this study were attached to nodules. This is similar to observations made in other studies (Simon-Lledó *et al.*, 2019b), and suggests that the impacts of mining activities will be significant, as the recovery of fauna that depend on nodules for attachment is expected to be extremely slow (Jumars, 1981). It is important to note that these were not all nodule-obligates, but included facultative morphotypes.

2.4.1.4 Ecological importance of xenophyophores

Xenophyophore densities were on average 42 times higher than the most abundant metazoan phylum observed in this study, and were much higher than recorded in other studies. Xenophyophore density varied between transects, with no particular pattern, except that low densities were recorded in transects that had overall low numbers of records, possibly as a result of high altitude images.

Levin's review (1991) on xenophyophore and metazoan interactions showed that xenophyophores play an important role in structuring deep-sea metazoan communities, and suggested that these organisms can represent hot spots of activity. Xenophyophores have been shown to support a range of infauna, including nematodes, copepods, ostracods, tanaids, and isopods, which occupy spaces in the varied and complex test structure (Gooday, 1984). In addition, sediments surrounding xenophyophores may support increased abundances of infaunal species due to improved access to food (Levin & Thomas, 1988). Although the relationship wasn't quantified, in this study ophiuroids were seen associated with xenophyophores, particularly plate-like and reticulate morphotypes, on many occasions, as observed by Levin and Thomas (1988). Xenophyophore tests may also provide refuge from predators, perform a nursery function and stabilise sediments (Levin, 1991). Finally, xenophyophores may even represent a food source for some metazoans (Tendal, 1985), although this role is thought to be relatively unimportant (Levin & Gooday, 1992). Some of these services are still provided even after xenophyophores are dead, for example provision of refugia (Gooday, 1984), and, given their importance in supporting faunal communities, understanding the distribution of xenophyophores could be important for spatial planning and biodiversity conservation (Ashford, Davies & Jones, 2014).

In this study, as in many others, a large proportion (on average 62%) of xenophyophores were observed attached to nodules. It is currently unclear how many species that occur on nodules are also able to live in surrounding sediments (Gooday, Goineau & Voltski, 2015). While some species can occur in both habitats, there are certainly other species that are hard substrate obligates, and these will be most vulnerable to nodule mining, as the availability of appropriate habitat is reduced (Gooday, Goineau & Voltski, 2015).

What is more, the dominant xenophyophore morphotypes observed in this study are hypothesised to be suspension feeding, and are all sessile. This combination of sessile, suspension feeding, and nodule-attached traits means that these xenophyophores are particularly vulnerable to mining impacts (Gooday *et al.*, 2017b). The production of a sediment plume, physical disturbance to the seafloor and the removal of nodules would all potentially affect xenophyophore populations, and this could have wider impacts beyond the xenophyophore assemblages themselves, to affect the benthic community as a whole, from bacteria to meio-, macro- and megafauna (Gooday *et al.*, 2017b). It has been suggested that xenophyophores could play an important role in the recovery of systems after mining, by supporting many other organisms (Kamenskaya, Melnik & Gooday, 2013), although this will likely apply only to sediment-dwelling species that are able to recolonise disturbed areas (Gooday *et al.*, 2017b). Nodule-associated xenophyophores are unlikely to recover due to their reliance on hard substrate for attachment, and the extremely slow growth rates of nodules.

Interestingly, the most abundant xenophyophore morphotypes observed in this study differed to two recent megafauna studies in the eastern and north-eastern CCZ. In this study, reticulate morphotypes were by far the most abundant, while Amon *et al.* (2016) observed mainly plate-like morphotypes and Simon-Lledó *et al.* (2019b) identified a tubular morphotype, *Aschemonella monile*, as the most abundant organism. Accepting that it is very difficult to distinguish xenophyophore species from image data, and that different levels of taxonomic resolution were used in these studies, this would suggest that there is turnover in the dominant xenophyophore forms from the eastern to central CCZ (or observer bias), and this may be linked to environmental conditions. This should be examined further and considered in spatial planning.

2.4.1.5 Regional diversity and comparisons with other abyssal plain sites

Comparisons of species richness and other diversity measures between studies is difficult due to a number of reasons, including different sampling equipment, levels of sampling effort, taxonomic resolution and data quality. In general, the metazoan morphotype richness observed in this study (86 morphotypes over a seafloor area of 7 200 m^2) was low compared to recent studies from eastern (110 morphotypes over 2 268 m^2, Amon *et al.*, 2016) and north-eastern (70 morphotypes over 1 322 m^2, Simon-Lledó *et al.*, 2019b) parts of the CCZ, when taking into account the area of seafloor analysed (see Figure 2.1 for study locations). Increasing the area explored, Amon *et al.* (2016) observed 136 morphotypes (metazoans and xenophyophores) over 4 202 m^2 across two sites in the eastern CCZ. This is greater than asymptotic estimates for the current study (134 morphotypes) in just over half the area, suggesting that morphotype richness in eastern parts of the CCZ is high (although many factors may have influenced this, including image quality). Morphotype richness in the current study was similar to a study in the central to eastern CCZ (70 morphotypes, Foell & Pawson, 1986), and high compared to a study in the western CCZ (38 morphotypes, Wang & Lu, 2002); however, information on the seafloor area analysed is not available for these studies and so comparison is futile.

Despite having lower morphotype richness, estimated Shannon diversity for megafauna in this study was slightly higher than recorded in the UK CCZ exploration contract area, called UK-1, and an adjacent site in the eastern CCZ (3.46 vs 3.25, Amon *et al.*, 2016), but metazoan Shannon and Simpson diversity estimates were lower than those recorded for flat abyssal plain areas in APEI 6 in the north-eastern CCZ (14.7 vs 29.7 and 10.1 vs 16.4, respectively, Simon-Lledó *et al.*, 2019b) (see Figure 2.1 for site locations).

Diversity estimates at abyssal plain sites outside the CCZ vary greatly. At a site called DISCOL, which is in a nodule province in the Peru Basin, the number of morphotypes observed was much higher than this study, at 170, but this was over a vast area of more than 700 000 m^2 (Bluhm, 2001). Similarly, in the north-east Pacific, 102 morphotypes were observed at a nodule-free, long-term monitoring station, Station M (Kuhnz *et al.*, 2014). Again, the area covered was nearly double the size of this study. On the Porcupine Abyssal Plain (PAP) in the North Atlantic, also nodule-free, just 39 morphotypes were observed, but the study area was nearly half the size of this study (Durden *et al.*, 2015). Shannon diversity in the current study was comparable to or higher than observed at PAP and Station M (1.8 - 2.7, Durden *et al.*, 2015; 2.5 - 3.3, Kuhnz *et al.*, 2014). It is important to note that neither PAP nor Station M is located in a nodule province, and that diversity is therefore expected to be different.

A more useful and objective comparison can be made by looking at the density of metazoans, although of course differences in sampling methods, image quality and observer bias will still have some effect. Mean metazoan density in this study (0.23 $ind.m^{-2}$) was higher than recorded by Morgan *et al.* (1993) (0.11 $ind.m^{-2}$) across OMCO sites in their preliminary analysis (sites not shown in Figure 2.1, but some overlap with OMCO sites in current study). This could be owing to examination of digital imagery rather than film, observer bias or sampling of different environmental conditions (Morgan *et al*. 1993 studied a far greater area of 66 709 m^2).

Metazoan density in the current study was low compared to estimates in the eastern (0.83 $ind.m^{-2}$, Amon *et al.*, 2016) and north-eastern CCZ (0.49 $ind.m^{-2}$, Simon-Lledó *et al.*, 2019b), similar to a combination of sites across the central to eastern CCZ (maximum 0.3 $ind.m^{-2}$, Vanreusel *et al.*, 2016), and substantially higher than other sites in the central (0.06 ind.m^{-2}, Tilot *et al.*, 2018) and western CCZ (0.01-0.02 ind.m^{-2}, Wang & Lu, 2002). A similar pattern of increasing metazoan abundance from west to east was observed by Morgan *et al.* (1993) in their analysis of OMCO sites. This pattern could be linked to the spatial gradient in POC across the CCZ, from high in the east to low in the west, and similar changes in abundance in response to POC have been observed in the CCZ (Smith *et al.*, 1997). Alternatively, long-term temporal change in environmental conditions could be driving the differences observed, as there are 30 to 40 years between studies (excluding Morgan *et al.*, 1993). Further comparison amongst megafauna studies in the CCZ is explored in Chapter 5.

Metazoan density in this study was also higher than recorded at other abyssal sites in the North Atlantic and South Pacific, including at the nodule province DISCOL site, where megafauna density ranged from 0.14 – 0.16 ind.m^{-2} (Bluhm, 1994). Metazoan density as sampled by otter trawl was just 0.007 ind.m^{-2} at PAP, and 0.001 ind.m^{-2} further south at the Madeira Abyssal Plain (MAP), also not located in a nodule province (Thurston, Rice & Bett, 1998). Density estimates at PAP in another study using image data were much more comparable to this study, ranging from 0.10 ind.m^{-2} - 0.36 ind.m^{-2}, with an average of 0.26 ind.m^{-2} (Durden *et al.*, 2015). At Station M, megafauna densities have been shown to change greatly in response to variable food supply, and thus ranged from much lower than this study (0.67 ind.m^{-2}), to much higher (6.46 ind.m^{-2}).

Finally, the dominant feeding strategy observed here differed from what has been seen in abyssal plain sites without nodules in the North Atlantic and north-east Pacific, and is likely linked to the presence of hard substrate. Unlike the CCZ, which is dominated by suspension feeders, sampling at PAP recorded 85% deposit feeders, while MAP was dominated by 70% predators and scavengers (Thurston, Rice & Bett, 1998). PAP is relatively eutrophic, experiencing high seasonal variation in phytodetritus input and was dominated by holothurians (Thurston, Rice & Bett, 1998). MAP, which is oligotrophic, was dominated by decapods, with a large proportion of Cephalopods (Thurston, Rice & Bett, 1998). A later study at PAP recorded higher densities of suspension feeders, but also higher biomass of surface deposit feeders (Durden *et al.*, 2015). In the north-east Pacific, the dominant feeding strategy at Station M has been shown to change over time from suspension feeding to deposit feeding (Kuhnz *et al.*, 2014).

Interestingly, the overall average ophiuroid density in this study was approximately ten times lower than observed in the CCZ at UK-1 and an adjacent site in the east (Amon *et al.*, 2016). Even the transect with the highest ophiuroid density in this study still had approximately 2.5 times lower density. This would suggest that ophiuroids are less important in benthic communities in the central CCZ than in the east, and could be linked to the POC gradient across the CCZ, which favours suspension feeders in low POC areas to the west and deposit feeders in high POC conditions to the east (Tilot *et al.*, 2018).

2.4.1.6 Local diversity

Local variability in diversity observed among transects is discussed in the section below in the context of potential environmental drivers of these patterns.

2.4.2 Links with environmental variables

Although the CCZ is a highly heterogeneous environment, based on the coarse, modelled environmental data available there were only minor differences between the transects in this study, despite being over 1 000 km apart. These subtle differences did, however, appear to drive some of the variation in megafauna and metazoan community composition and diversity observed.

2.4.2.1 Bathymetry and topography

Depth explained a significant proportion (20%) of the variation in megafauna community composition among transects and a close-to-significant proportion (19%) of the variation in metazoan community composition (p=0.077). In addition, it explained 21% of the variation in megafauna and metazoan morphotype richness, given BBPI. Factors that vary with depth are known to play a vital role in structuring faunal communities (Howell, Billet & Tyler, 2002), and it appears that the small changes in depth observed (up to 400 m) may account for subtle changes in the megafauna and metazoan communities. This could be due to the link between POC and depth. POC flux to the seafloor decreases as depth increases (Buesseler *et al.*, 2007), and there is a gradient of decreasing POC and increasing depth across the CCZ (Pushcharovsky, 2006; Smith *et al.*, 1997). These variables had a moderate negative correlation, and although POC was not a significant explanatory variable, it is possible that this is because the resolution of modelled POC data was poor, and that POC and not depth is responsible for the differences seen, as food input is expected to be more important in driving distribution in abyssal plains (Smith *et al.*, 2008a). Clustering of metazoan community composition data also produced two significant clusters, corresponding to relatively shallower and relatively deeper waters, although this was not formally tested.

Previous studies have shown that topography is important in structuring megafauna and bait-attending communities in abyssal plains (Durden *et al.*, 2015; Leitner *et al.*, 2017; Morris *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Stefanoudis, Bett & Gooday, 2016). Even small changes in topography can drive variation in local hydrodynamics (Thistle, Ertman & Fauchald, 1991), sediment composition (Durden *et al.*, 2015), and food availability (Morris *et al.*, 2016), which in turn have been shown to impact benthic communities.

BPI as measured with a scale factor of 1 – 10 km has been shown to exert control over megafauna (Morris *et al.*, 2016; Simon-Lledó *et al.*, 2019b) and bait-attending (Leitner *et al.*, 2017) communities. The current study suggests that relatively small changes in BPI at the scale of 100 kilometres (BBPI) may also be an important driver of megafauna and metazoan diversity at the scale of > 1 000 km. There were consistent, significant differences in diversity measures between several transects, and these appeared to be driven in part by differences in BBPI. BBPI explained a large, significant portion of the variation in both megafauna and metazoan morphotype richness (~53%), as well as megafauna Shannon and Simpson diversity (~60%). There appeared to be a negative relationship between BBPI and megafauna morphotype richness, Shannon diversity and Simpson diversity, so that areas that were relatively lower supported greater diversity.

It was surprising that it was BPI at a broad scale (100 km) and not fine-scale BPI (10 km) that was identified as important. Variation in abyssal megafauna communities with fine-scale topography has been linked to topographically-driven changes in local hydrodynamics, sediment conditions and food (Durden *et al.*, 2015; Morris *et al.*, 2016), but it is unclear what processes operate at the scale of BBPI used in this study. This suggests that changes in topography over large spatial scales is more important than fine-scale changes for communities up to 1 000 km apart, and provides support for the inclusion of BPI at a broad scale in the design of protected areas in abyssal plains. BPI should be considered not only in revision of the current CCZ EMP, but also in the development of future REMPs (for example in nodule fields in the Indian Ocean) and the design of high seas protected areas.

2.4.2.2 Nodule density

The coarse-scale, modelled nodule abundance data used in this study did not explain any significant variation in the community composition or diversity of megafauna or metazoans. However, the accuracy of this modelled data did not appear to be good. One transect with particularly high nodule abundance based on modelled data (T4) did not appear to have notably higher nodule coverage than other transects when based on image data, and vice versa for several other transects.

Existing studies on the relationship between nodule abundance and epibenthic megafauna communities are few, and are either not detailed (Amon *et al.*, 2016; Vanreusel *et al.*, 2016) or only consider areas of low to medium nodule cover (Simon-Lledó *et al.*, 2019b). Increasing nodule cover has been shown to increase the density of epibenthic megafauna, but the relationship with diversity is less clear (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016). The nodule cover in these studies has ranged from 1% - 20% (Simon-Lledó *et al.*, 2019c), <1% - 50% (Amon *et al.*, 2016), and <1% - 92% (Vanreusel *et al.*, 2016, but only 2 of 17 transects were >50%), and it is therefore also unclear how high nodule abundance affects the density, diversity and composition of megafauna communities.

Nodule cover in this study ranged greatly (see Figure 2.3), and further work is underway to quantify nodule density and surface area in images from this study. This will shed light on any potential relationships between nodule cover, and megafauna density, diversity and composition in areas of high nodule coverage. It may also provide insight into whether the high abundances of xenophyophores observed in this study when compared to others is linked to higher nodule cover. This work will help to understand how these habitats should be considered in spatial planning. Simon-Lledó *et al.* (2019c) and Vanreusel *et al.* (2016) argue that representative areas of all nodule cover classes should be preserved, including areas of high nodule abundance, and this would support a precautionary approach to environmental management.

2.4.2.3 POC

Although there was no significant relationship between megafauna or metazoan community composition and diversity and POC alone, there was a general pattern of higher proportions of deposit feeders (mainly ophiuroids) observed in the east, and higher proportions of suspension feeders (mainly anthozoans) in the west (Figure 2.6 & Figure 2.10, with transects named sequentially from west to east). This was also observed by Morgan *et al.* (1993) in their preliminary analysis of the OMCO imagery (although the deposit feeders were predominantly echinoids), and is in line with what would be expected given the POC gradient across the CCZ (Morgan *et al.*, 1993; Tilot *et al.*, 2018). In addition, given BBPI, POC explained 18% of variation in megafauna Shannon diversity, providing evidence that POC is important in structuring megafauna communities over scales of 2 - 1 000 km in the CCZ.

2.4.2.4 Limitations

From the small amount of data in this study it was possible to identify several environmental drivers that may be important in predicting the distribution of megafauna in the CCZ. However, more data, both biological and higher resolution environmental data, is required in order to carry out more robust regression modelling to build predictive models. The combination of multiple existing biological data sets to provide a larger data set would potentially allow this, but would require overcoming issues resulting from differences in sampling equipment, image quality and observer bias.

In future, a strategic sampling plan, undertaken by multiple parties, with standardised sampling and analysis techniques as well as collection of finer scale environmental data would help to better understand the relationship between the distribution of epibenthic megafauna in the CCZ and environmental variables. This should take the form of a Regional Environmental Assessment (REA) (ISA, 2017b; Jaeckel, 2015; Jones *et al.*, 2019), a model which has been used successfully in other offshore industries (e.g. Bett, 2001; BMAPA, 2019; Gov.UK, 2019; Narayanaswamy *et al.*, 2006; Tethys, 2019), and should be driven by the ISA. REA is a collaborative approach (discussed further in Chapter 3), that would bring together scientists and contractors to work under a strategic plan to collect data. This would provide a better understanding of baseline environmental conditions, and thus support the development of predictive models of megafauna distribution, which are likely to be vital in environmental management of seabed mining in the vast CCZ. In addition it would set the platform for monitoring and assessment of cumulative impacts.

2.5 Conclusions and implications for management

The results of this study have implications for the management of not only nodule mining, but also other high seas activities occurring in abyssal plain environments. The megafauna community appeared to be homogenous over large scales (> 1 000 km) in terms of the dominant taxa, but there was high turnover of rare species. Further research is required to understand the geographic ranges, genetic connectivity, and functional roles of both rare and common species, in order to better predict extinction risks and consequences. The outcomes of this research should inform spatial management of mining activities. The high abundance of rare species also has implications for appropriate sampling effort and strategies in the deep sea, and is discussed in Chapter 3.

The ability of sessile and suspension feeding organisms to resist and overcome stressors created by mining operations is currently unknown, as is the effect that loss of these organisms would have on population survival and exchange with mined areas (Gollner *et al.*, 2017). This study showed that these organisms form a large proportion of the morphotypes and individuals in epibenthic megafauna communities, suggesting that whatever the impacts of mining may be, they are likely to affect a large proportion of benthic communities and be widespread. Further work is required to understand the mortality points of sedimentation on suspension feeders, and the ecosystem-level impacts that loss of these species would have. This should inform the development of formal standards and guidelines to direct mining operations and thereby manage potential impacts.

A large proportion of morphotypes and individuals were also observed attached to nodules, indicating that there are many morphotypes that are at least facultative users of nodules, if not hard substrate obligate species. Recovery of nodule-specific fauna in areas that are mined is expected to take millions of years due to the slow growth of nodules, and the species ranges and dispersal of fauna reliant on nodules is not well understood (Gollner *et al.*, 2017). This study provides supporting evidence that a large proportion of epibenthic megafauna communities will be affected by loss of nodule habitat. Further work is also required to understand the relationship between nodule abundance and megafauna density, diversity and community composition in areas of high nodule coverage (> 50%). A precautionary management approach would involve the protection of all levels of nodule cover, which is not currently the case (Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016, and see Chapter 4).

Finally, this study provides support for the inclusion of BPI on a broad scale (1 000 km) in spatial plans for protection of fauna in abyssal plain areas targeted for nodule mining. This could be considered in any revision of the current CCZ EMP or designation of further APEIs, as well as in the development of future EMPs, for example in the Central Indian Basin. It also highlights the need for improved quality and quantity of environmental and biological data to better understand the drivers of megafauna distribution at different scales across the CCZ. This could be achieved through REA, and would support predictive modelling of species distributions, which is crucial in such a large area.

In summary, this study suggests that those organisms which are potentially most vulnerable to mining activities, rare species, nodule-specific species, suspension feeders and sessile organisms, form a large proportion of the CCZ epibenthic megafauna. Further research is urgently needed to understand the cumulative impacts of losing these organisms and the wider, ecosystem-level impacts this would have.

Chapter 3.

Assessing the sampling effort required to characterise abyssal epibenthic megafauna diversity in a nodule province

3.1 Introduction

3.1.1 Species-area relationships

The deep sea is increasingly being targeted by different human activities (Ramirez-Llodra *et al.*, 2011). To manage these activities and reduce potential impacts on marine life, information on the composition and distribution of faunal communities is required. This information is gathered by sampling the targeted communities, to describe and quantify them. Biological communities are associations of species that co-occur in a defined area at the same time, and have the potential to interact with one another and their habitat (Pyron, 2010). The spatial scales across which species occur differs depending on the community that is being examined (Gaston, 1990). This is because animals respond differently to the biotic and abiotic variables driving their distribution (Gaston, 1990), and means that the appropriate sampling effort to describe these communities will also differ (Clark, Consalvey & Rowden, 2016).

The question of how much sampling is required to adequately represent a community is not a new one. In the 1920s, terrestrial scientists worked on determining the minimum size a replicate sample would need to be in order to characterise a plant community (Arrhenius, 1921). The number of species in a community was observed to increase as the area sampled increased, but only until a certain point, after which stage asymptote was reached and additional species were no longer added (Arrhenius, 1921). Similar relationships have been observed between the number of individuals encountered and number of species (Fisher, Corbet & Williams, 1943). Equations have been developed to describe this relationship between species accumulation and sampling effort, to predict at what point the asymptote would be reached (e.g. Clench, 1979; Soberón & Llorente, 1993). Species accumulation curves can thus be used to determine if a particular sample has reached asymptote, or to determine the area (or number of samples) that would need to be sampled to adequately describe a community (Heck, van Belle & Simberloff, 1975).

Species accumulation curves estimate the number of species that are added to a sample, hence they measure species richness. Species richness is simply the number of species in a sample or community, and does not consider abundance. This is one of the key measures defining diversity of any biological community, and underpins many conservation strategies (Gotelli & Colwell, 2001). Accumulation curves can also be used to estimate increases in Shannon and Simpson diversity with increased sampling effort (Chao *et al.*, 2014). These diversity measures take into account the abundance and evenness of species and reduce the importance of relatively rarer species. Shannon diversity is a measure of the effective number of common or typical species, while Simpson diversity is the effective number of very abundant or dominant species in a community (Jost, 2006).

Similar to species accumulation curves are rarefaction and extrapolation (R/E) curves. Rarefaction refers simply to interpolating the reference sample to estimate diversity for a smaller number of individuals or samples, while extrapolation predicts what diversity estimates would be for a larger number of individuals or samples, based on the reference sample (Chao *et al.*, 2014). Sample-based R/E curves plot diversity estimates against sample size (for example, the number of images or area of seabed), and use species incidence frequency data (i.e. the number of samples a species occurs in). This effectively reduces the data set to presence/absence data. Individual-based R/E curves plot diversity estimates against the number of individuals encountered and thus use abundance data (i.e. the number of times a species occurs in a sample).

Another way of investigating how far off complete sampling a study is, is to determine the sample coverage, or sample completeness (Chao & Jost, 2012). When there are many species with very low abundances, it is thought that these will be difficult to detect during sampling, and it is therefore difficult to estimate species richness of an assemblage (Chao *et al.*, 2014). To combat this issue, Alan Turing and I. J. Good came up with a concept called sample coverage, or just coverage, which could be used to describe the proportion of the total community that is represented by the samples (Good, 1953; Good, 2000; Good & Toulmin, 1956). It can also be described as the proportion of all individuals in a community that belong to species that are represented in the sample, and gives a measure of sample completeness (Chao & Jost, 2012). Turing and Good's coverage estimator is calculated as one minus the proportion of singletons. When the coverage estimate is subtracted from 1, this gives insight into the proportion of the community that belongs to undetected species (Chao & Jost, 2012). It represents the probability that if an additional individual is encountered, it will be a new, previously undetected species (Chao & Jost, 2012). When sample coverage reaches 100%, this means that sampling is complete, for the given effort and sampling technique (Chao & Jost, 2012). This rarefaction and extrapolation approach overcomes some of the issues with comparing diversity across multiple communities or assemblages (Hsieh, Ma & Chao, 2016).

The practical benefits of understanding how species accumulate in a system include being able to plan for sampling expeditions, predict the number of species that occur in an area, and compare values between sites or ecosystems (Soberón & Llorente, 1993). These all have important applications in environmental management. The species-area relationship paradigm was the focus of much attention for the design of protected areas in the 1970s and 1980s (Neigel, 2003), and although it is no longer used to such an extent in this respect, it is still used to predict the loss of species from habitat destruction and fragmentation (e.g. Pimm *et al.*, 2006; Seabloom, Dobson & Stoms, 2002), and to identify biodiversity hotspots that should be prioritised for protection (e.g. Guilhaumon *et al.*, 2008; Wilson *et al.*, 2006). The species-area relationship can also be used to establish data-driven, ecologically relevant conservation targets (e.g. Desmet & Cowling, 2004; Foster, Foggo & Howell, 2013). This study is focussed on how the species-area relationship can be used to inform minimum sampling requirements for characterising megafauna diversity in the deep sea.

3.1.2 Sampling in the deep sea

The same fundamental questions around sampling methodology apply to sampling in the deep sea. Although sampling in different environments at different scales requires different sampling strategies, the general principles which need to be taken into account when sampling in the deep sea are similar to those in shallower waters (Tyler, Baker & Ramirez-Llodra, 2016). They include the scale of the study, the spatial arrangement of samples, how many replicate samples should be collected, and how to take into account environmental heterogeneity (Tyler, Baker & Ramirez-Llodra, 2016).

Very few studies have tackled the issue of identifying appropriate sample size in the deep sea (Simon-Lledó *et al.*, 2019b; Soetaert & Heip, 1990). It is particularly difficult in this environment because there is little known about the spatial differentiation of deep-sea communities (McClain & Hardy, 2010; Taylor & Roterman, 2017), and it is thus difficult to know at what scale they should be sampled. Fauna in the deep sea occur at lower densities and are spread over greater distances than in shallow waters (Rex *et al.*, 2006), and so larger sample sizes are presumably required to characterise these communities to the same degree. In the abyss, particularly, samples covering large surface areas, or multiple smaller areas, are required to obtain robust abundance estimates (Clark, Consalvey & Rowden, 2016). However, this is complicated by the fact that some species have very wide geographic ranges, while others show high endemicity (McClain & Hardy, 2010). What is more, many deep-sea fauna with seemingly wide distribution ranges display patchiness at various scales (McClain & Hardy, 2010), and this can impact comparative and descriptive studies of distribution and abundance (Morrisey *et al.*, 1992).

Current megafauna sampling techniques in the deep sea include trawl sampling and photographic and video surveys (Clark, Consalvey & Rowden, 2016). There are several issues with trawl sampling, including limited spatial interpretation due to pooled samples, difficulty in interpreting seafloor area sampled, damage to soft-bodied organisms, underrepresentation of some fauna, and benthic disturbance, and this has led to increased use of non-destructive methods, such as video and photographic surveys (Morris *et al.*, 2014). An aggregate of pooled or mosaicked images or segments of video are generally used as the sampling unit for studies using visual data (Jones *et al.*, 2009), and there is little definitive guidance available on the appropriate size this should be (i.e. number of images or area of seabed) (Durden *et al.*, 2016). Previous photographic surveys in the deep sea have used sampling units ranging in size from less than 5 m^2 to over 1 000 m^2, and sample sizes (number of replicates) from two to 139 replicate transects (e.g. Amon *et al.*, 2016; Durden *et al.*, 2015; Howell, Davies & Narayanaswamy, 2010; Howell *et al.*, 2016b; Jones, Bett & Tyler, 2007; Simon-Lledó *et al.*, 2019b; Vanreusel *et al.*, 2016). There have been few suggestions on the minimum number of individuals that a sampling unit should contain (e.g. McGill *et al.*, 2007).

Forcino *et al.* (2015) propose that a median of 58 individuals will be sufficient for multivariate analyses (i.e. will produce the same results as a larger sample size), although their study included data from a range of environments, not just the marine benthos. In the context of marine visual imaging, Durden *et al.* (2016) suggest that sampling units with lower than 100 individuals should be treated with caution.

In the deep sea, and in particular in areas targeted by human activities, it is important to get sampling effort right. Sampling in the deep sea is expensive, as this environment is remote and difficult to reach (Clark, Consalvey & Rowden, 2016; Glover & Smith, 2003), and over-sampling would have severe cost implications. Under-sampling, on the other hand, could lead to inaccurate and unsupported conclusions, and this would be problematic where the results have implications for environmental management (Durden *et al.*, 2017a; Levin *et al.*, 2016).

3.1.3 Environmental requirements of deep-sea mining

Deep-sea mining is emerging as an industry with potentially significant environmental risks, and there is thus a need for strict regulation regarding mining activities to safeguard the marine environment from any harmful effects. Existing environmental regulations aimed at protecting fauna in Areas Beyond National Jurisdiction (ANBJ) are contained in each of three sets of regulations developed by the International Seabed Authority (ISA), the intergovernmental body responsible for regulating mining in ABNJ. These regulations are for the exploration of polymetallic nodules (ISA, 2000), polymetallic sulphides (ISA, 2010a) and cobalt-rich ferromanganese crusts (ISA, 2012), and include a requirement for baseline studies, monitoring programmes and environmental impact assessment (EIA). Regulations for the exploitation of mineral deposits are still under development, but current draft regulations contain provisions on baseline studies, monitoring and EIA for the exploitation phase (ISA, 2019b).

Under the exploration regulations, contractors are required to carry out baseline studies, which are aimed at establishing an environmental baseline for the targeted area, and against which any impacts from mining-related activities can be measured (ISA, 2000, Regulation 31, para. 4). Baseline studies should include a description of the diversity and abundance of fauna, and "sufficient information from the exploration area to document the natural conditions that exist prior to test mining, to gain insight into natural processes such as […] benthic faunal succession…" (ISA, 2013, para. 13).

In addition, contractors are required to establish monitoring programmes in order to monitor the impacts of any mining-related activities in the exploration phase (ISA, 2000, Regulation 31, para. 6). Monitoring programmes are aimed at measuring change in baseline environmental conditions as a result of natural variation and exploration activities, and contractors are required to report annually to the ISA on the implementation and results of their monitoring programme (ISA, 2000, Regulation 31, para. 5). If a contractor applies for exploitation rights, the contractor is then required to establish impact and preservation reference zones (IRZs and PRZs) (ISA, 2000, Regulation 31, para. 7). The primary function of these areas is to monitor mining impacts within contractor areas.

Data gathered during baseline studies forms the basis of any EIA that the contractor is required to submit for mining-related activities carried out during the exploration phase (ISA, 2000, Regulation 18, para. 1 (c)) (Figure 3.1). EIA is an important component of planning and environmental management for any industrial or commercial activity (IAIA, 2019). EIAs support project planning and rollout by anticipating and assessing potential environmental and social risks, and implementing mitigation measures to reduce these (IAIA, 2019). Under the current legal framework for exploration, mining-related activities in ABNJ such as testing of equipment components, drilling or use of epibenthic sledges, dredges or trawls require an EIA (ISA, 2013, para. 19). Contractors are required to submit an EIA to the ISA with plans of work for environmental baseline studies and monitoring (amongst others); however, this EIA relates only to exploration activities, and should not be considered the whole EIA process for commercial deep-sea mining (Durden *et al.*, 2018).

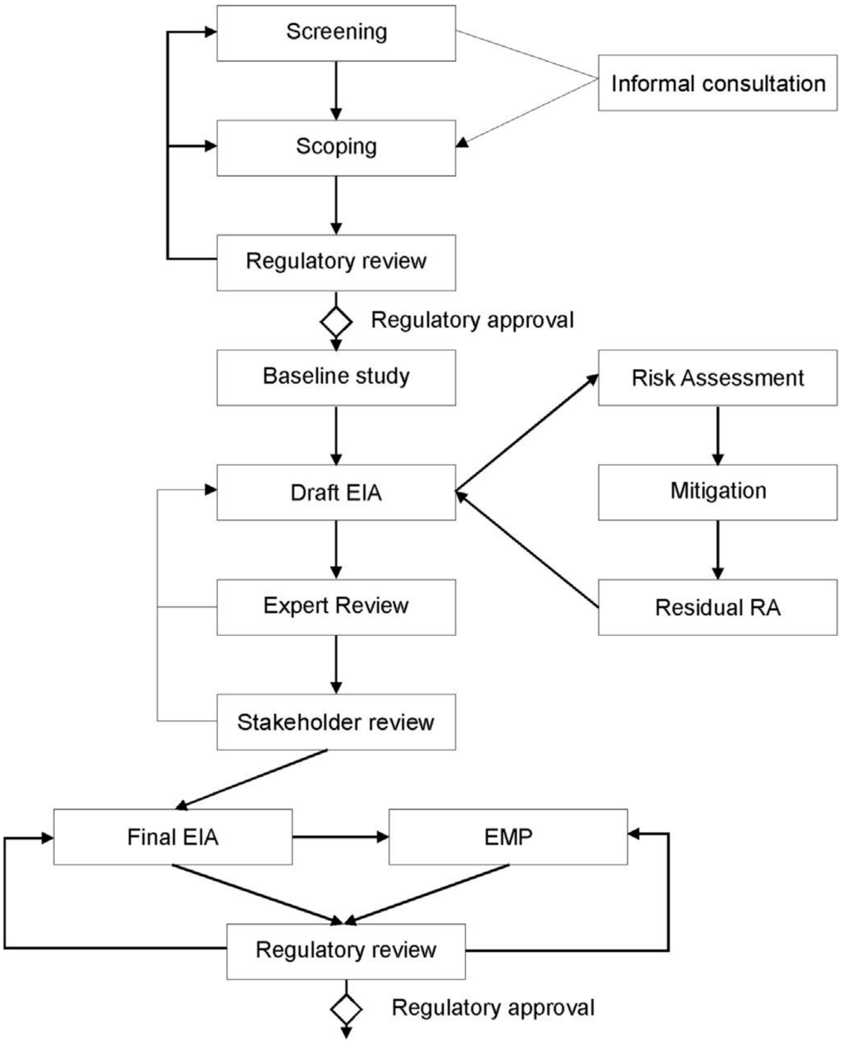

Figure 3.1 Environmental Impact Assessment (EIA) process proposed for deep-sea mining, indicating where baseline studies fit in. EMP = environmental management plan; RA = risk assessment. Monitoring programmes would form part of the EMP. Figure from Durden *et al.* (2018). Permission to reproduce this figure has been granted by Elsevier under the terms of the Creative Commons CC-BY license.

While there are provisions for environmental regulation in the exploration and draft exploitation regulations, these are fairly basic. They have thus been supplemented by several other documents from the Legal and Technical Commission (LTC), an advisory organ of the ISA, to provide more detailed (mandatory) guidance and recommendations on environmental requirements. To date, these are the “Environmental Management Plan for the Clarion-Clipperton Zone” (ISA, 2011), “Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area” (ISA, 2013), and “Recommendations for the guidance of contractors on the content, format and structure of annual reports” (ISA, 2015a). In addition, throughout the exploration and draft exploitation regulations and these supporting documents, there are frequent references to “standards and guidelines” and “best environmental practice”.

3.1.3.1 ISA guidance for sampling

The LTC recommendations for contractors in the exploration phase are generally identical for all three mineral deposits, although in some cases requirements are stated separately. In the recommendations for contractors on the assessment of environmental impacts, there is guidance for sampling the biological community, including specific recommendations for each size group of the fauna.

This study is concerned with sampling the epibenthic megafauna community, and while guidance is provided on the minimum size of organisms to be recorded and the minimum size of photographs to be taken (ISA, 2013, Annex I para. 36(a)), there are few recommendations on sampling design. Baseline requirements for the biological community include establishing “at least one station within each habitat type or region, as appropriate, to evaluate […] seabed communities” (ISA, 2013, Annex I para. 15 (e)(vi)).

However, there are no clear guidelines on how much sampling should be carried out at each station, including the size of sampling units (e.g. the number of photographs, length of video transects, or area of seabed) or the number of replicates (e.g. minimum number of transects) required to carry out a thorough baseline study.

Apart from the mandatory ISA recommendations for sampling during the exploration phase mentioned above, there are also references to best environmental practice, good industry practice, best available scientific evidence, best available techniques, and standards and guidelines throughout the exploration and exploitation regulations and other guidance documents (e.g. ISA, 2013, para. 14, 17, 34 & 55; ISA, 2019b, Regulation 47 para. 3 (d), Regulation 48 para. 3 (c), Regulation 51 (c), Regulation 58 para. 1 (f) & (g)). These standards and guidelines are intended to support the operationalisation of the core exploitation regulations, and are still under development (see ISA, 2019d).

3.1.4 Aims

As human activities are moving into deeper and more remote areas of the deep sea, where there is often very little known about the fauna, it is important to ensure that any sampling that is carried out to characterise baseline environmental conditions and support environmental monitoring is based on robust scientific design. Recommendations on the size and number of samples required needs to be grounded in science, and should be used to support the development of any standards and guidelines for sampling associated with deep-sea activities, including mining. With this in mind, this study aimed to:

1) Quantify the size of sampling units (number of individuals and area of seabed) and number of replicates required to capture different levels of epibenthic megafauna sample completeness in an abyssal nodule province; and

2) Use the results to inform discussions around minimum sampling requirements for baseline studies, monitoring and EIAs in the deep sea, and specifically relating to polymetallic nodule mining.

3.2 Methods

3.2.1 Study site

The Clarion-Clipperton Fracture Zone (CCZ) is a large area in the eastern central Pacific that is targeted for mining of polymetallic nodules. It is an abyssal plain area spanning approximately 6 million km^2 between the Clarion and Clipperton fracture zones, and has the highest global concentration of high grade polymetallic nodules (Thiel *et al.*, 1998). Although it is essentially an abyssal plain area, the CCZ also has topographic features, including abyssal hills, seamounts, troughs and ridges.

There are strong environmental gradients across the CCZ, in both an east-west and north-south direction. Particulate Organic Carbon (POC), depth and sediment characteristics vary across the region, resulting in differences in both nodule size and abundance as well as the composition of faunal communities (ISA, 2010b; Smith *et al.*, 1997; Wedding *et al.*, 2013). Megafauna in this area occur at low densities, with many rare species (Amon *et al.*, 2016). Sampling sites for this study were concentrated in flat abyssal plain areas in the central CCZ, between 11.6º N 136.3º W and 13.7º N 126.3º W (Figure 3.2).

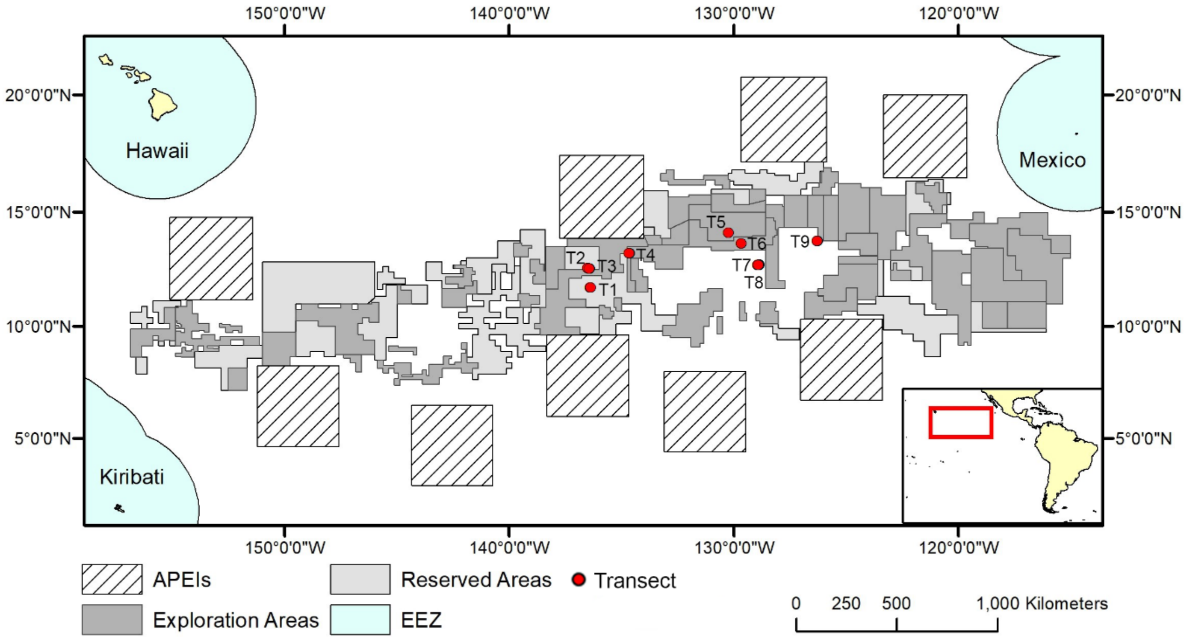

Figure 3.2 Location of image transects within CCZ management area. Land masses in cream, surrounded by Exclusive Economic Zones (EEZs) in pale blue. APEI = Area of Particular Environmental Interest.

3.2.2 Image analysis

The data set used in this study was a subset of a collection of archived images that were taken by Lockheed Martin and the Ocean Minerals Company (OMCO) consortium during exploration activities in the CCZ from 1978-1981, and made available by UK Seabed Resources Ltd (UKSRL). Images were taken using a Benthos 377 deep sea towed camera system with 35 mm film, and scanned into digital format by Woods Hole Oceanographic Institute (WHOI). Images were geolocated by UKSRL where information was available in archived cruise reports, environmental baseline reporting to U.S National Oceanographic and Atmospheric Agency (NOAA), and camera run logs. Metadata, including the altitude, compass heading and time stamp, were manually extracted from each image.

The location of transects is shown in Figure 3.2, with a minimum distance between transects of 2.5 km (between transects T7 and T8), and a maximum distance of 1 150 km (between transects T1 and T9). Images in each transect were filtered to remove blurred and overlapping images, including those with visible sediment plume (generated by impact of the camera system on the seafloor), and those outside the preferred altitude range of 2 - 4.6 m above the seabed. Where there was a low number of images per transect, the altitude range was increased to 1.8 - 5.5 m, with <5% sediment plume visible. Of this subset, images were then randomly selected (where possible) until a standardised area of 800 m^2 of seabed was reached. This resulted in a different number of images per transect (range 71 - 122, see Chapter 2, Table 2.1), as the field of view ranged from 1.4 m^2 to 18.5 m^2. The length of transect from which images were selected was also standardised as far as possible (ranging from 1.5 - 2.5 km), to reduce the likelihood of testing different types of diversity.

Epibenthic megafauna in each image were identified and annotated using the freely-available online software, BIIGLE 2.0 (Langenkämper *et al.*, 2017). Megafauna were defined as those individuals greater than 10 mm in size, and were identified to the lowest possible taxonomic ranking using morphotype labels. A megafauna morphospecies catalogue for the CCZ region developed by Simon-Lledó *et al.* (2019b) was used together with relevant literature to support identifications, and images across all transects were annotated in a random order to minimise bias (Durden *et al.*, 2016). Quality assessment was carried out by Dr Erik Simon-Lledó.

3.2.3 Rarefaction and extrapolation curves

To assess the level of sampling effort required to reach asymptotic biodiversity estimates and different levels of sample completeness, rarefaction and extrapolation (R/E) curves were fitted using epibenthic megafaunal and metazoan abundance and incidence data. This allowed an examination of the number of individuals that would need to be encountered (abundance data) and the area of seabed that would need to be sampled (incidence data) to reach different levels of sample coverage (SC).

To investigate the size of sampling units that would be appropriate to characterise diversity in the CCZ, R/E curves were fitted separately for every transect, using each image or individual within the transect as a data point (Figure 3.3). To determine the number of replicates of sampling unit that would be required to capture diversity, R/E curves were then fitted for all nine transects together, using each transect as a data point. It is important to note here that each replicate transect was ~3 500 megafauna individuals/~180 metazoan individuals or 800 m^2, and not the optimum/appropriate sampling units identified in the previous step.

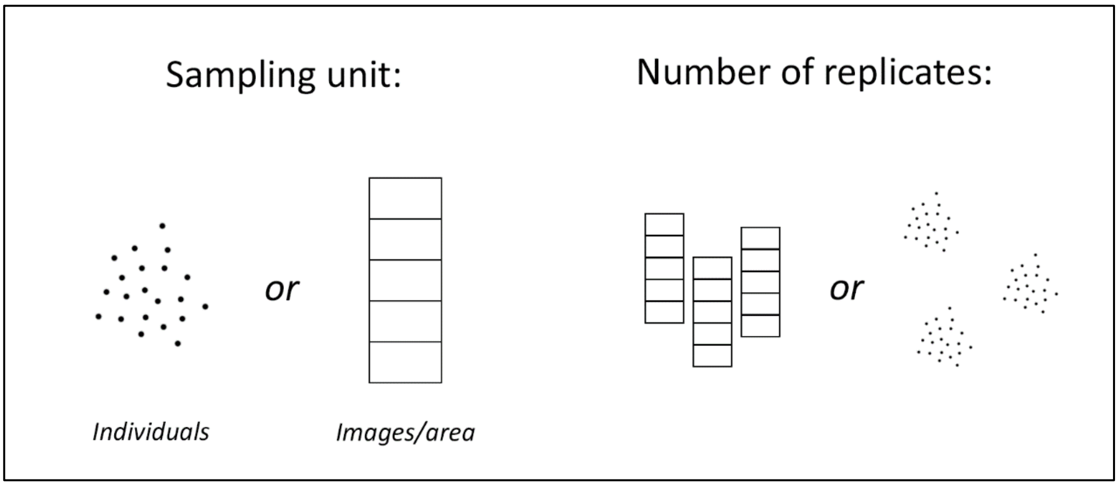

Figure 3.3 Schematic showing sampling units and number of replicates.

All analyses were carried out in R x64 3.5.1 using the iNEXT (iNterpolation/EXTrapolation) package (Hsieh, Ma & Chao, 2016). R/E curves were fitted for the first three Hill's numbers (morphotype richness, Shannon diversity and Simpson diversity; q = 0, 1, 2) for the reference (observed) sample size, and then extrapolated to 99% SC and asymptote, with confidence intervals of 95% obtained using a bootstrap method based on 1 000 replications. Estimates of morphotype richness extrapolated beyond double the reference sample size may be subject to large prediction bias, and may therefore be unreliable (Chao *et al.*, 2014); however, these were still included for completeness, but should be treated with caution.

Previous studies have found that including xenophyophores in analysis of community data can substantially affect the outcomes, and several reasons for this have been suggested, including difficulty in determining whether individuals are living and body size mismatch due to the very small size of xenophyophore protoplasm volume (Simon-Lledó *et al.*, 2019b). In this study, therefore, R/E curves were fitted for metazoans separately as well as megafauna (metazoans and xenophyophores) to determine the level of sampling required to understand both the megafauna and metazoan communities.

3.3 Results

It is important to note, before reporting any results, that diversity indices that are sensitive to rare species require higher sample sizes to estimate diversity than indices which are more reliant on common species (Soetaert & Heip, 1990). This was seen throughout the results, where the sampling units and number of replicates required to reach asymptotic diversity estimates were higher for morphotype richness than for Shannon and Simpson diversity (Table B.1 - Table B.6). However, it should also be noted that estimates of morphotype richness extrapolated beyond double the reference sample size may be subject to large prediction bias, and may therefore be unreliable (Chao *et al.*, 2014). The size and number of sampling units required to reach asymptotic morphotype richness are reported, for completeness, but these estimates should be treated with caution.

In addition, there can be differences in the sampling effort required to reach 99% sample coverage or asymptote when using individuals or area of seabed as the sampling unit, and this is due to spatial aggregation of species (Gotelli & Colwell, 2001). Where a community has a patchy distribution, for example, results can be biased by empty images or images with dense clumps of individuals. Using individuals as the sampling unit provides unbiased results, but may be less practical for recommendations on sampling (Moreno & Halffter, 2001).

3.3.1 Sampling unit size

There was high variability in the number of metazoan and megafauna individuals encountered in each transect (Table B.1, Table B.3). When considering the sampling unit as a number of individuals, megafauna R/E curves showed that the sample coverage of the reference samples (T1 – T9) was greater than 99.30% in all cases, meaning that the observed sampling units of 2 305 to 5 150 individuals were already achieving a sample coverage > 99% (Figure 3.4). The sample coverage of the reference metazoan sampling units were lower than those recorded for megafauna, ranging from 83.24% to 94.91% (Figure 3.5, Table B.3). When considering the sampling unit as the number of images or area of seabed, sampling units for both megafauna and metazoan data captured < 99% sample coverage, but were still high (Table B.2, Table B.4).

Based on the data set analysed in this study, sampling units for megafauna would need to be an average of 787 (± 185) individuals or 2 858 m^2 (± 1 455) to reach 99% sample coverage, and between 13 911 (± 13 528) - 27 178 (± 17 230) individuals or 5 348 m^2 (± 1 182) - 19 408 m^2 (± 10 800) to reach asymptotic diversity estimates. Sampling units for metazoans would need to be larger, with 962 (± 564) individuals or 4 643 m^2 (± 2 521) to reach 99% sample coverage, and between 2 541 (± 1 347) - 453 611 (± 413 199) individuals or 9 476 m^2 (± 2 556) - 18 530 m^2 (± 12 221) to reach asymptotic diversity estimates. The ranges of sampling units reported above for asymptotic diversity reflect the varying sampling effort required by different diversity indices based on sensitivity to rare species (see Table B.1 - Table B.4). Lower values are for Simpson diversity and are more reliable than the upper limits reported.

As expected, for both megafauna and metazoan data, asymptotic diversity estimates required much larger sampling units to obtain than at 99% sample coverage. In all cases, the majority of sample coverage was accounted for by a small number of abundant morphotypes, with the remaining coverage consisting of many rare morphotypes (Figure 3.4, Figure 3.5).

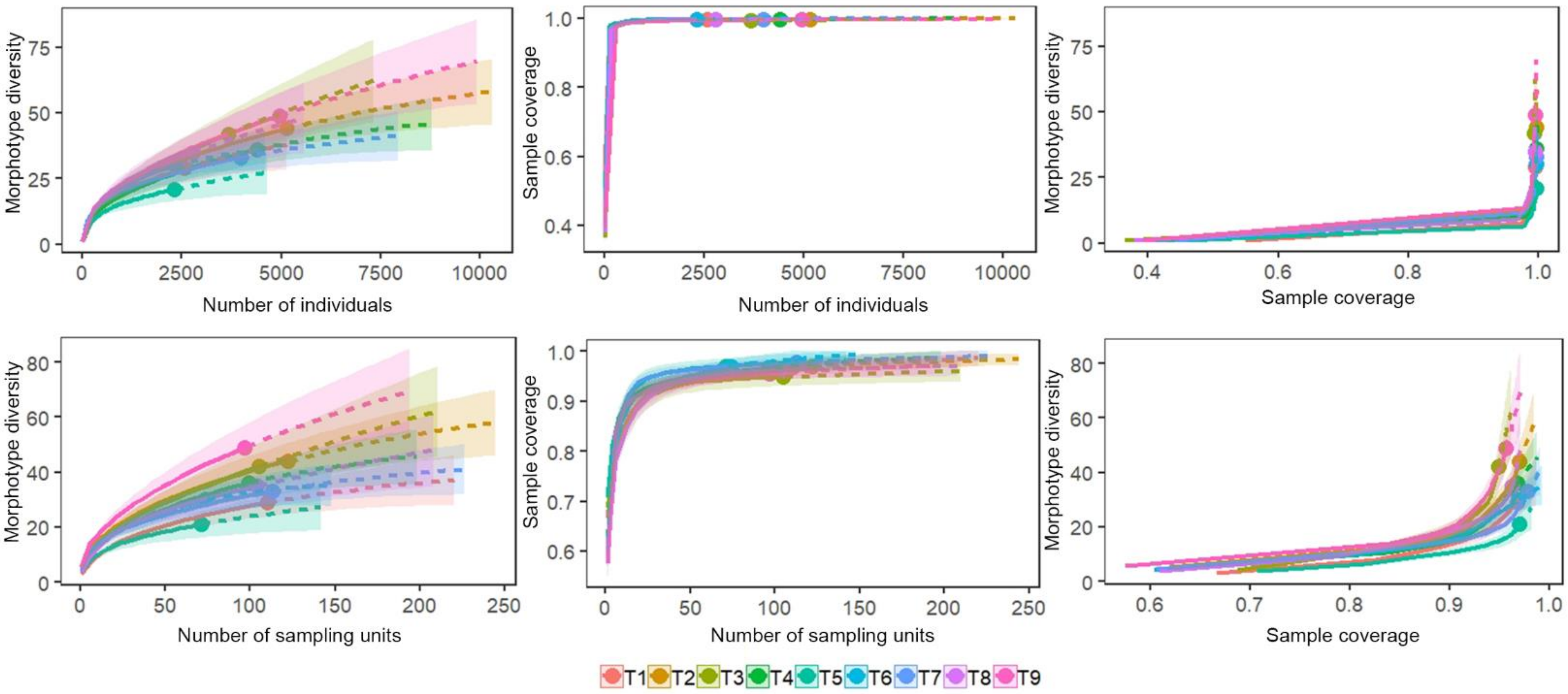

Figure 3.4 Individual- and sample-based sample unit and sample coverage rarefaction and extrapolation curves for morphotype richness of epibenthic megafauna data at the transect level. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolation to double the reference sample size. Sampling unit = individual/image, total area per transect = 800 m^2.

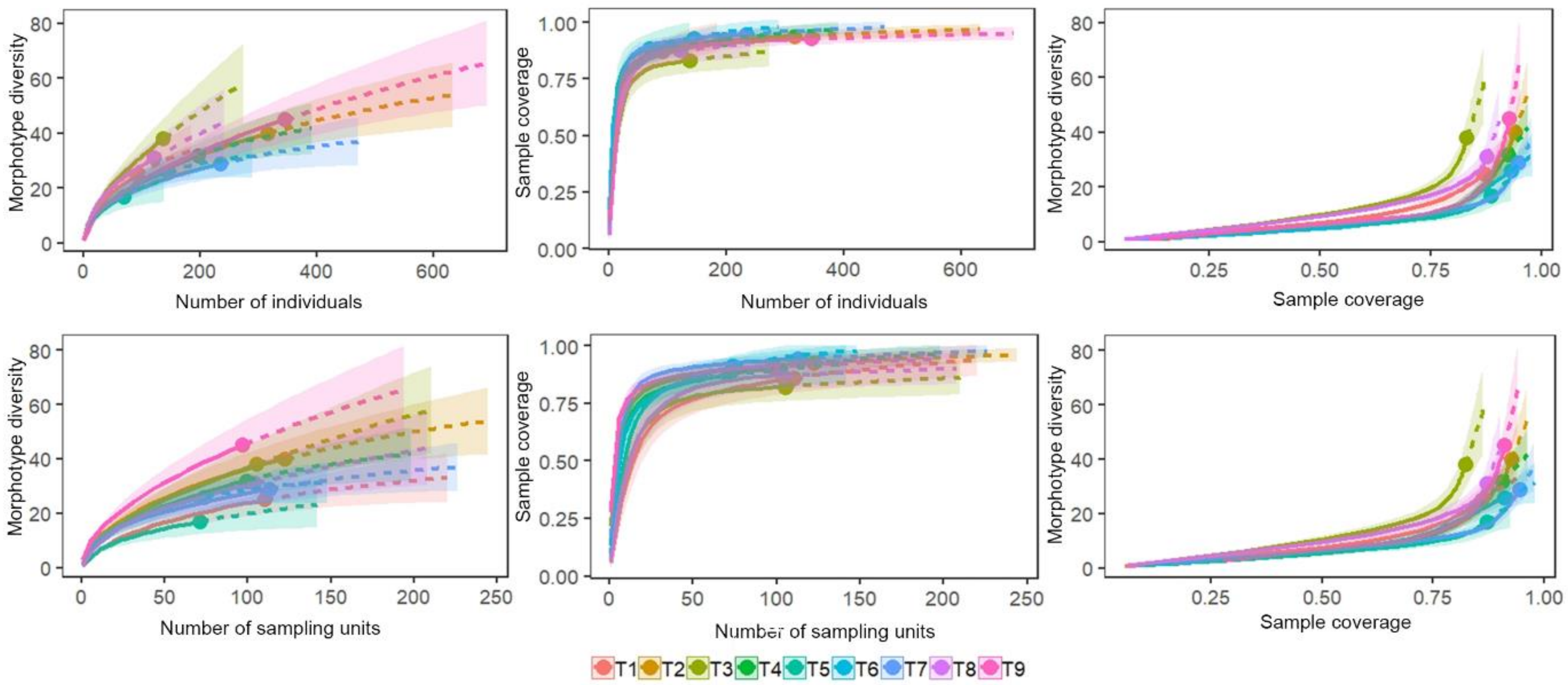

Figure 3.5 Individual- and sample-based sample unit and sample coverage rarefaction and extrapolation curves for morphotype richness of epibenthic metazoan data at the transect level. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolation to double the reference sample size. Sampling unit = individual/image, total area per transect = 800 m^2.

3.3.2 Number of replicates

The following numbers of replicates were determined based on the reference sampling units of this study (i.e. ~3 500 megafauna or ~180 metazoan individuals, or 800 m^2 per transect) (Table B.5, Table B.6).

The number of replicates used in this study captured >99% of the megafauna community when considering the number of individuals encountered, but further sampling was required to reach this same level when considering the replicates as an area of seabed (i.e. 800 m^2 transects). Similarly, the number of replicates in terms of both individuals and area of seabed failed to capture 99% of the metazoan community. A greater number of replicates was required in all cases to reach asymptotic diversity estimates, and both the megafauna and metazoan analyses revealed similar requirements for area of seabed sampling required to reach asymptote (Table B.5, Table B.6).

Based on this data set, the number of replicates required for megafauna sampling to reach 99% sample coverage would be 26 x 800 m^2 transects (or 20 800 m^2/909 individuals in total), and 130 – 535 transects (or 104 000 – 428 000 m^2/14 300 – 675 000 individuals in total) to reach asymptotic diversity estimates, depending on diversity index. Sampling effort for the metazoan community would require different numbers of individuals, but similar areas of seabed. To reach 99% sample coverage, 27 x 800 m^2 transects (or 21 600 m^2/2 872 metazoan individuals in total) would be required, with 128 – 520 transects (or 102 400 – 416 000 m^2/37 000 – 185 000 individuals in total) required to reach asymptotic diversity estimates. Lower estimates for the number of samples required to reach asymptotic diversity are for Simpson diversity and are more reliable than the upper limits reported.

Again, as expected, the effort to reach asymptotic estimates was very high. The majority of sample coverage was accounted for by a small number of abundant morphotypes, with the remaining coverage consisting of many rare morphotypes (Figure 3.6, Figure 3.7).

For the megafauna data, a high sample coverage of 99.3% was reached after just 1 700 megafauna individuals were encountered in individual-based R/E curves (Figure 3.6). For the first 1 700 individuals, 27 new morphotypes were recorded. For the same effort again, 10 new morphotypes were added, and this number continued to decrease steadily as the same number of individuals were encountered several times (Figure 3.8a). Based on extrapolation, after encountering 49 000 individuals, fewer than 1 new morphotype per 1 700 individuals was expected to be recorded. At this point, the increases in diversity estimates were very small for the level of sampling required to reach them.

For metazoans, using individuals as the sampling unit, over 86% of sample coverage was comprised of 24 common morphotypes, captured by just 92 individuals (Figure 3.8b, Figure 3.9). For the same effort again, 10 new morphotypes were added, and then 7 for the same effort again. The number of new morphotypes added to the data set continued to decrease steadily as the same number of individuals were encountered several times. After encountering 1 200 individuals, only 1 new morphotype was added for every 90 individuals encountered. Based on extrapolation, the number of new morphotypes decreased to less than 1 per 90 individuals after 2 600 individuals.

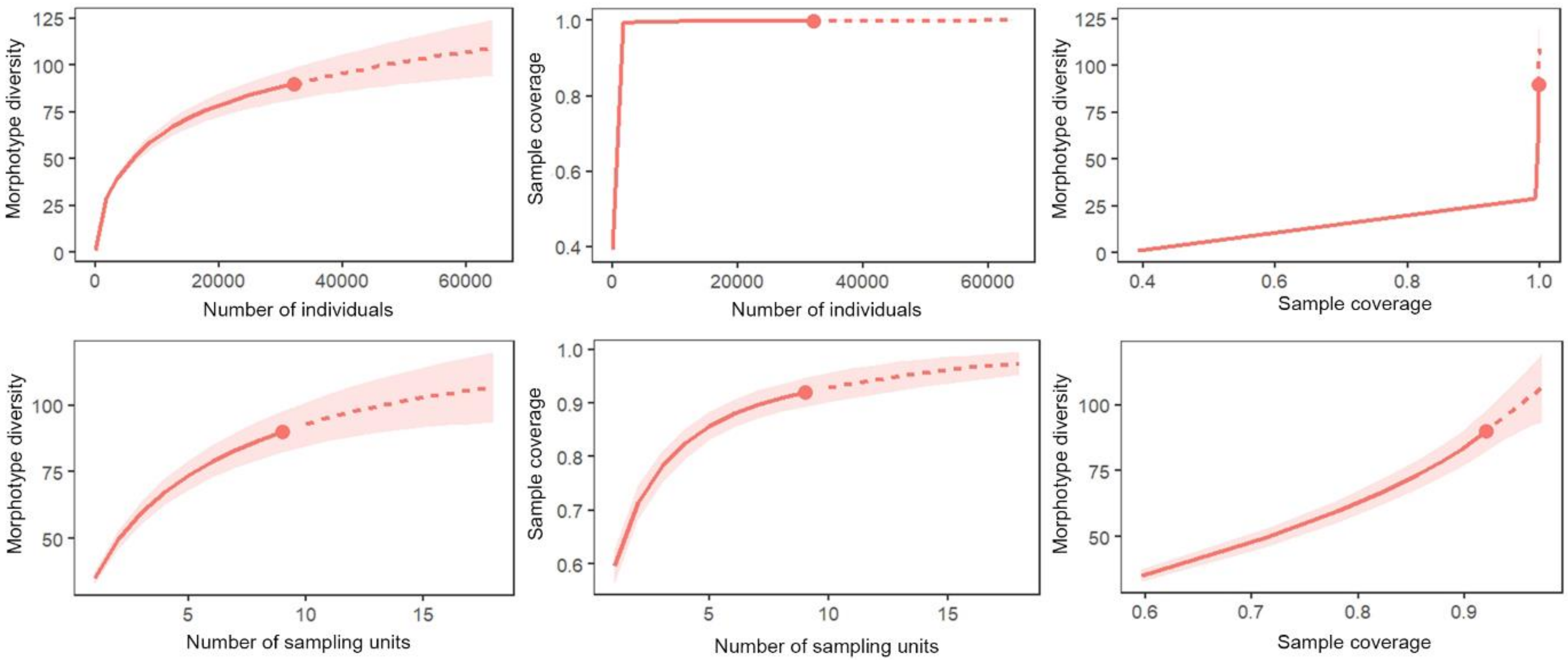

Figure 3.6 Individual- and sample-based sample unit and sample coverage rarefaction and extrapolation curves for morphotype richness of epibenthic megafauna data. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolation to double the reference sample size. Sampling unit = individual/800 m^2 transect.

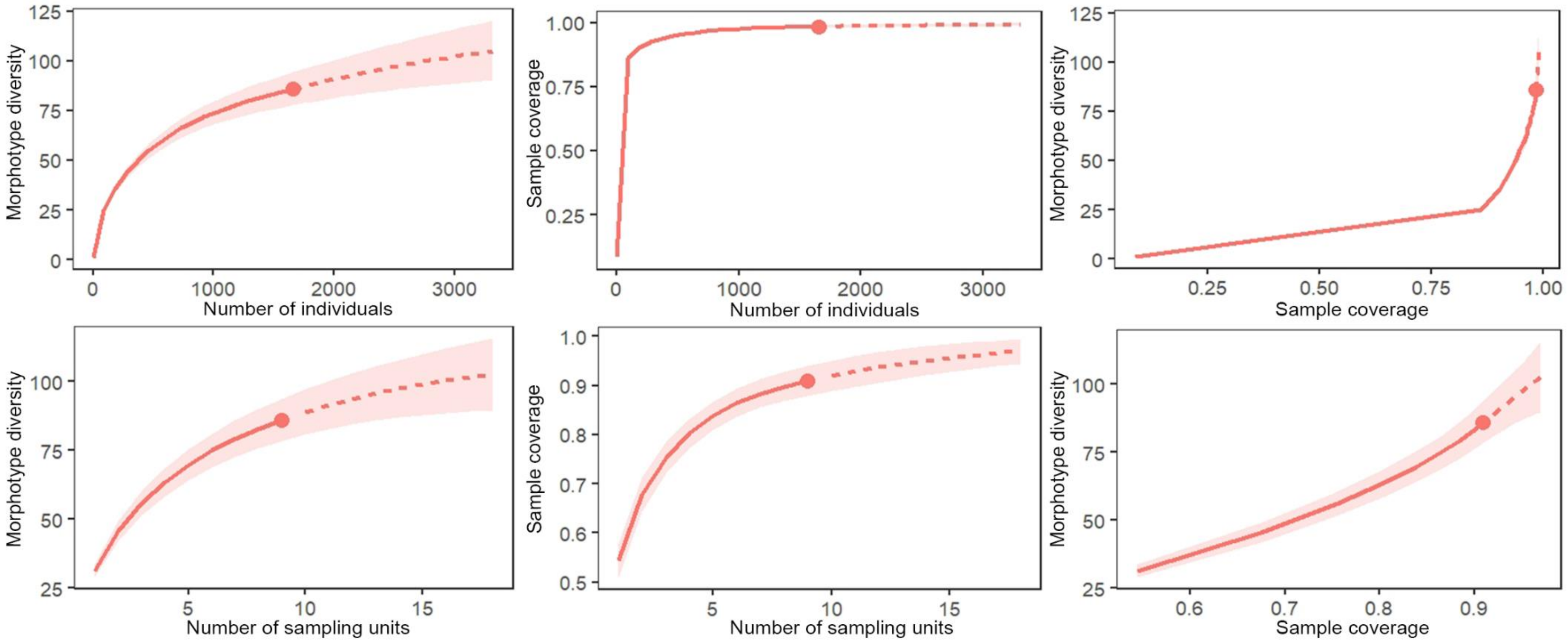

Figure 3.7 Individual- and sample-based sample unit and sample coverage rarefaction and extrapolation curves for morphotype richness of epibenthic metazoan data. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolation to double the reference sample size. Sampling unit = individual/800 m^2 transect.

Considering the replicate as an 800 m^2 transect, 35 megafauna and 31 metazoan morphotypes were encountered in the first transect. The number of new morphotypes halved for the next transect and dropped to 10 new morphotypes for the third transect (Figure 3.9). Based on extrapolation it was expected that after 12 transects (9 600 m^2) only 1 new megafauna or metazoan morphotype would be encountered in every additional transect, and after 18 transects (14 400 m^2) this became less than 1 new morphotype.

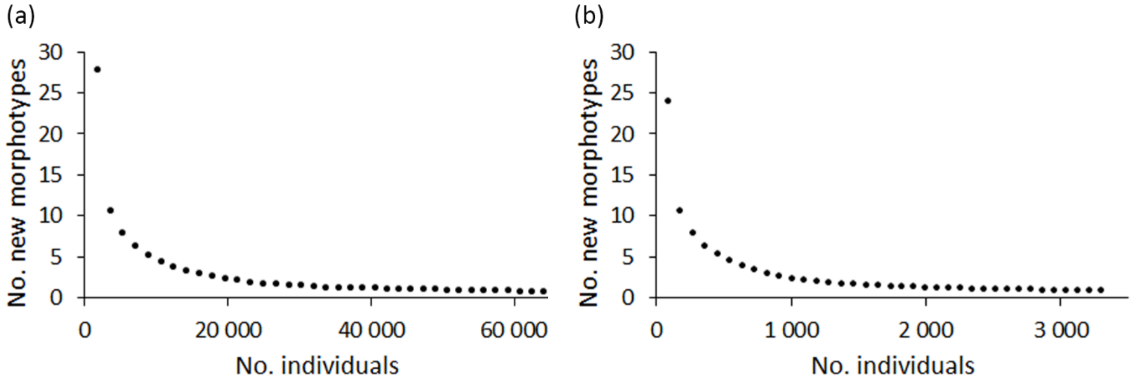

Figure 3.8 The number of new (a) megafauna, and (b) metazoan morphotypes encountered with additional individuals, based on individual-based R/E curves of double the reference sample.

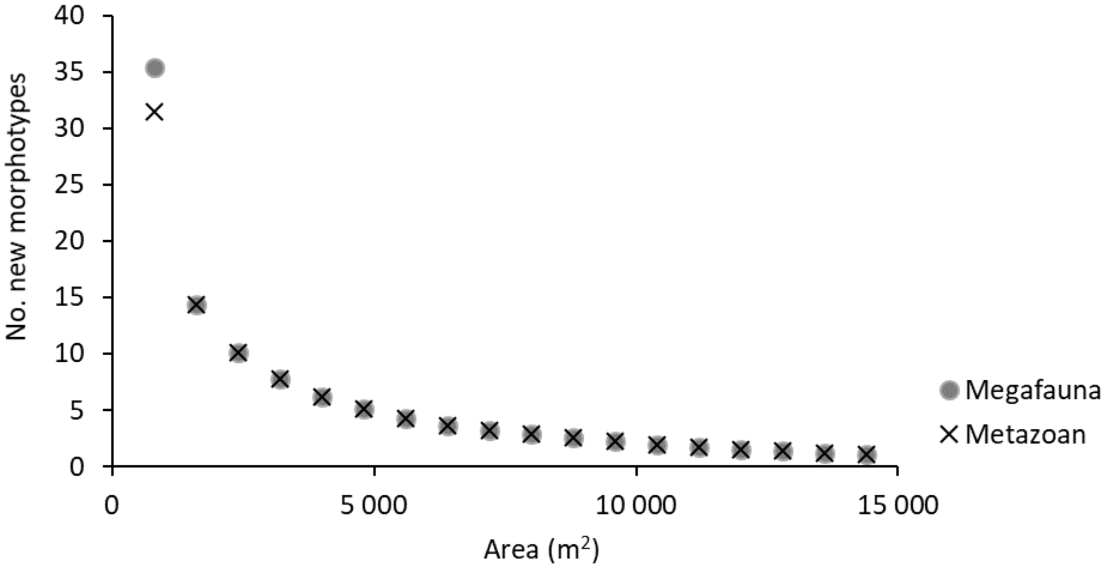

Figure 3.9 The number of new megafauna and metazoan morphotypes encountered with an increase in area, based on sample-based R/E curves of double the reference sample.

3.4 Discussion

3.4.1 Sampling effort assessment

High levels of sample coverage (>80%) were observed in this study for both the size and number of sampling units of megafauna and metazoan data before extrapolation, suggesting that a high proportion of the total diversity of the community was captured in the reference samples. However, to reach 99% sample coverage or asymptotic diversity estimates both sampling units and number of replicates generally required a great increase. This is typical of most studies, where species accumulation curves for megafauna do not reach asymptote, as this requires a high level of sampling (Clark *et al.*, 2016b).

Estimates for the size of sampling units (number of individuals or area of seafloor) that would be required for different levels of sample completeness differed depending on whether megafauna or metazoan data was used, with the metazoan community generally requiring a higher level of sampling. From the data set analysed in this study, sampling units ranged from a minimum of 787 (± 185) megafauna individuals or 2 858 m^2 (± 1 455) to reach 99% sample coverage, to a maximum of between 2 541 (± 1 347) - 453 611 (± 413 199) metazoan individuals or 9 476 m^2 (± 2 556) - 18 530 m^2 (± 12 221) to reach asymptotic diversity estimates (depending on transect and diversity index, with lower estimates more reliable). Interestingly, the number of megafauna individuals required to reach 99% sample coverage was lower than the reference sample (average of 787 individuals at 99% SC vs average of ~3 500 individuals in reference sample). This was likely because of spatial aggregation (i.e. patchy distribution) of the fauna.

The estimates reported here are far higher than the sampling units traditionally used in deep-sea studies (e.g. Amon *et al.*, 2016; Durden *et al.*, 2015; Howell *et al.*, 2016b; Jones, Bett & Tyler, 2007; Simon-Lledó *et al.*, 2019b; Vanreusel *et al.*, 2016), but the latter are aimed at characterising megafauna on a more general level, and do not necessarily intend to provide a thorough inventory of all species present. Simon-Lledó *et al.* (2019b), in their study on the effect of sampling unit size on the accuracy and precision of various ecological parameters, found that estimates of metazoan morphotype richness required 1 000 - 1 500 m^2 (> 500 individuals) to stabilise, while Shannon diversity required 700 - 1 000 m^2 (> 350 individuals) and Simpson diversity required just 400 - 600 m^2 (> 200 individuals). This is similar in terms of number of individuals to that observed in the current study, where diversity estimates tended to level off around 300 - 400 metazoan individuals, with only small increases in diversity observed after this point (Figure 3.5). However, the area required for metazoan diversity estimates to start to level off in this study was far greater, and this could be because of overall lower densities compared to those recorded by Simon-Lledó *et al.* (2019b).

Estimates of the number of replicates that would be required for different levels of sample completeness were more similar for megafauna and metazoans. These ranged from 26 x 800 m^2 transects (or 20 800 m^2/909 megafauna individuals in total) to reach 99% sample coverage, to 130 – 535 transects (or 104 000 – 428 000 m^2/14 300 – 675 000 megafauna individuals in total) to reach asymptotic diversity estimates, depending on diversity estimate, with lower estimates more reliable. Again, even the lower estimates are much higher than is generally used in deep-sea studies, and quantifies the huge increase in effort required to fully characterise the megafauna community.

It is important to point out that this study is not a power analysis, and thus does not give any insight into the amount of sampling required in order to have the statistical power to address scientific questions around variation in community composition (Cohen, 1977). It simply sheds light on the size and number of samples required to obtain different levels of information on the biodiversity of an abyssal plain area. This is important to understand for baseline studies and regional assessments, as is discussed below. In the context of power analysis, Jones *et al.* (2018) noted that depending on the variance of the indicator, the statistical power desired and the effect size being measured, 25 – 100 transects of over 1 kilometre length could be required to have a statistically robust design in the deep sea. This refers to the sampling effort required for monitoring and measuring disturbance events, rather than just characterising the fauna as here, but it is in the order of what was found in this study.

The very large number of large samples required to thoroughly sample the epibenthic megafauna and metazoan communities is a result of high morphotype richness, variability among transects, and very low faunal densities. There is high variation in sampling effort required to achieve certain levels of sample completeness amongst both transects and diversity indices. Variation among transects is a result of different observed number of individuals and diversity, while differences among diversity indices are a result of differential sensitivity to rare species.

Furthermore, the large differences in the number of individuals required for megafauna compared to metazoans is due to the highly abundant nature of the dominant xenophyophore morphotypes included in the megafauna estimates. Differences between megafauna and metazoan estimates could also be driven by the fact that xenophyophores were divided into just 4 groups, three of which were highly abundant, while in reality these are an extremely diverse fauna with many different species (Gooday *et al.*, 2017b). If it were possible to distinguish xenophyophore morphotypes to a lower taxonomic level, there would undoubtedly be far greater diversity estimates for this data set, and more sampling required to capture the full diversity of these organisms.

Finally, it must be pointed out that the biological data set analysed in this study comes from a relatively narrow range of environmental conditions for the CCZ (Figure B.1, and see Chapter 2). The sampling effort identified in this study refers only to characterising the environmental conditions sampled, and this means that separate analyses would need to be carried out to determine how much sampling is required to characterise other environments in the CCZ, such as abyssal hills, troughs and ridges. The CCZ has strong gradients in POC and depth, variable nodule abundance and topographic features (ISA, 2010b; Klitgord & Mammerickx, 1982; Pushcharovsky, 2006; Smith *et al.*, 1997), and habitat variation across the CCZ is explored further in Chapter 4. This suggests that a much greater sampling effort than is reported here may be required to assess diversity across the entire CCZ. These results are, however, most applicable to areas that will be targeted for nodule mining, as the transects were all located in flat areas with low to high nodule abundance.

3.4.2 Rare species and environmental management

The data presented here suggest that in the CCZ there are a few common morphotypes, and many very rare morphotypes. As discussed in Chapter 2, 25 of the morphotypes recorded were observed on only one occasion, and this number increased greatly for those observed on less than two occasions. The coverage-based curves showed that much of the diversity of the community could be captured by relatively few individuals or samples, but that it would take a huge amount of sampling to capture the remaining diversity. The amount of sampling effort required to increase sampling coverage from 99% to asymptote was very large. This reflects the rare morphotypes, that are not commonly encountered, but that may be as important as common species (see below).

Globally, the majority of species are rare (Gaston, 2008). Most species have populations that are restricted in geographic extent and comprise only few individuals (Gaston, 2008). This is particularly the case in the abyss, where "rare is common" and nearly all species are collected on only a few occasions (Ebbe *et al.*, 2010). Interestingly, these many rare species and those with narrow ranges are underrepresented in deep-sea data bases, which favour the few, common species (Higgs & Attrill, 2015). Rare species are more vulnerable to disturbances and short-term extinction than common species because of their small population sizes, restricted geographic distributions and often narrow environmental niches (Gaston, 1994). This has resulted in many rare species becoming priority species of conservation concern (Gaston & Fuller, 2007).

While biodiversity is widely accepted to support ecosystem function, the impact of loss of rare species on ecosystem functioning is not well understood (Jain *et al.*, 2014; Lyons *et al.*, 2005). It has been argued that rare species may possess traits that contribute new functional diversity to a community (e.g. Bracken & Low, 2012; Jain *et al.*, 2014; Leitão *et al.*, 2016; Lyons *et al.*, 2005), or they may have functionality which is redundant with common species, but which can complement and substitute for the latter if it were to suffer local extinction (Jain *et al.*, 2014). If the rare species lost performed functions that directly or indirectly affected ecosystem functioning, this could be important. In addition, biodiversity has been shown to support ecosystem functioning (e.g. Danovaro *et al.*, 2008; Lyons & Schwartz, 2001), and so removal or loss of rare species may reduce ecosystem resistance and/or resilience to change.

Some studies have shown that rare species do not, in fact, contribute significantly more than common species to the functional diversity of a system (e.g. Chapman, Tunnicliffe & Bates, 2018). For example, where rarity is defined by abundance (rather than geographic range or habitat specificity), the contribution of rare species may be significantly lower than that of common species (Jain *et al.*, 2014). Chapman, Tunnicliffe and Bates (2018) argue that the relative importance of rare species' contribution to functional diversity may be increased when there is human influence on a system. There is also a temporal aspect to the importance of rare species whereby they may become more important if their abundance increases due to environmental or anthropogenic-induced change (Lyons *et al.*, 2005; MacDougall *et al.*, 2013).

While there is debate around the contribution of rare species to ecosystem function and whether rare, keystone, flagship, or indicator species should be the focus of conservation attempts (Gaston & Fuller, 2007), a precautionary approach to conservation should involve protecting a large proportion of diversity in the targeted system, including rare species (Lyons *et al.*, 2005; Tekalign *et al.*, 2017). This greatly increases the logistical, intellectual and financial burden of sampling, which is particularly time consuming and expensive in the deep sea (Clark, Consalvey & Rowden, 2016). A United Nations report from 2017 estimates that it can cost anywhere between $10 000 - $40 000 per day to operate a research vessel (IOC-UNESCO, 2017). This cost can quickly accumulate to a large sum when considering the weeks required for a deep-sea cruise, particularly when visiting remote locations like the CCZ, and, as this study has shown, the amount of sampling required to capture all diversity of a community is expected to be high. It should be noted that sample collection is not always the bottleneck in data acquisition, but that image processing can be a limiting factor (Durden *et al.*, 2016). Staff time required for post-cruise analysis is therefore also expected to increase greatly with more sampling, and so will associated costs. Technological advancements in underwater imaging mean large amounts of image data can now be collected that are not feasible to annotate manually (Durden *et al.*, 2016; Schoening, Köser & Greinert, 2018), and this has led to studies on the application of artificial intelligence and computer vision to automate image analysis and overcome this bottleneck (Beijbom *et al.*, 2015; Piechaud *et al.*, 2019; Schoening *et al.*, 2012).

The cost of additional sampling also needs to be reconciled with the amount of information gained, and the effect that this additional information has on decision-making. This is called Value of Information (VoI) analysis (see Colyvan, 2016, for an overview and its application in conservation biology), and has been applied to environmental monitoring and management in the marine environment (e.g. Mäntyniemi *et al.*, 2009; Nygård *et al.*, 2016; Runting, Wilson & Rhodes, 2013). While power analysis can be used to determine the minimum level of sampling required to make statistically robust conclusions from data collected (Cohen, 1977), VoI analysis helps to understand the benefit of carrying out additional sampling with the cost of this sampling.

Estimating the potential cost of research efforts required to achieve certain levels of sampling completeness would be a relatively simple task. However, VoI analysis would also require an estimation of the economic benefit of achieving environmental goals or objectives through a monitoring or assessment programme (Nygård *et al.*, 2016). In addition, it would involve an investigation into how the accuracy of sampling information (for example, 99% sample completeness) affects decision-makers ability to choose optimum actions to achieve environmental goals and objectives and the gains from the improved environmental status that this would supposedly bring (Nygård *et al.*, 2016). This would have implications for standards and guidelines developed for baseline studies and monitoring carried out in any industry operating in the deep-sea environment.

In terms of deep-sea mining, specifically, the ISA could employ a Regional Environmental Assessment (REA) approach (discussed below) to achieve sampling required for certain levels of sample coverage (and thus understanding of baseline conditions), and this should be supported by a form of VoI analysis. There is an opportunity to shape environmental standards and guidelines around baseline sampling for deep-sea mining, as these are still being developed (ISA, 2019b). The importance of rare species has been noted in the ISA guidelines to contractors on sampling (ISA, 2013, Annex 1, para. 36(a)), and in their summary of environmental requirements for deep-sea mining, Bräger, Romero Rodriguez and Mulsow (2018) highlight that these are likely the species that will be most impacted by mining, either because they are ecologically more sensitive to mining impacts, or because they are K-selected species (i.e. with relatively stable populations and producing few offspring) or apex predators. They conclude by saying that “special emphasis should be put on rare species as they may be the first to be lost” (Bräger, Romero Rodriguez & Mulsow, 2018, p. 7). More research is required, firstly to fully characterise the baseline fauna in areas targeted for mining, and secondly to begin to understand the functional importance of rare species in the deep sea. This will be crucial in advancing environmental management of deep-sea activities, and specifically deep-sea mining.

3.4.3 Application to baseline studies

The application of this study is focussed more towards sampling for baseline environmental studies than monitoring or impact assessment, although both of the latter are highly dependent on good baseline data (Cordes *et al.*, 2016; Tunnicliffe *et al.*, 2018). Baseline studies quantify and describe the environmental conditions prior to any activity, including the physical, chemical, biological and geological features. They form the basis upon which other management measures depend, such as EIA and monitoring (Bräger, Romero Rodriguez & Mulsow, 2018).

This means that it is very important to establish robust environmental baselines, so that any changes in the environment caused by human activities can be measured. Through the EU Marine Strategy Framework Directive, for example, environmental status of EU member states' seabed and waters is measured against environmental baselines (Borja *et al.*, 2013). Baseline environmental surveys also form an integral component of environmental management in the oil and gas industry, although surprisingly they are not necessarily a requirement in all states (Cordes *et al.*, 2016). There is some guidance for baseline sampling for deep-sea mining (ISA, 2013), but this is generally focussed on the ecological components that should be sampled, rather than sample size or design.

The results of this study are applicable to deep-water industries, particularly those operating over very large areas with low faunal densities. The results suggest that a fairly good understanding of abyssal epibenthic megafauna diversity can be gleaned from the sampling units and sample sizes generally used, but show that in order to get a thorough inventory of the species present much more sampling is required. This study suggests that for abyssal plain environments, sampling units in the order of 2 858 m^2 - 4 643 m^2 (depending on whether megafauna or metazoans are examined) are required to get a very good (e.g. 99%) picture of the fauna present. Baseline studies should thus use transects in this size range, and ensure that the number of replicates covers a total seabed area of 20 800 m^2 - 21 600 m^2. It is important to note here again that this applies only to these environmental conditions sampled, and that additional sampling would be required to establish environmental baselines for other habitats. The large sampling effort required to characterise megafauna communities would be best tackled through a strategic approach, such as regional environmental assessment, as discussed below.

Robust baseline sampling is particularly important where activities are planned for areas with very little environmental data. In the context of deep-sea mining, the fauna associated with areas targeted for mining are still not well known (Glover *et al.*, 2018), nor are the potential impacts (Jones *et al.*, 2017), and so this becomes even more important. Establishing a good environmental baseline is one of the key ways to address uncertainty associated with activities (Jones *et al.*, 2019), and baseline studies carried out by contractors are expected to form the basis of regional Strategic Environmental Assessments, as discussed below (Bräger, Romero Rodriguez & Mulsow, 2018). At present, there is both a lack of enforcement of environmental regulations and a lack of environmental data to ensure that proper baseline studies and thus EIAs are carried out (Bräger, Romero Rodriguez & Mulsow, 2018).

3.4.4 Regional environmental assessment

The need for large sampling units and sample sizes to characterise megafauna diversity underscores the importance of Strategic Environmental Assessment (SEA) by the ISA. SEA refers to environmental management at scales greater than individual projects (Jones *et al.*, 2019). Unlike EIAs, SEAs have much broader spatial and temporal extent and are normally applied to a larger geographic extent or a whole sector (UNECE, 2003).

SEAs provide the basis for EIAs and marine spatial planning and should thus precede both of these (Warner, n.d.).

SEAs bring together the key stakeholders of an industry, including operators, government, regulators and the scientific research community, to design and implement a large-scale, coordinated, strategic environmental assessment of areas that are being targeted for commercial activity (e.g. Gov.UK, 2019). SEAs are used to improve understanding of poorly known areas, identify sensitive areas and ecological process that may be acting at a regional scale, identify potential cumulative impacts and develop protection measures (e.g. Bett, 2001). This broader approach promotes the merging of resources to ensure that all work carried out fits into an overarching strategic plan, avoids duplication of efforts, and provides a regional context for project-specific EIAs (BMAPA, 2019).

In the context of seabed mining in ABNJ, SEA refers to assessment at the scale of the Area as a whole, while Regional Environmental Assessment (REA) refers to assessment in a particular region, like the CCZ (Jones *et al.*, 2019). No REAs have yet been carried out for seabed mining, although a Regional Environmental Management Plan (REMP) has been developed and adopted for the CCZ (ISA, 2011). A tiered approach for environmental management of deep-sea mining in ABNJ is proposed, with an overarching environmental management strategy that would form the basis for regional environmental assessments and management plans (REAs and REMPs), which in turn would provide context for project-specific EIAs (Jaeckel, 2019; Jones & Weaver, 2017; Jones *et al.*, 2019).

The need for SEA or REA when managing multiple parties carrying out commercial activities in the marine environment has previously been identified in several countries, including the United States, Canada, Norway and Denmark (Doelle, 2009; Verheem & Tonk, 2000). In the EU, member states are required to conduct SEAs for new offshore energy developments (UNECE, 2003). Examples of successful SEA can be found in offshore industries in UK waters, for example in the oil and gas industry (Bett, 2001), aggregate dredging (BMAPA, 2019; Wallingford, 2010), and offshore energy development (Gill *et al.*, 2005; Gov.UK, 2019; Nedwell *et al.*, 2007). SEA has also been identified as an important factor in the negotiations for an international legally-binding instrument (ILBI) on the protection of biodiversity beyond national jurisdiction (BBNJ) (Warner, n.d.).

The outcomes of this study could be used together with power analyses and other design principles to inform the development of an REA sampling strategy for deep-sea mining. These results illustrate the minimum sampling effort required to achieve specific levels of sample coverage, and could be used to guide the choice of sampling units for epibenthic megafauna, as well as the number of replicates. Decisions would have to be made to define what constitutes “adequate” sampling and to balance the cost and benefit of additional sampling.

3.5 Conclusions

The fauna of the CCZ remains largely undiscovered (Glover *et al.*, 2018), and the extent of the potential environmental impacts of seabed mining are still unknown (Jones, Amon & Chapman, 2018; Jones *et al.*, 2017). This makes the ISA’s obligation to adopt the precautionary approach (ISA, 2000, Regulation 31, para. 2) even more important when managing mining activities, and robust baseline studies are a fundamental starting point for this. Questioning and fine-tuning the appropriate size and number of samples is extremely important to ensure that sampling is robust. This is particularly important where the results of studies will be used to inform environmental management of such a large-scale commercial activity as deep-sea mining (Durden *et al.*, 2017a; Levin *et al.*, 2016).

This study provides estimates for the sampling effort (both size and number of sampling units) required to capture diversity of epibenthic megafauna at different levels of sample coverage and asymptote. The outcomes of this study can be used to inform standards and guidelines for contractor baseline studies (within their area), as well as REA and best industry practice guidelines. The study highlights the need for a strategic approach to baseline sampling, in the form of REA, and this should be prioritised before any commercial activity commences.

Finally, this study reveals the high level of sampling effort required to capture rare morphotypes, and raises questions around sampling strategies going forward. Future work is needed to understand the functional importance of both rare and common megafauna species in abyssal plains, as well as VoI or cost-benefit analysis to understand the effect that additional information on rare species would have on decision-making.

Chapter 4.

Using a top-down, broad-scale habitat classification to assess representativity of the CCZ protected area network

4.1 Introduction

4.1.1 Marine spatial planning in ABNJ

Human activities and climate change have been shown to negatively impact the deep sea (Ramirez-Llodra *et al.*, 2011), and the influence of humans has been recorded in even the deepest part of the ocean, the Mariana Trench (Chiba *et al.*, 2018). With human activity continuing to increase and encroaching further and deeper into large, unknown areas, the global community needs to make decisions on how to manage these activities so as to minimise potential damage to deep-sea ecosystems, particularly in Areas Beyond National Jurisdiction (ABNJ) (Altvater, Fletcher & Passarello, 2019; Ardron *et al.*, 2008).

One of the most highly advocated approaches to managing human activities is through ecosystem-based management, an approach supported by various global (e.g. the 1982 United Nations Convention on the Law of the Sea (UNCLOS) and the Convention on Biological Diversity (CBD)) and regional conventions (e.g. the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR), the Convention on the Protection of the Marine Environment of the Baltic Sea Area (Helsinki, 1992), the Convention for the Protection of the Marine Environment of the North-east Atlantic (OSPAR, 1992) and the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean). Ecosystem-based management is an approach that considers all aspects of and interactions within an ecosystem, including humans, and follows a comprehensive strategy for managing activities that promotes conservation and sustainable use of resources (Ehler & Douvere, 2007). This approach is implemented through a number of different management measures, including standards, guidelines, permits, monitoring, enforcement, and Marine Spatial Planning (MSP).

MSP is an area-based planning tool that allocates space in the marine environment to different users based on ecological, economic and social objectives in order to balance demands for economic development with the need for environmental protection (Ehler & Douvere, 2007). It considers the ocean environment and all of its users as a whole, rather than through a piece-meal, sector-by-sector approach. MSP thus provides an integrated approach to managing multiple, overlapping activities with spatial coherence and looks for synergies amongst sectors, identifying compatible and conflicting activities to help minimise conflicts between overlapping users and reduce cumulative impacts (Ehler & Douvere, 2007; Maes, 2008). MSP also provides a framework for transparent, consistent decision-making so that stakeholders can engage in an open and planned way, and new activities can be incorporated into the system. In order to achieve ecological goals in a specific area, MSP may involve the allocation of protected areas to conserve biodiversity. Marine Protected Areas (MPAs) have been shown to have conservation benefits (Edgar *et al.*, 2014; Lubchenco *et al.*, 2003; Mumby & Harborne, 2010), and have gained political support (O'Leary *et al.*, 2012). Where MPAs were historically etablished on an ad hoc and individual basis (Toropova *et al.*, 2010; UNEP-WCMC, 2008), the focus is now on establishing networks of protected areas to advance protection (Dudley, 2008; Johnson *et al.*, 2014).

Although some nations have specific MSP requirements in their own national laws or policies (e.g. the EU: OJ, 2014; and South Africa: RSA, 2017; RSA, 2019), MSP is not mentioned explicitly in any international legislation pertaining to the high seas (Ardron *et al.*, 2008; De Santo, 2018; Maes, 2008). Under the current legal frameworks, not only are states or institutions not obliged to undertake MSP in ABNJ, there is in fact no mechanism for them to do so (Altvater, Fletcher & Passarello, 2019; UNEP-WCMC, 2018). There are several mechanisms through which area-based management tools (ABMTs), spatial closures with “more stringent regulation of one or more or all human activities, for one or more purposes” (Molenaar, 2013), can be implemented in ABNJ. Areas of Particular Environmental Interest (APEIs) have been designated by the International Seabed Authority (ISA) through UNCLOS to conserve regional biodiversity from seabed mining in the Clarion Clipperton Fracture Zone (CCZ) (ISA, 2011), and closures of Vulnerable Marine Ecosystems (VMEs) to bottom-fishing have been implemented in some areas through Regional Fisheries Management Organisations (RFMOs) (WGDEC, 2006; Wright *et al.*, 2015). Twelve high seas MPAs have also been declared, two in the Southern Ocean under CCAMLR, and ten in the North-east Atlantic through the OSPAR Convention and the North-east Atlantic Fisheries Commission (NEAFC) (De Santo, 2018). Emission Control Areas/Special Areas and Particularly Sensitive Sea Areas (PSSAs) can also be put in place through the International Maritime Organization's (IMO) International Convention for the Prevention of Pollution from Ships (MARPOL). Finally, the CBD has established criteria for identifying ecologically or biologically significant areas (EBSAs) that are important for maintaining healthy oceans and should therefore be protected (CBD, 2009).

All of these measures are, however, single sector, do not always follow similar criteria to identify protected areas, are contained in a number of different agreements and, in some cases, are voluntary. There is no overarching process for MSP, and MPA designation, on the high seas (Altvater, Fletcher & Passarello, 2019; De Santo, 2018).

This has led, after more than a decade of informal discussions, to the adoption in June 2015 of a Resolution by the UN General Assembly (UNGA resolution 72/249) on the conservation and sustainable use of marine biological diversity in ABNJ (UNGA, 2017). A new, legally-binding instrument is currently being negotiated to address marine management and conservation on the high seas. This will be an integrated, cross-sectoral, overarching framework to manage activities in ABNJ, and will ensure that ABMTs are implemented in a strategic, coherent and organised manner to ensure protection of biodiversity on the high seas. MSP is widely accepted as a pragmatic tool to support planning within national jurisdiction, and has thus been highlighted as an integral component to be included in negotiations (Altvater, Fletcher & Passarello, 2019; Ardron *et al.*, 2008; Maes, 2008; UNEP-WCMC, 2018; Wright *et al.*, 2018). Several models for incorporating MSP in ABNJ management have been suggested, including planning at a regional level with international oversight, and there are expected to be challenges with implementing MSP in ABNJ, including the lack of a coordinating body to develop and implement plans, a paucity of data in the high seas and a large, diffuse stakeholder group (Altvater, Fletcher & Passarello, 2019; Wright *et al.*, 2018).

4.1.2 Habitat classification as a tool to support MSP

One of the most common and useful tools to support MSP and area-based management is habitat classifications. The identification and delineation of different types of marine habitats and the communities they contain is required, so that planning can take place on how and where to protect habitats (Ehler & Douvere, 2007; Roff, Taylor & Laughren, 2003). Habitat classifications are used to partition an area into distinct groups or classes that contain environmental properties and a biological community that is unique to that class (Howell, 2010; UNESCO, 2009). These classifications provide insight into the distribution of different species, communities, or ecosystems, and can be used to identify habitats that are relatively rare or fragmented (Howell, 2010; UNESCO, 2009).

Habitat classifications are a versatile tool for environmental managers. They provide a science-based framework that can be used as a tool by decision-makers (e.g. governments or international institutions) to support planning concerning what activities should (or should not) be allowed where (UNESCO, 2009). Maps produced through classification systems underpin MSP by providing an ecological context for planning and management (Carpenter *et al.*, 1999). They can, and should, be used together with other geographically-relevant layers of information, such as past and current human use, to support spatial planning (Carpenter *et al.*, 1999).

One of the common uses of habitat classifications is in the design of MPAs (CBD, 2009), as a fundamental step in MPA design is to identify or predict areas that contain certain habitats or communities (Carpenter *et al.*, 1999). With the use of planning software, classifications can be used to identify alternative options for MPAs, or to assess the effectiveness of existing MPA networks (e.g. Douglass *et al.*, 2014; Evans, Peckett & Howell, 2015; Foster *et al.*, 2017). A well accepted criteria of MPAs is that they should protect a representative sample of the habitats in the area of interest (OSPAR, 2008). Under the CBD, the current target for global representation of coastal and marine areas is 10% by 2020 (CBD, 2010), with a more ambitious target of 30% advocated at the 2003 World Parks Congress (IUCN, 2005). Habitat classifications can be used together with ecological sensitivity assessments and socio-economic data to identify optimum areas for protection that fulfil these conservation targets, while minimising impacts on other users of the sea (e.g. South Africa's offshore MPA planning, Sink *et al.*, 2011). Alternatively, they can be used to assess the habitat representativity of existing MPA networks. Evans, Peckett and Howell (2015) used a biophysical classification scheme in the North-east Atlantic high seas to show that the current MPA network is neither efficient nor representative, and identified areas for consideration to improve the design.

4.1.3 Habitat classifications in ABNJ

The scale of habitat classifications varies greatly, from relatively fine-scale classifications focussed on national waters (e.g. Connor *et al.*, 2004; Last *et al.*, 2010; Leathwick, Dey & Julian, 2006; Lombard *et al.*, 2004; Lundblad *et al.*, 2006; Ramos, Puente & Juanes, 2015; Roff & Taylor, 2000; Snelder *et al.*, 2006; Verfaillie *et al.*, 2009) to regional-scale approaches (e.g. Davies, Moss & Hill, 2004; Douglass *et al.*, 2014; Evans, Peckett & Howell, 2015; Grant *et al.*, 2006; Howell, 2010; Ramos *et al.*, 2012) and finally global biogeographic classifications (e.g. Clark *et al.*, 2011; Harris & Whiteway, 2009; Sayre *et al.*, 2017; Sherman, 1986; Spalding *et al.*, 2007; Sutton *et al.*, 2017; UNESCO, 2009).

Most habitat classifications have been carried out in shallow and coastal waters to support management of activities at a national level (see examples above); however, in recent years, these methods have been applied to deep waters (> 200 m) in ABNJ to classify offshore benthic (e.g. Douglass *et al.*, 2014; Evans, Peckett & Howell, 2015; Howell, 2010) and pelagic (e.g. Sayre *et al.*, 2017; Sutton *et al.*, 2017; UNESCO, 2009) habitat types, as well as specific features (e.g. seamounts, Clark *et al.*, 2011).

In the equatorial Pacific, the CCZ is being targeted for mining of polymetallic nodules (see Chapter 1). Global classification efforts relevant to this area have identified broad biogeographic provinces, which are large, continuous areas unique to one another (see Watling et al. (2013) for a full review of deep-sea benthic classification schemes). The

Global Open Oceans and Deep Seabed (GOODS) biogeographic classification used environmental and, where available, biological data to divide the global oceans into 68 provinces, 30 pelagic and 38 benthic (UNESCO, 2009). Through this study 14 abyssal provinces were identified, two of which overlapped within the CCZ management area. The abyssal and bathyal portions of this bioregionalisation were then improved by Watling et al. (2013), who included additional relevant environmental variables to further refine the classification, resulting again in 14 abyssal provinces. Harris and Whiteway (2009) also proposed a global benthic classification, based on physical and chemical variables, and identified 11 biogeographic provinces, which they called seascapes. Finally, the most recent global biogeographic classification was carried out by Sayre et al. (2017), and put forward 37 different Ecological Marine Units (EMUs), identified using a classification of physical and chemical variables stratified from surface waters to the seafloor.

In each case, the CCZ management areas falls within one or two of the biogeographic provinces identified through these studies. While a useful starting point, these global level classifications do not offer the resolution needed to make regional management decisions. Therefore, a more regional approach is needed.

In terms of regional habitat classifications, several areas in ABNJ have received a lot of attention, with more detailed classifications carried out, for example the North-east Atlantic (Evans, Peckett & Howell, 2015; Howell, 2010) and Southern Ocean (Douglass et al., 2014; Grant et al., 2006). This is a result of high levels of industrial activity in these areas and legislative requirements for MSP (OSPAR and CCAMLR conventions, respectively). These classifications are aimed at aiding the management of high seas activities occurring at regional scales. In the equatorial Pacific, where the CCZ is located, there have been no such regional classification efforts to date.

4.1.4 Habitat classification methods

4.1.4.1 Classification approaches

There are a number of different approaches for performing habitat classification. The most popular methods can be generally divided into (1) thematic, hierarchical classifications, (2) numerical or multivariate classifications, and (3) expert-derived classifications (Clark *et al.*, 2011).

Thematic classifications involve the categorisation and combination of individual thematic layers into a hierarchical structure. The output is a conceptual hierarchical framework that can be applied by marine managers to an area of interest that needs to be classified and mapped. This approach is subjective, and may lead to unnatural groupings; however, it is generally easy to understand and work through, and is therefore popular for marine management purposes (Clark *et al.*, 2011). Some examples using this approach are the European Nature Information System (EUNIS) classification scheme (Davies, Moss & Hill, 2004), a global classification of seamounts (Clark *et al.*, 2011), a benthic classification for the Southern Ocean (Douglass *et al.*, 2014), a benthic classification for the North-east Atlantic (Howell, 2010) and classifications of Australian (Last *et al.*, 2010; Mount, Bricher & Newton, 2007), Canadian (Roff & Taylor, 2000) and South African (Lombard *et al.*, 2004) waters.

Multivariate classifications apply statistical methods to multiple (normally continuous) variables to identify groupings in the data, which are treated as distinct habitats. This is a quantitative statistical approach that relies on analysis and interpretation of variables.

It is generally considered a more objective and statistically robust approach, which recognises natural groupings in the data (Clark *et al.*, 2011). The output of this classification approach is a map identifying where the different habitat types are. Some examples include classifications of, *inter alia*, the North-east Atlantic both within (Ramos *et al.*, 2012) and beyond (Evans, Peckett & Howell, 2015) national jurisdiction, the Belgian North Sea (Verfaillie *et al.*, 2009), the New Zealand Exclusive Economic Zone (EEZ) (Snelder *et al.*, 2006), the Australian margin (Whiteway *et al.*, 2007), South African unconsolidated sediments (Karenyi, Sink & Nel, 2016), and the global oceans (Harris & Whiteway, 2009; Sayre *et al.*, 2017).

One of the downfalls of multivariate classifications is that conceptually they are more complicated than thematic classifications, and there are many different types of analyses that can be used to identify groupings in the data, with no standard procedure established (Clark *et al.*, 2011). The multivariate approach also often produces a classification with a large number of groups, and application in different regions produces different end classes making it very difficult to combine multiple regional maps. This method is thus not widely adopted by environmental managers (Clark *et al.*, 2011).

Finally, an expert-derived classification approach makes use of expert knowledge to delineate boundaries of habitat types based on patterns in environmental variables and biological data (where available). Examples of expert-derived classifications include the GOODS classification (UNESCO, 2009) and it's refined bathyal and abyssal version (Watling *et al.*, 2013), the Large Marine Ecosystems (LMEs) (Sherman, 1986) and Marine Ecoregions of the World (MEOW) (Spalding *et al.*, 2007) classifications, and a global mesopelagic biogeographic classification (Sutton *et al.*, 2017). This approach does not allow for rigorous replication (Spalding *et al.*, 2007).

4.1.4.2 Surrogates

In the deep sea, and even coastal areas, there is often a paucity of biological data, and the use of surrogates to represent biological diversity has become an established method for mapping both benthic and pelagic habitats (e.g. Clark *et al.*, 2011; Evans, Peckett & Howell, 2015; Harris & Whiteway, 2009; Howell, 2010; Roff & Taylor, 2000). The surrogates used are generally chemical, physical or environmental variables that are more readily available and, critically, are identified as important factors driving the distribution and turnover of species and communities (Evans, Peckett & Howell, 2015). Habitat maps produced through classifications using surrogates are used to infer the distribution of a species, community or ecosystem. These are hypothesis-driven approaches, whereby the classes or groups produced through a classification are hypothesised to contain different biological communities, based on the fact that the environmental properties of the classes are distinct.

The five most common surrogates used in benthic classifications are biogeography, depth, geomorphology, substrate and biological assemblage, although data for the latter is often severely lacking for the deep sea (Howell, 2010). Importantly, the divisions or categories used within each surrogate should represent variation in faunal composition (Howell, 2010).

4.1.5 Biological validation

One of the fundamental assumptions of biophysical habitat classifications performed using surrogates is that environmental heterogeneity reflects variation in biological communities, and that the habitat maps produced using the classification are biologically meaningful. Biological validation, or ground-truthing, of habitat classifications is key, and should be carried out (where biological data sets are available) before a classification is used for spatial planning purposes (Evans, Peckett & Howell, 2015; Howell, 2010; Kenny *et al.*, 2003; Valesini *et al.*, 2010).

Only a handful of studies have used biological data to test the validity of their classifications, and demonstrate statistically that their groupings are biologically relevant. These have mostly been in shallow waters, within national jurisdiction, on classifications produced using multivariate analyses (e.g. Bowden *et al.*, 2011; Ramos, Puente & Juanes, 2015; Ramos *et al.*, 2014; Snelder *et al.*, 2006; Verfaillie *et al.*, 2009; Whiteway *et al.*, 2007). There have been some attempts to validate classifications in deep waters at a global scale, although these have been far fewer (e.g. Clark *et al.*, 2011; Watling *et al.*, 2013). Clark *et al.* (2011) tested their seamount classification using biological data from the North Atlantic. Their cluster analysis of octocoral data showed similar groupings to the hierarchical seamount classification, suggesting the classification may be biologically realistic. (Watling *et al.*, 2013) used knowledge of faunal distributions (where large enough data sets were available) to support and refine the boundaries of their expert-derived global seafloor classification.

A number of different methods have been used to validate classifications. The most common approach involves clustering biological data to determine whether patterns in the biological clusterings reflect those found through the physical classification. Clustering-type approaches include group averaging hierarchical clustering (Bowden *et al.*, 2011; Clark *et al.*, 2011), multi-dimensional scaling (MDS) (Ramos, Puente & Juanes, 2015; Ramos *et al.*, 2014), and Similarity Profile Analysis (SIMPROF) (Clark *et al.*, 2011).

Multivariate analyses such as Analysis of Similarities (ANOSIM) and Permutational multivariate analysis of variance (PERMANOVA) have also been used to investigate whether there are significant differences in biological data among different habitat types (e.g. Bowden *et al.*, 2011; Ramos, Puente & Juanes, 2015; Ramos *et al.*, 2014; Snelder *et al.*, 2006), and Similarity Percentage (SIMPER) has been used to identify the species contributing to the differences (Ramos, Puente & Juanes, 2015; Ramos *et al.*, 2014). Finally, an indicator species analysis approach has also been used to determine whether significant indicator species (identified using an index that is highest when all individuals of a species are found in a single habitat and when the species occurs in all sites of that habitat) can be identified for each habitat type in a classification, thus giving it a form of ecological relevance (Verfaillie *et al.*, 2009).

Non-statistical approaches have also been used, for example Watling *et al.* (2013) tested some of the boundaries of their proposed classification by examining previously described patterns in bathyal and abyssal fauna distribution, and comparing this to their classification where the distributions cross boundaries of the proposed biogeographic provinces.

While not all habitat classifications based on abiotic variables have been tested using these validation techniques, those that have, have shown varying degrees of confidence in their groupings. Problems may arise when classifications are not used at the scales for which they are intended (Bowden *et al.*, 2011).

4.1.6 Aims

Deep-sea mining is an emerging industry in ABNJ, that requires balancing of exploitation and commercial activity with protection of the marine environment. MSP is essential to support environmental management associated with mining activities (Lodge et al., 2014; Vanreusel et al., 2016; Wedding et al., 2015), and some MSP has been undertaken in the CCZ through the CCZ Environmental Management Plan (EMP) (ISA, 2011), with the designation of an APEI network to protect regional biodiversity and discussions around impact and preservation reference zones (IRZs and PRZs) within contract areas to facilitate monitoring of mining impacts. There have been calls for habitat mapping in the CCZ to support the spatial management of mining activities (e.g. De Smet *et al.*, 2017; ISA, 2017b); however, in the equatorial Pacific, where the CCZ is located, no regional habitat classifications have been carried out, and the only classifications relevant to the CCZ are thus global biogeographic regionalisations, which are intended only to provide a broad-scale overview within which regional classifications can nest.

The overall goal of this study was to support spatial planning associated with seabed mining, and bridge the gap between policy demands (e.g. ISA needs to make decisions on APEIs, IRZs and PRZs) and available science. Specifically, this study aimed to:

1) Produce a broad-scale habitat map of the CCZ management area using a top-down habitat classification system of environmental surrogates developed through non-hierarchical cluster analysis;
2) Determine whether this classification was successful in representing patterns in the distribution of epibenthic megafauna; and
3) Use the classification produced to test the representativity of the current network of protected areas in the CCZ, and make recommendations to fill any gaps identified.

4.2 Methods

This study used a two step process to classify environmental surrogates that were identified as being potentially important in driving species distributions in the CCZ: first, a *k*-medoids clustering algorithm was used to perform an unsupervised non-hierarchical clustering on each group of environmental variables; then, the outputs of the clustering were combined to give a final habitat classification. Several ground-truthing analyses were then performed on biological data to determine the ability of the classification method to identify ecologically relevant habitats in the CCZ. Finally, the classification was used to test the habitat representativity of the current APEI network in the CCZ, described in Chapter 1.

4.2.1 Study site

The site for this study was the entire CCZ management area, as defined by the boundaries in the CCZ EMP (ISA, 2011) (shifted slightly east to encompass all licence areas comfortably): 0°-23°30′ N x 114° W-159° W (Figure 1.1).

The CCZ is a large area of approximately 6 million km^2, located between the Clarion and Clipperton fracture zones in the equatorial Pacific. It is an abyssal plain area, with topographic features including seamounts, troughs and ridges, and the highest global concentration of high-grade nodules (Thiel *et al.*, 1998).

4.2.2 Habitat classification

The classification approach followed these steps:

- Identify variables that describe key biophysical and ecological patterns and processes in the CCZ (restricted to data sets available for the region)
- Collect relevant data sets

- Pre-process data and apply clustering algorithm
- Evaluate clustering outputs and expert review
- Combine and finalise habitat classification

4.2.2.1 Identification of key variables

Some guiding principles for variable selection, from Ramos, Puente and Juanes (2015), were:

- Spatial variability at the regional level in the study
- Proposed in other classifications of similar scales
- Related to the geographic distribution of the communities of interest
- Possibility of obtaining continuous quantitative and standardised data across the study area
- No mutual influence (i.e. correlation) between variables

While the list of variables chosen for this study did not include all of those driving species distribution in the CCZ, it was believed to include the most important variables that would have the strongest influence on biological communities in this area, and it was expected that these drivers would be correlated with other parameters that were not included that may also influence species distribution.

4.2.2.1.1 Water mass structure

Water mass structure is an important factor in determining the distribution of organisms in the marine environment (Howell, Billet & Tyler, 2002; Miller *et al.*, 2011; Tyler & Zibrowius, 1992). The extent and distribution of water masses can be used to gain insight into species connectivity, due to transport and mixing processes within water bodies (Miller *et al.*, 2011). In their global classification Watling *et al.* (2013) used water mass structure to infer connectivity, assuming that seafloor regions overlain with a particular water mass are more likely to share species (as there are fewer barriers to dispersal), and thus fall within the same biogeographic province.

Knowledge on water mass structures below 800 m is extremely poor, but it can be estimated using temperature and salinity (Evans, Peckett & Howell, 2015). Although salinity does not have direct biological significance in the deep sea (as salinity variations are very low) (Roff & Taylor, 2000), the combination of temperature and salinity (amongst others) can be used to distinguishing water mass structure (Bryan & Lewis, 1979; Emery & Meincke, 1986; Roff & Taylor, 2000; Watling *et al.*, 2013). Mapping combined salinity and temperature provides insight into the extent and distribution of different water masses, and thus provides a broad biogeographic context for the habitat classification. Based on existing global biogeographic classifications (Harris & Whiteway, 2009; Sayre *et al.*, 2017; UNESCO, 2009; Watling *et al.*, 2013) it was anticipated that this classification would overlap with two biogeographic provinces.

4.2.2.1.2 Topography

Depth and topography play an important role in determining species distribution in the deep sea, with species showing preference for certain depth ranges and topographic conditions (Hecker, 1990; Rice, Thurston & New, 1990; Rowe & Menzies, 1969). In abyssal regions, and specifically the CCZ, where there are seamounts, troughs and ridges, topographic variation has been shown to influence biological communities (Durden *et al.*, 2015; Leitner *et al.*, 2017; Simon-Lledó *et al.*, 2019b; Stefanoudis, Bett & Gooday, 2016). Bathymetry and derived variables representing different topographic conditions can therefore be used to identify areas that are likely to support different communities.

Although depth is a crucial factor driving species distribution and depth data is readily available, this parameter acts as an indirect surrogate for variables such as temperature, pressure, oxygen, water mass structure and food supply (Evans, Peckett & Howell, 2015), and was therefore excluded from this study. Rather, derivatives of bathymetry were used to represent topographic features of the region: slope, broad-scale bathymetric position index (BBPI) and fine-scale bathymetric position index (FBPI).

Slope is a first-order derivative of bathymetry, and provides information on the gradient of the seafloor in degrees. It acts as a surrogate for local hydrodynamics (Guinan *et al.*, 2009), as benthic currents are directed by seafloor terrain and current velocity is expected to be strong where there is a steep slope (Genin *et al.*, 1986). This influences the flow of food and impacts suspensions feeders particularly (Gage & Tyler, 1996).

Bathymetric position index (modified from topographic position index) is a second-order derivative of bathymetry, and gives the relative elevation of a point in relation to the overall landscape (Lundblad *et al.*, 2006). Positive values indicate features rising above the surrounding terrain (e.g. peaks and crests), while negative values indicate depressions such as valleys and troughs. Areas with constant slope or flat areas are represented by near-zero values. BPI is a scale dependent phenomenon that can be calculated on both a broad- (BBPI) and fine-scale (FBPI), thus identifying topographic features that are relevant to the study (Weiss, 2001). BPI acts as a surrogate for various environmental factors that affect species distribution, such as exposure to current, current speed and sedimentation, without confounding the effects of other variables (e.g. temperature and salinity) as depth does (Evans, Peckett & Howell, 2015).

4.2.2.1.3 Particulate Organic Carbon

Food availability plays arguably the most important role in determining the distribution of organisms in the deep sea (Cartes & Sarda, 1993; Gooday, 2002; Levin *et al.*, 2001; Ruhl & Smith, 2004; Smith *et al.*, 1997; Smith *et al.*, 2008a; Wei *et al.*, 2011; Woolley *et al.*, 2016). In this food-poor environment, organisms are dependent on detrital matter in the form of Particulate Organic Carbon (POC) sinking from surface waters to the seafloor, except for cases of organic food falls and chemosynthetic habitats (Gooday *et al.*, 1990; Smith & Baco, 2003; Turner, 1973; Van Dover, 2000). As a result, community structure, function and diversity, life history patterns, body size, and feeding type, amongst others, are all strongly dependent on POC flux to the seafloor (Smith *et al.*, 2008a).

In the CCZ there is a strong gradient in POC, decreasing from east to west, and from south to north (Lutz *et al.*, 2007; Pennington *et al.*, 2006; Smith *et al.*, 1997). Equatorial upwelling results in high productivity and moderate to high POC flux to the abyssal seafloor in south-eastern parts of the CCZ. To the north, however, the North Pacific Gyre leads to oligotrophic conditions and very low POC flux. This spatial gradient in POC across the CCZ has been linked to differences in faunal communities (e.g Smith *et al.*, 1997; Smith *et al.*, 2008a; Vanreusel *et al.*, 2016; Veillette *et al.*, 2007), and flux of POC to the seafloor was thus identified as a key driver of faunal distribution in the CCZ. There is very low seasonal variation in POC across the central Pacific (Lutz *et al.*, 2007) and although POC responds to El Niño warming events (Barber *et al.*, 1996), it is not clear how this affects POC flux to the seafloor in the CCZ (Barber *et al.*, 1996; Smith *et al.*, 1997).

4.2.2.1.4 Substrate

Substrate type plays a role in determining species distributions in the deep sea, and the use of substrate as a surrogate to represent biological diversity in deep-sea habitat classifications is well established (Howell, 2010). Although there is no global map of seabed substrate, in the CCZ polymetallic nodules provide most of the hard substrate in an otherwise soft sediment environment and nodule abundance thus gives a general idea of substrate across the region.

Nodules provide habitat for encrusting and epifaunal species that depend on hard surface for attachment (e.g. Amon *et al.*, 2016; Dugolinsky, Margolis & Dudley 1977; Gooday, Goineau & Voltski, 2015), and support a range of fauna in the crevices of individual nodules (Bussau, Schriever & Thiel, 1995; Thiel *et al.*, 1993). The increased habitat heterogeneity provided by nodules has been suggested to influence faunal standing stocks and distribution compared to other nodule-free abyssal environments (Amon *et al.*, 2016; Gooday *et al.*, 2017b; Vanreusel *et al.*, 2016). A recent study provided the first quantified evidence that variation in nodule cover in the CCZ influenced faunal standing stock, faunal composition, functional group composition, the distribution of individual species and some biodiversity attributes (Simon-Lledó *et al.*, 2019c). Nodules thus play an important role in determining the distribution of benthic fauna in the CCZ.

4.2.2.2 Description of data sets

All variables were interpolated to 1 km^2 and converted into rasters in WGS 1984 PDC Mercator projection, an equal-area projection suitable for use in the Pacific Ocean. An equal-area projection was used so that estimates of the area of each habitat identified through the classification could be calculated. Mercator is also a popular projection for navigation as it uses straight line segments that represent true bearings, and outputs from the classification could therefore be used to support the implementation of spatial management measures.

4.2.2.2.1 Water mass structure

Oceanographic variables used to represent water mass structure were obtained from the National Oceanic and Atmospheric Administration (NOAA) World Ocean Atlas (WOA) 2013 data series. Measurements of mean annual temperature and mean annual salinity were obtained for the period 2005-2012 at 1 degree resolution and various depths throughout the water column (0, 50, 100 m etc).

The CCZ is an abyssal plain region with water depths of up to 5 500 m, and the NOAA WOA 2013 data do not extend all the way to the seafloor in some parts of this area. A random forest spatial model was therefore used to predict measures of salinity and temperature at the seafloor. A 3 dimensional (latitude*longitude*depth) random forest model was created in R 3.3.1 using the NOAA data available in the CCZ from surface waters to a depth of 2 000 m. Although NOAA data is available at greater depths in some areas of the CCZ, these values were excluded from the model as the majority of data was in the first 2 000 m and the sparsity of deeper values weakened the model.

The random forest model was then trained on the General Bathymetric Chart of the Oceans (GEBCO) bathymetry layer (at 1 km^2 resolution) to obtain a grid of values of the variable (salinity or temperature) at the seabed in each GEBCO cell (Figure 4.1). The model was built with 500 trees, and explained 99.24% of the variance.

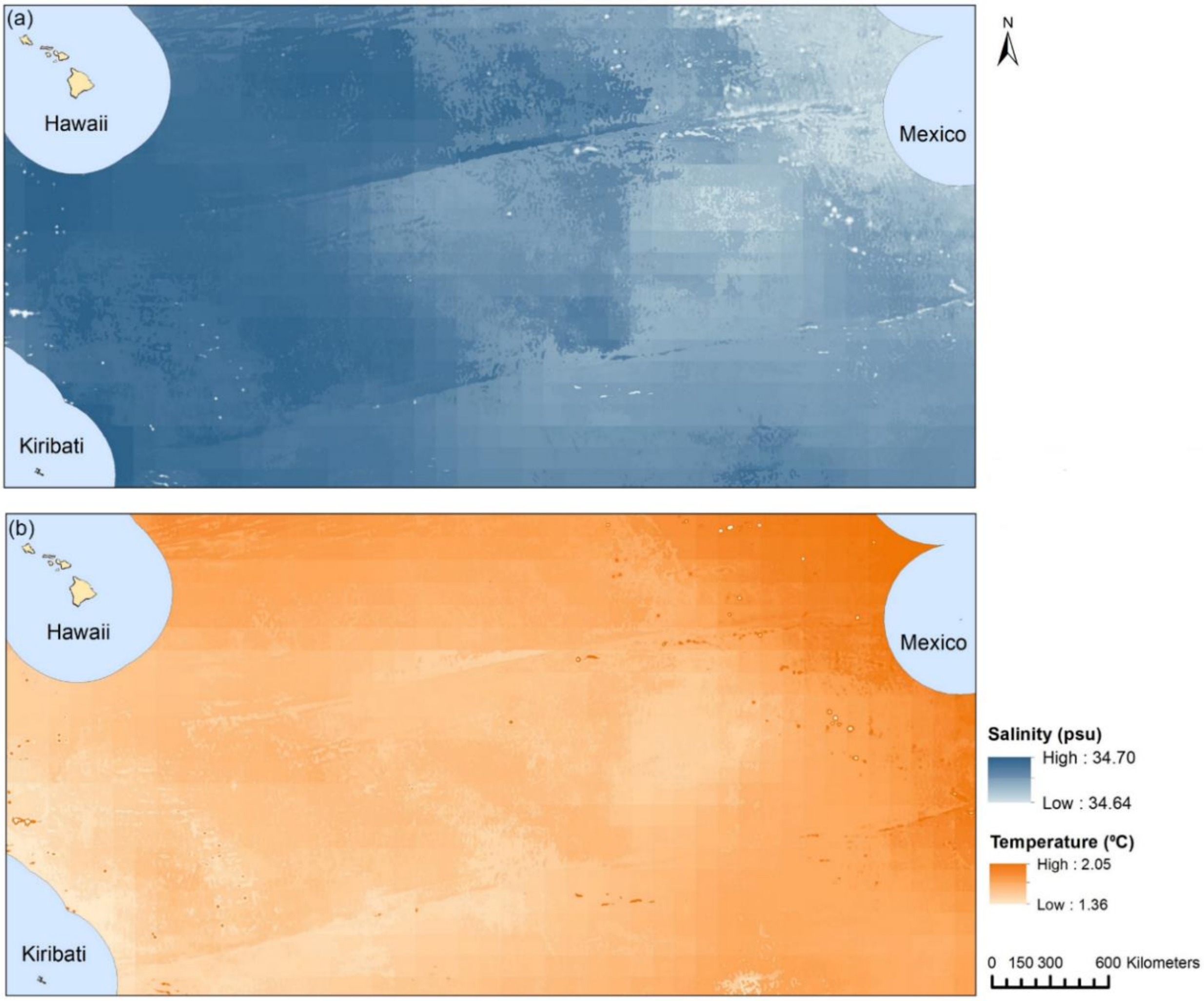

Figure 4.1 Mean annual (a) salinity and (b) temperature for the period 2005-2012, modelled to the seafloor at 1 km^2 resolution across the CCZ. Continents shown in cream, with EEZs in pale blue.

4.2.2.2.2 Topography

Topographic variables were derived from the GEBCO bathymetry layer (Figure 4.2). All variables were generated in ArcMap 10.4 using the Benthic Terrain Modeler extension (Wright *et al.*, 2005). Slope is determined as the largest change in elevation between a cell and its 8 nearest neighbours. BPI was derived at both broad and fine scale, to capture topographic features at different scales across the region. BBPI was derived with an inner radius of 1 and an outer radius of 100, with a scale factor of 100 km. This broad scale layer identified large geomorphological units, such as abyssal plains, steps and troughs. This choice of scale draws from the US Federal Geographic Data Committee's Coastal and Marine Ecological Classification Standard (CMECS) (FGDC, 2012). FBPI was derived with an inner radius of 1 and an outer radius of 10, with a scale factor of 10 km. This finer scale layer identified smaller megahabitats or features on the scale of kilometres to tens of kilometres, as defined in Greene *et al.* (1999). These features include seamounts, abyssal hills, canyons, plateaux, large banks and terraces.

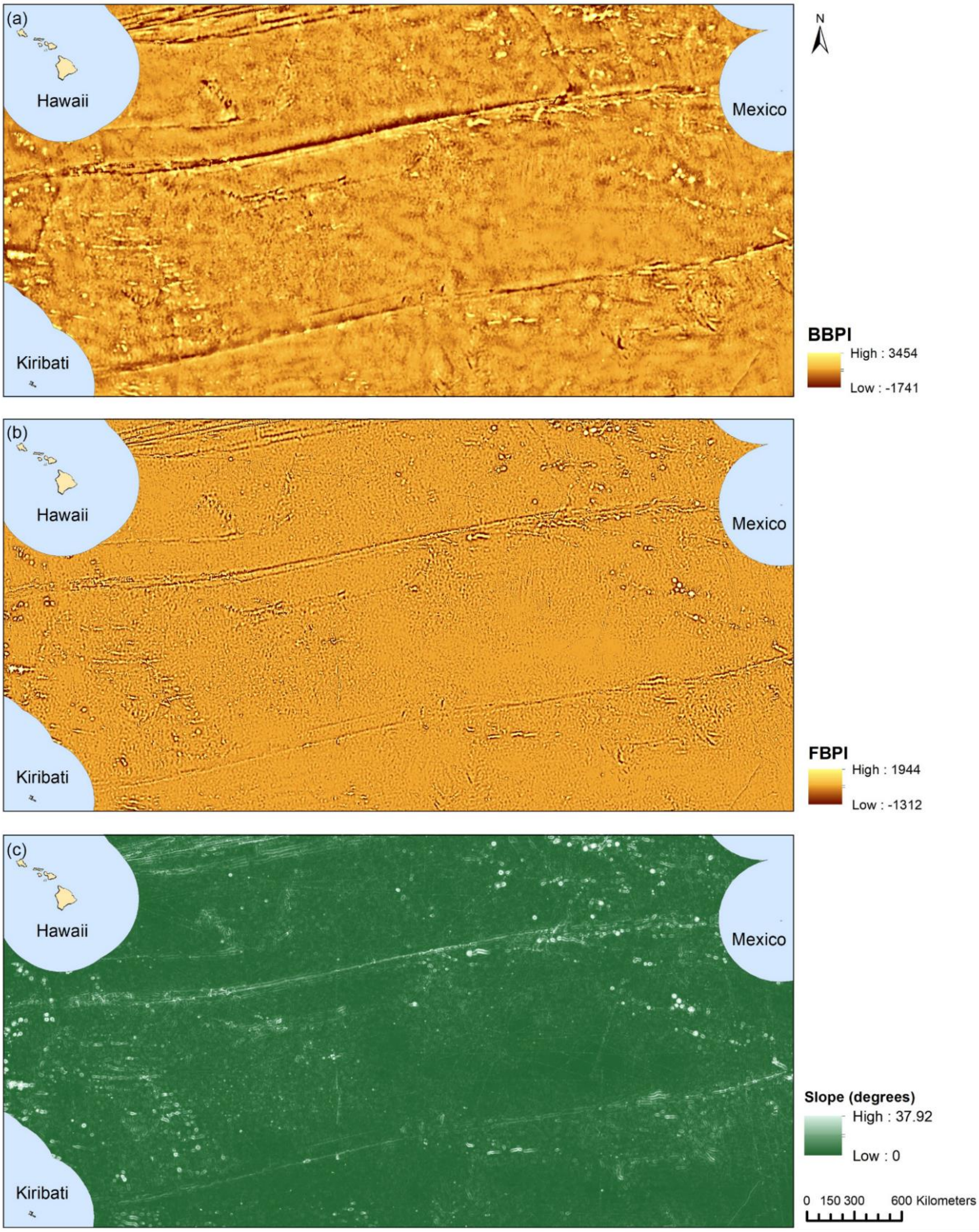

Figure 4.2 Topographic variables, (a) broad-scale bathymetric position index (BBPI), (b) fine-scale bathymetric position index (FBPI) and (c) slope, derived from GEBCO bathymetry at 1 km^2 across the CCZ.

4.2.2.2.3 POC

Estimates for POC in the CCZ were obtained from a global model produced by Lutz *et al.* (2007). Lutz *et al.* (2007) modelled flux of POC to the seafloor based on water depth and seasonal variability in remote-sensed net primary productivity between 19 August 1997 and 24 June 2004. These estimates were interpolated to a 1 km^2 resolution in the CCZ using kriging (Figure 4.3).

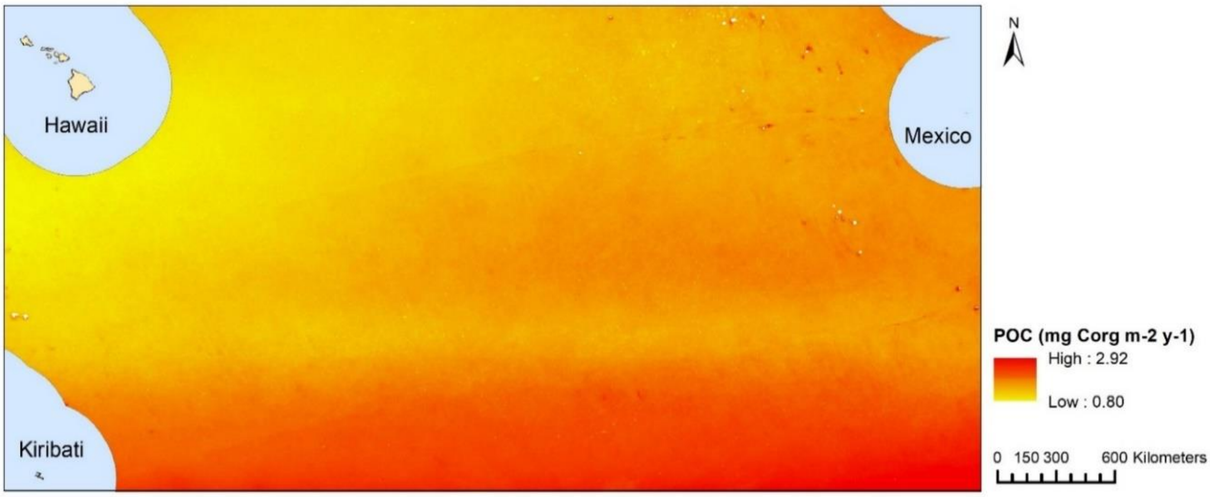

Figure 4.3 Particulate organic carbon (POC) modelled across the CCZ by Lutz *et al.* (2007) and interpolated to 1 km^2 resolution.

4.2.2.2.4 Nodule abundance

Modelled estimates of nodule abundance across the CCZ region were obtained from *ISA Technical Study No. 6: A Geological Model of Polymetallic Nodule Deposits in the Clarion Clipperton Fracture Zone* (ISA, 2010b). This model was developed by a group of technical experts in response to a need identified by the ISA for better resource assessments across the CCZ. The model used both publically available and proprietary data, with the latter provided by the Lockheed Martin Cooperation (Ocean Minerals Company, OMCO), the Government of the Republic of Korea, the China Ocean Mineral Resources Research and Development Association (COMRA) of China and the Inter Ocean Metal Joint Organization (IOM), composed of Bulgaria, Cuba, the Czech Republic, Poland, the Russian Federation and Slovakia.

Data sets comprised mainly of free-fall grab samples, with some box cores. Nodule abundance (weight of nodules per unit of seafloor area in kg.m^{-2}) was estimated by dividing the weight of recovered nodules by the surface area covered by the sampling equipment, and was expected to be an underestimate. In order to protect proprietary concerns, each sampling station was assigned to a grid cell (one-tenth of a degree) and summary statistics for all data within a cell were calculated. The entire study area was then divided into four sections (to improve efficiency of data processing) and the data for average nodule abundance per grid cell were interpolated for each section using Ordinary Kriging. The area of each cell was multiplied by the abundance estimate for that cell, and this gave the final estimate, which was then extrapolated across the whole CCZ management area. Although this modelled nodule layer was at a broad scale and so did not capture the small-scale heterogeneity of nodule fields (Peukert *et al.*, 2018), it represented broad patterns in nodule abundance across the region.

Modelled nodule abundance was obtained from the technical study at a quarter degree resolution in WGS 1984 projection. This layer was then converted to WGS 1984 PDC Mercator projection, at 1 km^2 resolution (Figure 4.4).

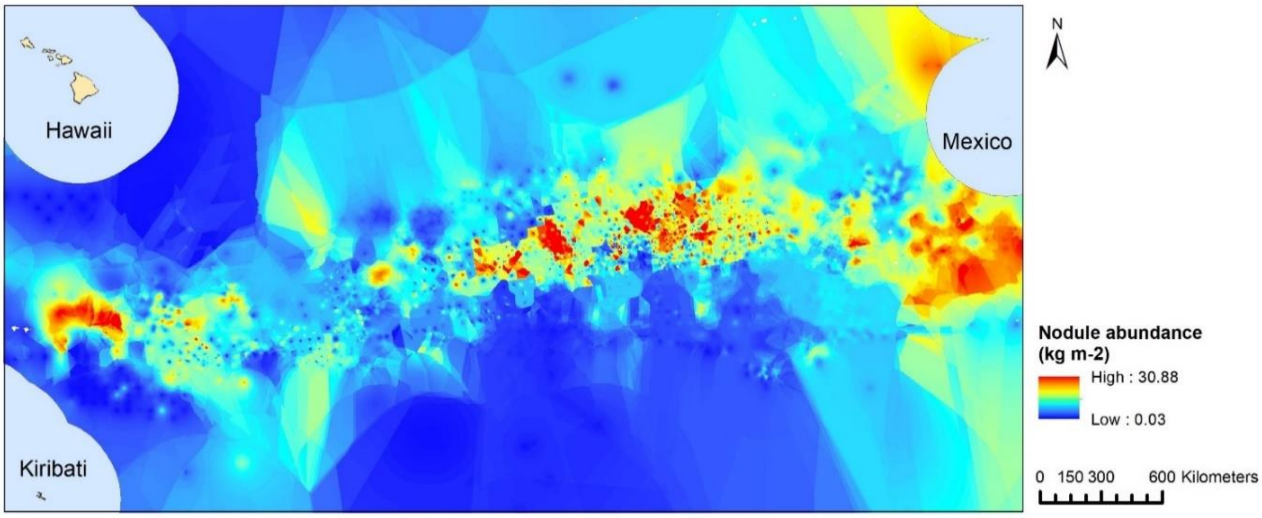

Figure 4.4 Nodule abundance modelled across the CCZ (ISA, 2010b), interpolated to 1 km^2 resolution.

4.2.2.3 Pre-processing and cluster analysis

This study made use of an unsupervised, non-hierarchical clustering algorithm called Clustering Large Applications (CLARA) (Kaufman & Rousseeuw, 1990). CLARA is a *k*-medoids clustering technique that clusters data points around the medoids and is appropriate for use with large data sets.

A separate CLARA cluster analysis was used to carry out unsupervised clustering on each group of variables: (1) oceanographic variables (salinity and temperature), (2) topographic variables (FBPI, BBPI and slope), (3) POC, and (4) nodule abundance. This provided an overview of the different water mass structures and topographic features within the region, and areas of high to low POC and nodule abundance.

All pre-processing and clustering was carried out in R 3.3.1 using the “fpc” package and CLARA function. As the focus of this study is on the deep sea in international waters, the EEZs of countries were excluded from the analysis. Prior to clustering, all variables were normalised to have equal variance and a common scale of 0-1. The dissimilarity matrix was calculated using Euclidean distances, as this is the appropriate distance measure for abiotic variables (Legendre & Legendre, 1998). CLARA requires the user to define the number of clusters the data set should be split into, and for each analysis clustering was therefore run a number of times, using a different number of clusters in each iteration, in order to identify the best number of clusters (see below).

4.2.2.4 Evaluation of clusters

The next step was to choose the most appropriate number of clusters for oceanography, topography, POC and nodule abundance, using a combination of cluster evaluation statistics and expert opinion. This is the most subjective stage of the process, but there are a number of different statistical tests and indices that can be used to help to determine the optimal number of clusters, as well as the strength of clustering. These include assessing cluster stability through resampling, visual cluster validation and separation-based validation indices (e.g. Hennig, 2007; Hsu, 2015). In an ideal scenario multiple indices should be considered in order to make a decision on the number of clusters. However, most of these statistics have extremely high computational time, space and power requirements for calculation and are thus inappropriate for use on such a large data set.

For this study a method called average silhouette width (ASW) was used. This is a popular method with low time, space and power requirements that can be applied to any clustering approach and is built into the R CLARA function. This method compares the ASW of clustering iterations with different numbers of clusters (Kaufman & Rousseeuw, 1990). The silhouette width of an object is a measure of how similar it is to its own cluster, compared to other clusters. Silhouette width values range from -1 to 1, with a high silhouette width indicating that an object is well matched to its assigned cluster and poorly matched to neighbouring clusters, and a low value (close to -1) indicating that an object is poorly matched to its own cluster.

ASW gives the average of all silhouette widths in a data set, with the output being one value representing the overall silhouette width for the whole cluster analysis. The ASW method compares ASW among cluster analyses carried out on the same data set with different numbers of clusters, and the optimum number of clusters is the one with the highest average silhouette width (Kaufman & Rousseeuw, 1990). Expert judgment was also used to further refine the final choice of number of clusters.

4.2.2.5 Finalisation of classification

Once the number of clusters was finalised for each variable grouping (oceanography, topography, POC and nodule abundance), the four layers were combined in ArcGIS 10.4 using the “Combine” tool to produce the final habitat classification. This was a layer of polygons, each a different set of oceanographic, topographic, POC and nodule cover conditions, representing habitat heterogeneity across the region.

Although both the clustering algorithm and the final combination allowed weighting of each variable to account for its importance in determining species distributions, the current state of knowledge of these relationships in the deep sea is poor, and it was thus unwise to allocate weightings to each variable.

4.2.3 Biological validation

The habitat classification produced was tested using epibenthic megafauna data from the CCZ to determine if patterns in habitat distribution identified through the classification represented spatial variability in the megafauna community. The biological data used for this ground truthing has been described in Chapter 2 (Figure 2.1), and consisted of nine transects (each 800 m^2) overlapping with three different habitat types. Metazoans and xenophyophores were analysed separately, as previous studies have found that including xenophyophores in analyses of community data can substantially affect the outcomes (Simon-Lledó *et al.*, 2019b). Several reasons for analysing them separately have been suggested, including difficulty in determining whether individuals are living (Hughes & Gooday, 2004) and body size mismatch due to the very small size of xenophyophore protoplasm volume (Levin & Gooday, 1992).

Several approaches were used to address the validation step. The first approach used ANOSIM to determine whether biological data with *a priori* groupings according to the habitat type within which they fell, were significantly different (Clarke, 1993). The second approach used SIMPROF to explore whether biological data with no *a priori* groupings fell into the same clusters given through the classification (Clarke, Somerfield & Gorley, 2008), and finally, SIMPER analysis was used to identify those taxa contributing to differences in metazoan distribution between habitat classes. ANOSIM and SIMPROF were carried out on Bray-Curtis similarity matrices of square-root transformed metazoan and log transformed xenophyophore community composition data. SIMPER was only carried out on square-root transformed metazoan data using Bray-Curtis distances. All analyses were performed using the statistical software package PRIMER 6.1.13 (Clarke & Gorley, 2006; Clarke & Warwick, 2001).

4.2.4 APEI representativity

In order to test the representativity of the current APEI network, the proportion of each habitat class from the classification located in both the APEIs and exploration and reserved areas of the CCZ management area was calculated. Exploration areas are those under contract for exploration activities, while reserved areas are areas of equal commercial value to exploration contracts that are set aside for exploration by developing states or the ISA.

All analyses were carried out in ArcGIS 10.6. Shape files of the APEIs, exploration areas and reserved areas were overlaid on the classification and used to extract all habitats within those designated areas. For each habitat the percent protected in the APEI network, as well as the percent located within the exploration and reserved areas was then calculated.

4.3 Results

4.3.1 Initial clustering

In most cases, the clustering iteration with the highest ASW was chosen for the habitat classification, and this decision was supported by literature and expert review (Table C.1). Oceanographic variables showed the best clustering with two groups (ASW = 0.51). These variables represented water mass structure, providing a broad biogeographic context for the classification, and this clustering aligned with Harris and Whiteway (2009) biogeographic classification in this region. The two oceanography clusters broadly split the CCZ region longitudinally into two large water masses, one with lower salinity and higher temperature, the other with higher salinity and lower temperature (Table 4.1, Figure 4.5a). It is important to note here that the difference in salinity and temperature between these two clusters is not large (0.06 psu and 0.69^{o}C, respectively).

Topographic variables also clustered best in two groups (ASW = 0.55), essentially representing flat and sloping areas. The two topographic clusters distinguished areas that were relatively flat (0 - 5.69 degrees slope), with small topographic features, from those with steeper slope and (0.04 – 37.92 degrees) more prominent peaks and troughs (Table 4.1, Figure 4.5b).

The highest ASW for POC was with eight clusters (ASW = 0.66). However, this broad pattern of POC across the region was also captured using just three clusters (ASW = 0.60) and in order to simplify the classification this number of clusters was chosen. The three POC clusters captured the gradient in POC flux to the seafloor across the region, from low in the west (mean 1.18 g C_{org} m^{-2} y^{-1}) and medium in the east (mean 1.53 g C_{org} m^{-2} y^{-1}), to high in the south (mean 2.09 g C_{org} m^{-2} y^{-1}) (Table 4.1, Figure 4.5c).

Finally, nodule abundance showed the best clustering with four groups (ASW = 0.66). These four clusters highlighted areas of very low (mean of 1.18 kg m^{-2}) to high (mean of 11.10 kg m^{-2}) nodule abundance (Table 4.1, Figure 4.5d). Areas of medium to high nodule abundance were concentrated in the central CCZ, with some medium to high nodule abundance also predicted in the north- and south-east (Figure 4.5).

Table 4.1 Properties of clusters selected for the final habitat classification, produced through CLARA clustering of environmental variables.

Variable group	**Cluster**	**Variable**	**Min**	**Max**	**Mean**	**St Dev**	**Description**
Oceano-graphy	Cluster 1	Sal (psu)	34.64	34.69	34.69	0.00	Low salinity, high temperature
		Temp (^{o}C)	1.39	2.05	1.47	0.04	
	Cluster 2	Sal (psu)	34.69	34.70	34.70	0.00	High salinity, low temperature
		Temp (^{o}C)	1.36	1.52	1.44	0.03	
Topo-graphy	Cluster 1	Slope (^{o})	0.0	5.69	0.78	0.47	Flat, with some small topographic features
		BBPI	-1700	1230	-17.21	114.29	
		FBPI	-1256	555	-4.10	43.79	
	Cluster 2	Slope (^{o})	0.04	37.92	3.64	2.35	Sloped, with more prominent peaks and troughs
		BBPI	-1741	3454	135.48	325.05	
		FBPI	-1312	1806	37.77	139.82	
POC (g C_{org} m^{-2} y^{-1})	Cluster 1		0.80	1.36	1.18	0.11	Low POC
	Cluster 2		1.36	1.78	1.53	0.10	Med POC
	Cluster 3		1.78	2.92	2.09	0.19	High POC
Nodule abundance (kg m^{-2})	Cluster 1		0.03	2.64	1.18	0.68	V low nodule abundance
	Cluster 2		2.64	5.29	3.98	0.65	Low nodule abundance
	Cluster 3		5.29	8.57	6.56	0.83	Med nodule abundance
	Cluster 4		8.57	30.88	11.10	2.19	High nodule abundance

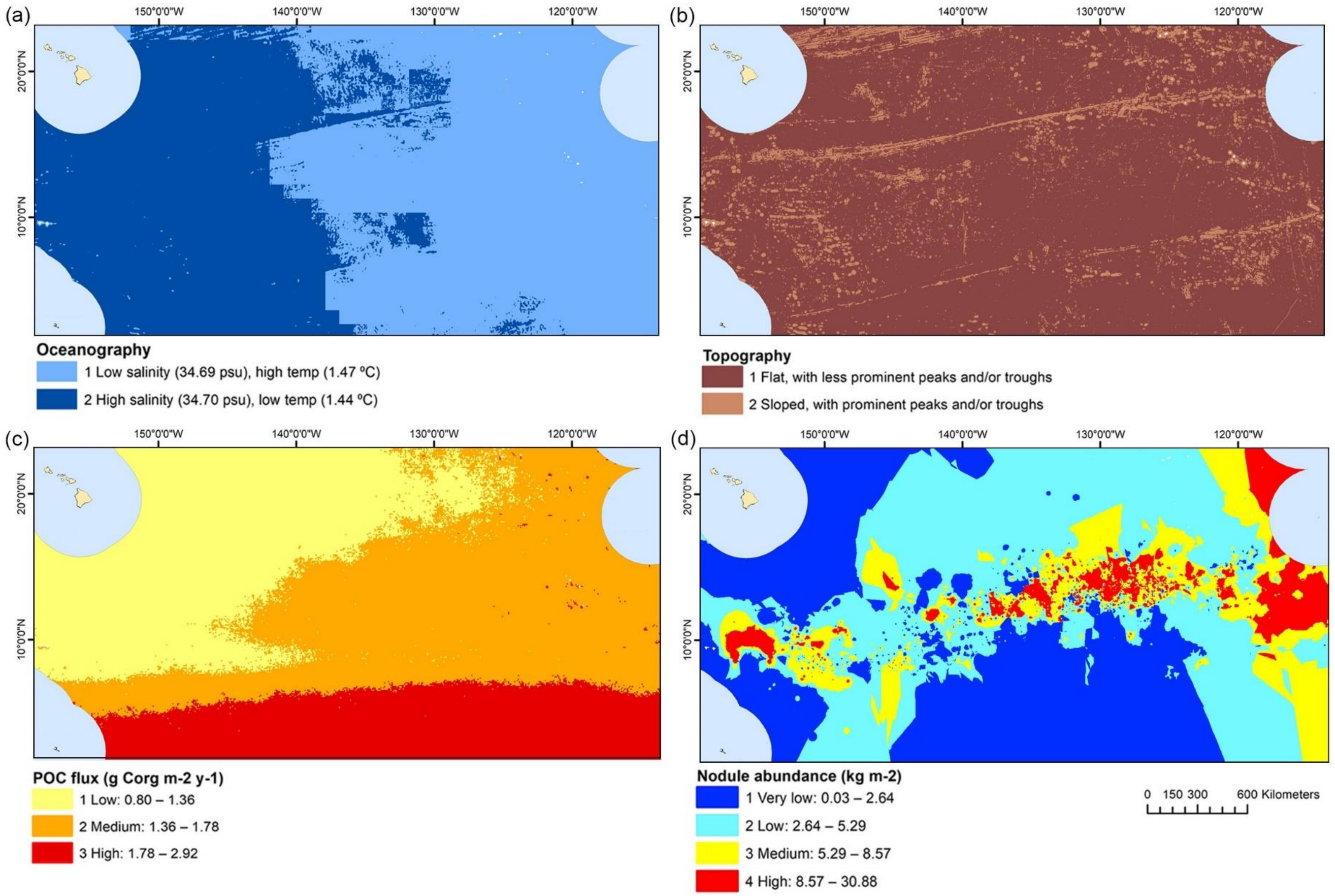

Figure 4.5 Outputs of initial CLARA clustering on groups of variables to represent (a) oceanography, (b) topography, (c) POC and (d) nodule abundance in the CCZ region. Land masses are shown in cream as labelled in Figure 4.1, surrounded by EEZs in pale blue.

4.3.2 Final habitat classification

The final classification produced by combining layers of clustered environmental variables proposed 46 habitat classes (Figure 4.8). Each habitat represented a different set of environmental conditions (see Table C.2 and Table C.3 in Appendices), and was hypothesised to support a distinct biological community.

Habitats in the east fell within a water mass with low salinity and high temperature, and were characterised by medium to high POC in the north- and south-east respectively. In the west, habitats fell within a different water mass, characterised by higher salinity and lower temperature. These habitats were mainly areas of low POC, with medium to high POC in the south-west. Habitats with medium to high nodule abundance were concentrated in the central CCZ, bounded by the Clarion and Clipperton fracture zones and overlapping with exploration contracts and reserved areas. In the periphery were areas of low and very low nodule abundance, although some medium and high nodule abundance habitats were also proposed to the south- and north-east respectively. Habitats with high nodule cover made up only 6.6% of the area, which was otherwise dominated by low and very low nodule abundance (42% and 36% of the CCZ, respectively). The CCZ habitats were also predominantly flat abyssal plain, with pockets of habitats distinguished by sloping topographic features like seamounts, peaks and troughs. In fact, 89% of the area was composed of habitats that were flat or of constant slope, with small topographic features, with only 11% sloped with larger peaks and troughs.

Based on this classification the CCZ management area appeared to be dominated by several very large habitats, with many smaller pockets of different habitat spread across the area (Figure 4.6). The three most abundant habitats identified in the CCZ (each with

> 1 million km^2) were all flat with some small topographic features, low to medium POC flux to the seafloor and low to very low nodule abundance. These top three largest habitats occurred mainly in the periphery of the central CCZ area, in the north-east and north-west, and fell in both water masses. In total they made up 33% of the area of the CCZ. In fact, out of 46 habitats, the largest five formed nearly 50% of the area of the CCZ (Figure 4.7). These five largest habitats differed in terms of the water mass within which they fell, the level of POC input and nodule cover, but were all characterised by the same topographic conditions: flat or constant slope, with some small topographic peaks and depressions.

In terms of the smallest habitat types, the five smallest all contained high nodule abundance and were scattered across the CCZ, in the centre, to the east and north-east, and where there were small pockets of high nodule cover in the south-west.

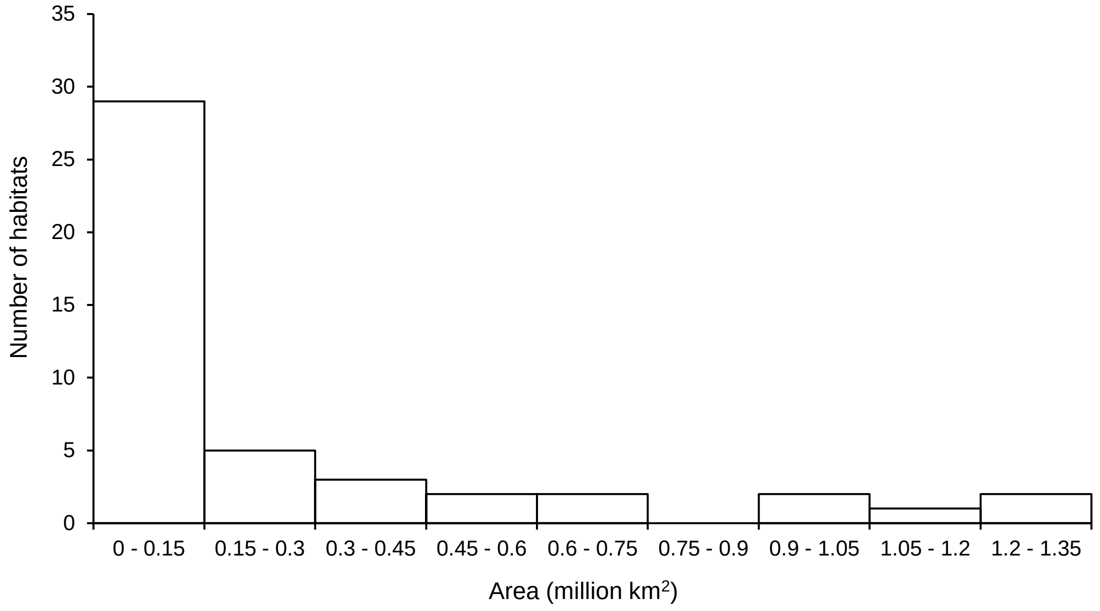

Figure 4.6 Number of habitats of different sizes.

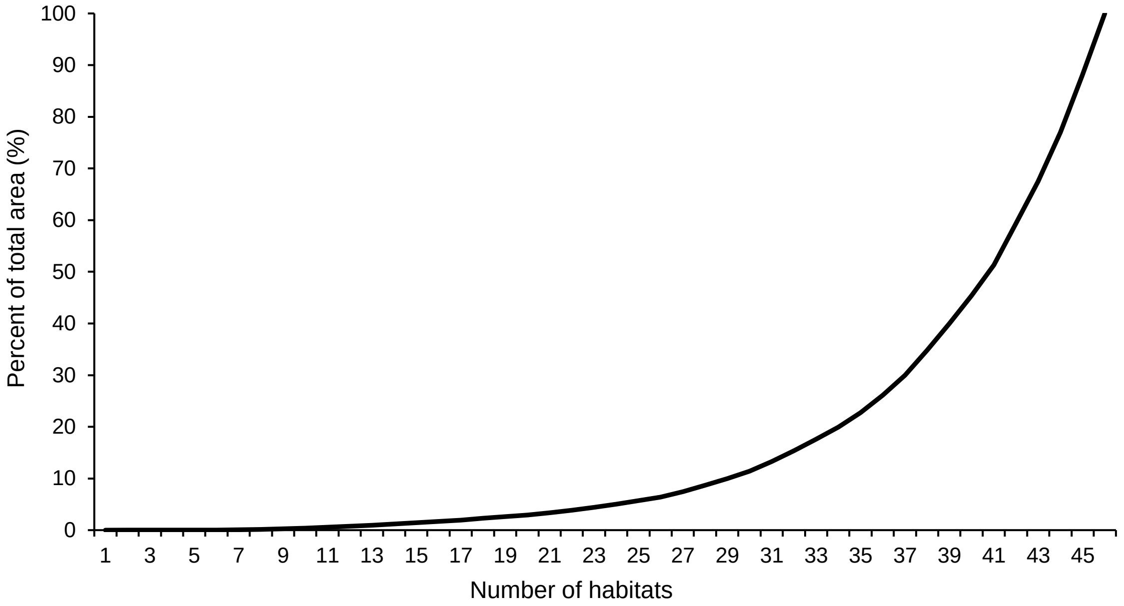

Figure 4.7 The contribution of an increasing number of habitats to total area.

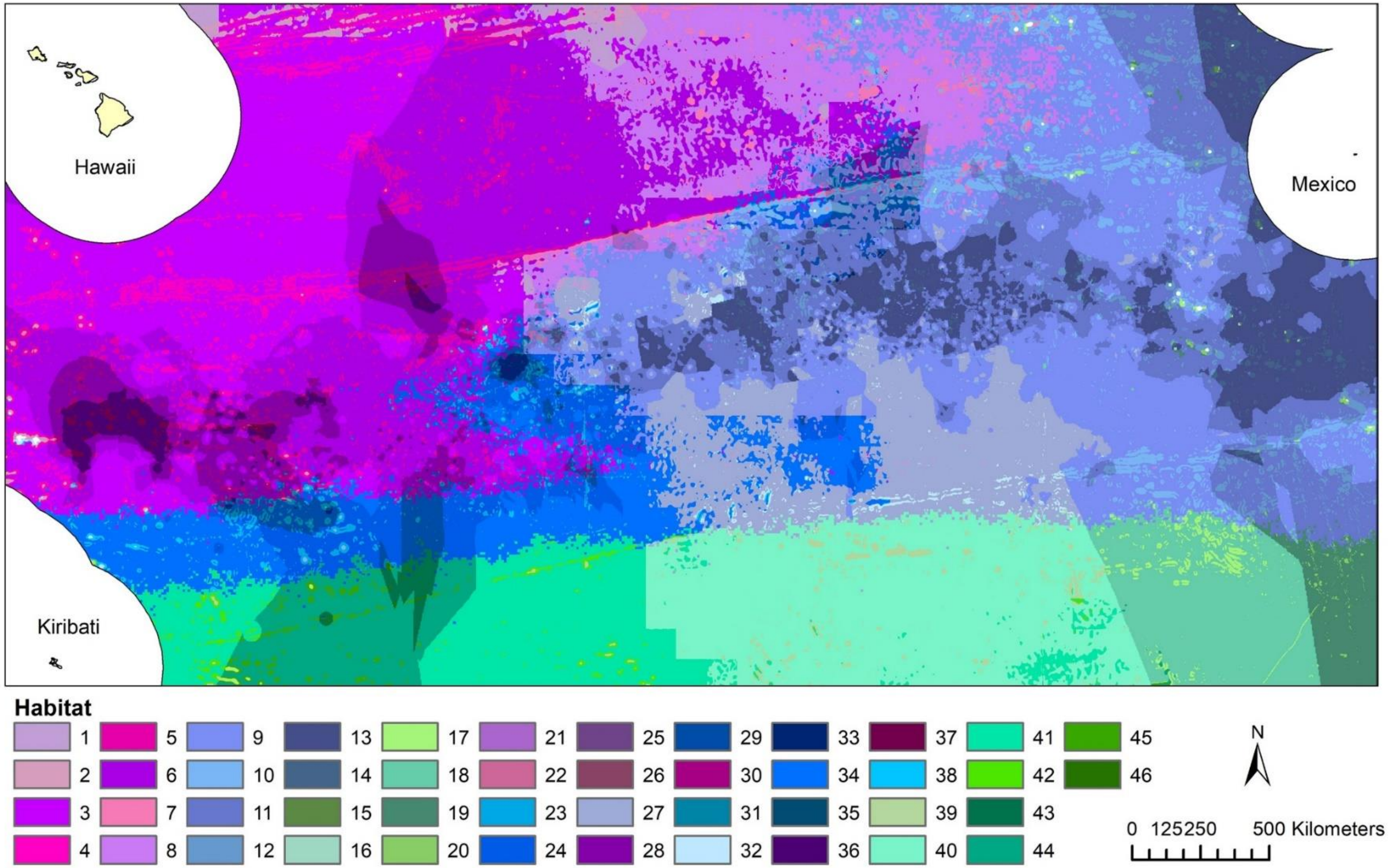

Figure 4.8 Final habitat classification produced by combining layers of clustered environmental variables. Areas of low POC are shown in purple, areas of medium POC in blue, and high POC in green. Pastel colours in the east represent habitats in water mass 1 (low salinity, high temperature), with bright colours in the west indicating water mass 2 (high salinity, low temperature). Within a colour block (purple, blue or green), pale to dark colours represent a gradient of very low to high nodule abundance, and within these divisions light colours indicate sloped areas, while dark colours indicate flat or constant slope. Land masses are shown in cream, surrounded by EEZs in white.

4.3.3 Biological validation

The nine image transects used to validate the final habitat classification fell within three habitat types: Habitats 9, 11 and 13 (Table 4.2, Figure 4.9). These three habitat types differed only in terms of nodule cover, with Habitat 9 having low nodule abundance, Habitat 11 medium abundance, and Habitat 13 high abundance. All three habitats fell within the same water mass, were flat with some small topographic features, and had medium POC flux. Habitat 9 was the largest, covering over 1 million km^2 across the CCZ. Habitat 11 was slightly smaller, and Habitat 13 was the smallest at nearly 600 000 km^2.

Table 4.2 Description of the three habitats overlapped by biological transects

Transects	**Habitat**	**Total area (km^2)**	**Description**
T2, T6, T8	9	1 244 357	Low salinity, high temperature; Flat, with some small topographic features; Medium POC flux; Low nodule abundance
T1, T7, T9	11	901 232	Low salinity, high temperature; Flat, with some small topographic features; Medium POC flux; Medium nodule abundance
T3, T4, T5	13	583 859	Low salinity, high temperature; Flat, with some small topographic features; Medium POC flux; High nodule abundance

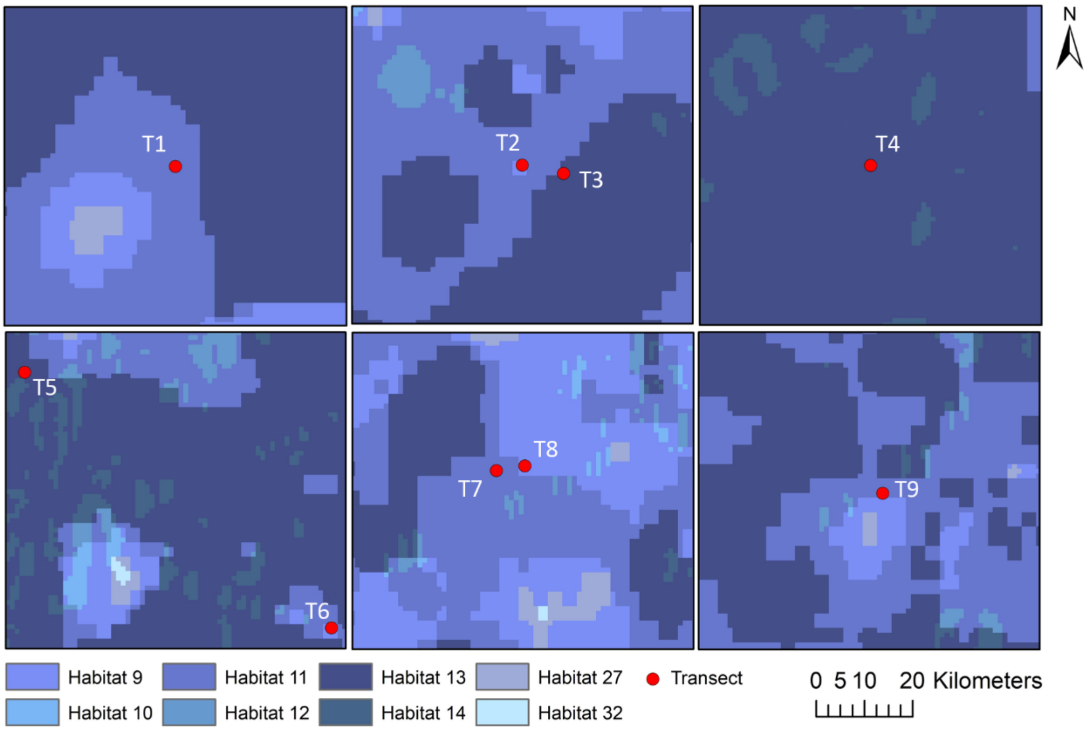

Figure 4.9 Location of transects used for biological validation with respect to habitat types identified through the habitat classification. For location of transects within CCZ see Chapter 2, Figure 2.1.

The following results relate to analyses carried out on all morphotypes, analysed separately for metazoans and xenophyophores.

4.3.3.1 ANOSIM

No significant groupings by habitat were found for any habitat with metazoan or xenophyophore data. Exploratory Multidimensional Scaling (MDS) plots on Bray-Curtis similarity matrices of square-root and log transformed data showed no grouping of transects by habitat (Figure 4.10).

The ANOSIM test statistic (Global R) was not significant for metazoan or xenophyophore community composition data, indicating that there was no grouping of biological transects by habitat (R = -0.309 and -0.284 respectively, $p>0.05$). In both cases a histogram of the frequency of the test statistic calculated in a number of permutations, each with randomly allocated habitat labels, showed that the R value obtained for samples with their correct labels was lower than that calculated with labels randomly assigned (Figure 4.11), implying that transects are more interspersed than would be expected if habitat was randomly allocated.

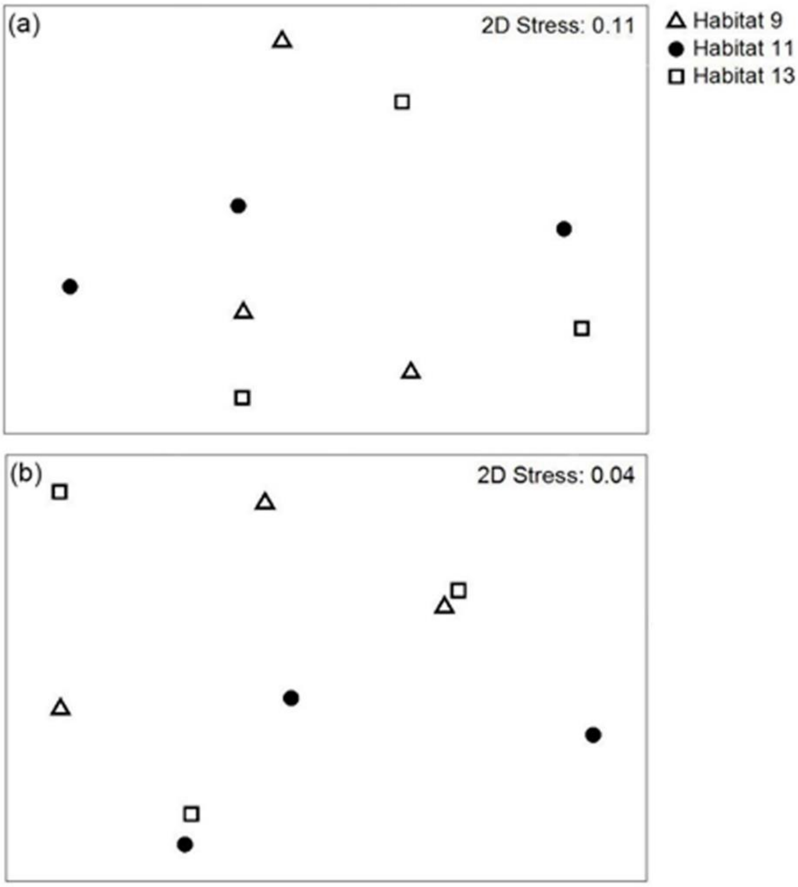

Figure 4.10 Multidimensional Scaling (MDS) plots on a Bray-Curtis similarity matrix of (a) square-root transformed metazoan and (b) log transformed xenophyophore community composition data showed no grouping of biological transects by habitat.

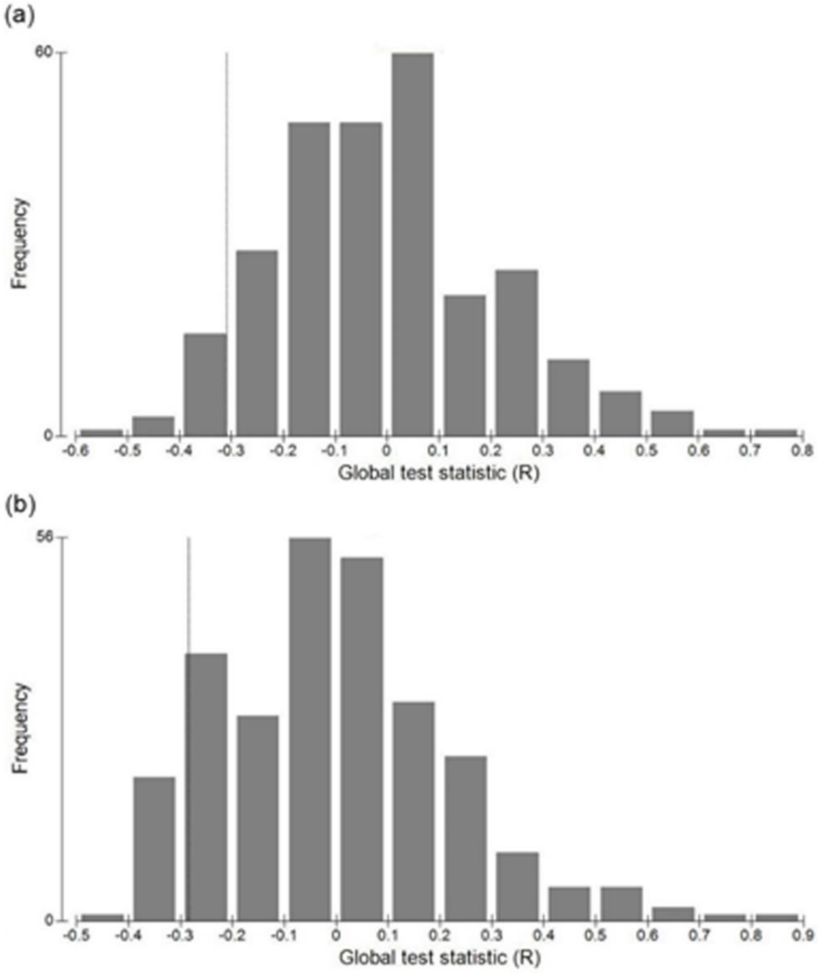

Figure 4.11 Distribution of ANOSIM test statistic (R) calculated in all possible permutations where transects are assigned a habitat randomly, for (a) metazoan and (b) xenophyophore community composition data. R ranges from -1 to 1, with 1 indicating perfect separation among groups. Dotted line indicates R value calculated for samples with correct labels.

4.3.3.2 SIMPROF

There was no structure to the biological data based on habitat type when it was clustered through SIMPROF. SIMPROF analysis on a Bray-Curtis similarity matrix of square-root transformed metazoan community composition data produced a dendrogram where two transects grouped together for Habitat 11 and otherwise all other transects were grouped with those of another habitat (Figure 4.12). This shows the same clustering as Chapter 2 (see Figure 2.7), but reflects habitat type rather than depth.

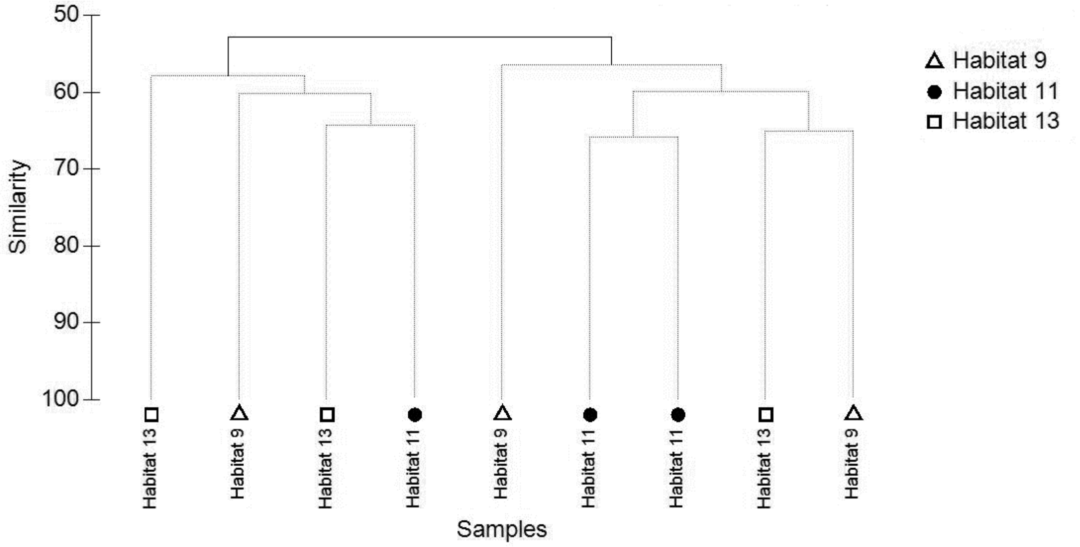

Figure 4.12 SIMPROF analysis on a Bray-Curtis similarity matrix of square-root transformed metazoan community composition data, with 999 permutations. Solid black lines indicate significant clustering ($p < 0.05$); dashed grey lines indicate non-significant clustering.

These results were further supported by the rank and resemblance graphs below (Figure 4.13), which showed that the actual resemblance curves fell close to the mean and within the 99% confidence band, indicating that the resemblance of samples was the same as if habitat was randomly assigned.

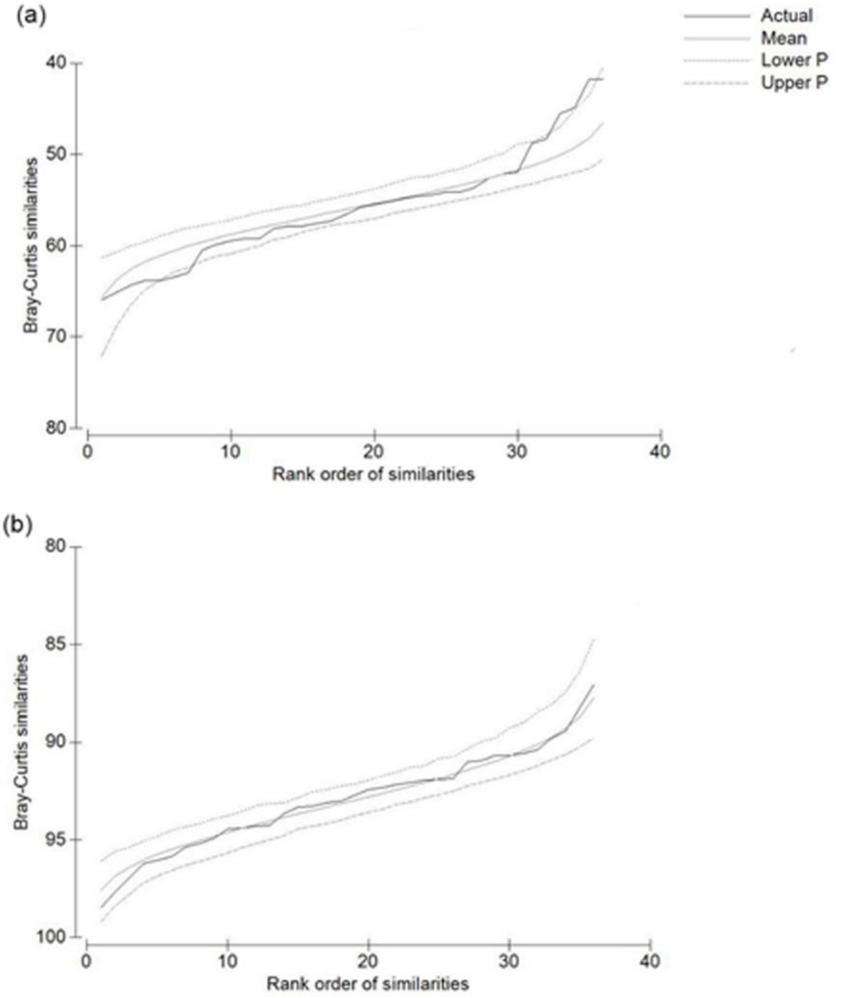

Figure 4.13 Similarity profile of (a) square-root transformed metazoan and (b) log transformed xenophyophore community composition data. Dashed grey lines indicate 99% confidence envelope.

4.3.3.3 SIMPER

SIMPER analysis on metazoan community composition data showed the top seven most abundant taxa were the same for all habitats, in different orders of importance (Table C.4). These morphotypes were general groups of alcyonaceans, sponges, actiniarians and annelids, two anemone morphospecies and an urchin. In all transects the top five to six most abundant taxa accounted for 50% of the total abundance.

Habitat 9 had an average Bray-Curtis dissimilarity of 42.96% and 43.44% to Habitats 11 to 13, respectively, while Habitat 11 and Habitat 13 were, on average, 44.00% dissimilar (Table C.5). The main difference between Habitat 9 and Habitat 11 was driven by the abundance of two ophiuroid species, an urchin, and general sponge and alcyoncean groups. These differences in abundance contributed collectively to over 20% of the difference between the two habitats, and abundances for these morphotypes were always higher in Habitat 11 than Habitat 9 (Figure 4.14a).

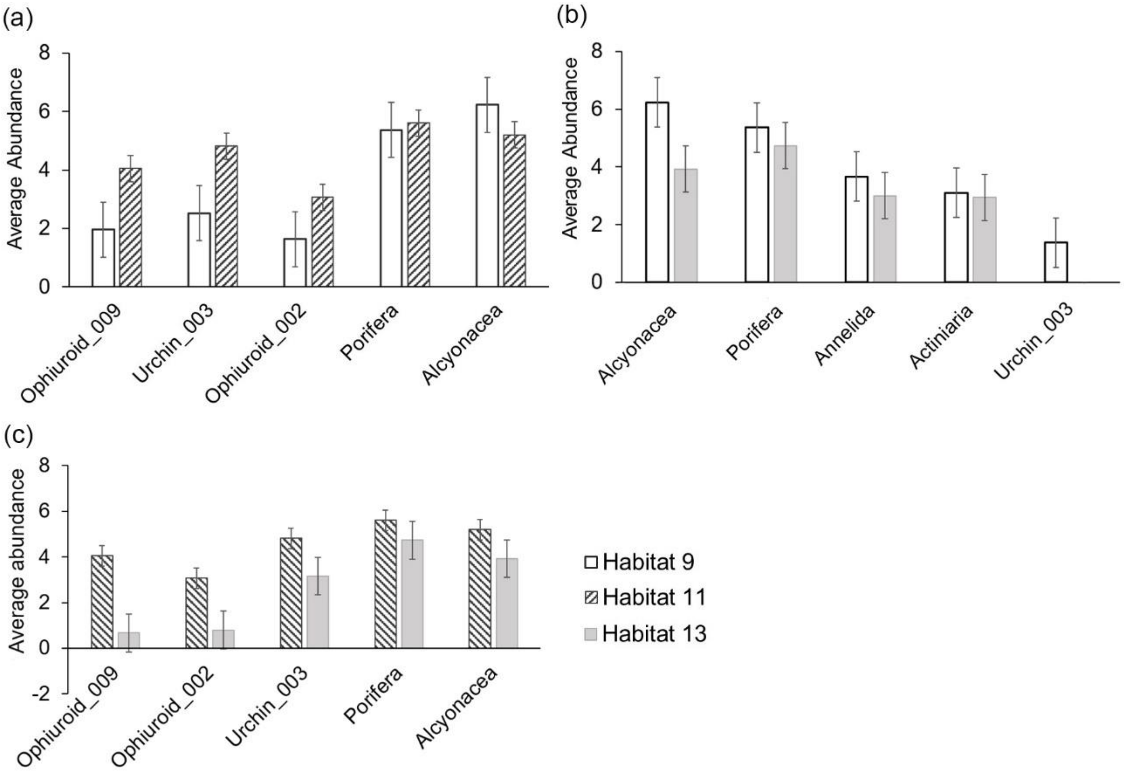

Figure 4.14 Pairwise comparisons showing the average abundance (individuals per transect) of the five morphotypes with the highest contributions to dissimilarity between (a) Habitats 9 and 11, (b) Habitats 9 and 13, and (c) Habitats 11 and 13.

The most important morphotypes for distinguishing Habitats 9 and 13 were general groups of alyconaceans and sponges, which accounted for 4.77% and 4.51% dissimilarity respectively, with lower abundances observed in Habitat 13. The next 10% dissimilarity was accounted for by differences in the abundance of annelids, actiniarians and an urchin species (Figure 4.14b). Finally, Habitats 11 and 13 differed principally in the abundance of two ophiuroid morphospecies, an urchin and a general group of sponges, contributing 6.98%, 5.24%, 5.09% and 4.06% respectively to dissimilarity, again with lower abundances observed in Habitat 13 (Figure 4.14c). Based on these pairwise comparisons, it appeared to be mainly differences in the abundances of the most common morphotypes that drove dissimilarity between the habitat types.

4.3.4 APEI representativity

A test for the habitat representativity of the CCZ APEI network showed that 39 habitats (of a total of 46) were included in the APEIs, with 36 habitats in exploration areas and 33 in reserved areas (or 38 habitats collectively in the exploration and reserved areas) (Table C.6). The following results present combined results for the exploration and reserved areas (subsequently referred to as mining areas) as these are both ultimately intended for mining activities, and are up-to-date with all contracts awarded as of June 2019.

In the current APEI network 25 habitats (over 50% of the total number of habitats in the area) were represented by less than 10%, and 20 of these were represented by less than 5%. These 25 habitats had a range of environmental conditions including both water masses, both topographic conditions, and all POC and nodule abundance levels. Thirteen habitats were represented by less than 1%, and of these seven received no protection in the APEI network at all. Those habitats with > 0% but < 1% in the APEIs were fairly large, ranging in total size across the CCZ from 17 000 – 580 000 km^2, and were generally located in large patches in the central CCZ. Those habitats with no protection at all were mainly much smaller (< 2 000 km^2 total habitat size), with one larger habitat (> 100 000 km^2), and were found in peripheral areas with some small pockets in the central CCZ. All of these habitats with no protection had medium to high nodule abundance, either low or high POC and were predominately located in the water mass characterised by low salinity and high temperature in the east.

Of the 25 habitats with < 10% representation in the APEIs, 14 of these were found to have > 10% of their total area in mining areas, 12 had > 20% and seven had > 50% (Table C.6). Some habitats with 0% or nearly 0% representation in APEIs had a large proportion of their total extent located in exploration and/or reserved areas, some as much as 70%. These were all habitats with high nodule abundance located in the central CCZ (Figure 4.15), and ranged from very small habitats of only 152 km^2 in total, to larger habitats of up to nearly 600 000 km^2. Of habitats represented by < 1% in the APEI network, eight occurred at > 20% in mining areas and six at > 50%.

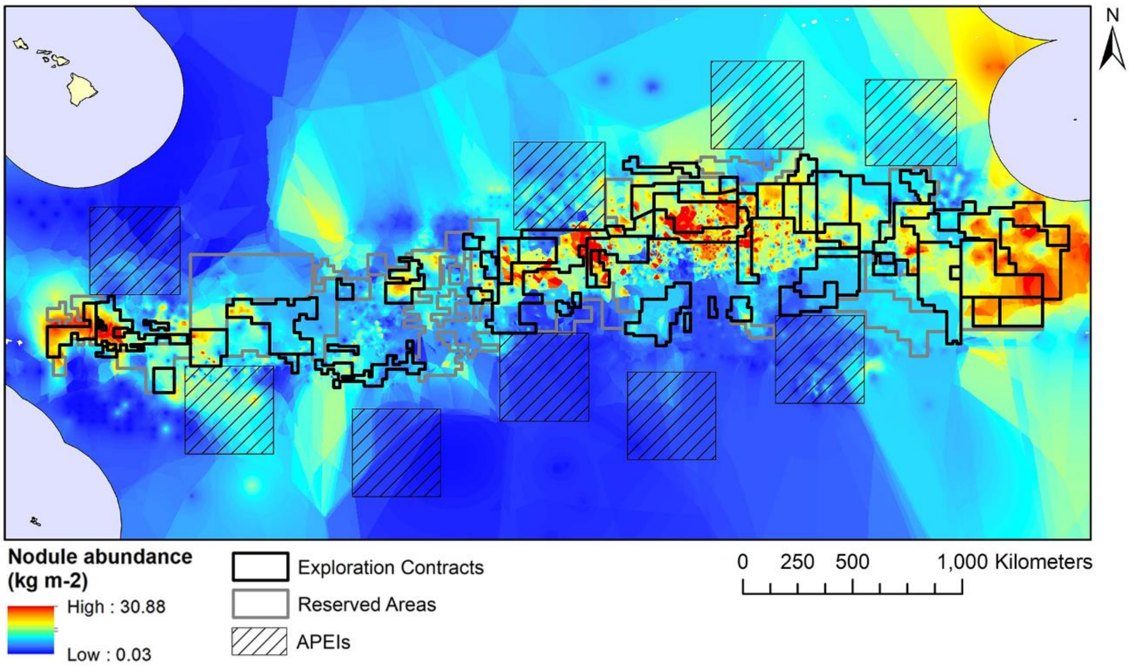

Figure 4.15 Overlap of exploration areas, reserved areas and Areas of Particular Environmental Interest (APEIs) with modelled nodule abundance in the CCZ. Note areas of high nodule abundance are generally located in the central CCZ, in exploration and/or reserved areas. Land masses are shown in cream, as labelled in Figure 4.1, surrounded by EEZs in pale blue.

When the conservation target was increased to 30% protection, just 5 habitats were found to be adequately represented in the current APEI network. This means that 89% (41 habitats) were not considered adequately protected. This included habitats of all environmental conditions that were spread across the peripheral areas around the APEI network and in the central CCZ. Seventeen of these habitats had > 20% of the total extent located within mining areas, while eight had > 50%.

Some habitats were not found in mining areas, but were represented in the APEI network. These were all habitats that were found in the periphery of the central exploration and reserved areas, and had low or very low nodule abundance (with other conditions varying). Three habitats had > 20% (but < 30%) of their total area in APEIs, and only 0 - 5% of their area in mining areas. These habitats ranged in size from a total area of 50 000 km^2 - 420 000 km^2, and were located in the north in central areas and in the south in central to western areas.

Finally, there were several habitats that were represented by < 1% in both APEIs and mining areas. Two habitats, of sizes < 2000 km^2 and < 30 000 km^2, were not found in the designated areas at all, while another two were found at < 1%. The former differed only in terms of topography and were located next to each other in a pocket of medium nodule abundance in the south west. The latter included a large habitat in the south-east with high POC flux and low nodule abundance.

4.4 Discussion

4.4.1 Comparisons with other global classifications

The final habitat classification consisted of 46 benthic habitats that were hypothesised to support different fauna. Each of these habitats represented a different set of environmental conditions, based on the water mass within which the habitat was located, the topographic features of the area, POC flux to the seafloor and the abundance of nodules. According to this classification, the CCZ is dominated by several large habitats, all flat or with constant slope and relatively small topographic features, with many smaller pockets of different habitats interspersed across the region. Areas of high nodule abundance are located in the central CCZ, with a patch of high nodule abundance in the north east.

No similar regional scale habitat classifications have been carried out in the CCZ region, but the current classification can be compared with other global efforts that cover the region. These include the GOODS classification (UNESCO, 2009), Watling *et al.* (2013), Harris and Whiteway (2009) and Sayre *et al.* (2017). These are all larger scale, global classifications but provide a broad biogeographic context for this classification. Both GOODS and Watling *et al.* (2013) proposed two biogeographic provinces overlapping the CCZ, split in a north-south direction, and the boundaries for these were driven primarily by differences in POC across the region. This split was captured in the current classification, which identified habitats falling within areas of different POC flux, mainly in a latitudinal direction. Although a similar pattern is observed in the pelagic portions of the classification by Sayre *et al.* (2017), at the benthic level the CCZ falls within a single EMU. The classification produced by Harris and Whiteway (2009), on the other hand, splits the equatorial Pacific longitudinally, proposing different seascapes in the east and west. This split echoes the east-west split of the two different water masses identified in the current classification.

Possible shortcomings of the classification methods used include missing an important explanatory variable, not weighting input variables, and issues with scale, modelled input data and selection of the number of clusters. For example, the large habitats in the periphery of the CCZ could be a result of less detail in the modelled nodule abundance input data in peripheral areas outside of the central CCZ. Although it is likely that POC is the most important factor driving species distribution in a deep-sea abyssal plain area like the CCZ (Smith *et al.*, 2008a), there is insufficient knowledge on the drivers of faunal communities in these environments to quantify or weight the varying levels of importance of input variables. Chapter 2 has shown that much more data, both biological and environmental, is needed to try and understand this by modelling the relationship between species distributions and environmental drivers. There is also insufficient information on the scales of megafaunal distribution across the CCZ. It is highly likely that the scale and intensity of current sampling efforts are not adequate for capturing species turnover (see Chapter 3), and this hinders our understanding of community ecology both in the area of interest and in deep sea areas in general.

4.4.2 Biological validation

The biological validation of the final classification did not support the habitats delineated through the classification process. There are possible shortcomings of both the classification methods and validation data collection that may have contributed to this. Shortcomings of the classification methods have been mentioned briefly above, and issues with the validation data set are principally related to relatively low resolution image data, which introduced challenges with detection rates and the resolution of taxonomic identification. Some morphotypes, like holothurians, were relatively easy to identify at the given resolution, while others, such as alcyonaceans, were much more difficult. In addition, the habitats tested by biological data in this study differed only in terms of nodule cover. Based on visual inspection of images, the accuracy or reliability of the modelled nodule abundance data used in this classification isn’t clear. Nodule abundance as observed in images from the transects in this study did not always match modelled estimates from ISA (2010b). For example, transect T2 is classified as a low abundance habitat according to the habitat classification, but appears to have extremely high nodule abundance throughout, on inspection of images from this transect (see Figure 4.16a). What is more, the input nodule data was broad-scale, and did not reflect the variability in nodule abundance observed at small spatial scales (Peukert *et al.*, 2018), as seen in Figure 4.16b-c.

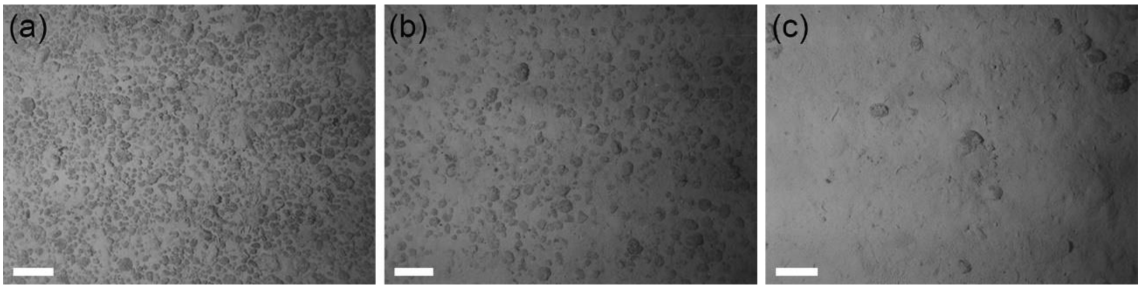

Figure 4.16 Nodule abundance as observed in images from the current study showing (a) high nodule abundance in transect T2, classified as a low abundance habitat by the habitat classification, and (b-c) high variation in nodule abundance observed on small spatial scales, i.e. within a transect (T3 shown here). White scale bars = 20cm.

4.4.2.1 Power analysis

Despite the above challenges, it is more likely that the validation data set did not have the power to conclusively accept or reject the classification. Deep-sea systems and associated fauna are highly heterogeneous on both large and small scales (Levin *et al.*, 2001; Smith *et al.*, 2008b; Vanreusel *et al.*, 2010). In the CCZ, the environment and fauna are heterogeneous on scales of metres to 100s and 1 000s of kilometres (Amon *et al.*, 2016; Glover *et al.*, 2002; ISA, 2010b; Simon-Lledó *et al.*, 2019b; Smith *et al.*, 1997). In order to detect a difference between habitats in this classification, sampling would need to be sufficient to determine whether within transect variability was greater or less than between transect variability. In other words, sampling (size and replication) needs to be enough to detect unusual patterns of change (Lan & Lian, 2010). In this study, given the heterogeneity of the CCZ, the scale of megafauna distributions, the high diversity and number of rare species, and the low densities of megafauna, three replicate transects per habitat of the size used were likely far too few to test this.

This comes down to whether or not the sampling design has the power to detect the desired effect. Although not carried out here, power analysis can be used to determine how much sampling is required to make statistically robust conclusions about a certain ecological question (Cohen, 1977). Power analyses should ideally be done in advance, in order to determine the size and quantity of samples required to produce a statistically robust design (Lan & Lian, 2010; Osenberg *et al.*, 1994). Paradoxically, they require comprehensive baseline data, but where this is not available, other existing data should be used to guide the process (Osenberg *et al.*, 1994).

Power analysis related to deep-sea studies is very much in its infancy. Because of the low abundances and high diversity of fauna associated with nodule fields, the statistical power to detect change in all but the most common species is expected to be low (Jones *et al.*, 2018). This means that very large sample sizes and replicates are required in order to improve the statistical power (Jones *et al.*, 2018). Jones *et al.* (2018) propose that depending on a number of factors, anywhere between 25 and over 100 replicates of transects over a kilometre in length may be required to test for changes in deep-sea communities resulting from seabed mining (based on Lan & Lian, 2010). In the current study, both the size and replication of samples was likely incomplete. This was explored further using species accumulation curves in Chapter 3.

4.4.2.2 Addressing a lack of data in the deep sea

To effectively test the classification developed, more biological data is clearly needed to improve the validation data set and build on the analyses carried out here. In addition, there is still much work to be done to understand the relationships between fauna and environmental drivers in the CCZ (see Chapter 2). It is possible that additional data could be gathered by combining individual data sets collected by contractors and scientists across the CCZ region. However, much work would be required on standardisation and quality control before a large, combined data set would be useable.

This highlights the challenges of ocean management at very large scales, in areas of the high seas that are vast and data-poor. In these cases the only area-based tools available to support management decisions are surrogate-based classification and mapping techniques. It is crucial when making decisions on activities, particularly at these large scales, that tools used to support planning are underpinned by ecological relevance; however, many of these tools are not validated or have only minimal evidence to support their ecological relevance, and this is often due to a lack of sufficient biological data that can be used for validation. Sampling in the deep sea is expensive, resources are limited and the areas targeted are generally large (Glover & Smith, 2003). There is thus a global need for a more cohesive approach to sampling and data management that can support collation of data from multiple sources to produce larger data sets with statistically robust sampling designs, and thus support broad-scale management.

In the deep-sea mining industry there have been calls for the adoption of an overarching environmental management strategy by the ISA, to provide the framework and context for regional assessments and project-specific environmental studies like Environmental Impact Assessments (EIAs) (Jaeckel, 2015; Jaeckel, 2019; Jones & Weaver, 2017; Jones *et al.*, 2019). This would ensure that any sampling is guided by an overarching strategy. Within this tiered approach to environmental management should sit Regional Environmental Assessments (REA), used to identify and evaluate potential environmental impacts of a plan, programme or policy at a regional level, and Regional Environmental Management Plans (REMPs), an output of REA (Jones *et al.*, 2019).

REA has been carried out successfully by other offshore industries, including the UK oil and gas (e.g. Bett, 2001), renewable energy (Gill *et al.*, 2005; Nedwell *et al.*, 2007), and aggregate dredging sectors (e.g. BMAPA, 2019; Wallingford, 2010), to support environmental management using an holistic approach. Instead of each contractor carrying out independent, small-scale studies in areas of immediate commercial interest, REA encourages a cooperative approach whereby a large-scale, coordinated, strategic regional environmental survey is undertaken to improve understanding of poorly known areas, identify sensitive areas, assess potential cumulative impacts, develop protection measures and assess regional-scale ecological processes (e.g. Bett, 2001). This approach in the CCZ would make the best use of limited resources (e.g. ship time, and staff time for labour intensive sample processing and analysis) to ensure that research efforts formed part of a strategic, statistically robust sampling plan to address some of the key questions that are currently impeding progress in environmental management of deep-sea mining. This would also ensure that habitats falling outside of mining areas, for example in the APEI network, are sampled in a systematic, coordinated manner.

Looking beyond just sector or area-specific management, there is a need for a global strategic, integrated, cohesive approach to data collection to provide the fundamental basis for decision-making regarding human activities in the deep sea, and in the high seas in particular. This is an issue that requires cooperation on a global scale and could be addressed by an initiative like the United Nations Decade of Ocean Science for Sustainable Development (2021-2030). While there are platforms like the Ocean Biogeographic Information System (OBIS) and the World Register of Marine Species (WoRMs) that store data and provide standardised identification guides, an overarching strategy for data collection is required to address the big questions surrounding ocean management. Improved engagement with industry would also help to ensure that all existing non-confidential data is used, thus unlocking data sources to support environmental management.

4.4.3 APEI representativity

4.4.3.1 Representativity test

Although I could not demonstrate validation of the classification, this could be due to a lack of statistical power to conclusively reject or accept the classification, and thus discussion of the representativity test is still warranted. One of the key considerations of MPAs is that they should conserve representative examples of all of the types of habitats and species in an area of interest, to try and capture the diversity and heterogeneity that supports biodiversity in that area (Roberts *et al.*, 2003). By being representative, MPAs should support the systems that generate and sustain the biodiversity that is the focus of protection (Young & Carr, 2015). Habitat representativity is a well-accepted requirement for MPA design (CBD, 2009; O'Leary *et al.*, 2012; OSPAR, 2006) and is one of the principle criteria for an MPA network to be considered ecologically coherent (Ardron, 2008; OSPAR, 2010). In addition, habitat types within an MPA network should be represented by areas of adequate size and replication to ensure long-term viability of habitats and associated species (Foster *et al.*, 2017; Rondinini, 2010; Young & Carr, 2015), although this was not addressed in the current study. In this study, the CBD conservation target of 10% was applied, as well as the larger World Park's Congress target of 30%. Other studies have used a threshold of 20% (e.g. Foster *et al.*, 2017; as recommended by OSPAR, 2006).

The current APEI network does not meet representativity requirements, based on either the CDB or World Park's congress conservation targets. A large proportion of habitats (over half) were represented by less than 10% of the total extent, with the vast majority (89%) represented by less than 30% area. Many habitats were represented by < 1%, and some were not located within the APEI network at all. Of the least protected habitats, many occurred in large areas across the central CCZ, while those receiving no protection were found in smaller pockets, some also within the central CCZ, in areas of high nodule abundance. Some of these were very small habitats, which is of concern as smaller, less common habitats require greater protection than large, widely distributed ones (Johnson *et al.*, 2014).

Of the habitats with low (< 10%) representation in the APEIs, seven of these were found to have a large proportion (> 50%) of the total area in mining areas. Most of these habitats were represented by less than 1% in the APEI network. This only increased to eight habitats when considering a conservation target of 30%. Of the habitats with no protection in the APEI network whatsoever, three had over 50% of their area in mining areas, and these were all very small habitats.

Considering this from another angle, if all areas outside of the mining areas are "safe" from mining impacts, there were no habitats with 90% of the total extent in mining areas, and thus no habitats that were less than 10% "safe". There was one habitat that had 88% of the total extent in mining areas, and this habitat also had less than 10% in the APEI network. It was a fairly small habitat, at just 9 372 km^2, that was flat or constant slope, in an area of medium POC, with high nodule abundance, located in the central CCZ. When the more ambitious target of 30% protection was adopted, two habitats had over 70% of the total extent in mining areas, and thus less than 30% area "safe".

Considering areas outside of the exploration and reserved areas as safe is potentially unwise, and would not constitute a precautionary approach. Exploration licences for nodule mining are still being awarded (e.g. ISA, 2017c), and some impacts from mining may extend beyond the boundaries of a contract area (Aleynik *et al.*, 2017).

4.4.3.2 Revisiting the CCZ APEI design

This study suggests that additional measures for protection are required in the CCZ, and that the APEI network should potentially be augmented by additional APEI(s). Protected areas are currently located in the periphery of the central, high nodule abundance areas and thus fail to protect these habitats. Such a network will inevitably fall short of representing all of the unique habitats of the area and thus fully protecting biodiversity in the region (O'Leary *et al.*, 2012).

The CCZ APEI network was designed using the principles of compatibility with the existing legal framework for protection, minimising socio-economic impacts, maintaining sustainable, intact and healthy populations, accounting for regional ecological gradients, habitat representativity, creating buffer zones and using straight line boundaries to ease compliance (Wedding *et al.*, 2013). The CCZ management area was split into nine representative sub regions based on environmental surrogates (nodule abundance, nitrogen flux, seamount distribution, bathymetry) and macrobenthic abundance, and an APEI was placed within each sub region (Wedding *et al.*, 2013). When the APEI network was adopted by the ISA, some of the proposed MPAs within the central CCZ were shifted to the periphery to avoid existing exploration and/or reserved areas, resulting in the current APEI scenario (Wedding *et al.*, 2013). The shortfall of the current APEI network has been acknowledged by the ISA, with calls for two additional APEIs to the north-west and south-east of the current network noted (Figure 4.17) (ISA, 2016).

The classification produced through this study provides support for the establishment of additional APEIs in these locations, and could be used to identify the optimum positioning of these areas. Although not formally quantified in this study, these APEIs would provide protection for several key habitats that currently receive little to no protection but have >50% of the total extent in mining areas. The addition of APEIs based on improved knowledge of the CCZ would represent the operationalisation of the adaptive management approach that has been advocated by many for deep-sea mining (e.g Jaeckel, 2016).

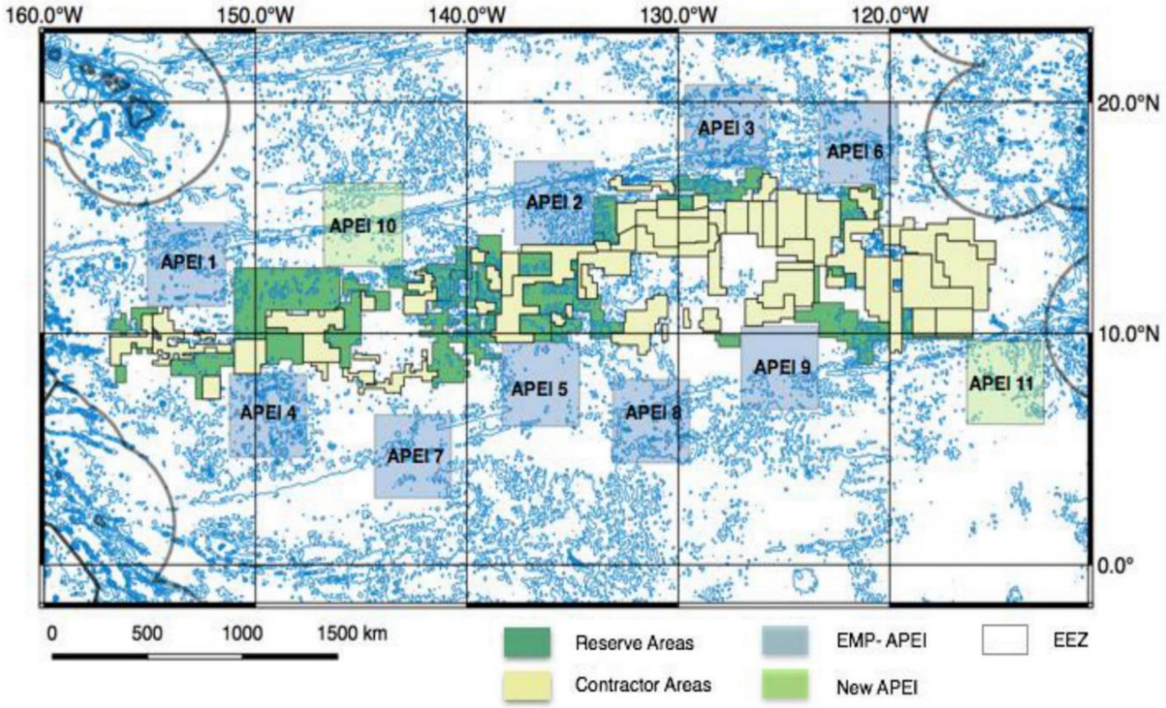

Figure 4.17 Two new Areas of Particular Environmental Interest (APEIs) have been proposed for inclusion in the current Clarion-Clipperton Fracture Zone APEI network. The proposed APEIs are numbers 10, to the north-west, and 11, to the south-east. Figure from ISA (2016).

This study also highlights that alternative ways of protecting high nodule abundance habitats in the central CCZ will be required. Several habitats are located only within the central CCZ, and conservation of these unique habitats would thus require protection measures within mining areas. These could take the form of PRZs with a conservation function, as suggested by Jones *et al.* (2018), and this study certainly supports this. PRZs appear to have a dual function of providing a control site for monitoring mining impacts and ensuring preservation of biodiversity. In the ISA regulations for nodule exploration, PRZs are described as:

> *"…areas in which no mining shall occur to ensure representative and stable biota of the seabed in order to assess any changes in the flora and fauna of the marine environment."* (ISA, 2000, Regulation 31, para. 7)

This definition doesn't mention a conservation function for PRZs, but the CCZ EMP does:

> *"Contractors will provide in their environmental management plans the designation of the required impact and preservation reference zones for the primary purposes of ensuring preservation and facilitating monitoring of biological communities impacted by mining activities."* (ISA, 2011)

The main function of PRZs appears to be to provide a control site for monitoring mining impacts, and this means that these areas will be sampled and thus most probably disturbed. Jones *et al.* (2018) propose that separate PRZs that are not sampled could be put aside to fulfil a conservation function, also alluded to by Vanreusel *et al.* (2016) and Simon-Lledó *et al.* (2019c). Due to their proximity to mining areas, these PRZs could act as stepping stones to connect the APEI network to mined areas and thus improve chances of recolonisation (Jones *et al.*, 2018). Whatever their name, this study highlights the need for conservation areas within high nodule abundance central CCZ habitats in order to have a fully representative protected area network. Careful consideration would need to be given to the size, connectivity and distance from mining of these areas, as well as how they interact with the existing APEI network.

The proposal to establish additional protected areas within the central CCZ raises some interesting questions about how this would be carried out. Contractors are required to designate PRZs within their contract areas for monitoring purposes, but it is unclear whether they also have this obligation for conservation purposes (see definitions above). If contractors are obliged to establish conservation-focussed PRZs within their mining areas, it is likely the resultant protected areas would be selected in an ad-hoc, piece-meal, contractor-by-contractor approach, unless the ISA establishes a regional-level programme to identify where these areas are most needed using a broader, more strategic approach. Focus areas for protection could be identified and then boundaries refined through stakeholder consultation. This would help to establish a more spatially efficient and coherent network of protected areas that are well connected and protect features of interest.

However, this would also undoubtedly raise conflict around the positioning of protected areas if certain areas were of greater conservation concern than others. A regional approach, while preferable, would require buy-in from all stakeholders to ensure its success. Over time, contractors are required to relinquish parts of the area allocated to them (ISA, 2000, Regulation 25), and while contractors could not be forced to relinquish specific parts of their area, it may be possible to work with them to identify areas for relinquishment that contribute to conservation objectives. Engaging with contractors through ISA processes and fostering relationships would support this process and improve the likelihood of buy-in to the process. Lessons learnt in other industries and national waters on retrospectively establishing protected areas that may overlap with commercial interests and balancing conservation and exploitation needs could also be applied (e.g. RSA, 2014). If a regional approach were adopted, it would be prudent to rename these protected areas to avoid confusion with contractor-specific monitoring-focussed PRZs, as these areas would have a very different function.

4.4.4 Future applications

This study provides an example of a classification method that can be used to support spatial planning in large, data-poor areas. Specifically, it could be used to direct the development of further EMPs in other areas targeted by seabed mining, as encouraged by UNGA Resolution 68/70 (UNGA, 2014, para. 51). The only other area in ABNJ targeted for polymetallic nodule mining is in the Central Indian Basin (CIB)[3]. Although the ISA has awarded a contract to the Government of India for exploration of nodules in this area, no EMP has been developed for this region, and no spatial planning has been carried out to pre-emptively support the protection of regional biodiversity before exploratory or commercial activities commence. India's initial exploration contract expired in 2017, and was then extended until 2022 (ISA, 2019c). After this they may apply for an exploitation contract, if the exploitation regulations are finished by then as planned (IISD, 2018). The low level of activity in the CIB suggests that this area is of less concern than the CCZ, but India has recently announced that it will be testing mining equipment at 6 000 m in the CIB by the end of 2019, with a full prototype expected ready for testing by 2022 (Tejonmayam, 2019).

A benthic classification approach similar to the one in this study could be used to support spatial planning in the CIB, including the design of a network of protected areas. A

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[3] See https://www.isa.org.jm/deep-seabed-minerals-contractors

proactive approach to designating protected areas could help to avoid a scenario like the CCZ where certain commercially valuable habitats are all contained within mining areas. This would be a precautionary approach and would require a large collaboration between scientists, contractors and the regulator. Although any network of protected areas that is designed would undoubtedly require revision as new scientific knowledge becomes available, the development of a benthic classification and an APEI network proposal would be a positive first step for this area, and would ensure that any future allocation of exploration contracts meets both conservation and exploitation objectives.

Conservation planning to identify a network of protected areas has already begun for an area on the mid-Atlantic Ridge where there are several existing contracts for polymetallic sulphide mining at hydrothermal vents (Dunn *et al.*, 2018). The authors have made use of available, relevant environmental surrogates to identify different scenarios for an APEI network. This could be complemented by a top-down habitat classification scheme to fine tune appropriate locations for APEIs in this area. Although consideration of APEI size, spacing and replication will be different from this study due to the very different nature of hydrothermal vents compared to abyssal plains, the approach for carrying out a top-down benthic classification could be replicated.

Finally, under the ISA recommendations to contractors on biological sampling to establish environmental baselines, contractors are required to take samples of fauna that are “representative of variability of habitats” in their area and to establish at least one station “within each habitat type or region” for monitoring purposes (ISA, 2013, para.15 (e)). A similar top-down benthic classification approach could be used at a finer-scale within contractors’ exploration areas to support these requirements.

4.5 Conclusions

The top-down benthic classification presented here identified 46 habitat classes within the CCZ management area. More biological data is required to determine whether patterns in habitat distribution identified through the classification represent spatial variability in the megafauna community. This is an extremely important step in the process, and highlights the need for a strategic, regional approach to sampling in the CCZ, but also the global need for large-scale, coordinated sampling strategies.

Assuming that the classification is biologically meaningful, the current APEI network in the CCZ does not adequately represent all of the unique habitats found in this area, particularly those high nodule abundance habitats in the central CCZ. Implementing both adaptive management and the precautionary approach, additional protection measures should be developed to establish a fully representative APEI network. These should include additional APEIs in peripheral areas and smaller protected areas within mining areas. It is recommended that these should be designed using a regional approach to ensure that these areas are spatially coherent.

Finally, a number of future applications for this method have been identified, including in the CIB to support the development of an APEI network, in other areas targeted for mining, and in individual contractor exploration areas.

Chapter 5.

General discussion

The studies carried out in this thesis were performed to increase knowledge on epibenthic megafauna communities associated with polymetallic nodule fields, to support spatial planning in the CCZ and to provide recommendations on environmental management of deep-sea mining.

Although the deep sea is a vast and remote environment, it is still impacted by human activities, and operations are moving into deeper waters (Ramirez-Llodra *et al.*, 2011). At the same time, we still know very little about the fauna that inhabit these environments (Glover *et al.*, 2016a), and increased knowledge on this is fundamental to strengthen environmental management of deep-sea activities. Deep-sea mining, in particular, has the potential for large-scale environmental impacts (Glover & Smith, 2003), and is receiving a lot of attention as technological challenges associated with mining at great depths are being overcome (Petersen *et al.*, 2016) and exploitation regulations for mining in ABNJ are being developed (ISA, 2019b).

This thesis began by reviewing the current status of polymetallic nodule mining activities and regulations, and knowledge on mining impacts and nodule-associated fauna (Chapter 1). It then explored the composition, life history traits and diversity of epibenthic megafauna communities associated with nodule fields and identified potential environmental drivers of these patterns (Chapter 2). Using the same data, the thesis investigated the size of sampling units and number of replicates required to achieve different levels of sample completeness when characterising the epibenthic megafauna community (Chapter 3). Finally, a top-down classification of the CCZ was presented, and used to assess the representativity of the current APEI network (Chapter 4).

This general discussion briefly summarises the outcomes of each chapter and then considers how these findings contribute to the field of research, highlighting implications for environmental management and future research going forward. It finishes with a brief discussion of the limitations of the thesis and deep-sea studies in general, as well as the benefits of repurposing industry data sets.

5.1 Synthesis

Using the CCZ in the eastern equatorial Pacific as a case study for deep-sea mining, the composition, life history traits and diversity of the epibenthic megafauna community associated with polymetallic nodules was described at both regional (> 1 000 km) and local (2 km) scales (Chapter 2). This study found that 98% of the megafauna observed were suspension feeders, 63% were attached to nodules, 98% were sessile and 76% had fewer than 10 records. This showed that those organisms which are potentially most vulnerable to mining activities formed a large proportion of the CCZ epibenthic megafauna, suggesting that mining impacts could be extensive. The community appeared to be homogenous over large scales (> 1 000 km) in terms of the dominant taxa, but there was very high turnover of rare species, suggesting that many species either have a very limited geographic extent or occur at very low densities. The study also found that BBPI may be an important driver of megafauna and metazoan diversity at regional scales for predictive models, although more data is required to properly investigate this.

Chapter 3 then explored the sampling effort required to capture diversity of epibenthic megafauna at different levels of sample coverage and at asymptote. Sample coverage was >80% for the observed sampling units of 800 m^2 used, and numbers of individuals sampled, but in order to reach 99% sample coverage and asymptotic diversity estimates, much greater levels of sampling effort were required. Sampling units ranging from 2 858 m^2 (or 787 individuals) for megafauna to 4 643 m^2 (or 962 individuals) for metazoan data were required to reach 99% sample coverage, while the number of 800 m^2 transects required to reach 99% sample coverage for the CCZ habitat type sampled ranged from 26 for megafauna to 27 for metazoans. The low densities of organisms and high numbers of rare species described in Chapter 2 were responsible for driving this, greatly increasing the size and number of sampling units required. The benefits of the increased knowledge gained through such sampling would have to be weighed against the cost and time required to obtain and analyse the data.

Finally a potential spatial management tool for large, data-poor areas was explored in Chapter 4, as a way to overcome biological data shortages in areas where management decisions on commercial activities need to be made. A top-down, broad-scale habitat classification of the CCZ identified 46 habitats across the CCZ, which were hypothesised to support different fauna. Although not validated, the classification was used to identify gaps in the APEI network in the CCZ. The classification highlighted that several unique habitats with high nodule abundance in the central CCZ are not currently protected and showed that many of these habitats overlap with exploration and/or reserved areas. This study suggests that additional APEIs should be established in peripheral areas and conservation-focussed PRZs in mining areas, to protect habitats receiving less than 10% protection. However, more biological data is required to ground-truth the classification.

5.2 Contribution to knowledge

5.2.1 Contextualising CCZ megafauna observations

While there are a number of caveats within which to frame comparisons between different studies on abyssal megafauna in the CCZ (including the possibility of observer bias and differing sampling equipment, image quality and taxonomic resolution), these discussions can provide a useful context for the findings of this thesis and its contribution to baseline knowledge on the CCZ epibenthic megafauna. Approximate locations of megafaunal studies in the CCZ are shown in Figure 5.1.

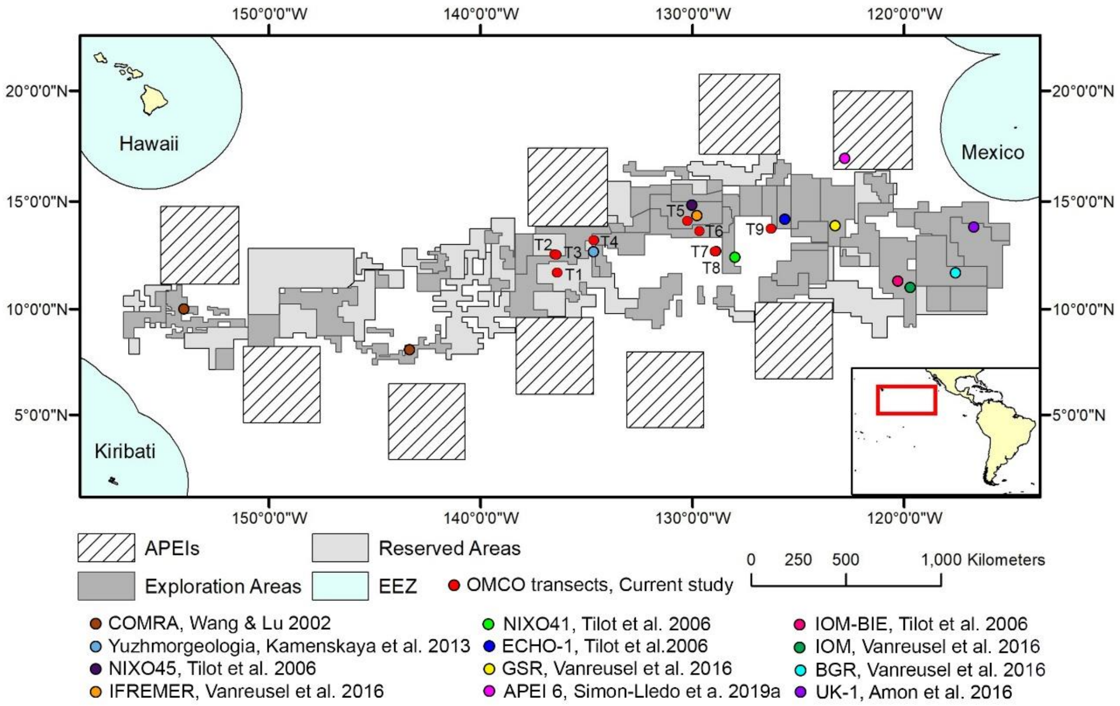


Figure 5.1 Approximate locations of megafauna study sites across the CCZ, including relevant references.

The key faunal groups observed in the current study reflected what has been seen in other sites across the CCZ, both in terms of rank abundance and morphotype richness. Cnidarians, echinoderms and Porifera appear to dominate the metazoan megafauna in this region, as was observed here and by others (e.g. Amon *et al.*, 2016; Morgan *et al.*, 1993; Simon-Lledó *et al.*, 2019b; Tilot *et al.*, 2018; Vanreusel *et al.*, 2016; Wang & Lu, 2002). While there were several new morphotypes recorded, which had not been included previously in the CCZ megafauna morphospecies catalogue used (see Figure 2.16), most morphotypes observed were similar to, or appeared the same as, those already known from the CCZ (Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b). As in many other studies, there was a high number of rare morphotypes, and the community was dominated by xenophyophores (e.g. Amon *et al.*, 2016; Foell & Pawson, 1986; Kamenskaya, Melnik & Gooday, 2013; Simon-Lledó *et al.*, 2019b; Tilot, 2006; Vanreusel *et al.*, 2016).

Variation amongst studies emerges when examining the densities and diversity of megafauna metazoans and xenophyophores recorded. The most striking finding of the current study was the extremely high xenophyophore densities when compared to other studies in the CCZ (Figure 5.2). As the data were quality controlled using multiple observers and photographs were at lower resolution than other studies, the high densities of xenophyophores observed here likely represent a true difference between studies. This could be linked to the generally high nodule abundance observed in transects of this study (see Figure 2.3), which could increase habitat availability for hard substrate obligate or facultative xenophyophores. However, this is speculative and further work is required to understand the relationship between increasing nodule cover and both metazoan and xenophyophore responses in areas of high nodule cover. A global habitat suitability model for Xenophyophorea suggests that more suitable habitat is located in the eastern CCZ, when compared to central and western areas (Ashford, Davies & Jones, 2014), which contradicts the observations made here (see Figure 5.1 for location of the current study in relation to those in the eastern CCZ).

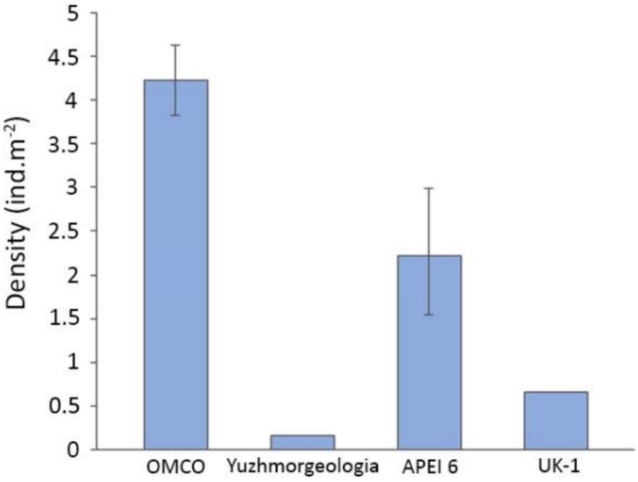


Figure 5.2 Density of xenophyophores from different study sites across the CCZ. Studies from left to right reflect a progression from west to east. Locations and references of studies shown in Figure 5.1. Standard error provided where available.

Another possible reason for the differences in xenophyophore abundance could relate to the historic nature of the OMCO data set. Long-term changes in environmental conditions in this area could potentially be driving the differences between studies. For example, if we look at POC, there is not expected to be much seasonal variation in POC flux to the seafloor in the CCZ (Lutz *et al.*, 2007); however, POC may vary on a larger time scale. Annual and longer term variability in productivity has been observed in the CCZ, equatorial Pacific and north-east Pacific related to upwelling and El Niño and La Niña events (Barber *et al.*, 1996; Dunne *et al.*, 2000; Dymond & Collier, 1988; Smith *et al.*, 2018). Xenophyophores are generally accepted to occur in greatest densities in areas of high productivity (Tendal, 1972), have been shown to respond to phytodetritus input (Drazen, Baldwin & Smith, 1998), and a model predicting their distribution using a proxy for surface productivity has performed well (see Ashford, Davies & Jones, 2014). It could therefore be that the high densities of xenophyophores observed in this study relate to annual or decadal scale changes in POC flux to the seafloor in the CCZ. There is also the possibility that some other environmental conditions driving xenophyophore distribution (e.g. oxygen parameters, nitrate concentration, carbon-chemistry parameters or temperature) could have changed in the time between the studies reported here, which amounts to nearly 40 years.

In contrast to the high abundance of xenophyophores observed, xenophyophore diversity was low compared to other studies (e.g. Amon *et al.*, 2016; Gooday *et al.*, 2017b; Simon-Lledó *et al.*, 2019b). This relates to poor image quality in the current study, and the decision to use broad, generic groupings based on the difficulty of distinguishing different xenophyophore morphotypes (see section 2.3.1.1).

Similarly, metazoan diversity in the current study was low, in so far as it can be compared to other studies (see section 2.4.1.5). Although studies examined different areas of seafloor, the morphotype richness observed here appeared to be low when compared to studies in the eastern CCZ. This is likely a result of poor image quality limiting the taxonomic resolution of image annotations, but could also be linked to spatial and temporal variability in environmental conditions, discussed below in the context of density differences.

A more reliable comparison can be made between metazoan densities across different studies. The densities observed here were a lot higher than some (e.g. those reported by Tilot, 2006; Wang & Lu, 2002, see Figure 5.1 for locations of studies), and lower than others (e.g. Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019b; Vanreusel *et al.*, 2016). Overall, there appears to be a broad pattern of increasing metazoan abundance from west to east (Figure 5.3). This corresponds to a longitudinal gradient of increasing POC flux to the seafloor across the region (see Figure 4.3). A strong correlation between mega- and macrofaunal abundances has been observed with the latitudinal gradient in POC across the CCZ (Smith *et al.*, 1997), and similar responses in faunal distribution have been observed in megafauna at other abyssal sites (Thurston, Rice & Bett, 1998). It is important to note, however, that not all studies examined fit this pattern of increasing abundance from west to east. Densities observed by Tilot (2006), for example, appear very low in comparison to other studies in the eastern CCZ. This could be due to one or more of the caveats mentioned above introducing artificial variation amongst studies, or other environmental variables driving the patterns observed (e.g. nodule abundance, see Amon *et al.*, 2016; Simon-Lledó *et al.*, 2019c; Vanreusel *et al.*, 2016).

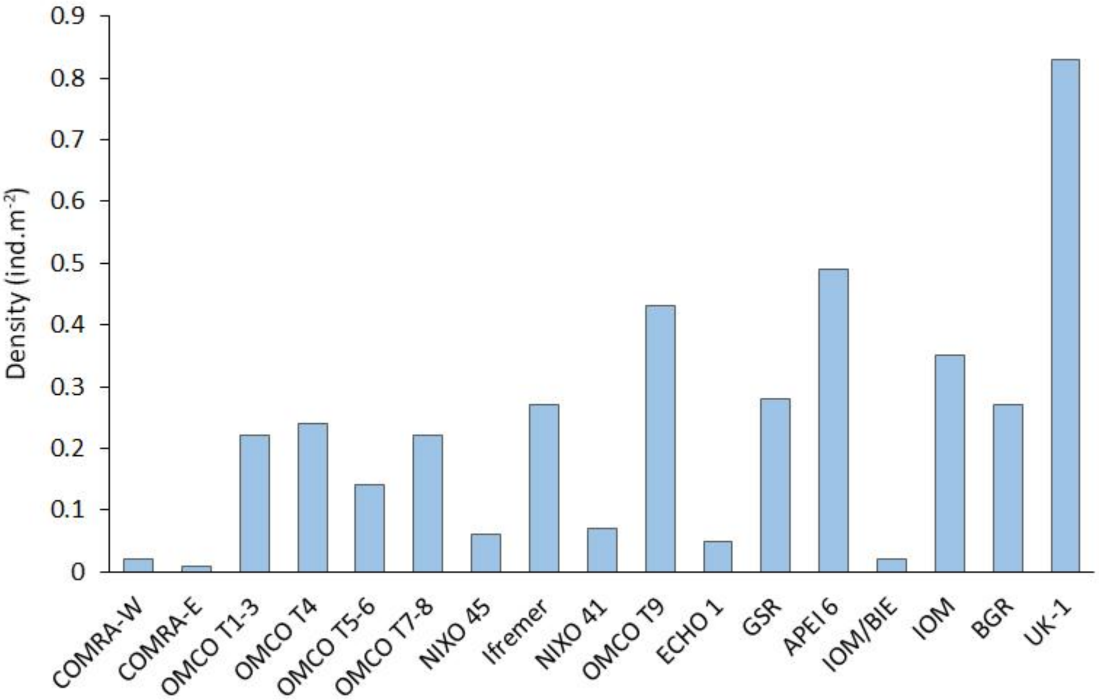


Figure 5.3 Density of metazoan megafauna from different study sites across the CCZ. Studies from left to right reflect a progression from west to east. Locations and references of studies shown in Figure 5.1. OMCO transects (i.e. those from this study) have been grouped where they occur in close proximity. Standard error not available for most studies and thus excluded.

As discussed above with reference to patterns in the xenophyophore community, the differences in metazoan abundance and diversity observed in the current study could also relate to temporal variation in environmental conditions. Smith *et al.* (1997) suggested that megafaunal abundance in the CCZ could be used as a proxy for seafloor POC flux on time scales of years to decades, as this broadly reflects the lifespans of abyssal megafauna (Gage & Tyler, 1996) and could therefore capture megafaunal responses to POC variability. At Station M, in the north-east Pacific, decadal scale changes in the megafauna community have been observed. Although these were first linked to temporal variation in seafloor POC flux (Kuhnz *et al.*, 2014; Ruhl & Smith, 2004), it is now less clear what is driving those shifts (Kuhnz *et al.*, 2020). What is apparent, is that abyssal megafaunal communities vary on far shorter time scales than previously expected (Kuhnz *et al.*, 2014; Kuhnz *et al.*, 2020). It could therefore be that the differences in metazoan density and diversity observed here relate to annual or longer term changes in environmental conditions in the CCZ, or a combination of both temporal and spatial variability in conditions.

5.2.2 Deep-sea sampling design

From Chapter 3 it is clear that the standardisation of sampling design needs further attention. Design of a robust sampling strategy would need to include consideration of the size of sampling units and number of replicates. This thesis has provided estimates of the sampling effort required to capture different levels of diversity of the epibenthic megafauna communities, and these could be used to inform sampling recommendations or requirements, in the form of standards and guidelines. The outcomes of this study are particularly relevant for characterising baseline communities, while sampling for monitoring purposes must be informed by power analyses. A regional environmental assessment approach is advocated for to support sufficient environmental sampling within a robust, strategically designed programme (discussed below).

5.2.3 Spatial planning

Finally, this thesis suggests further allocation of protected areas is required if the ISA wishes to meet the internationally recognised conservation targets of 10% habitat representativity. Chapter 4 identified many habitats that received less than 10% protection in the current APEI network, and some of these with > 70% of their total extent within exploration and/or reserved areas. The APEI network needs improving, through the establishment of additional areas protecting habitats that currently receive little protection. In addition, smaller, conservation-focussed PRZs may be required within the central CCZ and overlapping with mining areas in order to protect those unique high nodule abundance habitats found only in the central CCZ. If, with further biological data, the classification proves to be biologically meaningful, this could be used to identify appropriate locations for these protected areas.

5.3 Future work

This thesis highlights many areas of research that require further attention before human activities, and particularly deep-sea mining, can be managed properly. What is made apparent through all of the studies, is that there is a significant lack of biological data across the CCZ region. Figure 5.1 shows the vast size of the CCZ and highlights those areas where the megafauna community has not yet been explored (although this map does not include all studies with information on megafauna). In addition, many of the studies shown in this figure represent just one or two video transects.

The scientific knowledge required to adequately manage mining activities in the deep-sea lags far behind the development of mining technology and the regulatory framework. Fundamental, baseline knowledge on the taxonomy, ecology, geographic ranges, connectivity and functional roles of the megafauna communities that will be affected by mining is still woefully lacking. Chapter 2 showed that those species that are thought to be most vulnerable to mining impacts formed a large proportion of the epibenthic megafauna, and highlighted that more research on these species particularly is needed, although of course more baseline information is needed even to just identify these species.

Further research is needed to understand the vulnerability of organisms to mining impacts, to predict extinction risks and support the development of thresholds, standards and guidelines for mining operations. Experimental studies on, for example, filtering rates of nodule-associated suspension feeders would allow an estimation of sedimentation mortality thresholds and would thus support the development of standards and thresholds to prevent significant adverse impacts. These experiments could be undertaken in laboratory conditions using shallow water counterparts (if these exist) or with specimens collected from depth (if these survive). Collection of specimens from the CCZ or similar nodule provinces would require dedicated ROV dives, and it may be challenging to obtain sufficient specimens to allow representative experiments due to the generally low densities of abyssal megafauna. Laboratory experiments could be complemented by in-situ experiments (e.g. see Grant *et al.*, 2018), if these are possible at such depths. Studies are also needed on geographic ranges of megafauna to understand if rare species really are rare, or just occur at low densities, and on genetic connectivity to understand how mining might impact populations of rare or low density species. This information would support spatial planning (e.g. Taboada *et al.*, 2018).

Chapter 2 also highlighted that studies on the functional roles of nodule-associated organisms and the ecosystem services they provide are vital, together with information on vulnerability to mining, to predict the impact that loss of these organisms would have on a system. This is particularly important to understand for those species that are most vulnerable to mining impacts, and is crucial to understanding the ecosystem-level impacts that cumulative mining operations could have. This is fundamental for supporting spatial planning and other management tools like environmental standards and guidelines.

Likewise, studies to better understand the relationship between nodule density and megafauna community composition and diversity in areas of high nodule cover (> 50%) are urgently needed. Chapter 4 revealed that many of the habitats receiving < 10% protection were areas of high nodule abundance overlapping with mining areas in the central CCZ. It is thus critical to investigate this relationship to be able to predict the impact that loss of high nodule areas would have on regional diversity. There are plans to address this using data gathered in the thesis. Data on nodule cover (as a percent of the total area in each image) are being extracted from the images analysed for megafauna in this study. This is a time-consuming process, involving manual outlining of nodules in BIIGLE 2.0 (Langenkämper *et al.*, 2017), and thus 20% of images in each transect are being randomly selected and analysed to provide an estimate. Once these data are available, correlations between percent nodule cover and megafauna abundance and diversity will be explored. It is anticipated that this will shed light on the relationship between nodule cover and megafauna distribution in areas of high nodule abundance, and thus the importance of conserving these areas.

Finally, bigger biological data sets are required to ground-truth classification methods, such as used in Chapter 4, and to build and validate predictive models. The validation of approaches using environmental surrogates is extremely important, as one of the fundamental assumptions of biophysical habitat classifications performed using surrogates is that environmental heterogeneity reflects spatial variability in the biological community. Chapter 3 showed that the biological data set obtained in this study was insufficient to fully characterise the megafauna community in terms of both the size and number of samples, and this data did not provide evidence of support for the classification in Chapter 4. More data is needed to ground-truth this classification before it can be confidently used for spatial planning purposes.

This thesis has also highlighted that the paucity of both biological data and fine-scale environmental data prohibits the use of predictive modelling in the CCZ. Species distribution maps, produced through predictive modelling, have proven to be a useful tool in supporting management decisions on issues relating to resource use and conservation planning (e.g. Buhl-Mortensen *et al.*, 2015; Howell *et al.*, 2016a), and there have been calls for habitat mapping and predictive modelling in the CCZ to support the spatial management of mining activities (De Smet *et al.*, 2017). Chapter 2 provided some insight into environmental variables that may be driving species distribution at a broad (> 1 000 km) scale, but this study was based on broad-scale, modelled environmental data and just nine biological samples. Higher resolution environmental data and a much greater quantity of biological data are required to carry out species distribution modelling.

All of these areas of research would help to bridge the gap between what is required for decision-making and the science that is currently available, and would ultimately support environmental management of mining activities to ensure that regional biodiversity is protected.

5.4 Regional environmental assessment

While some of the issues raised above could be addressed through the collation of multiple data sets, this is difficult due to differences in sampling effort, taxonomic resolution and data quality between studies. A regional, strategic and coordinated approach to sampling, in the form of regional environmental assessment (REA), would overcome this and would ensure that environmental management has a robust scientific foundation. Both Chapters 2 and 4 highlight the need for REA, while Chapter 3 provides insight into sampling considerations for REA.

REA would bring together the key stakeholders of the industry, to design and implement a large-scale, coordinated, strategic environmental assessment of areas that are being targeted for commercial activity. More data collected in this way would serve so many purposes, from improving our understanding of the basic ecology of fauna associated with nodule fields to validating habitat classifications and building predictive models of species distribution. In addition, it would ensure that habitats falling outside of exploration areas were sampled in a systematic, co-ordinated manner so that MSP could take place with a better understanding of fauna both within and outside of areas directly impacted by mining operations.

In undertaking a regional assessment, consideration would need to be given to sampling design, data collection protocols, standardised morphospecies catalogues and data storage. The ISA has taken some steps towards this. They have issued guidance and recommendation documents which are intended to support the implementation of environmental requirements by contractors (ISA, 2011; ISA, 2013; ISA, 2015a) and have identified various standards and guidelines that should be developed to inform mining operations, including those for baseline studies, impact assessment and monitoring (ISA, 2019d). The ISA has also recently released an online platform that will act as a central repository for data collected by contractors, and non-proprietary data will be made freely available (ISA, 2019a). Contractors are required to send data to this repository for regional management purposes, including conservation (ISA, 2013, Annex I, para. 2 (d));

however, different sampling methods are employed between contractors and their data does not fit into any regionally designed study as advocated here.

If contractors were to employ the same sampling methods to support a REA approach, this would require buy-in from, and a high level of coordination between, all contractors. This would require consultations and negotiations to establish agreed upon sampling methods, and the development of more detailed standards and guidelines for sampling. Issues would be likely to arise where contractors have access to different sampling equipment, and where different sampling methods or regimes have financial implications. This process would require oversight by the ISA.

Finally, the ISA has developed a megafauna catalogue for the CCZ, although this is no longer available online and would need to be further developed and kept up-to-date as new morphotypes are observed. All of these actions are steps in the right direction to building a more comprehensive view of the biological communities in the CCZ; however, further work is required to ensure that sufficient data is collected using a statistically robust design.

While there are many issues that would need clarification while developing a REA, including questions around who would develop and carry out the REAs, what the procedures for conducting assessments would be, and what common criteria would be applied to all REAs and REMPs (Jaeckel, 2019), the ISA could draw on experiences in other industries to guide the process. Although the situation is undoubtedly more complicated with seabed mining in ABNJ, because the targeted areas are very large, and there are many different countries involved, examples from other industries show how key stakeholders can work together to carry out strategic, co-ordinated work to the benefit of everyone.

The CCZ is much larger and diplomatically more complicated than, for example, UK waters, but there are 14 contractors working in this area with an obligation to carry out baseline studies (ISA, 2019c). If, for arguments sake, it was decided that a target sample coverage of 99% was adequate, 26 transects of 800 m^2 would be required to characterise the megafauna in flat areas of medium POC with variable nodule coverage. This would be less than two transects per contractor. Of course this is an oversimplification because it applies only to a limited environmental range and group of fauna, but it demonstrates that such sampling could be feasible with strategic planning. A thought-out, overarching strategic approach to sampling would optimise the use of limited resources (e.g. ship time, man-hours of processing samples and analysis), allow collation of data sets, and ensure that research efforts form part of a strategic sampling plan intended to support regional environmental management, including MSP, in the area.

5.5 Limitations and opportunities

There were some common limitations experienced throughout the thesis that were generally linked to a lack of data, both biological and environmental, and there are limitations with the use of image data for environmental assessment, which should be addressed. However, this thesis also highlights opportunities for improved understanding of biological communities through repurposing of industry data sets. These limitations and opportunities are discussed below.

5.5.1 Limitations

While the use of imagery is a non-destructive sampling method, allowing in-situ observations of organisms, it is not without limitations. The resolution of taxonomic identifications from images can vary greatly, both within and between studies. This is affected by image quality, but also the sensitivity of different taxa to image quality, with more distinctive taxa identified to higher taxonomic resolution. In addition, the reliability of observations (both in terms of taxonomic accuracy and detection of individuals) varies greatly between observers (Durden *et al.*, 2016; Schoening *et al.*, 2020). Still images and videos also do not provide information on meio- and macro-fauna, as these classes are too small to be seen (unless high resolution, targeted sampling is carried out in the style of the Nautilus Live Expeditions, see https://nautiluslive.org), nor do they provide insight into infaunal communities. Finally, imagery collected through different means (e.g. remotely operated vehicles, ROVs, vs autonomous underwater vehicles, AUVs) may show different components of the faunal community (Schoening *et al.*, 2020).

Despite these limitations, imagery can be extremely useful for providing an overview of a site in a non-destructive manner, as large areas can be covered, particularly if using an AUV. Image data also provides finer spatial resolution to community data, as samples are not aggregated as with a dredge. This method should be used as part of a suite of tools in environmental assessments, complemented by methods like box cores and grabs, which provide insight into other faunal components and allow collection of physical samples for taxonomic identification and genetic studies. In the future, the use of imagery may become even more important in rapid assessments of large areas as Artificial Intelligence (AI) and Computer Vision (CV) methods are developed (see below).

When considering a lack of biological data, the principle bottlenecks with image analysis from deep-sea data are in the collection of sufficient imagery, and in the time needed to process this imagery. Autonomous technologies like AUVs have already proven capable of collecting large quantities of data, and can be used to achieve the levels of sampling identified in this thesis (Piechaud *et al.*, 2019). Global sampling programmes are needed to coordinate a strategic plan of sampling the deep sea. Initiatives like the DOSI Decade Working Group (DOSI, 2019), which has been established to ensure that priorities for deep ocean science, and particularly ecology, are included in the United Nations Decade of Ocean Science for Sustainable Development (2021-2030), will hopefully move this forward.

In addition, this study showed that limited human capacity to manually annotate images greatly restricted the amount of data that could be analysed from the large OMCO data set. New image processing techniques developed through AI and CV have proven somewhat successful in the automated annotation of images, and may help to overcome the bottleneck associated with manual annotation in the future (Piechaud *et al.*, 2019). AI and CV methods may also help to support the collation of multiple data sets by providing a method that is more consistent and shareable. Standardised reference image databases are also needed to support this. Better biological data would help to address some of the key issues limiting environmental management in the deep sea, and could be used for purposes like ground-truthing top-down classifications and building predictive models.

This thesis also highlighted the lack of high resolution environmental data. In terms of identifying environmental drivers of megafauna distribution, there was very little environmental data to work with, and it was coarse-scale. New observer programmes like the Global Ocean Observing System (GOOS) under the UN Decade of Ocean Science for Sustainable Development may help to provide better environmental data (Ryabinin *et al.*, 2019), as well as initiatives like the General Bathymetric Chart of the Ocean

(GEBCO) Seabed 2030 project, which aims to improve bathymetric data through collation of existing data sets and focussed seafloor mapping (GEBCO, 2019). Improved environmental data would support classifications and predictive modelling by providing a wider variety of higher resolution explanatory variables.

5.5.2 Repurposing industry data sets

Finally, this thesis highlights the opportunities and benefits of industry collaboration and working with industry data sets. Accessing industry data can play an important role in increasing our knowledge of areas like the CCZ, where much resource-related exploration has been undertaken, but biological data lags behind and is desperately needed. Data that are not necessarily intended for scientific research can be repurposed to unlock unseen biological data for an area, as was the case with the OMCO data set used in this thesis.

Levin *et al*. (2019) talk about the value of repurposing industry data and the benefits that flow from this, including improved surveying, monitoring and observing approaches and increased value of data collected. Use of industry data sets can greatly decrease or even remove costs associated with biological sampling, as it involves using existing data sets. In addition, it not only provides new information of operational importance (e.g. for regulators and operators), but can also contribute to the broader understanding of deep-sea ecology (Levin *et al*., 2019). Collaboration can therefore lead to improved scientific knowledge of an area, thereby decreasing uncertainty, supporting monitoring and even improving environmental management (Gates *et al.*, 2017). This approach has been successfully used in other industries, particularly the oil and gas sector (see Gates *et al.*, 2017, and papers therein).

Industry collaboration requires investment in relationship-building between scientists and industry operators, but where trust is developed this can become a mutually beneficial relationship, where industry can improve data collected for scientific reporting and scientists can gain access to data that may otherwise remain confidential (Levin *et al.*, 2019). While there are challenges with working with industry data, including gaining access to data or not having control over sampling design, the current study provides an example of how these data can be repurposed to provide new insights into faunal communities, and hopefully improve environmental management.

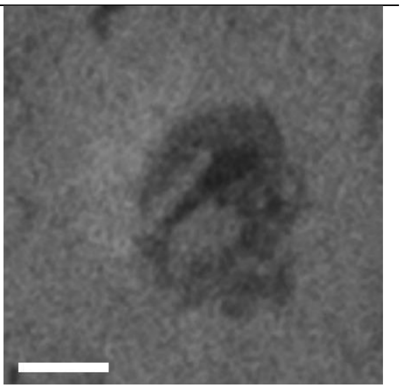
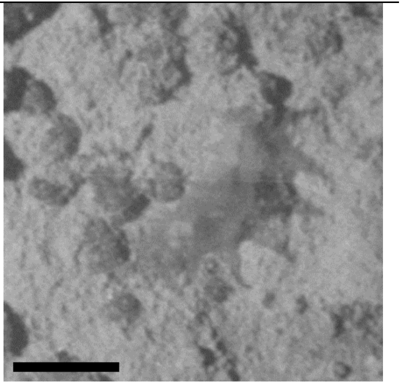
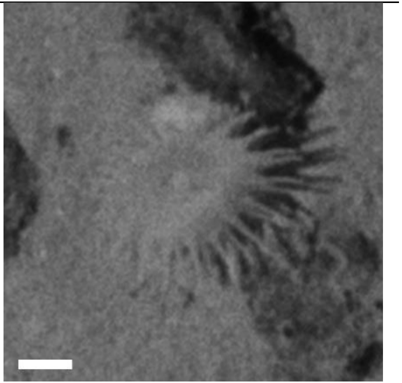
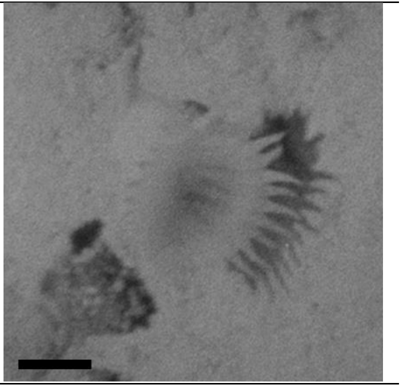
Appendix A

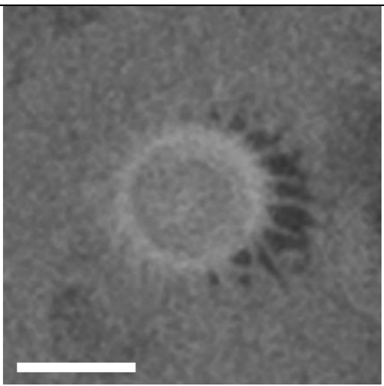
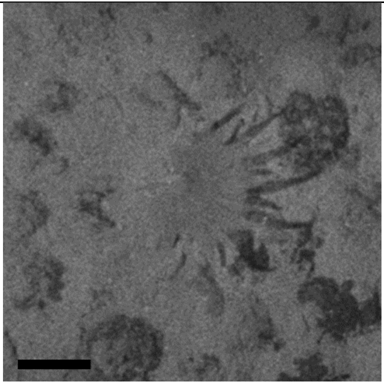
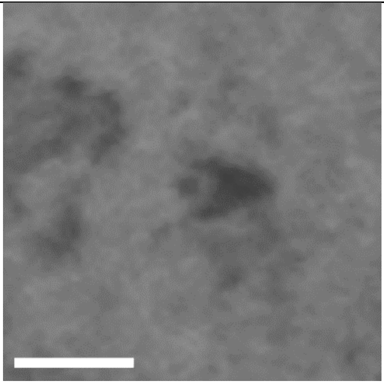
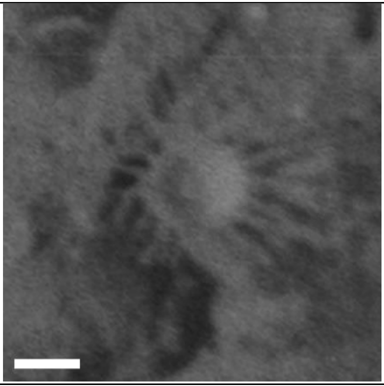
The following figures and tables accompany Chapter 2:

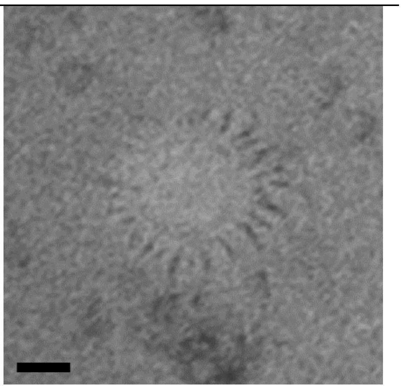
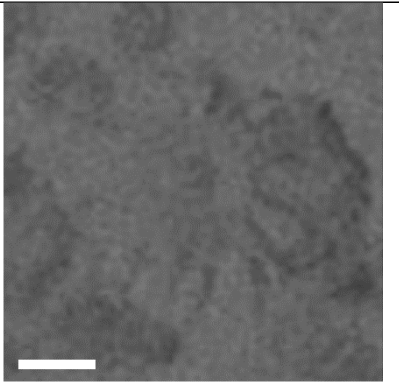
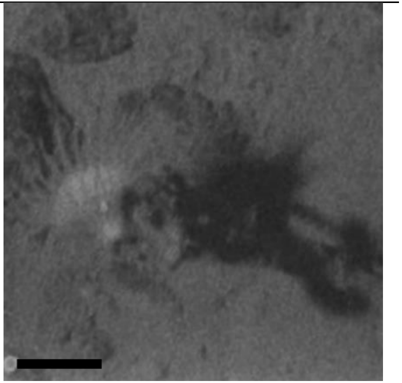
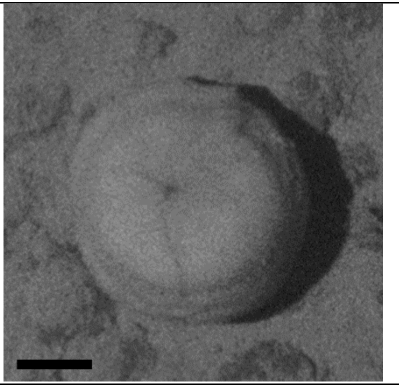
Table A.1 Megafaunal morphotypes and abundance observed in images from nine transects, collected using a Benthos 377 deep-sea towed camera system by Lockheed Martin and the Ocean Minerals Company (OMCO) consortium during exploration activities in the CCZ from 1978-1981

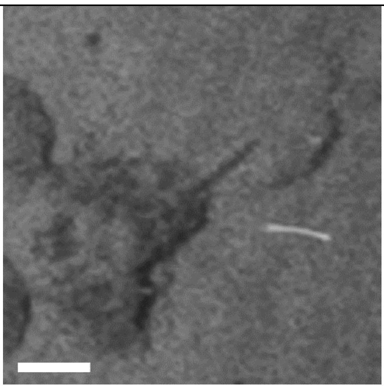
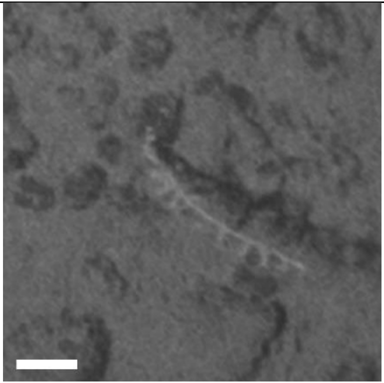
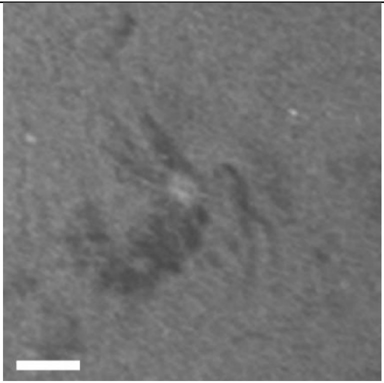
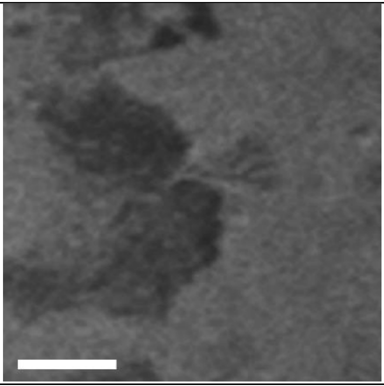
Phylum	Class	Subclass/Order	Morphotypes (n)	T1	T2	T3	T4	T5	T6	T7	T8	T9
Annelida			1	2	24	9	16	4	19	11	3	16
Cnidaria	Anthozoa	Actiniaria	11	42	85	54	48	30	41	60	30	42
		Alcyonacea	2	13	92	14	23	11	31	37	16	36
		Antipatharia	7	1	1	3			2	5	1	6
		Ceriantharia	1					1				1
		Corallimorpharia	1				1					
		Pennatulacea	5	3		2	4					3
	Hydrozoa		2	1		1	1					
Bryozoa			4		3		1			1		3
Chordata	Actinopterygii		3	2	1		1		2			
	Ascidiacea		3		2			1				
Arthropoda			2						1		1	
		Decapoda	2				1					2
Echinodermata	Asteroidea		5		3	3		1		2	6	7
	Crinoidea		4				2	1	2	1	5	10
	Echinoidea		3	3	5	8	26	3	8	18	14	73
	Holothuroidea		21	10	18	20	12	2	10	9	13	19
	Ophiuroidea		2		6	1	3	1	3	63	17	54
Mollusca			3		2		1			1	1	1
Porifera			1	14	64	11	53	13	26	23	9	69
	Demospongiae		1		2	2	1			1		1
	Hexactinellida		2	1	8	9	2	1		3	5	2
Foraminifera	Xenophyophorea		4	2479	4834	3526	4200	2254	2160	3736	2662	4613
		TOTAL (metazoans)	86	92	316	137	196	69	145	235	121	345
		TOTAL (megafauna)	90	2571	5150	3663	4396	2323	2305	3971	2783	4958

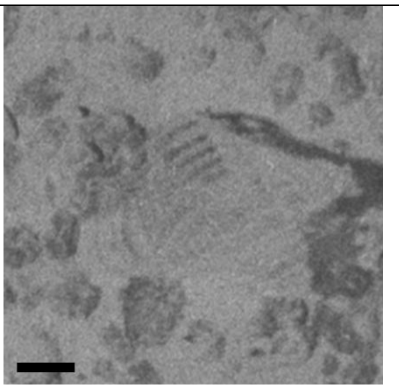
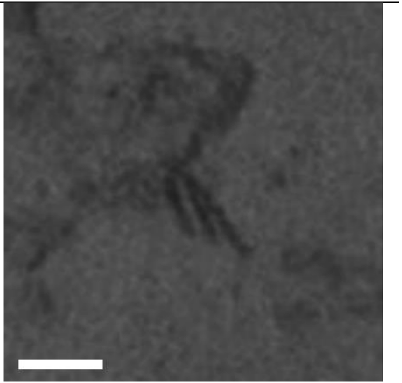
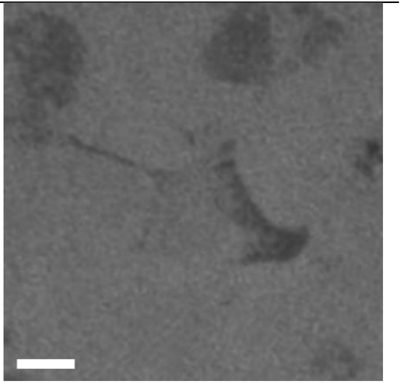
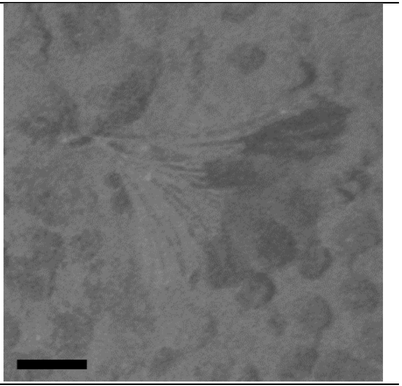
Table A.2 Morphotype list with example images. White scale bar = 2cm, black scale bar = 5cm, mtp = morphotype. Morphotype numbers based on Dr Erik Simon-Lledó's megafauna catalogue for the CCZ region, as at July 2019 (Simon-Lledó *et al.*, 2019b).

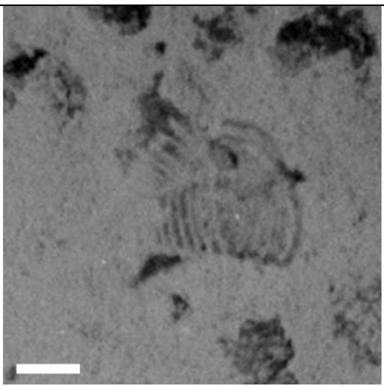
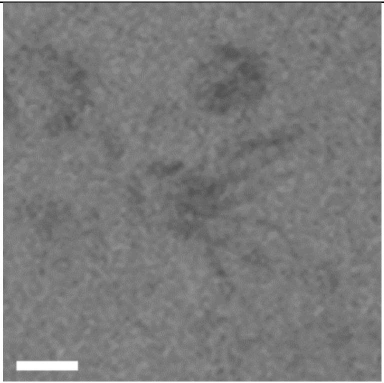
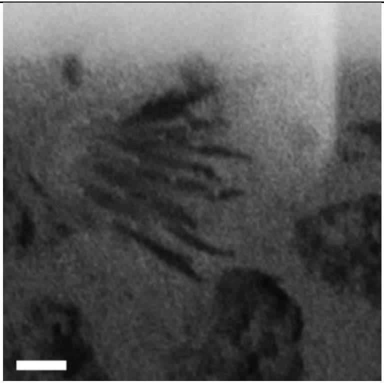
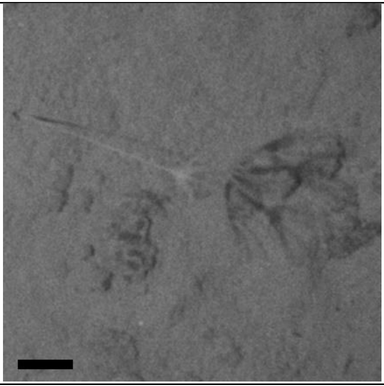
Phylum	Class	Subclass/Order	Morphotype	Example image
ANNELIDA			Annelida (general)	
CNIDARIA	Anthozoa	Actiniaria	Actiniaria (general)	
		Actiniaria	Actiniaria mtp 002	
		Actiniaria	Actiniaria mtp 003	

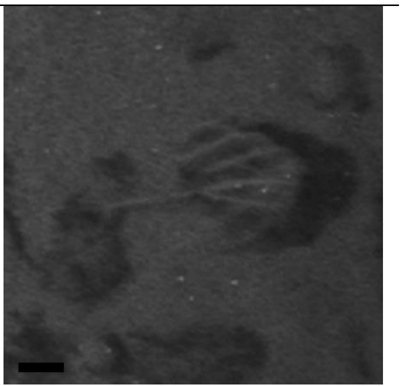
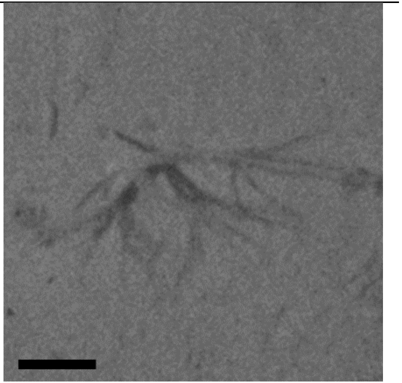
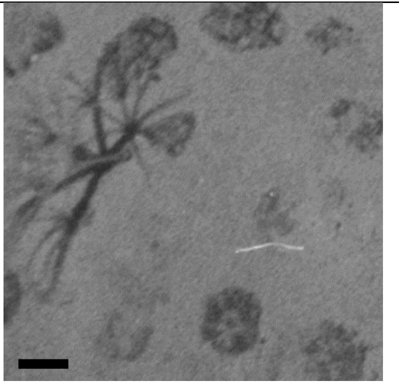
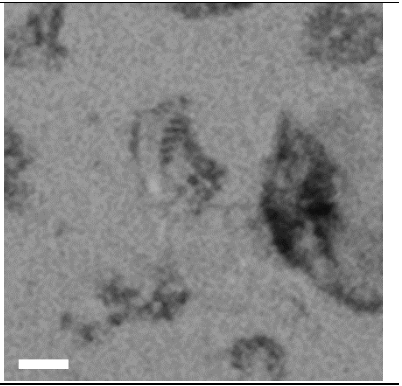
Phylum	Class	Subclass/Order	Morphotype	Example image
		Actiniaria	Actiniaria mtp 004	
		Actiniaria	Actiniaria mtp 010	
		Actiniaria	Actiniaria mtp 018	
		Actiniaria	Actiniaria mtp 022	

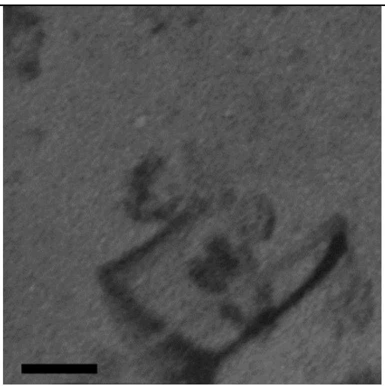
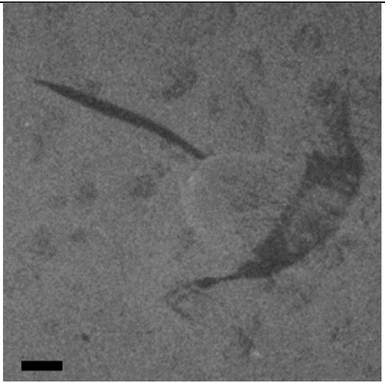
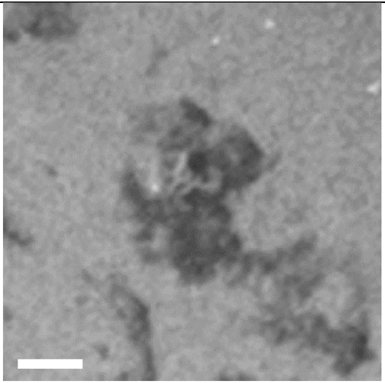
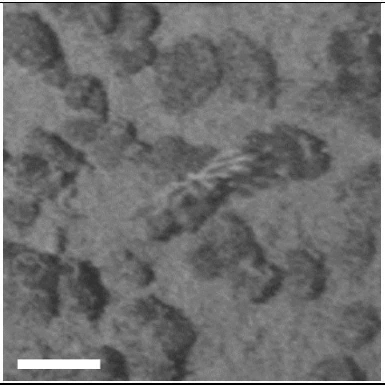
Phylum	Class	Subclass/Order	Morphotype	Example image
		Actiniaria	Actiniaria mtp 028	
		Actiniaria	Actiniaria mtp 035	
		Actiniaria	Actiniaria mtp 042	
		Actiniaria	Actiniaria mtp 048	

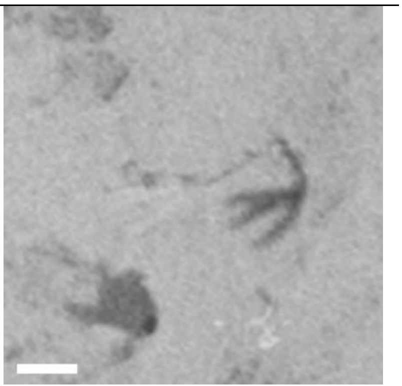
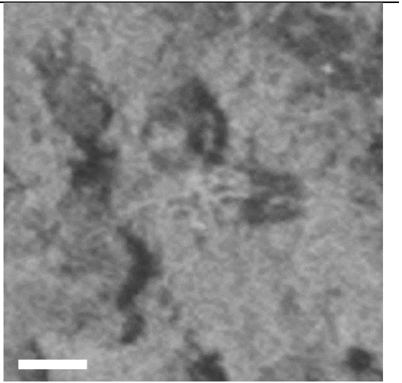
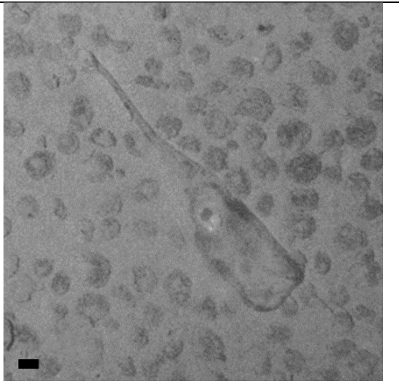
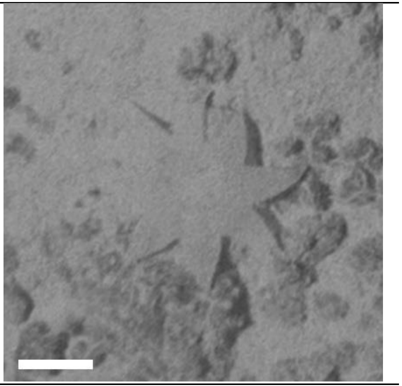
Phylum	Class	Subclass/Order	Morphotype	Example image
		Alcyonacea	Alcyonacea (general)	
		Alcyonacea	Alcyonacea mtp 004	
		Antipatharia	Antipatharia (general)	
		Antipatharia	Antipatharia mtp 001	

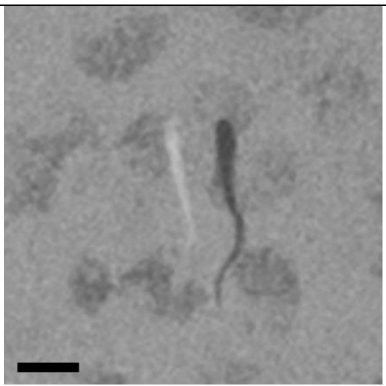
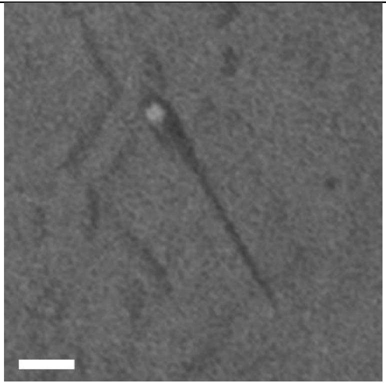
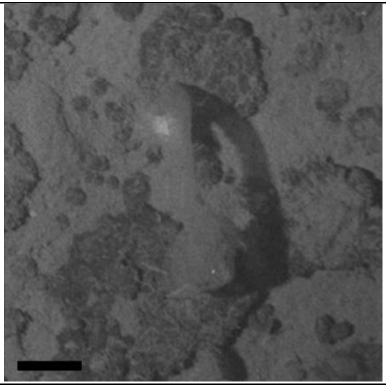
Phylum	Class	Subclass/Order	Morphotype	Example image
		Antipatharia	Antipatharia mtp 002	
		Antipatharia	Antipatharia mtp 003	
		Antipatharia	Antipatharia mtp 006	
		Antipatharia	Antipatharia mtp 008	

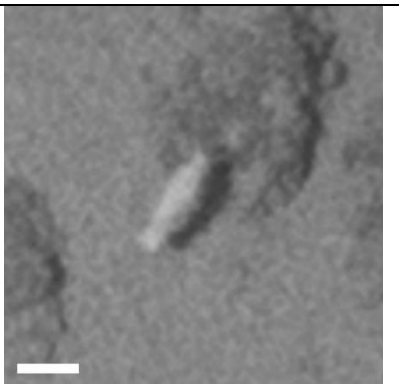
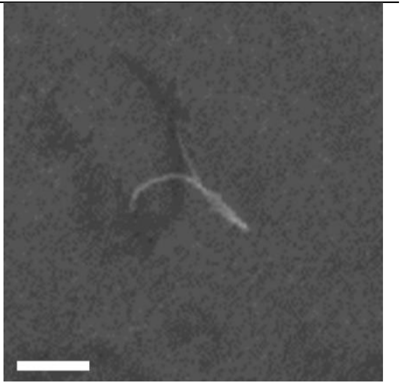
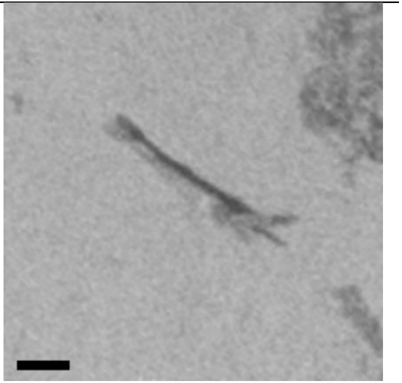
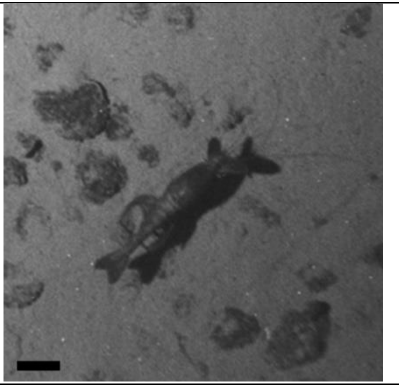
Phylum	Class	Subclass/Order	Morphotype	Example image
		Antipatharia	Antipatharia mtp 011	
		Ceriantharia	Ceriantharia (general)	
		Corallimorpharia	Corallimorpharia (general)	
		Pennatulacea	Pennatulacea mtp 003	

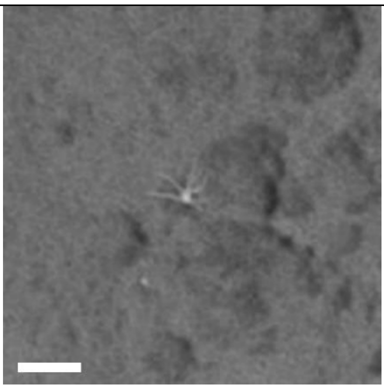
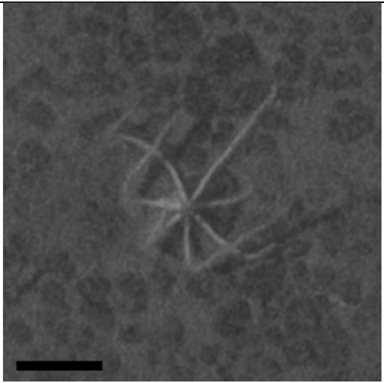
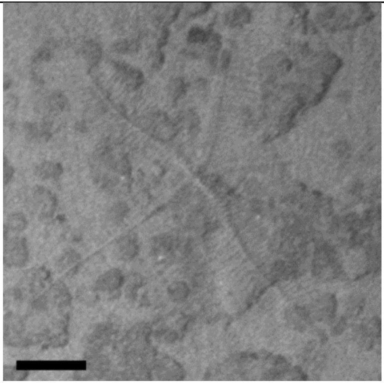
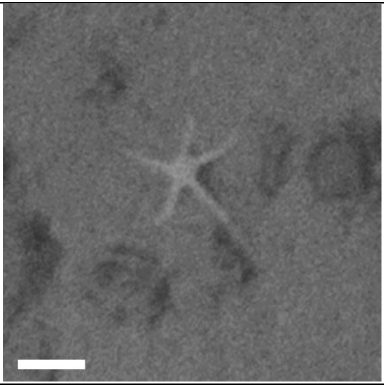
Phylum	Class	Subclass/Order	Morphotype	Example image
		Pennatulacea	Pennatulacea mtp 009	
		Pennatulacea	Pennatulacea mtp 010	
		Pennatulacea	Pennatulacea mtp 015	
		Pennatulacea	Pennatulacea mtp 016	

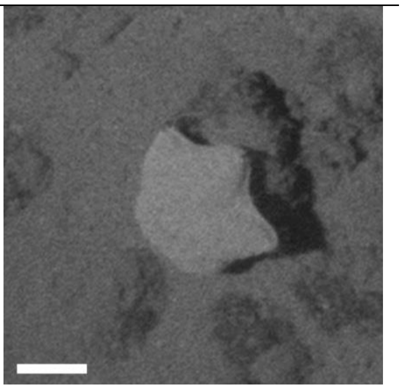
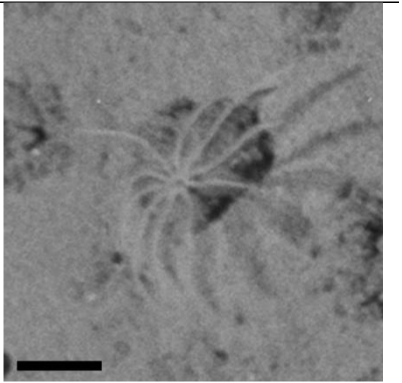
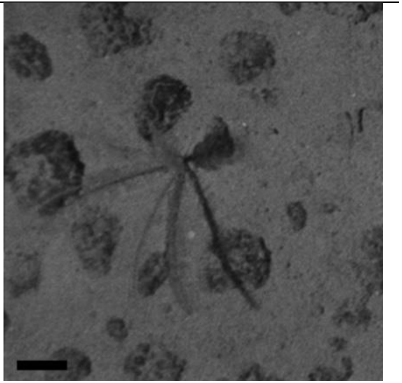
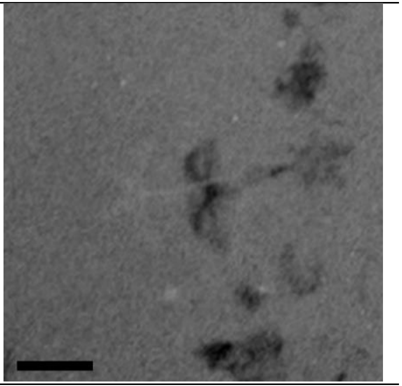
Phylum	Class	Subclass/Order	Morphotype	Example image
	Hydrozoa		Hydrozoa mtp 006	
	Hydrozoa		Hydrozoa mtp 008	
BRYOZOA			Bryozoa (general)	
			Bryozoa mtp 002	

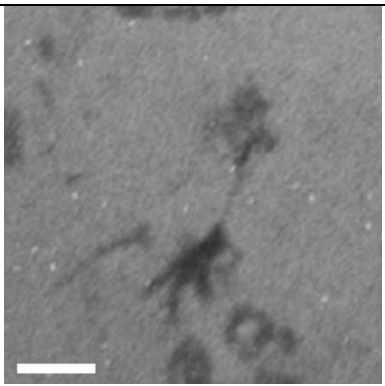
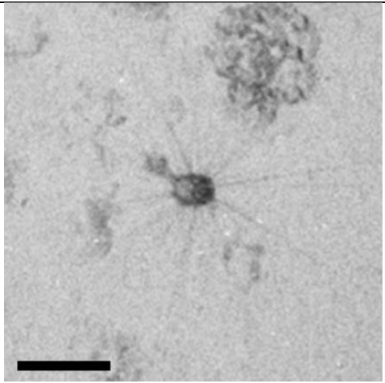
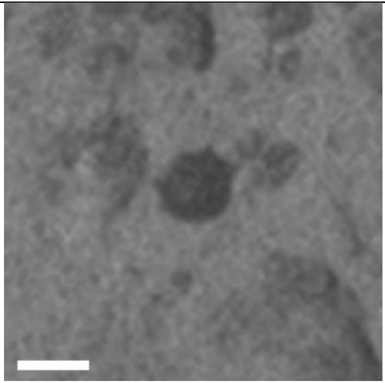
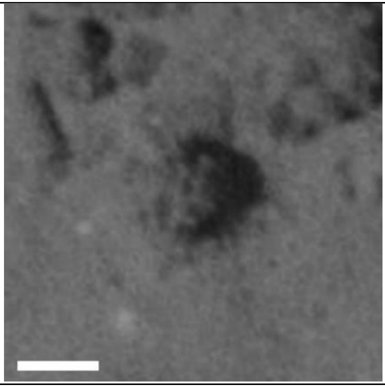
Phylum	Class	Subclass/Order	Morphotype	Example image
			Bryozoa mtp 003	
			Bryozoa mtp 004	
CHORDATA			Chordata mtp 001	
			Chordata mtp 006	

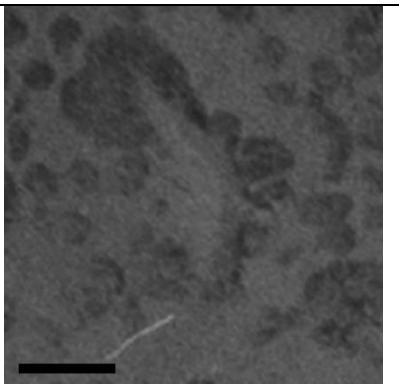
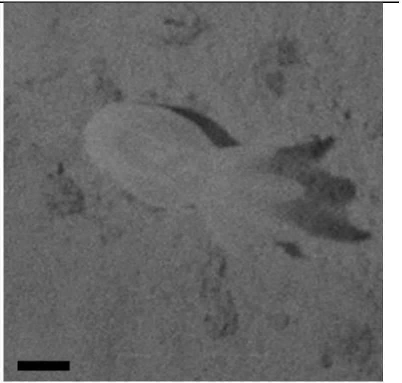
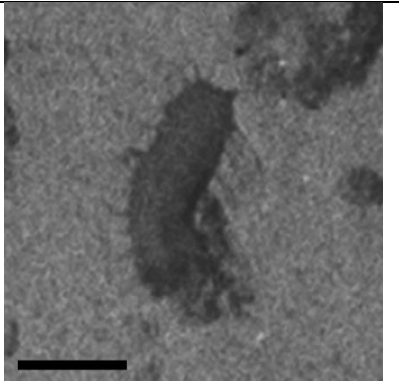
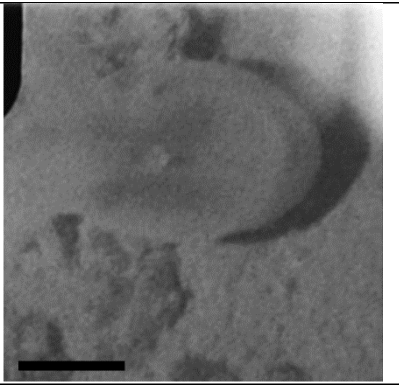
Phylum	Class	Subclass/Order	Morphotype	Example image
	Actinopterygii		Actinopterygii (general)	
			Actinopterygii mtp 004	
			Actinopterygii mtp 010	
	Ascidiacea		Ascidiacea (general)	

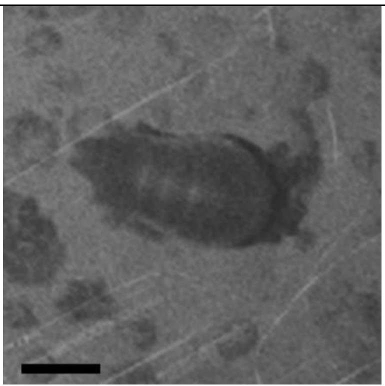
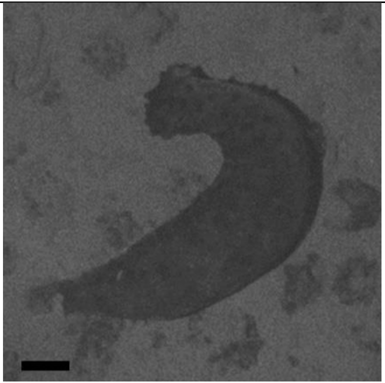
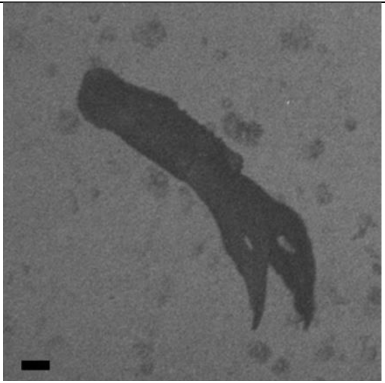
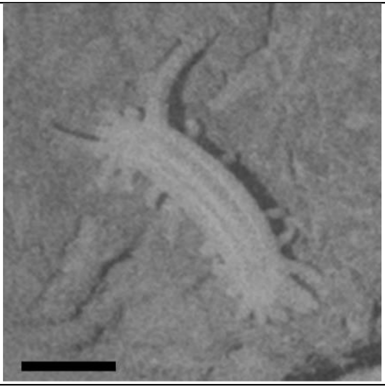
Phylum	Class	Subclass/Order	Morphotype	Example image
ARTHROPODA			Crustacea (general)	
			Arthropoda mtp 008	
		Decapoda	Decapoda (general)	
		Decapoda	Decapoda mtp 001	

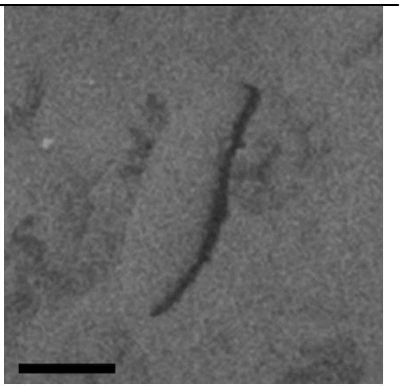
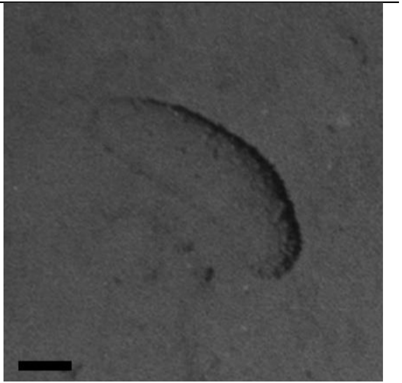
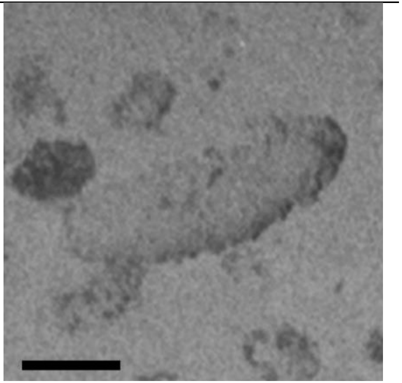
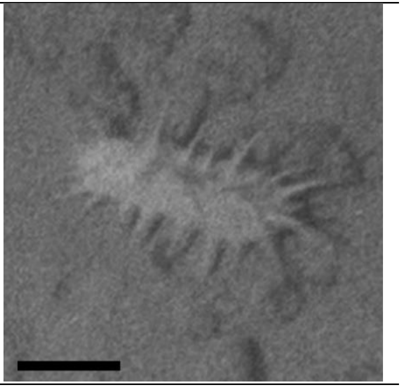
Phylum	Class	Subclass/Order	Morphotype	Example image
ECHINODERMATA	Asteroidea		Asteroidea (general)	
	Asteroidea		Asteroidea mtp 001	
	Asteroidea		Asteroidea mtp 002	
	Asteroidea		Asteroidea mtp 005	

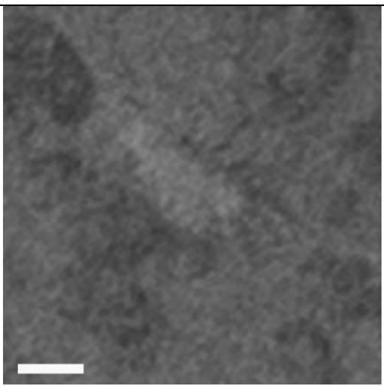
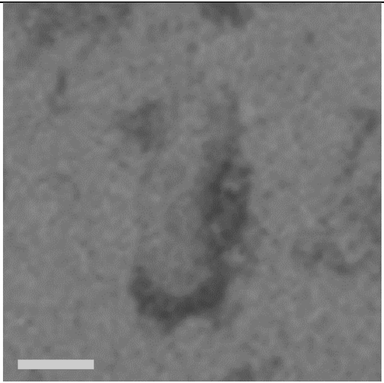
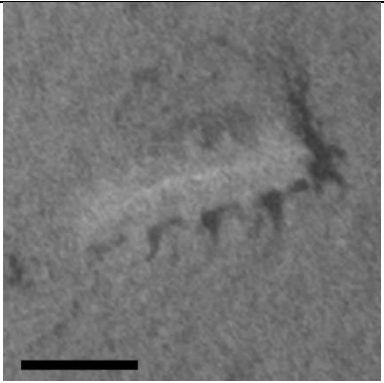
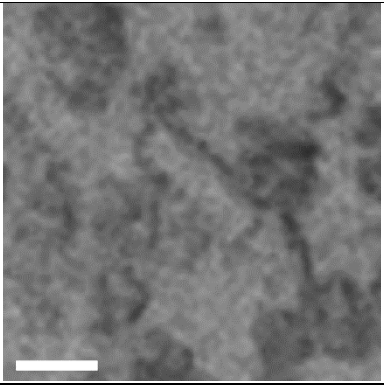
Phylum	Class	Subclass/Order	Morphotype	Example image
	Asteroidea		Asteroidea mtp 015	
	Crinoidea		Crinoidea mtp 001	
	Crinoidea		Crinoidea mtp 002	
	Crinoidea		Crinoidea mtp 005	

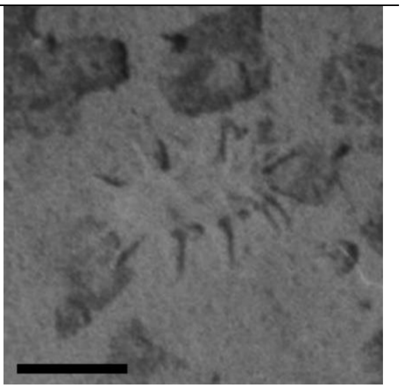
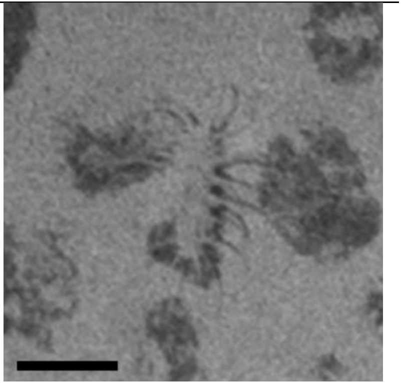
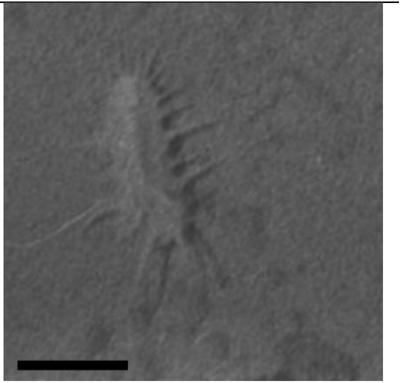
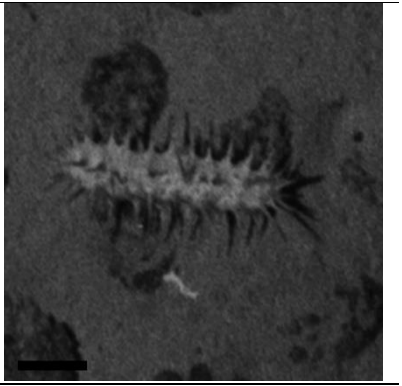
Phylum	Class	Subclass/Order	Morphotype	Example image
	Crinoidea		Crinoidea mtp 006	
	Echinoidea		Echinoidea mtp 003	
	Echinoidea		Echinoidea mtp 006	
	Echinoidea		Echinoidea mtp 011	

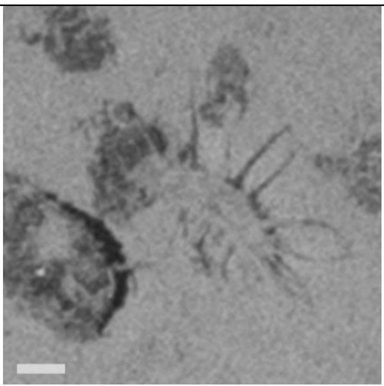
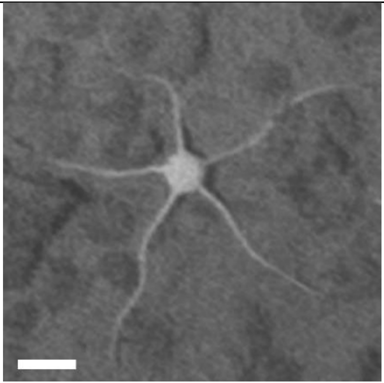
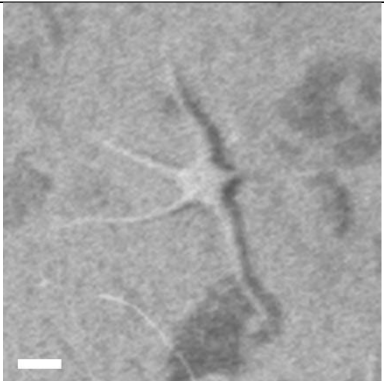
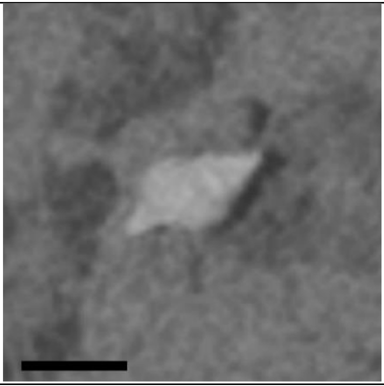
Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea		Holothuroidea (general)	
	Holothuroidea	Elasipodida	Amperima (general)	
	Holothuroidea	Elasipodida	Benthodytes (general)	
	Holothuroidea	Elasipodida	Elpidiidae (general)	

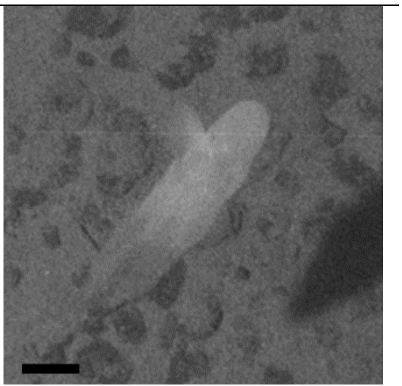
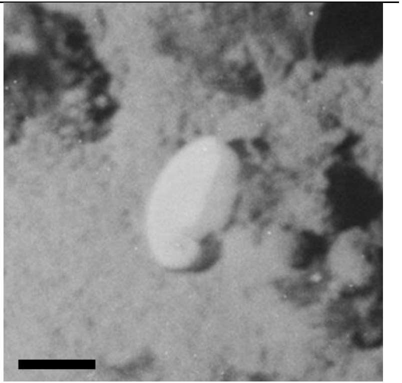
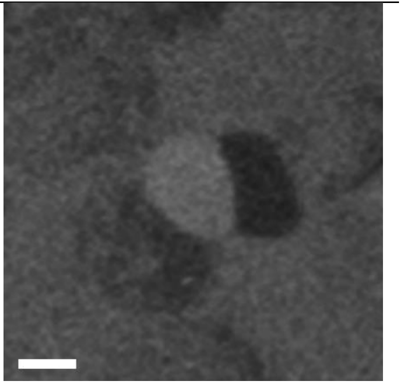
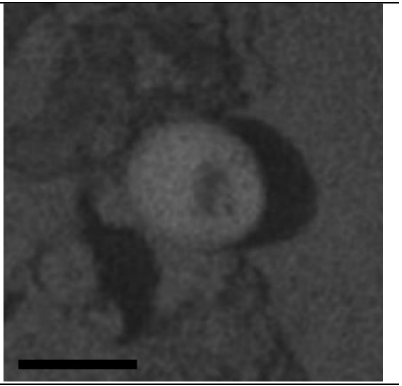
Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea	Elasipodida	Peniagone (general)	
	Holothuroidea	Elasipodida	Psychronaetes (general)	
	Holothuroidea	Elasipodida	Psychropotes (general)	
	Holothuroidea	Elasipodida	Scotoplanes (general)	

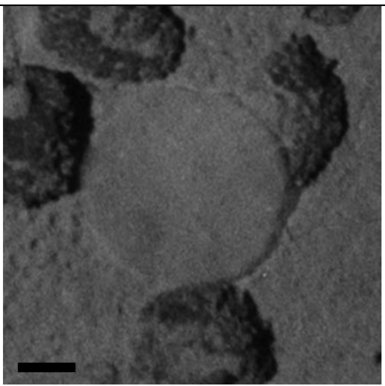
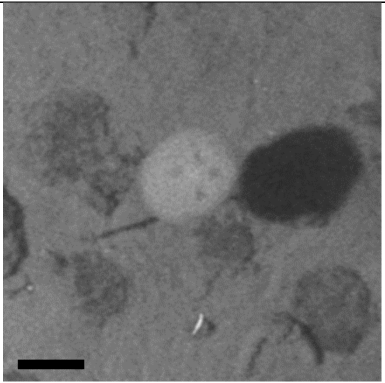
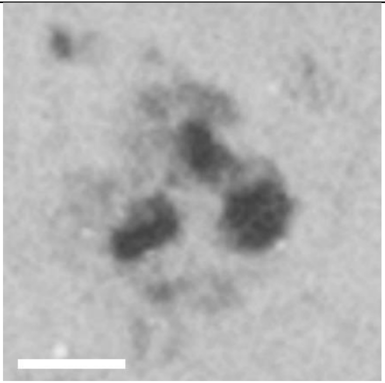
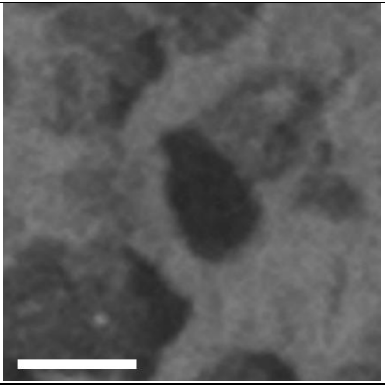
Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea	Holothuriida	Mesothuria (general)	
	Holothuroidea	Persiculida	Molpadiodemas (general)	
	Holothuroidea	Persiculida	Pseudostichopus (general)	
	Holothuroidea	Synallactida	Deima (general)	

Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea	Synallactida	Deimatidae (general)	
	Holothuroidea	Synallactida	Paelopatides (general)	
	Holothuroidea	Synallactida	Synallactes (general)	
	Holothuroidea		Holothuroidea mtp 016	

Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea		Holothuroidea mtp 054	
	Holothuroidea		Holothuroidea mtp 058	
	Holothuroidea		Holothuroidea mtp 060	
	Holothuroidea		Holothuroidea mtp 063	

Phylum	Class	Subclass/Order	Morphotype	Example image
	Holothuroidea		Holothuroidea mtp 068	
	Ophiuroidea		Ophiuroidea mtp 002	
	Ophiuroidea		Ophiuroidea mtp 009	
MOLLUSCA			Mollusca mtp 003	

Phylum	Class	Subclass/Order	Morphotype	Example image
			Mollusca mtp 010	
			Mollusca mtp 011	
PORIFERA			Porifera (general)	
	Demospongiae		Demospongiae (general)	

Phylum	Class	Subclass/Order	Morphotype	Example image
	Hexactinellida		Hexactinellida (general)	
	Hexactinellida		Hyalonema (general)	
FORAMINIFERA	Xenophyophorea		Xenophyophorea (general)	
	Xenophyophorea		Plate-like Xenophyophorea	

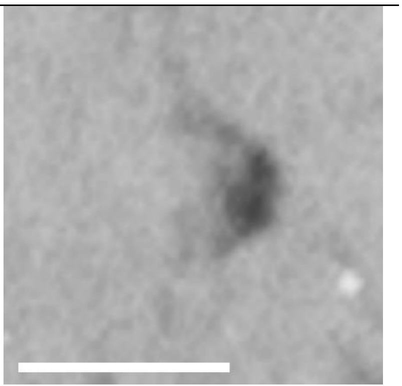
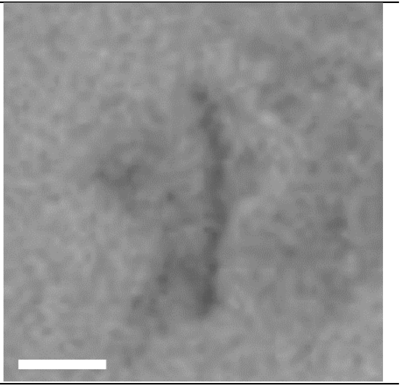
Phylum	Class	Subclass/Order	Morphotype	Example image
	Xenophyophorea		Reticular Xenophyophorea	
	Xenophyophorea		Tubular Xenophyophorea	

Table A.3 Morphotype richness (MR), Shannon diversity (H’), Simpson diversity (1/D), and Chao richness estimates calculated using rarefaction and extrapolation curves for individual-based and sample-based epibenthic megafauna and metazoan data in the CCZ, for the observed reference sample size, double the reference sample size (RS) and at asymptote. Sample unit for sample-based curves = 800 m^2 transect.

	Megafauna					**Metazoans**				
	MR (±s.e)*	**H’ (±s.e)**	**1/D (±s.e)**	**Chao 1 (±s.e)**	**Chao 2 (±s.e)**	**MR (±s.e)***	**H’ (±s.e)**	**1/D (±s.e)**	**Chao 1 (±s.e)**	**Chao 2 (±s.e)**
Individual-based										
Observed	90	3.46 (0.05)	2.55 (0.02)	90	-	86	19.83 (1.47)	11.38 (0.76)	86	-
Double RS	109 (14)	3.47 (0.05)	2.55 (0.02)	NA	-	105 (14)	20.30 (1.43)	11.42 (0.76)	NA	-
Asymptote	134 (25)	3.47 (0.02)	2.55 (0.01)	134 (25)	-	130 (25)	20.65 (0.78)	11.45 (0.38)	130 (25)	-
Sample-based										
Observed	90	67.37 (5.51)	55.34 (4.06)	-	90	86	64.85 (5.27)	52.86 (4.25)	-	86
Double RS	106 (13)	73.47 (5.82)	57.35 (4.41)	-	NA	102 (12)	71.50 (6.16)	55.22 (4.60)	-	NA
Asymptote	114 (12)	77.94 (3.66)	59.43 (2.57)	-	114 (12)	110 (12)	76.43 (3.40)	57.69 (2.80)	-	113 (13)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

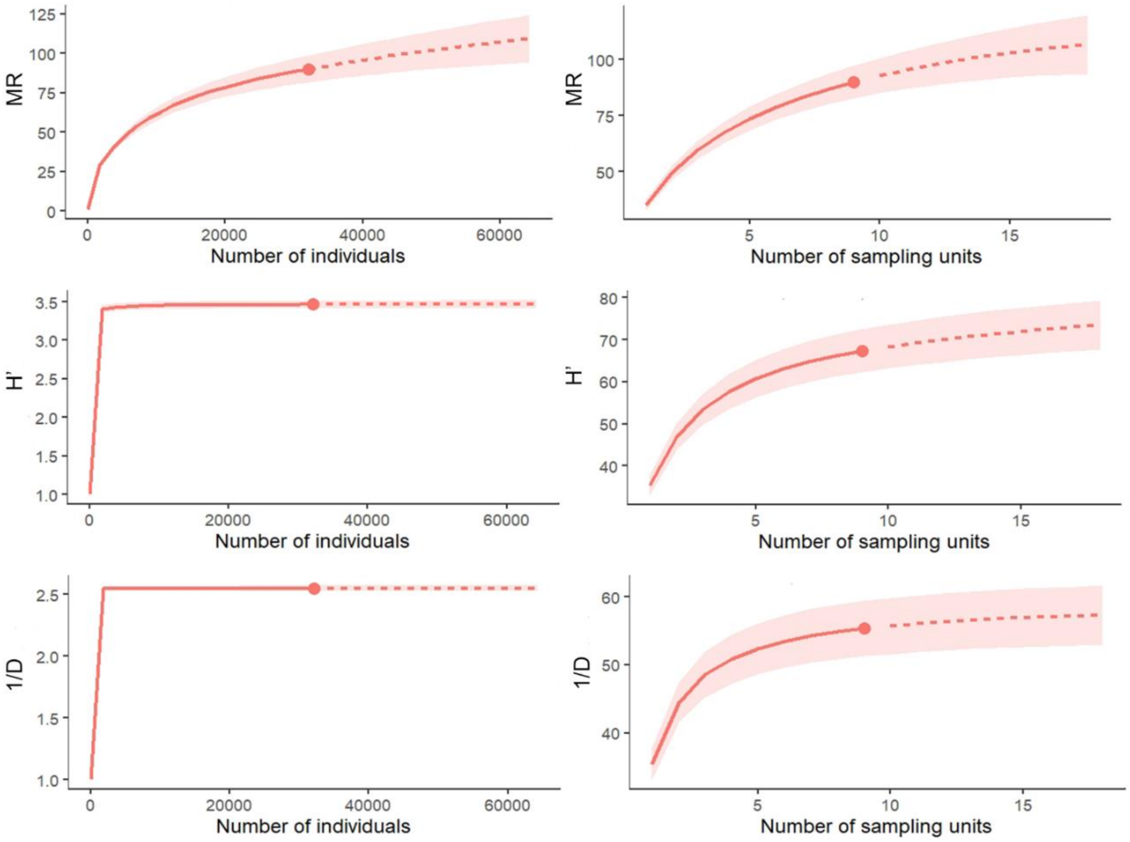


Figure A.1 Rarefaction and extrapolation curves with 95% confidence intervals for morphotype richness (MR), Shannon diversity (H') and Simpson diversity (1/D) for individual- and sample-based epibenthic megafaunal data in the CCZ at the reference sample size and double the reference sample size. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolated data. Sampling unit = 800 m^2 transect.

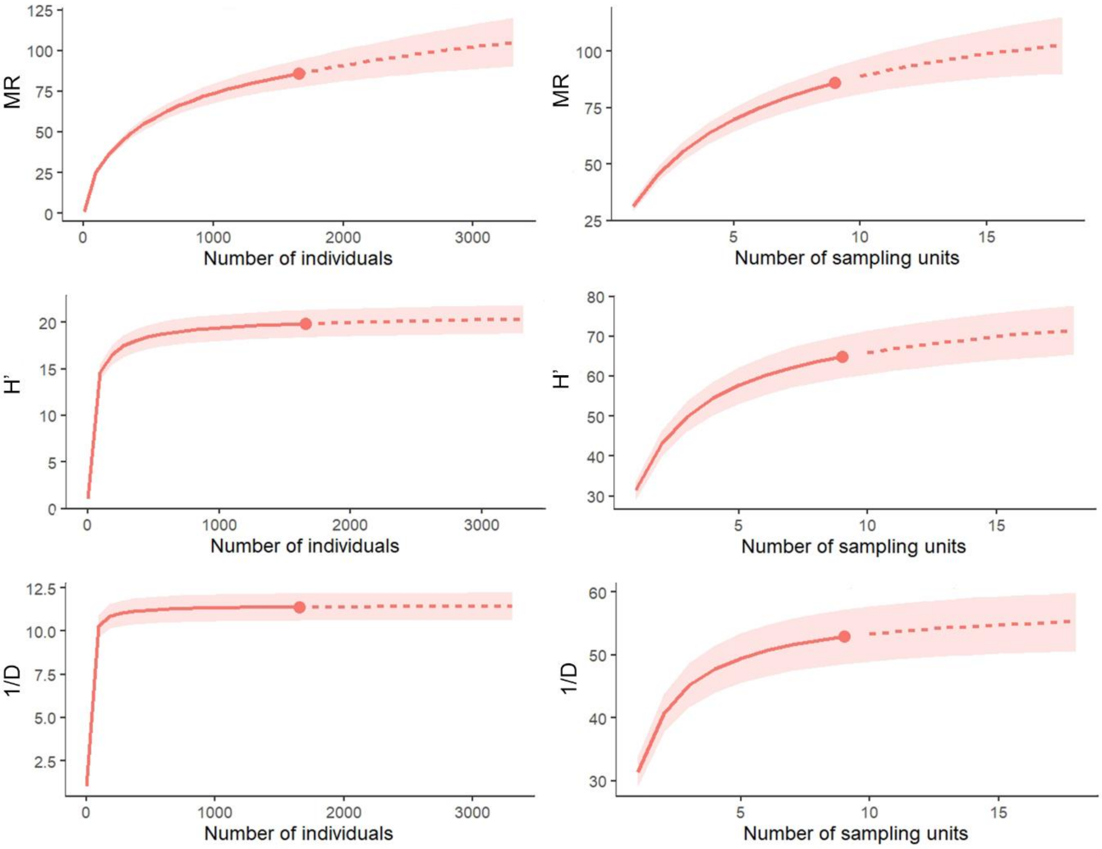


Figure A.2 Rarefaction and extrapolation curves with 95% confidence intervals for morphotype richness (MR), Shannon diversity (H’) and Simpson diversity (1/D) for individual- and sample-based epibenthic metazoan data in the CCZ at the reference sample size and double the reference sample size. Observed estimates are represented by a point, solid lines represent interpolated data, and dashed lines represent extrapolated data. Sampling unit = 800 m^2 transect.

Table A.4 Bray-Curtis similarity matrix of log transformed megafauna density data.

	T1	T2	T3	T4	T5	T6	T7	T8
T1								
T2	71.64							
T3	67.09	81.39						
T4	78.14	90.56	81.60					
T5	89.92	71.24	73.97	77.75				
T6	86.04	71.96	74.43	77.72	93.90			
T7	81.03	86.63	75.49	87.69	80.71	80.24		
T8	73.19	77.98	87.58	80.60	81.46	81.35	82.10	
T9	72.97	86.46	78.04	92.45	72.76	73.48	85.11	76.94

Table A.5 Bray-Curtis similarity matrix of square-root transformed metazoan density data.

	T1	T2	T3	T4	T5	T6	T7	T8
T1								
T2	48.30							
T3	63.41	58.06						
T4	56.61	65.12	54.44					
T5	64.30	45.51	54.59	48.76				
T6	60.51	57.53	55.78	57.86	59.82			
T7	54.13	63.81	59.21	59.18	55.20	62.93		
T8	51.96	54.12	57.84	55.49	53.59	54.79	63.84	
T9	41.72	57.31	44.84	59.46	41.76	52.09	65.90	52.59

Table A.6 Observed and extrapolated diversity estimates for individual-based megafauna abundance data. Chao 1, morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (RS) and asymptote. Significant differences denoted with letters a-f.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	29^{ab}	44^{a}	42^{a}	36^{ab}	21^{b}	30^{a}	33^{ab}	35^{a}	49^{a}
H' (±s.e)	$2.56\ (0.12)^{a}$	$3.53\ (0.12)^{b}$	$3.53\ (0.14)^{b}$	$3.10\ (0.11)^{cd}$	$2.87\ (0.14)^{c}$	$3.51\ (0.19)^{b}$	$3.25\ (0.13)^{d}$	$3.46\ (0.16)^{b}$	$3.51\ (0.13)^{b}$
1/D (±s.e)	$1.82\ (0.07)^{a}$	$2.55\ (0.06)^{bc}$	$2.74\ (0.07)^{d}$	$2.39\ (0.05)^{e}$	$2.20\ (0.08)^{f}$	$2.47\ (0.09)^{be}$	$2.27\ (0.07)^{f}$	$2.64\ (0.07)^{cd}$	$2.54\ (0.05)^{bc}$
Double RS									
MR (±s.e)	37 (9)	58 (12)	62 (16)	45 (10)	27 (8)	35 (7)	41 (9)	48 (13)	69 (15)
H' (±s.e)	2.57 (0.12)	3.54 (0.12)	3.55 (0.14)	3.12 (0.11)	2.88 (0.14)	3.53 (0.19)	3.26 (0.13)	3.48 (0.16)	3.53 (0.13)
1/D (±s.e)	1.81 (0.07)	2.55 (0.06)	2.74 (0.07)	2.39 (0.04)	2.20 (0.08)	2.47 (0.09)	2.27 (0.07)	2.64 (0.07)	2.54 (0.05)
Asymptote									
MR (±s.e)*	46 (14)	74 (19)	130 (63)	52 (11)	36 (16)	38 (6)	47 (11)	91 (49)	111 (38)
H' (±s.e)	2.58 (0.07)	3.55 (0.06)	3.58 (0.07)	3.12 (0.06)	2.89 (0.07)	3.54 (0.09)	3.27 (0.07)	3.50 (0.08)	3.54 (0.07)
1/D (±s.e)	1.82 (0.03)	2.55 (0.03)	2.74 (0.03)	2.40 (0.02)	2.20 (0.04)	2.47 (0.05)	2.27 (0.03)	2.64 (0.04)	2.54 (0.02)
Chao 1	46 (14)	74 (19)	130 (63)	52 (11)	36 (16)	38 (6)	47 (11)	91 (49)	111 (38)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

Table A.7 Observed and extrapolated diversity estimates for sample-based megafauna incidence frequency data. Chao 2, morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (RS) and asymptote. Significant differences denoted with letters a-f.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	29^{ac}	44^{bd}	42^{bd}	36^{abc}	21^{c}	30^{acd}	33^{acd}	35^{abc}	49^{b}
H' (±s.e)	7.92 (1.03)^{a}	12.83 (1.12)^{bcd}	10.63 (1.30)^{c}	11.45 (1.14)^{c}	7.74 (1.04)^{ab}	10.98 (1.23)^{c}	11.84 (1.00)^{c}	10.40 (1.31)^{c}	15.28 (1.45)^{d}
1/D (±s.e)	4.85 (0.45)^{a}	8.30 (0.58)^{b}	6.18 (0.57)^{cd}	7.47 (0.61)^{b}	5.29 (0.54)^{ac}	7.06 (0.69)^{bde}	7.80 (0.58)^{b}	6.16 (0.61)^{ce}	10.13 (0.68)^{f}
Double RS									
MR (±s.e)	37 (9)	57 (11)	62 (15)	45 (9)	27 (8)	35 (7)	41 (9)	48 (13)	69 (15)
H' (±s.e)	8.21 (1.09)	13.25 (1.19)	11.19 (1.44)	11.86 (1.20)	8.01 (1.12)	11.39 (1.29)	12.15 (1.05)	10.87 (1.44)	15.96 (1.60)
1/D (±s.e)	4.86 (0.46)	8.32 (0.58)	6.20 (0.57)	7.49 (0.61)	5.31 (0.54)	7.09 (0.69)	7.82 (0.58)	6.18 (0.61)	10.17 (0.69)
Asymptote									
MR (±s.e)*	43 (11)	73 (19)	129 (62)	51 (10)	36 (16)	37 (5)	47 (11)	90 (49)	110 (37)
H' (±s.e)	8.42 (0.54)	13.59 (0.64)	11.92 (0.80)	12.14 (0.65)	8.25 (0.57)	11.64 (0.69)	12.37 (0.62)	11.40 (0.81)	16.68 (0.99)
1/D (±s.e)	4.87 (0.22)	8.34 (0.31)	6.20 (0.29)	7.51 (0.32)	5.32 (0.28)	7.11 (0.33)	7.84 (0.30)	6.20 (0.30)	10.20 (0.34)
Chao 2	43 (11)	73 (19)	129 (62)	51 (10)	36 (16)	37 (5)	47 (11)	90 (49)	110 (37)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

Table A.8 Observed and extrapolated diversity estimates for individual-based metazoan abundance data. Chao 1, morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (RS) and asymptote. Significant differences denoted with letters a-c.

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	T9
Observed									
MR	25^{ab}	40^{a}	38^{b}	32^{a}	17^{a}	26^{a}	29^{a}	31^{ab}	45^{ab}
H' (±s.e)	$15.07\ (3.30)^{ab}$	$12.72\ (2.08)^{a}$	$21.36\ (4.58)^{b}$	$12.58\ (2.22)^{a}$	$10.77\ (2.71)^{a}$	$12.26\ (2.32)^{a}$	$14.12\ (1.83)^{a}$	$19.66\ (3.90)^{b}$	$14.16\ (2.14)^{a}$
1/D (±s.e)	$10.91\ (2.61)^{ac}$	$7.31\ (1.18)^{b}$	$14.84\ (3.02)^{c}$	$7.69\ (1.51)^{ab}$	$8.40\ (2.01)^{ab}$	$8.24\ (1.57)^{ab}$	$10.56\ (1.34)^{a}$	$14.83\ (2.85)^{c}$	$8.55\ (1.23)^{ab}$
Double RS									
MR (±s.e)	33 (9)	54 (11)	58 (14)	41 (9)	23 (8)	31 (8)	37 (9)	44 (12)	65 (15)
H' (±s.e)	17.25 (4.11)	13.57 (2.30)	25.12 (6.16)	13.62 (2.49)	12.18 (3.42)	13.29 (2.62)	14.96 (2.03)	22.55 (5.09)	15.23 (2.44)
1/D (±s.e)	11.54 (2.91)	7.38 (1.21)	15.63 (3.35)	7.82 (1.57)	8.88 (2.25)	8.45 (1.65)	10.78 (1.40)	15.74 (3.20)	8.4 (1.26)
Asymptote									
MR (±s.e)*	42 (14)	69 (19)	125 (63)	47 (10)	32 (16)	34 (6)	43 (11)	86 (49)	107 (38)
H' (±s.e)	19.25 (2.53)	14.29 (1.18)	30.91 (4.94)	14.40 (1.43)	13.61 (2.20)	13.97 (1.39)	15.59 (1.16)	26.45 (3.59)	16.39 (1.42)
1/D (±s.e)	12.24 (1.54)	7.46 (0.61)	16.52 (1.88)	7.96 (0.83)	9.42 (1.23)	8.68 (0.89)	11 (0.80)	16.77 (1.89)	8.74 (0.59)
Chao 1	42 (14)	69 (19)	125 (63)	47 (10)	32 (16)	34 (6)	43 (11)	86 (49)	107 (38)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

Table A.9 Observed and extrapolated diversity estimates for sample-based metazoan incidence frequency data. Chao 2, morphotype richness (MR), Shannon's diversity (H') and Simpson's diversity (1/D) extrapolated to double the reference sample (RS) and asymptote. Significant differences denoted with letters a-d.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	25^{ac}	40^{bd}	38^{bd}	32^{abd}	17^{c}	26^{abc}	29^{abc}	31^{abcd}	45^{d}
H' (±s.e)	$15.71\ (3.47)^{ab}$	$15.57\ (2.41)^{ab}$	$22.17\ (4.58)^{a}$	$15.15\ (2.65)^{ab}$	$11.39\ (2.74)^{b}$	$14.83\ (2.63)^{ab}$	$14.72\ (2.00)^{ab}$	$19.93\ (4.02)^{a}$	$16.47\ (2.65)^{ab}$
1/D (±s.e)	$11.56\ (2.60)^{ab}$	$9.41\ (1.43)^{a}$	$15.57\ (3.35)^{b}$	$10.00\ (1.79)^{ab}$	$9.02\ (2.16)^{a}$	$10.54\ (2.04)^{ab}$	$11.05\ (1.40)^{ab}$	$14.98\ (3.03)^{b}$	$10.02\ (1.36)^{ab}$
Double RS									
MR (±s.e)	33 (9)	54 (11)	58 (15)	41 (9)	23 (8)	31 (7)	37 (8)	44 (12)	65 (16)
H' (±s.e)	18.14 (4.34)	16.83 (2.72)	26.32 (6.22)	16.69 (3.07)	13.06 (3.50)	16.44 (3.04)	15.68 (2.25)	22.98 (5.27)	17.99 (3.10)
1/D (±s.e)	12.35 (2.96)	9.55 (1.47)	16.49 (3.74)	10.28 (1.89)	9.67 (2.46)	11.00 (2.22)	11.30 (1.46)	15.95 (3.40)	10.16 (1.40)
Asymptote									
MR (±s.e)*	39 (11)	69 (19)	125 (62)	47 (10)	32 (16)	33 (5)	43 (11)	86 (49)	106 (37)
H' (±s.e)	20.20 (2.65)	17.93 (1.47)	32.80 (6.04)	17.87 (1.68)	14.80 (2.72)	17.49 (1.85)	16.40 (1.23)	27.14 (3.83)	19.70 (1.77)
1/D (±s.e)	13.11 (1.68)	9.66 (0.79)	17.41 (2.29)	10.51 (1.01)	10.26 (1.62)	11.41 (1.21)	11.51 (0.75)	16.90 (2.05)	10.27 (0.66)
Chao 2	39 (11)	69 (19)	125 (62)	47 (10)	32 (16)	33 (5)	43 (11)	86 (49)	106 (37)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

Table A.10 Outputs of hierarchical agglomerative clustering on log transformed megafauna and square-root transformed metazoan community composition data.

Community	**Bray-Curtis similarity**	**Pi**	**p**
Megafauna	66.85	1.15	0.002
Metazoans	52.86	1.38	0.013

Table A.11 Spearman's rank-order correlation (r_s) between paired environmental variables: depth (m), transformed BBPI, FBPI, POC (g C_{org} m^{-2} y^{-1}) and log nodule abundance (kg m^{-2}).

Variable	**Depth**	**BBPI**	**FBPI**	**POC**
BBPI $(+112)^2$	-0.22			
FBPI	-0.25	-0.02		
POC	-0.65	0.30	0.03	
Log nodule abundance	0.17	0.07	-0.23	-0.68

Appendix B

The following figures and tables accompany Chapter 3:

Table B.1 The number of individuals for sampling units corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for individual-based megafauna data. MR = morphotype richness, H’ = Shannon’s diversity, 1/D = Simpson’s diversity.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	29	44	42	36	21	30	33	35	49
H’ (±s.e)	2.56 (0.12)	3.53 (0.12)	3.53 (0.14)	3.10 (0.11)	2.87 (0.14)	3.51 (0.19)	3.25 (0.13)	3.46 (0.16)	3.51 (0.13)
1/D (±s.e)	1.82 (0.07)	2.55 (0.06)	2.74 (0.07)	2.39 (0.05)	2.20 (0.08)	2.47 (0.09)	2.27 (0.07)	2.64 (0.07)	2.54 (0.05)
Indiv (n)	2 571	5 150	3 663	4 396	2 323	2 305	3 971	2 783	4 958
SC (%)	99.53	99.63	99.37	99.66	99.66	99.57	99.70	99.46	99.50
99% SC									
MR (±s.e)	17 (2)	19 (2)	22(2)	15 (1)	11 (1.43)	20 (2)	16 (1.30)	22 (2)	22 (2)
H’ (±s.e)	2.52 (0.13)	3.46 (0.13)	3.48 (0.13)	3.04 (0.11)	2.82 (0.14)	3.46 (0.18)	3.18 (0.14)	3.41 (0.15)	3.44 (0.12)
1/D (±s.e)	1.82 (0.06)	2.55 (0.06)	2.74 (0.07)	2.39 (0.05)	2.20 (0.08)	2.47 (0.10)	2.26 (0.06)	2.64 (0.07)	2.54 (0.05)
Indiv (n)	771	856	1 044	616	460	861	578	939	957
SC (%)	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00
Asymptote									
MR (±s.e) *	46 (14)	74 (19)	130 (63)	52 (11)	36 (16)	38 (6)	47 (11)	91 (49)	111 (38)
H’ (±s.e)	2.58 (0.07)	3.55 (0.06)	3.58 (0.07)	3.12 (0.06)	2.89 (0.07)	3.54 (0.09)	3.27 (0.07)	3.50 (0.08)	3.54 (0.07)
1/D (±s.e)	1.82 (0.03)	2.55 (0.03)	2.74 (0.03)	2.40 (0.02)	2.20 (0.04)	2.47 (0.05)	2.27 (0.03)	2.64 (0.04)	2.54 (0.02)
Indiv (n)†	3 300-43 500	5 850-90 000	52 000-200 000	15 000-57 000	5 700-50 000	11 500-23 000	3 150-51 500	5 970-51 000	4 200-144 000
SC (%)†	99.60-100 (0.2)	99.70-100 (0-0.2)	100 (0-0.1)	100 (0-0.1)	100 (0-0.2)	100 (0-0.1)	99.60-100 (0-0.1)	99.60-100 (0.3)	99.50-100 (0-0.2)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H’ & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

Table B.2 The number of images and area (m^2) for sampling units corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for sample-based megafauna data. MR = morphotype richness, H' = Shannon's diversity, 1/D = Simpson's diversity.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	29	44	42	36	21	30	33	35	49
H' (±s.e)	7.92 (1.03)	12.83 (1.12)	10.63 (1.30)	11.45 (1.14)	7.74 (1.04)	10.98 (1.23)	11.84 (1.00)	10.40 (1.31)	15.28 (1.45)
1/D (±s.e)	4.85 (0.45)	8.30 (0.58)	6.18 (0.57)	7.47 (0.61)	5.29 (0.54)	7.06 (0.69)	7.80 (0.58)	6.16 (0.61)	10.13 (0.68)
Images (n)	110	122	105	99	71	74	113	104	97
Area (m^2)	800	800	799	802	800	801	801	799	799
SC (%)	96.70	97.00	94.90	96.80	97.00	96.90	97.80	96.20	95.60
99% SC									
MR (±s.e)*	38 (10)	63 (16)	112 (63)	46 (10)	31 (13)	34 (7)	40 (9)	76 (40)	96 (33)
H' (±s.e)	8.28 (1.02)	13.40 (1.36)	11.88 (1.83)	11.91 (1.24)	8.15 (1.18)	11.34 (1.29)	12.14 (1.09)	11.36 (1.49)	16.56 (1.87)
1/D (±s.e)	4.86 (0.44)	8.33 (0.58)	6.21 (0.58)	7.49 (0.64)	5.31 (0.53)	7.08 (0.71)	7.82 (0.59)	6.20 (0.61)	10.19 (0.68)
Images (n)	268	333	757	223	224	133	220	621	452
Area (m^2)	1 950	2 184	5 762	1 806	2 523	1 441	1 559	4 773	3 725
SC (%)	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00
Asymptote									
MR (±s.e)*	43 (25)	73 (19)	129 (62)	51 (10)	36 (16)	37 (5)	47 (11)	90 (49)	110 (37)
H' (±s.e)	8.42 (0.54)	13.59 (0.64)	11.92 (0.80)	12.14 (0.65)	8.25 (0.57)	11.64 (0.69)	12.37 (0.62)	11.41 (0.81)	16.68 (0.99)
1/D (±s.e)	4.87 (0.22)	8.34 (0.31)	6.20 (0.29)	7.51 (0.32)	5.32 (0.28)	7.11 (0.33)	7.84 (0.30)	6.20 (0.30)	10.20 (0.34)
Images (n)†	500-1 500	950-2 400	600-5 000	750-1 200	340-1 700	530-570	850-1 800	600-4 550	900-3 300
Area (m^2)†	3 638-10 913	6 230-15 740	4 567-38 056	6 073-9 717	3 830-19 149	5 740-6 174	6 023-12 755	4 612-34 974	7 417-27 196
SC (%)†	99.80-100 (0.2-1)	100 (0.1-0.4)	98.50-100 (0.1-2)	100 (0.2-0.4)	99.60-100 (0-1.2)	100 (0.3-0.4)	100 (0.1-0.4)	98.90-100 (0-1.5)	99.8-100 (0.1-0.6)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H' & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

Table B.3 The number of individuals for sampling units corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for individual-based metazoan data. MR = morphotype richness, H’ = Shannon’s diversity, 1/D = Simpson’s diversity.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	25	40	38	32	17	26	29	31	45
H’ (±s.e)	15.07 (3.30)	12.72 (2.08)	21.36 (4.58)	12.58 (2.22)	10.77 (2.71)	12.26 (2.32)	14.12 (1.83)	19.66 (3.90)	14.16 (2.14)
1/D (±s.e)	10.91 (2.61)	7.31 (1.18)	14.84 (3.02)	7.69 (1.51)	8.40 (2.01)	8.24 (1.57)	10.56 (1.34)	14.83 (2.85)	8.55 (1.23)
Indiv (n)	92	316	137	196	69	145	235	121	345
SC (%)	87.05	94.00	83.24	92.38	88.49	93.16	94.91	87.63	92.76
99% SC									
MR (±s.e)*	41 (22)	64 (23)	120.30 (86)	45 (13)	31.39 (19)	33 (10)	40 (12)	82 (63)	98 (41)
H’ (±s.e)	19.00 (4.81)	14.11 (2.30)	30.87 (9.40)	14.11 (2.59)	13.51 (3.96)	13.58 (2.83)	15.30 (2.14)	26.41 (7.73)	16.31 (2.65)
1/D (±s.e)	11.94 (3.27)	7.42 (1.24)	16.36 (3.49)	7.87 (1.65)	9.23 (2.36)	8.51 (1.61)	10.85 (1.44)	16.56 (3.45)	8.70 (1.28)
Indiv (n)	443	1 211	1 608	621	402	377	693	1 254	2 048
SC (%)	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00	99.00
Asymptote									
MR (±s.e)*	42 (14)	69 (19)	125 (63)	47 (10)	32 (16)	34 (6)	43 (11)	86 (49)	107 (38)
H’ (±s.e)	19.25 (2.53)	14.29 (1.18)	30.91 (4.94)	14.40 (1.43)	13.61 (2.20)	13.97 (1.39)	15.59 (1.16)	26.45 (3.59)	16.39 (1.42)
1/D (±s.e)	12.24 (1.54)	7.46 (0.61)	16.52 (1.88)	7.96 (0.83)	9.42 (1.23)	8.68 (0.89)	11 (0.80)	16.77 (1.89)	8.74 (0.59)
Indiv (n)†	1 470-520 000	3 400-71 000	3 000-1 150 000	2 300-81 500	900-425 000	1 400-85 000	2 800-370 000	2 200-1 100 000	5 400-280 000
SC (%)†	100 (0.7)	100 (0.2-0.5)	99.90-100 (0.2)	100 (0.4)	100 (0.2)	100 (0.5-0.6)	100 (0-0.4)	99.90-100 (0-0.1)	100 (0-0.6)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H’ & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

Table B.4 The number of images and area (m^2) for sampling units corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for sample-based metazoan data. MR = morphotype richness, H’ = Shannon’s diversity, 1/D = Simpson’s diversity.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Observed									
MR	25	40	38	32	17	26	29	31	45
H’ (±s.e)	15.71 (3.47)	15.57 (2.41)	22.17 (4.58)	15.15 (2.65)	11.39 (2.74)	14.83 (2.63)	14.72 (2.00)	19.93 (4.02)	16.47 (2.65)
1/D (±s.e)	11.56 (2.60)	9.41 (1.43)	15.57 (3.35)	10.00 (1.79)	9.02 (2.16)	10.54 (2.04)	11.05 (1.40)	14.98 (3.03)	10.02 (1.36)
Images (n)	110	122	105	99	71	74	113	104	97
Area (m^2)	800	800	799	802	800	801	801	799	799
SC (%)	85.99	92.62	82.22	90.60	87.19	91.08	94.35	87.10	91.01
99% SC									
MR (±s.e)*	38 (19)	65.80 (24)	120 (91)	46 (16)	31 (20)	32 (9)	40 (12)	82 (58)	99 (49)
H’ (±s.e)	19.82 (4.86)	17.71 (3.19)	32.75 (10.65)	17.50 (3.20)	14.68 (4.86)	16.86 (3.37)	16.12 (2.41)	27.10 (7.46)	19.60 (3.75)
1/D (±s.e)	12.81 (3.02)	9.62 (1.42)	17.35 (3.81)	10.40 (1.94)	10.15 (2.64)	11.10 (2.14)	11.39 (1.64)	16.82 (3.85)	10.25 (1.29)
Images (n)	457	506	1 254	335	429	189	346	1 093	625
Area (m^2)	3 325	3 318	9 544	2 713	4 832	2 047	2 452	8 401	5 151
SC (%)	99	99	99	99	99	99	99	99	99
Asymptote									
MR (±s.e)*	39 (11)	69 (19)	125 (62)	47 (10)	32 (16)	33 (5)	43 (11)	86 (49)	106 (37)
H’ (±s.e)	20.20 (2.65)	17.93 (1.47)	32.80 (6.04)	17.87 (1.68)	14.80 (2.72)	17.49 (1.85)	16.40 (1.23)	27.14 (3.83)	19.70 (1.77)
1/D (±s.e)	13.11 (1.68)	9.66 (0.79)	17.41 (2.29)	10.51 (1.01)	10.26 (1.62)	11.41 (1.21)	11.51 (0.75)	16.90 (2.05)	10.27 (0.66)
Images (n)†	1 400-1 500	1 180-2 400	1 800-5 000	1 050-1 200	730-1 700	570-840	1 280-1 800	1 750-4 550	1 000-3 200
Area (m^2)†	10 185-10 913	7 739-15 740	13 700-38 056	8 503-9 717	8 223-19 149	6 174-9 098	9 070-2 755	13 452-34 974	8 241-26 372
SC (%)†	100 (0.9)	100 (0.3-0.8)	99.80-100 (1.2-0.2)	100 (0.5-0.6)	99.99-100 (0.1-0.5)	100 (0.5-0.7)	100 (0.3-0.5)	99.80-100 (0.1-0.2)	99.80-100 (0.2-0.9)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H’ & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

Table B.5 The number of individuals, replicate transects and area (m^2) corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for individual- and sample-based megafauna data at the regional level. MR = morphotype richness, H’ = Shannon’s diversity, 1/D = Simpson’s diversity. Sample unit for individual-based curves = megafauna individual; sample unit for sample-based curves = 800 m^2 transect.

	MR (±s.e)*	H’ (±s.e)	1/D (±s.e)	No. samples (n)†	Area (m^2)†	SC (%) (±s.e)†
Individual-based curves						
Observed	90	3.46 (0.05)	2.55 (0.02)	32 120	-	99.92
99% SC	21 (1)	3.38 (0.04)	2.54 (0.02)	909	-	99.00
Asymptote	134 (25)	3.47 (0.02)	2.55 (0.01)	14 300-675 000	-	99.80-100.00 (0.10)
Sample-based curves						
Observed	90	67.37 (5.51)	55.34 (4.06)	9	7 200	92.00
99% SC	111 (15)	75.86 (6.14)	58.00 (4.59)	26	20 800	99.00
Asymptote	114 (12)	77.94 (3.66)	59.43 (2.57)	130-535	104 000-428 000	100.00

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H’ & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

Table B.6 The number of individuals, replicate transects and area (m^2) corresponding to diversity estimates both observed and extrapolated to 99% sample coverage (SC) and asymptote for individual- and sample-based metazoan data at the regional level. MR = morphotype richness, H’ = Shannon’s diversity, 1/D = Simpson’s diversity. Sample unit for individual-based curves = metazoan individual; sample unit for sample-based curves = 800 m^2 transect.

	MR (±s.e)*	**H’ (±s.e)**	**1/D (±s.e)**	**No. samples (n)†**	**Area (m^2)†**	**SC (%) (±s.e)**
Individual-based curves						
Observed	86	19.83 (1.47)	11.38 (0.76)	1 656	-	98.49
99% SC	101 (11)	20.21 (1.39)	11.41 (0.78)	2 872	-	99.00
Asymptote	130 (25)	20.65 (0.78)	11.45 (0.38)	37 000-185 000	-	100.00
Sample-based curves						
Observed	86	64.85 (5.27)	52.86 (4.25)	9	7 200	90.93
99% SC	108 (16)	74.33 (7.05)	56.05 (4.74)	27	21 600	99.00
Asymptote	110 (12)	76.43 (3.40)	57.69 (2.80)	128-520	102 400-416 000	100.00 (0)

* Extrapolated estimates of MR exceeding double the reference sample size may be subject to large prediction bias.

† Asymptotic estimates of MR, H’ & 1/D diversity reached after a different number of samples, with different levels of sample coverage.

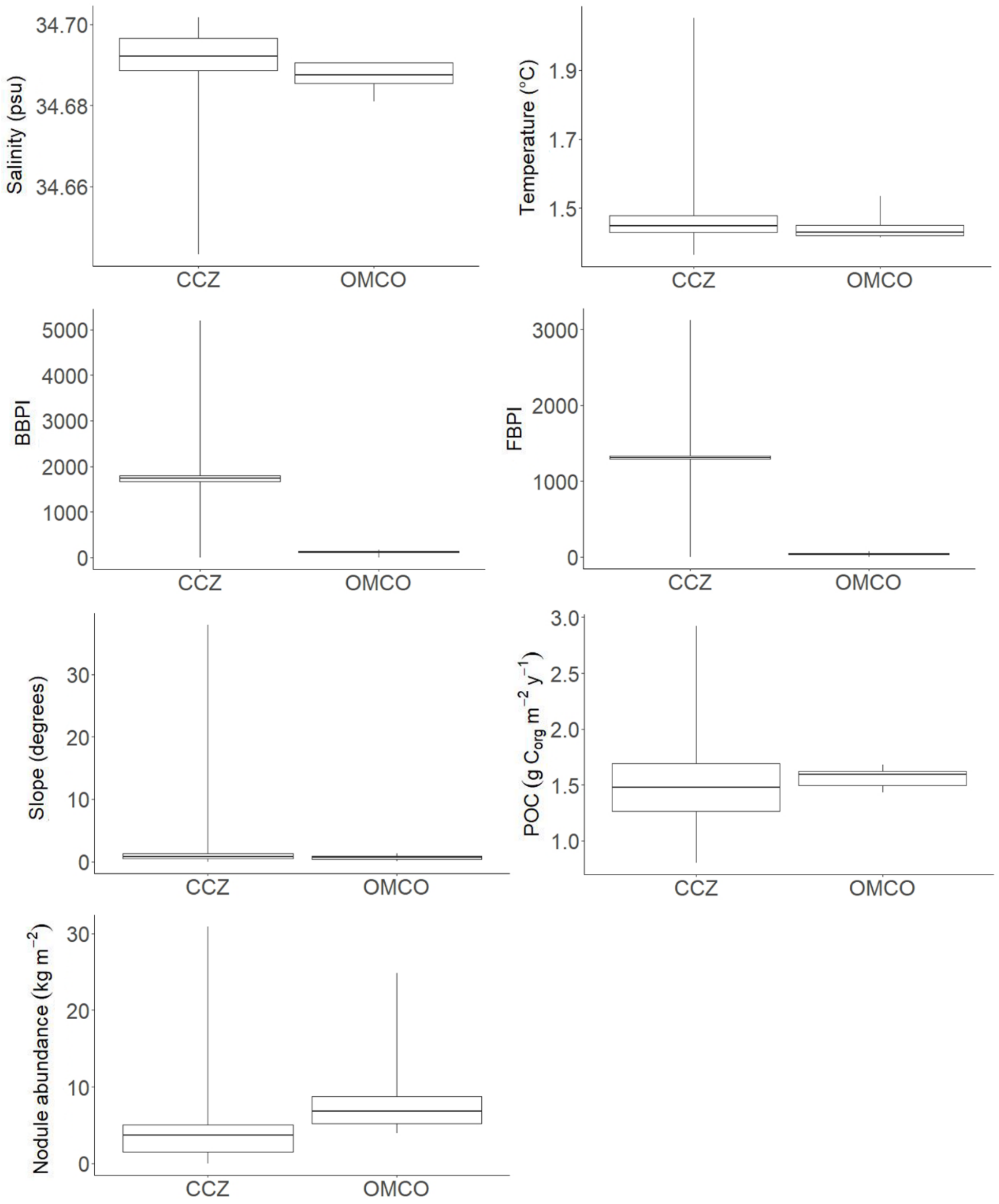


Figure B.1 The range of environmental conditions across the entire Clarion Clipperton Fracture Zone (CCZ) management area compared to the Ocean Minerals Company (OMCO) data analysed in this study.

Appendix C

The following figures and tables accompany Chapter 4:

Table C.1 Average silhouette width (ASW) for CLARA clustering iterations with 1-10 clusters for each group of environmental variables. Bold starred text indicates the iteration selected for the final habitat classification. ASW close to 1 indicates good clustering, close to 0 indicates observations lie between two clusters and close to -1 indicates observations are in the wrong cluster.

Variable group	**ASW for cluster iterations 1-10**										**Justification for choice of number of clusters**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Oceanography	0	**0.51***	0.37	0.48	0.44	0.47	0.43	0.40	0.37	0.41	- Highest ASW - Aligns with Harris and Whiteway (2009)
Topography	0	**0.55***	0.19	0.21	0.27	0.28	0.31	0.35	0.24	0.27	- Highest ASW - Highly simplified interpretation of topography of the region, but captures most important aspects
POC	0	0.65	**0.60***	0.55	0.60	0.56	0.56	0.66	0.64	0.61	- ASW for 3 clusters is relatively high, and clusters capture broad POC gradient without increasing number of habitats greatly - Aligns with GOODS (UNESCO, 2009) and Watling *et al.* (2013)
Nodule abundance	0	0.61	0.60	**0.66***	0.61	0.59	0.60	0.60	0.56	0.58	- Highest ASW - Reflects modelled nodule abundance well

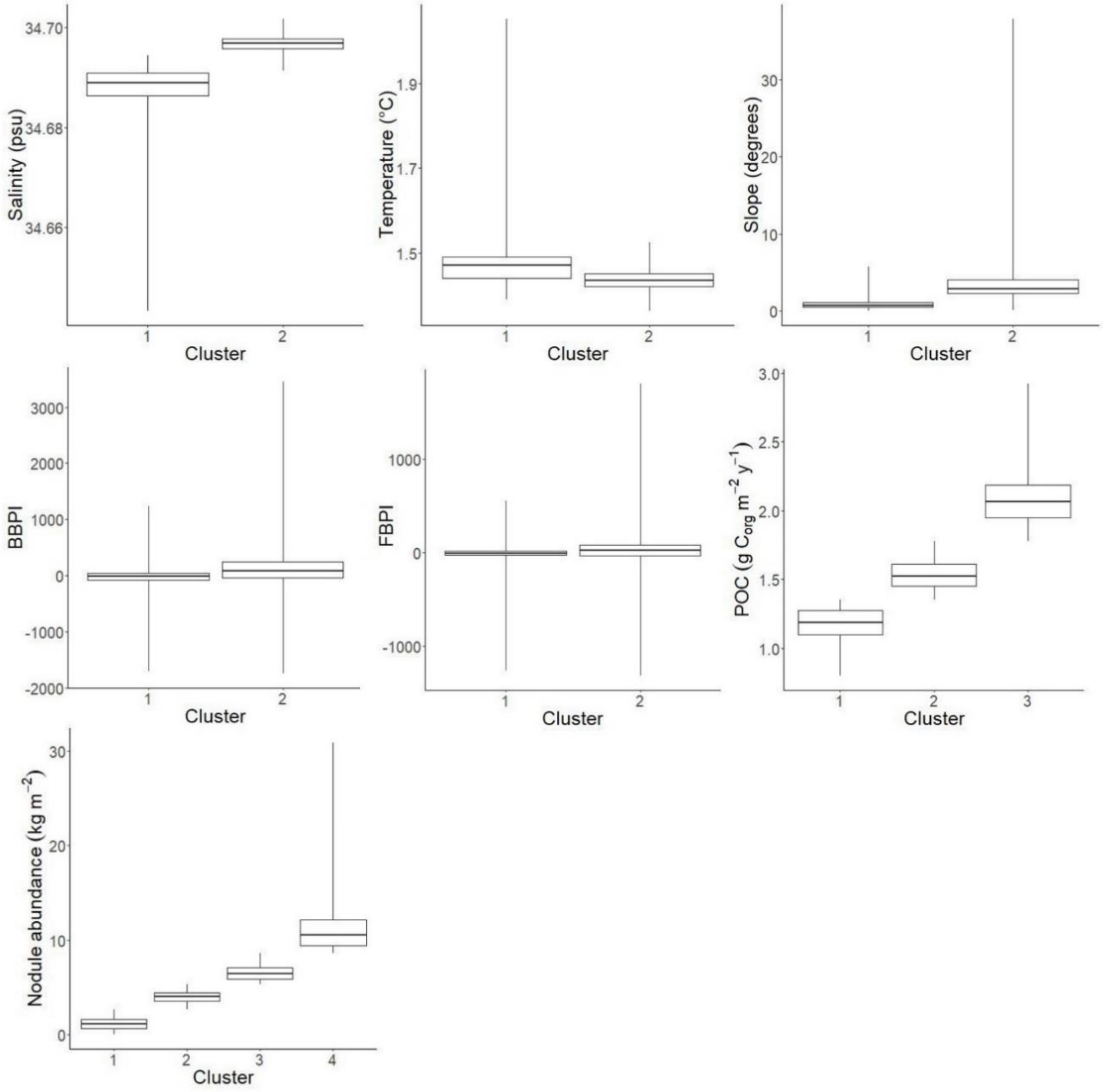


Figure C.1 Properties of the clusters of each environmental variable selected for the final habitat classification, showing median, with upper and lower quartiles, minimum and maximum values.

Table C.2 Properties (mean) of each habitat type identified through top-down habitat classification system.

Habitat	Area (km^2)	Salinity (psu)	Temp (^{0}C)	Slope (^{0})	BBPI	FBPI	POC (g C_{org} m^{-2} y^{-1})	Nod Abund (kg m^{-2})
1	72 910	34.69	1.47	0.78	-17.21	-4.10	1.18	1.18
2	32 858	34.69	1.47	3.64	135.48	37.77	1.18	1.18
3	1 069 595	34.70	1.44	0.78	-17.21	-4.10	1.18	1.18
4	207 882	34.70	1.44	3.64	135.48	37.77	1.18	1.18
5	162 165	34.70	1.44	3.64	135.48	37.77	1.18	3.98
6	1 324 870	34.70	1.44	0.78	-17.21	-4.10	1.18	3.98
7	79 164	34.69	1.47	3.64	135.48	37.77	1.18	3.98
8	603 402	34.69	1.47	0.78	-17.21	-4.10	1.18	3.98
9	1 244 357	34.69	1.47	0.78	-17.21	-4.10	1.53	3.98
10	228 713	34.69	1.47	3.64	135.48	37.77	1.53	3.98
11	901 232	34.69	1.47	0.78	-17.21	-4.10	1.53	6.56
12	145 482	34.69	1.47	3.64	135.48	37.77	1.53	6.56
13	583 859	34.69	1.47	0.78	-17.21	-4.10	1.53	11.10
14	57 070	34.69	1.47	3.64	135.48	37.77	1.53	11.10
15	1 125	34.69	1.47	3.64	135.48	37.77	2.09	11.10
16	714	34.69	1.47	0.78	-17.21	-4.10	2.09	11.10
17	35 602	34.69	1.47	3.64	135.48	37.77	2.09	3.98
18	380 817	34.69	1.47	0.78	-17.21	-4.10	2.09	3.98
19	137 318	34.69	1.47	0.78	-17.21	-4.10	2.09	6.56
20	7 384	34.69	1.47	3.64	135.48	37.77	2.09	6.56
21	19 778	34.69	1.47	0.78	-17.21	-4.10	1.18	6.56
22	4 816	34.69	1.47	3.64	135.48	37.77	1.18	6.56
23	36 027	34.70	1.44	3.64	135.48	37.77	1.53	3.98
24	312 846	34.70	1.44	0.78	-17.21	-4.10	1.53	3.98
25	341	34.69	1.47	0.78	-17.21	-4.10	1.18	11.10
26	152	34.69	1.47	3.64	135.48	37.77	1.18	11.10
27	662 686	34.69	1.47	0.78	-17.21	-4.10	1.53	1.18
28	260 709	34.70	1.44	0.78	-17.21	-4.10	1.18	6.56
29	117 953	34.70	1.44	0.78	-17.21	-4.10	1.53	6.56
30	61 539	34.70	1.44	3.64	135.48	37.77	1.18	6.56
31	19 598	34.70	1.44	3.64	135.48	37.77	1.53	6.56
32	36 554	34.69	1.47	3.64	135.48	37.77	1.53	1.18
33	9 372	34.70	1.44	0.78	-17.21	-4.10	1.53	11.10
34	424 125	34.70	1.44	0.78	-17.21	-4.10	1.53	1.18
35	927	34.70	1.44	3.64	135.48	37.77	1.53	11.10
36	70 558	34.70	1.44	0.78	-17.21	-4.10	1.18	11.10
37	17 771	34.70	1.44	3.64	135.48	37.77	1.18	11.10
38	26 579	34.70	1.44	3.64	135.48	37.77	1.53	1.18
39	46 280	34.69	1.47	3.64	135.48	37.77	2.09	1.18
40	901 996	34.69	1.47	0.78	-17.21	-4.10	2.09	1.18
41	541 520	34.70	1.44	0.78	-17.21	-4.10	2.09	1.18
42	26 584	34.70	1.44	3.64	135.48	37.77	2.09	1.18
43	29 160	34.70	1.44	0.78	-17.21	-4.10	2.09	6.56
44	255 833	34.70	1.44	0.78	-17.21	-4.10	2.09	3.98

Habitat	Area (km^2)	Salinity (psu)	Temp (^{0}C)	Slope (^{0})	BBPI	FBPI	POC (g C_{org} m^{-2} y^{-1})	Nod Abund (kg m^{-2})
45	20 878	34.70	1.44	3.64	135.48	37.77	2.09	3.98
46	1 844	34.70	1.44	3.64	135.48	37.77	2.09	6.56

Table C.3 Description of the oceanography, topography, POC and nodule abundance properties of each habitat type identified through top-down habitat classification system.

Habitat	Area (km^2)	Oceanography	Topography	POC	Nodule Abundance
1	72 910	Low salinity, high temp	Flat, some small topographic features	Low	Very low
2	32 858	Low salinity, high temp	Sloped, with peaks and troughs	Low	Very low
3	1 069 595	High salinity, low temp	Flat, some small topographic features	Low	Very low
4	207 882	High salinity, low temp	Sloped, with peaks and troughs	Low	Very low
5	162 165	High salinity, low temp	Sloped, with peaks and troughs	Low	Low
6	1 324 870	High salinity, low temp	Flat, some small topographic features	Low	Low
7	79 164	Low salinity, high temp	Sloped, with peaks and troughs	Low	Low
8	603 402	Low salinity, high temp	Flat, some small topographic features	Low	Low
9	1 244 357	Low salinity, high temp	Flat, some small topographic features	Medium	Low
10	228 713	Low salinity, high temp	Sloped, with peaks and troughs	Medium	Low
11	901 232	Low salinity, high temp	Flat, some small topographic features	Medium	Medium
12	145 482	Low salinity, high temp	Sloped, with peaks and troughs	Medium	Medium
13	583 859	Low salinity, high temp	Flat, some small topographic features	Medium	High
14	57 070	Low salinity, high temp	Sloped, with peaks and troughs	Medium	High
15	1 125	Low salinity, high temp	Sloped, with peaks and troughs	High	High
16	714	Low salinity, high temp	Flat, some small topographic features	High	High
17	35 602	Low salinity, high temp	Sloped, with peaks and troughs	High	Low
18	380 817	Low salinity, high temp	Flat, some small topographic features	High	Low
19	137 318	Low salinity, high temp	Flat, some small topographic features	High	Medium
20	7 384	Low salinity, high temp	Sloped, with peaks and troughs	High	Medium
21	19 778	Low salinity, high temp	Flat, some small topographic features	Low	Medium
22	4 816	Low salinity, high temp	Sloped, with peaks and troughs	Low	Medium
23	36 027	High salinity, low temp	Sloped, with peaks and troughs	Medium	Low

Habitat	Area (km^2)	Oceanography	Topography	POC	Nodule Abundance
24	312 846	High salinity, low temp	Flat, some small topographic features	Medium	Low
25	341	Low salinity, high temp	Flat, some small topographic features	Low	High
26	152	Low salinity, high temp	Sloped, with peaks and troughs	Low	High
27	662 686	Low salinity, high temp	Flat, some small topographic features	Medium	Very low
28	260 709	High salinity, low temp	Flat, some small topographic features	Low	Medium
29	117 953	High salinity, low temp	Flat, some small topographic features	Medium	Medium
30	61 539	High salinity, low temp	Sloped, with peaks and troughs	Low	Medium
31	19 598	High salinity, low temp	Sloped, with peaks and troughs	Medium	Medium
32	36 554	Low salinity, high temp	Sloped, with peaks and troughs	Medium	Very low
33	9 372	High salinity, low temp	Flat, some small topographic features	Medium	High
34	424 125	High salinity, low temp	Flat, some small topographic features	Medium	Very low
35	927	High salinity, low temp	Sloped, with peaks and troughs	Medium	High
36	70 558	High salinity, low temp	Flat, some small topographic features	Low	High
37	17 771	High salinity, low temp	Sloped, with peaks and troughs	Low	High
38	26 579	High salinity, low temp	Sloped, with peaks and troughs	Medium	Very low
39	46 280	Low salinity, high temp	Sloped, with peaks and troughs	High	Very low
40	901 996	Low salinity, high temp	Flat, some small topographic features	High	Very low
41	541 520	High salinity, low temp	Flat, some small topographic features	High	Very low
42	26 584	High salinity, low temp	Sloped, with peaks and troughs	High	Very low
43	29 160	High salinity, low temp	Flat, some small topographic features	High	Medium
44	255 833	High salinity, low temp	Flat, some small topographic features	High	Low
45	20 878	High salinity, low temp	Sloped, with peaks and troughs	High	Low
46	1 844	High salinity, low temp	Sloped, with peaks and troughs	High	Medium

Table C.4 SIMPER analysis identifying the seven most abundant species in Habitats 9, 11 and 13. Average abundance is the square-root transformed average number of individuals per transect.

Species	**Average abundance**	**Percent contribution (%)**	**Cumulative contribution %**
Habitat 9			
Alcyonacea	6.23	13.09	13.09
Porifera	5.37	10.65	23.74
Actinian_018	4.29	9.77	33.51
Annelida	3.66	7.42	40.92
Actinian_002	3.02	6.44	47.37
Urchin_003	2.52	5.64	53.00
Actiniaria	3.10	5.26	58.26
Habitat 11			
Alcyonacea	5.20	12.05	12.05
Porifera	5.61	11.45	23.50
Actinian_018	4.14	10.63	34.13
Actiniaria	3.65	9.95	44.08
Urchin_003	4.82	6.88	50.96
Actinian_002	2.73	6.19	57.15
Annelida	2.91	5.50	62.66
Habitat 13			
Actinian_018	4.21	13.36	13.36
Alcyonacea	3.92	12.92	26.28
Porifera	4.73	12.88	39.16
Actiniaria	2.94	10.65	49.81
Actinian_002	2.69	9.22	59.04
Annelida	3.00	8.55	67.59
Urchin_003	3.16	7.45	75.04

Table C.5 SIMPER analysis showing the five morphotypes with the highest contributions to dissimilarity between Habitats 9, 11 and 13 in pairwise comparisons. Average abundance is the square-root transformed average number of individuals per transect.

Species	Av abund (1st habitat)	Av abund (2nd habitat)	Av Dissimilarity	Percent contribution	Cumulative contribution (%)
Habitat 9 and Habitat 11 - 42.96% dissimilarity					
Ophiuroid_009	1.96	4.05	2.60	6.05	6.05
Urchin_003	2.52	4.82	2.00	4.66	10.71
Ophiuroid_002	1.63	3.07	1.98	4.60	15.31
Porifera	5.37	5.61	1.73	4.03	19.34
Alcyonacea	6.23	5.20	1.61	3.75	23.09
Habitat 9 and Habitat 13 - 43.44% dissimilarity					
Alcyonacea	6.23	3.92	2.07	4.77	4.77
Porifera	5.37	4.73	1.96	4.51	9.28
Annelida	3.66	3.00	1.36	3.14	12.42
Actiniaria	3.10	2.94	1.28	2.95	15.37
Urchin_003	1.38	0.00	1.26	2.90	18.27
Habitat 11 and Habitat 13 - 44.00% dissimilarity					
Ophiuroid_009	4.05	0.67	3.07	6.98	6.98
Ophiuroid_002	3.07	0.80	2.31	5.24	12.22
Urchin_003	4.82	3.16	2.24	5.09	17.31
Porifera	5.61	4.73	1.79	4.06	21.37
Alcyonacea	5.20	3.92	1.30	2.94	24.31

Table C.6 Percent of each habitat class located in the Areas of Particular Environmental Interest (APEI) network, exploration areas, reserved areas and combined exploration and/or reserved areas as of June 2019. * = Habitats with less than 30% of the total extent within APEIs; ** = Habitats with less than 10% of the total extent within APEIs; † = Habitats with greater than 50% of the total extent located within exploration and/or reserved Areas.

Habitat	Total area (km^2)	% In APEIs	% In exploration areas	% In reserved areas	Combined % in exploration & reserved areas
1**	72 910	2.23	0.00	0.03	0.03
2**	32 858	2.61	0.09	0.07	0.16
3*	1 069 595	11.12	0.68	3.38	4.06
4*	207 882	16.59	0.51	3.29	3.80
5**	162 165	4.89	4.65	10.42	15.07
6**	1 324 870	3.89	5.57	12.69	18.26
7*	79 164	22.76	0.17	0.36	0.53
8*	603 402	18.43	0.03	0.21	0.24
9*	1 244 357	17.55	17.48	14.47	31.95
10*	228 713	26.65	3.97	4.42	8.40
11**	901 232	7.51	33.26	9.83	43.09
12*	145 482	12.09	15.62	6.19	21.80
13**†	583 859	0.39	57.04	4.40	61.44
14**†	57 070	0.76	49.51	2.98	52.49
15**	1 125	0.00	20.98	0.00	20.98
16**	714	0.00	48.60	0.00	48.60
17**	35 602	0.75	0.06	0.00	0.06
18**	380 817	0.05	0.08	0.02	0.10
19**	137 318	0.00	0.84	0.00	0.84
20**	7 384	1.22	7.75	0.00	7.75
21	19 778	33.83	2.58	6.00	8.58
22*	4 816	23.65	2.14	14.26	16.40
23	36 027	34.98	4.25	15.36	19.62
24*	312 846	22.24	8.52	17.16	25.68
25**†	341	0.00	0.00	64.81	64.81
26**†	152	0.00	43.42	26.97	70.39
27	662 686	40.99	8.00	9.18	17.17
28**	260 709	5.96	18.26	18.73	36.99
29*	117 953	24.41	12.94	8.46	21.41
30**	61 539	8.14	16.95	15.12	32.07
31	19 598	45.04	10.78	7.14	17.92
32	36 554	49.42	7.94	8.81	16.75
33**†	9 372	4.01	74.39	13.19	87.58
34*	424 125	27.35	1.33	3.62	4.95
35*†	927	22.01	49.84	4.21	54.05
36**†	70 558	0.34	54.40	14.88	69.28
37**†	17 771	0.25	50.16	6.23	56.39
38*	26 579	19.12	1.36	7.17	8.53
39*	46 280	28.85	0.00	0.00	0.00
40**	901 996	9.91	0.00	0.00	0.00

Habitat	Total area (km^2)	% In APEIs	% In exploration areas	% In reserved areas	Combined % in exploration & reserved areas
41*	541 520	18.75	0.00	0.00	0.00
42*	26 584	12.48	0.00	0.00	0.00
43**	29 160	0.00	0.00	0.00	0.00
44**	255 833	7.25	0.00	0.00	0.00
45**	20 878	2.51	0.00	0.00	0.00
46**	1 844	0.00	0.00	0.00	0.00

List of acronyms

ABNJ	Areas Beyond National Jurisdiction
APEI	Area of Particular Environmental Interest
BPI	Bathymetric Position Index
CBD	Convention of Biological Diversity
CCZ	Clarion-Clipperton Fracture Zone
CIB	Central Indian Basin
CLARA	Clustering Large Algorithms
DISCOL	Disturbance and Recolonization Experiment
EMP	Environmental Management Plan
EEZ	Exclusive Economic Zone
ISA	International Seabed Authority
MPA	Marine Protected Area
MSP	Marine Spatial Planning
OMCO	Ocean Minerals Company
POC	Particulate Organic Carbon
R/E	Rarefaction/extrapolation
REA	Regional Environmental Assessment
REMP	Regional Environmental Management Plan
SEA	Strategic Environmental Assessment
SEMP	Strategic Environmental Management Plan
UNCLOS	United Nations Convention on the Law of the Sea

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