Shallow Soft Sediment Communities in the Central Red Sea: Revealing
Patterns in Community Structure across Space and Time

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ABSTRACT

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Due to intensive coastal development, a combination of local (e.g. pollution, fishing) and global pressures (such as climate change) is affecting marine habitats worldwide. This is a pressing issue in Saudi Arabia, particularly considering the plans for the expansion of sea-related activities within the Saudi Arabia's Vision 2030 framework. Sustaining some of those activities, such as tourism, is dependent upon the maintenance of good ecosystem health. National monitoring programs in Saudi Arabia are scarce and a lack of sound knowledge on how marine organisms change in space and time and what the main factors driving their responses are, limits the contribution of scientists to the management and conservation of the Red Sea. Here we provide baseline knowledge, that can be critical for assessing changes associated with current and future coastal development as well as climate change by collecting data across multiple spatial (including multiple habitats) and temporal scales for the analysis of macroinvertebrate organisms and environmental drivers. One of the most striking findings is related to the low densities observed for macroinvertebrates, making populations potentially vulnerable to disturbance. We also highlight the contribution of different habitats within the seascape and the need to prioritize the features of the bottoms for management and conservation purposes. Each habitat has a unique ecological signature but they are connected to adjacent habitats through a subset of species able to utilize different biotopes within the seascape. Disrupting this ecological network may affect biodiversity patterns from local to regional levels. Within each habitat, temporal variability should be taken into account as patterns change on a seasonal and annual scale. The aim of the thesis is to contribute to the sustainable development of the Red Sea, a unique resource shared among several countries, which will result in a long-term benefit to the Kingdom of Saudi Arabia, and other countries. Information provided is critical as previous knowledge for the region was almost inexistent and allows for future studies to investigate and predict the impacts of intense coastal development and inform conservation and management decisions.
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LIST OF ABBREVIATIONS

AMBI  Marine biotic index
ANOVA  Analysis of variance
BENTIX  Biotic index Bentix
BIBI  Benthic Index of Biotic I
BOPA  Polychaeta Amphipoda Index
CBD  Convention on Biological Diversity
Chl a  Chlorophyll a
CTD  Conductivity, Temperature, and Depth
dbRDA  Distance-based redundancy analysis
βsor  Sørensen dissimilarity
Eh  Sediment oxidation/reduction potential
ES_{50}  Hurlbert’s rarefaction index determines the expected number of species
H’  Shannon-Wiener diversity index
IndVal  Indicator value
ISA  Indicator species analysis
J’  Pielou’s Evenness
LCBD  Local Contribution to Beta Diversity
LOI  Loss on ignition
M-AMBI  Multivariate AZTI Marine Biotic Index
MEPA  Meteorology and Environmental Protection Administration
MPAs  Marine Protected Areas
MSP  Marine spatial planning
N  Abundance
NCWCD  National Commission for Wildlife Conservation and Development
nMDS  Non-metric multidimensional scaling
OTUs  Operational taxonomic units
PCA  Principal Components Analysis
PERMANOVA  Permutational multivariate analysis of variance
PERSGA  Regional Organization for the Conservation of the Environment of the Red Sea and the Gulf of Aden
POC  Particulate organic carbon
qPCR  Quantitative Real-Time Polymerase Chain Reaction
S  The total number of species
SAP  Strategic Action Program
SIMPER  Similarity Percentage analysis
TOC  Total organic carbon
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The Red Sea. Photo credits: Yasser Katta
Chapter 1

1. Introduction

1.1. Degradation of the marine environment: biodiversity loss and ecosystem changes

Marine ecosystems provide invaluable ecological and socio-economic goods and services (Fig. 1.1), including food, nutrient recycling, leisure and recreation, fossil fuels, renewable energy resources, raw materials and minerals, as well as millions of jobs worldwide (Naeem et al., 2009; Koundouri and Giannouli, 2015; Barbier, 2017). These goods and services are estimated to be worth $125 trillion to the global economy (Costanza et al., 2014). Nevertheless, there is a strong agreement within the scientific community that marine ecosystems around the world are subject to increasing challenges, under continuous human pressure (Watson et al., 2001; Halpern et al., 2008; Visbeck et al., 2014). Indeed, more than 40% of the human population lives in coastal areas (Martínez et al., 2007) placing huge pressure on the marine environment. Natural ecosystems are being altered, driven by human needs, resulting in the: i) overexploitation of resources, namely from fishing, production of freshwater, food, and energy; ii) habitat destruction, for example from coastal defense operations (land-claim, breakwater walls along the shores and in harbours), aquaculture and coastal construction; and iii) pollution and eutrophication from, among others, oil spills, industrial and urban waste discharges, radioactive wastes (Valiela et al., 2001; Lotze et al., 2006; Fulweiler and Nixon, 2009; Waycott et al., 2009; Andrady, 2011).

The degradation of marine habitats has resulted in remarkable declines and losses of critical habitats, such as mangroves (Gilbert and Janssen, 1998; Valiela et al., 2001; Alongi, 2002), seagrass beds (Orth et al., 2006; Airoldi and Beck, 2007; Waycott et al., 2009), and coral reefs (Gardner et al., 2003; Côté et al., 2005). Habitat loss has detrimental consequences for marine communities worldwide (Thrush and Dayton, 2002; Gray et al., 2006). The loss of essential habitats is considered a major threat to marine biodiversity and food webs, which may result in a decline in ecosystem functions and services delivery (Dobson et al., 2006; Thrush et al., 2006; Airoldi et al., 2008).
Our oceans are affected locally via anthropogenic pressures and globally via impacts of climate change. Average global sea surface temperatures have risen by close to 1°C, over the last century (Hartmann et al., 2013). According to the Intergovernmental Panel on Climate Change (IPCC), sea surface temperatures projected to further increase by 1.1–6.4°C by the year 2100, depending on the state of global greenhouse gas emissions (Vuuren et al., 2008). This ongoing increase in atmospheric carbon emissions leads to changes in the physical and chemical properties of the oceans. For instance, ocean-surface pH is currently lower by 0.1 compared to the pre-industrial values, and a further reduction by 0.3–0.4 units is expected by the end of the century (Orr et al., 2005; Andersson and Mackenzie, 2011). An increase in sea surface temperature and decrease in pH are major threats to marine biodiversity. For instance, reduced shell growth and severe mortality events among benthic invertebrates are already being recorded (Coma and Ribes, 2003; Shirayama and Thornton, 2005; Garrabou et al., 2009).

The combined influence of local pressures resulting from human activities and those of a global nature associated with climate change will likely exacerbate impacts on the ocean chemistry and the distribution, metabolic dysfunctions, physiological performance (Somero, 2002; Harley et al., 2006; Doney et al., 2011) and even death (Garrabou et al., 2009) of marine
organisms. Frequency and intensity of these impacts are expected to result in a major biodiversity crisis (Bianchi and Morri, 2000; Jackson et al., 2001b; Sala, 2004; Garrabou et al., 2009) and, ultimately, affect health, functioning, and productivity of coastal ecosystems (Walther et al., 2002; Doney et al., 2012; Tett et al., 2013). According to the first World Ocean’s Assessment (2016), “most of the oceans are now seriously degraded, with changes and losses in the structure, function and benefits” https://en.unesco.org/node/268984.

1.2. The case of the Red Sea

In 1960, the Red Sea was classified as in “virgin condition” (Ormond, 1987). However, due to rapid coastal development and industrial expansion that occurred as a result of petroleum exploration, Red Sea ecosystems have been degraded and are now far from their original pristine status (Gladstone, 2009) and scientists expect this to continue in the future. Currently in the Saudi Arabian Red Sea, where new cities have been built and the population of most of the cities increased exponentially, there is an increased demand for fresh water, food and energy. To face the demand for food, expansion of aquaculture in the Red Sea is ongoing, which might be particularly harmful to the environment considering the original oligotrophic status of the system (Caddy, 1993; Loya et al., 2004). Aquaculture has the potential to change the biogeochemistry of the water and sediment with significant negative impacts on ecosystems (Crawford et al., 2003; Hargrave et al., 2008). Frequent and continuous nutrient inputs into the marine environment may lead to oxygen depletion and hydrogen sulphide accumulation (Stenton-Dozey et al., 2001; Cromey et al., 2002; Islam, 2005). Benthic community patterns can change in terms of the numbers of species, total abundance, individual fitness and biomass in response to eutrophication (Wu, 2002; Loya et al., 2004), especially in oligotrophic habitats (Kon et al., 2009). Environmental impacts resulting from aquaculture activities are mainly related to effluent discharges (onshore facilities) with heavy loads of organic materials (carbon, nitrogen and phosphorus) being released into the coastal area. Studies in the Red Sea by Kürten et al. (2014, 2015) demonstrated that Chlorophyll a, total nitrogen, total phosphorus, dissolved silicate concentrations, as well as abundance of zooplankton and phytoplankton significantly increased at the Al Lith region along the Saudi Arabian Red Sea. Kürten et al. (2014) also showed that potentially harmful or toxic phytoplankton taxa (e.g. *Heterosigma akashiwo*) were present in the vicinity of the shrimp farm (Mohamed and Al-Shehri, 2012). Al Lith hosts the main aquaculture facility in the Middle
East. It began operating in 1983 and has an estimated annual capacity of 100,000 tones. However, knowledge about the responses of Red Sea marine communities to aquaculture-related stressors is still very limited.

1.3. **Biodiversity loss and challenges expected for the Saudi Arabian Red Sea marine ecosystems**

More than 50 phyla are recorded in the world oceans and scientists have estimated that about 1 to 1.4 million species inhabit marine habitats (Costello et al., 2010; Thrush and Dayton, 2002). As a result of a worldwide collaboration of marine scientists (from 2000 to 2010) targeting mainly invertebrates and fishes, more than 1,200 new species to science were recently discovered (Costello et al., 2010). However, scientists also recognize that there is still a considerable underestimation of the total marine biodiversity with tropical regions and particularly invertebrate fauna of soft sediments being poorly described (Guzmán-Alvis and Carrasco, 2005; Kenchington et al., 2017).

For a long time, limited research facilities in tropical regions, coupled with low sampling efforts and insufficient taxonomic expertise, have led to poor discovery rates when compared to countries in temperate regions (Costello et al., 2006; Butler et al., 2010). One of the central issues in ecological studies, critical for management and conservation, is the loss of biodiversity in response to natural and anthropogenic disturbances. Taking into consideration the high number of taxa that are estimated to be unknown to science, and the escalating pressures faced by marine ecosystems over the last century, it is currently accepted that a large number of species could have been lost before being described (Costello et al., 2010). The alarm has been raised because the loss of biodiversity (Fig. 1.2) could affect ecosystem functioning and the delivery of services that are crucial for economic growth and human well-being (Duarte, 2000; Symstad and Tilman, 2001; Worm et al., 2006). Understanding the linkages between biodiversity and processes operating at multiple scales in the marine ecosystem is, therefore, needed to assess the consequences of loss or shift in diversity and its ability to the delivery of goods and services (Butler et al., 2010; Snelgrove et al., 2014).

Biodiversity loss is also a pressing topic in Saudi Arabia. Along with changes expected from an expansion of marine aquaculture, an increase in waste via sewage discharges, brine discharges from desalination plants, and petroleum products from refineries is anticipated.
from current and planned development. With those discharges, an increasing cocktail of pollutants will be expelled into the sea, ending up in either in the water, sediments, or taken up in marine life like fish that may eventually be consumed by humans. Marine animals have different sensitivities to contaminants. Some will be able to thrive even under conditions of stress, but others may die and if the pressing situation continues, species may even disappear (Wu, 2002; Lancellotti and Stotz, 2004). Currently, under Saudi Arabia’s Vision 2030, the plan of the kingdom to diversify the economy, there is a clear message associated with blue growth. Projects and activities along the Red Sea should be carefully managed to support long-term sustainability associated with fisheries, aquaculture, oil and gas development and extraction, and coastal tourism. Unhealthy habitats cannot support sustained development of those activities. Consequently, an urgent effort is needed to protect diversity, essential habitats and natural heritage of the Red Sea. However, we cannot protect what we do not know or understand. There is still a considerable gap in knowledge about the physical, chemical and biological processes in this unique system, as well as in the existing natural resources and how natural and anthropogenic events affect them. Therefore, research efforts need to be intensified and planning of current and future developments of the Red Sea carefully analysed. Sound environmental impact assessment and monitoring will additionally help managers to make decisions to allow for sustained economic growth beyond 2030.
1.4. The need for monitoring the marine environment

Macrobenthos, i.e. organisms living in or on the sediment that are retained on 0.5-1.0 mm mesh sieves play a crucial functioning role in the marine ecosystems (Pearson and Rosenberg, 1978; Snelgrove, 1997, 1998). Macrofauna contribute to the recycling of organic material at the ocean floor (such as detritus, fecal pellets, and animal bodies) and to the transfer of energy via marine food webs, linking the benthic and the pelagic realms. They are important food sources to many fish species that are commercially and ecologically important (Pearson and Rosenberg, 1978; Gray and Elliott, 2009). Macrobenthic organisms also contribute to the recycling of nutrients and other chemical elements (Weisberg et al. 1997; Levin 2000; Rosenberg 2001; Lu et al. 2008), as well as to increased oxygen levels in sediments through bioturbation, i.e. turning over the sediment when crawling or making tubes and galleries (Snelgrove, 1997, 1998; Gaston et al., 1998). Overall, macrofauna affect nitrogen, carbon, and sulphur cycling at a global scale, and therefore are critical to global carbon inventories and atmospheric CO₂ regulation as well as to nitrogen availability for oceanic primary productivity.
(Webb and Eyre, 2004). The loss of macrobenthic biodiversity, i.e. the loss of species, may, therefore, alter the functioning of coastal marine ecosystems.

Long-term changes in the marine ecosystem are reflected in the benthic communities. Indeed, marine benthic communities have been successfully integrated in national monitoring programs as bioindicators of environmental quality as a response to multiple human activities (Borja et al., 2009; Birk et al., 2012). As most of contaminants will settle on the seabed, several studies in different regions have demonstrated that quality assessment of ecological changes in the coastal area and transition zones can be effectively evaluated by analysing sedimentary habitat and benthic fauna (Carvalho et al., 2006; Pinto et al., 2009; Joydas et al., 2012). This partially results from the characteristics exhibited by benthic organisms that make them valuable bioindicators (Bilyard, 1987; Weisberg et al., 1997; Levin, 2000; Rosenberg, 2001). That is, benthic organisms are: i) mainly sedentary with low mobility that prevents them from avoiding deteriorations in local conditions; ii) have long-life spans, providing an integrated response to environmental changes over time; and iii) consist of a diversity of species with a wide range of tolerances and responses to natural and anthropogenic stresses such as organic and inorganic pollutants.

From the literature available worldwide, it is known that the distribution and structure of soft-bottom organisms are driven by several factors, such as: sediment type (Dye and Barros, 2005), organic matter content (Lardicci et al., 1997; Tagliapietra et al., 1998), depth (Cusson and Bourget, 2005; Macdonald et al., 2012), latitude (Roy et al., 2000; Witman et al., 2004), nutrients and food availability (Josefson and Rasmussen, 2000; Rossi and Lardicci, 2002), pH (Riedel et al., 2012), temperature and salinity (Pearson and Rosenberg, 1978; Ganesh and Raman, 2007), as well as species interactions, namely predation and competition (Wilson, 1990; Constable, 1999). Overall, benthic assemblages are distributed according to their capability to cope with physical, chemical, hydrodynamics and biological factors (Smith and Rule, 2001; Carvalho et al., 2012). However, discrepancies in the responses of benthic communities at different spatial scales have been reported (Gray, 2002) and may need to be taken into account. Importantly, generalizations should be made with caution. Indeed, responses in community structure to different environmental drivers may vary depending on the way structure is described, i.e. composition (taxonomy), animal size, biomass spectra or biological traits (Gray and Elliott, 2009).
1.5. The state of knowledge of soft bottom diversity studies in the Saudi Arabian Red Sea

The Red Sea is approximately 2100 km long and 280 km wide, with 1800 km of the eastern coast belonging to Saudi Arabia. The average depth is about 500 m, reaching in some places more than 2000 m. It is a semi-enclosed basin connected to the Mediterranean Sea through the Suez Canal and to the Indian Ocean through the Strait of Bab el Mandab (Sofianos and Johns, 2002). The Red Sea is surrounded by desert environments where rivers are almost non-existent. With the absence of fresh water supply and high evaporation rates, the Red Sea is one of the most saline (Bruckner et al., 2013). The average salinity in the south of the Red Sea is about 38 and can reach more than 40 in the North. Water temperature ranges from 21 to 27°C in the North and from 26 to 32 °C in the South (Kürten et al., 2014). The Red Sea is a unique ecosystem with optimal conditions for coral reef habitats, which are known to harbour high rates of endemism (DiBattista et al., 2016). Along most of its extent, it also contains substantial mangrove habitats and seagrass beds, the latter particularly flourishing in the south (Sawall et al., 2014).

Although Red Sea sediments have been studied for a long time, knowledge on sedimentary habitats is very limited compared to what is known about coral reefs in the region. Studies have been focused on mineralogy (Bischoff, 1969); paleontology (Almogi-Labin et al., 1993); geochemistry (Mohamed et al., 2011); and heavy metal concentration (Badr et al., 2009). Research on coastal soft-sediment benthic communities is mainly restricted to the northern tip of the Red Sea. The first studies of the “littoral benthic fauna” were carried out by Ruppell (1828/1830), Ehrenberg (1834), and Klunzinger (1877/1879), as documented in Fishelson (1971). Fishelson (1971) described communities within a variety of shallow benthic habitats in the Gulf of Aqaba. The first data on deep-sea benthos were provided by Thiel (1979) in the central western region of the Red Sea (25°N). The study was focused on meiofauna from a depth of 500 to 2000 m, as part of an environmental assessment of the potential for mining the metalliferous muds from the deep brine pools of the Red Sea. The mining sector is again attracting the attention of the government and some companies along with the global interest in expanding this activity worldwide (Thiel et al., 2015).

More recently, studies conducted in Egypt have focused on the impacts of environmental conditions and water quality on the distribution of macrobenthic fauna (Hassan et al., 2015;
Belal et al., 2016). Also, macrofauna communities along the Sudanese Red Sea coast have been studied to investigate the potential effect of mangrove clearance on community structure and the composition of the trophic structure (Sabeel et al., 2015). The results from this study showed a significant effect of mangrove clearance on the trophic structure of the associated fauna and suggested that macrofauna can be a valuable biological indicator of changes in ecosystems resulting from human disturbance.

In Saudi Arabia, the limited research conducted so far addressed the impacts of sewage disposal on coastal sediments off Jeddah (Basaham et al., 2009; Al-Farraj et al., 2012). This research indicated an increase in algal growth in the vicinity of the effluent discharges, along with degradation of the nearby mangrove stands. The composition and structure of polychaete assemblages were also altered as a function of distance from the sources of pollutants, with the opportunistic polychaete worms *Capitella capitata*, *Heteromastus filiformis* and *Notomastus* spp. being the most abundant species near to the sewage stations (Al-Farraj et al., 2012). Recently, a study conducted in the southern Red Sea showed high levels of species diversity in soft sediment communities (Ellis et al., 2017), namely an increase in bivalves associated with higher primary productivity in this region. Factors influencing species composition, species richness, abundance, biomass and diversity of soft bottom macrofauna and the resulting patterns in relation to environmental drivers (such as sediment characteristics, depth and seawater temperature) are particularly poorly understood. A better knowledge across multiple spatial and temporal scales is needed. Without understanding how these organisms change in space and time, one cannot optimize the use of this valuable biological component of the Red Sea ecosystem when making management and conservation decisions, such as the design and establishment of Marine Protected Areas. Therefore, priority should be given to research in soft sediment diversity, as sediments should be considered in any monitoring program as they can provide a long-term picture of changes in a given area.

Considering the limited knowledge in the region, and lessons from other areas (Costello et al., 2010), many species might have been lost even before we were able to identify and protect them. The likelihood of species new to science in the Red Sea has been evident by recent discoveries both in soft sediments (Ravara and Carvalho, 2017) and coral reefs (e.g. Berumen
and Rotjan, 2010) and emphasizes that the Red Sea remains one of the largely unexplored marine basins.

1.6. Objectives and thesis structure

The present PhD thesis aims to tackle several knowledge gaps by providing baseline information that can be used to assess environmental impacts caused by urban and industrial development in the Red Sea. It also aims to provide relevant information about the drivers (natural and anthropogenic) influencing the response of the marine communities in this unique environment and serve as a foundation for other sub-tropical and oligotrophic regions. To better explain the spatial and temporal patterns of biodiversity, abundance and community structure of soft-sediment macrobenthic assemblages in tropical regions, this study is organized into the following three research chapters:

1. Consistent variability in beta-diversity patterns contrasts with changes in alpha-diversity along an onshore to offshore environmental gradient: the case of Red Sea soft-sediment macrobenthos.

2. Composition, uniqueness and connectivity among habitats in a tropical coastal lagoon.

3. The combined effect of vegetation and local environmental conditions on shaping bacterial and macroinvertebrate benthic communities in the Red Sea.
1.7. Main research questions of the study

In order to respond to the main questions, a major effort in this thesis was dedicated to collect and analyse sediment samples to understand macrobenthic diversity and composition changes at multiple temporal and spatial scales (Fig 1.3). Within the spatial scale, different habitats, such as seagrass beds, mangroves, unvegetated soft-sediments in lagoon and offshore areas, representative of the region, were sampled. There are several scientific questions that we will attempt to answer:

- Given the harsh environmental conditions, particularly the low availability of food, high temperature and salinity, do the Red Sea shallow-water sediments harbour assemblages with low diversity and low abundance?
- Given the changes in the environmental characteristics observed in different habitats, how will composition, structure species richness and abundance of macrobenthic change across mangrove, seagrass meadows and unvegetated sediments? Also, how are benthic assemblages connected across lagoon habitats?
- Due to the limited variability in sea surface temperature (SST) throughout the year, how will macrofauna diversity, abundance and structure vary over seasons and inter-annually across different habitats?
- Will seagrass meadows support richer and more stable communities as observed for several temperate and tropical regions compared to other lagoon habitats?
- How will the fragmentation of vegetated habitats affect the diversity and structure of macrofaunal and bacterial communities in shallow water lagoons of the Red Sea?
Fig 1.3. Structure and research plan of the thesis, considering spatial and temporal patterns of biodiversity, abundance and community structure of soft-sediment macrobenthic assemblages.
1.8. References


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Chapter 2

2. Consistent variability in beta-diversity patterns contrasts with changes in alpha-diversity along an onshore to offshore environmental gradient: the case of Red Sea soft-sediment macrobenthos

Photo credits: Susana Carvalho, Arm Gusti and Zahra Alsaffar

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2.1. Abstract

Patterns of variability in diversity (alpha and beta), abundance, and community structure of soft-sediment macrobenthic assemblages were investigated across an inshore/offshore environmental gradient in the central Red Sea. A total of three distinct biotopes were identified through multivariate techniques: seagrass meadows, nearshore and offshore unvegetated sediment. While the seagrass biotope was associated with higher organic matter content, the two coastal biotopes presented higher redox potential in the sediments and dissolved oxygen in the water. Depth and medium sand increased toward the offshore, while the percentage of fine particles was a determinant of the nearshore communities. Regardless of the prevailing environmental conditions, the three biotopes were characterized by high numbers of exclusive taxa (occur in one biotope only), most of which were singletons. Changes in species richness were not related to depth or organic matter, peaking at intermediate depths (nearshore biotope). However, the number of taxa increased exponentially with abundance. On the other hand, density decreased exponentially with increasing depth and decreasing organic matter in sediments, probably linked to the reduced availability of food. One of the most conspicuous features of the macrobenthic assemblages inhabiting soft-sediments in the central oligotrophic Red Sea is the low level of dominance resulting from high species: abundance ratios. Despite the differences observed for alpha-diversity across the three biotopes, beta-diversity patterns were rather consistent. These findings suggest that mechanisms driving biodiversity are similar across the depth gradient. The partitioning of beta-diversity into turnover (or replacement) and richness (or nestedness) also shows that assemblages are mainly driven by the substitution of species (turnover or replacement) most likely as a result of environmental filtering. The heterogeneity of the seafloor in shallow waters of the Red Sea promoted by the co-existence of coral reefs interspaced by sedimentary habitats may increase the regional pool of colonizers and potentiate the random the distribution patterns.

Keywords: macrobenthic assemblages, biotopes, seagrass, coastal area, spatial distribution, tropics
2.2. Introduction

A fundamental topic in ecology is to understand factors shaping species distribution (Holt et al., 2005). Benthic assemblages are distributed according to their capability to cope with physical, chemical, hydrodynamics, and biological factors (Smith and Rule, 2001; Bolam et al., 2008; Labrune et al., 2008; Carvalho et al., 2012). Among the environmental drivers, sediment particle size (Gray, 2002; Carvalho et al., 2012; Khedhri et al. 2016; Ellis et al. 2017), organic matter (Rosenberg, 1995; Carvalho et al., 2006a; Ramey and Bodnar, 2008; Silva et al., 2011), depth (Karakassis and Eleftheriou, 1997; Hughes et al., 2009; Carvalho et al., 2012; Joydas et al., 2012), and salinity (Teske and Wooldridge, 2004; Lu et al., 2008; Hughes et al., 2009) have been reported as significantly affecting the spatial distribution of benthic macroinvertebrates. On the biological side, predation and competition (André et al., 1993; Martinetto et al., 2005; Matias et al., 2012), phytoplankton productivity (Zhang et al., 2015), and the presence of vegetation (Carvalho et al., 2006b; Duarte et al., 2008; Carvalho et al., 2011; Włodarska-Kowalczuk et al., 2014; Sokołowski et al., 2015) have also been found to influence the distribution and abundance of macrobenthos in coastal embayments (e.g. lagoons) and open waters.

Despite a few recent studies addressing the distribution patterns of macrobenthic assemblages in Red Sea in relation to environmental gradients (Al-Farraj et al., 2012; Sabeel et al., 2015; Pearman et al., 2016; Ellis et al., 2017), factors influencing species composition, abundance, biomass and diversity of soft-sediment macrofauna communities are still poorly understood. Overall, this is a consistent pattern across sub-tropical and tropical latitudes. Indeed, despite various studies addressing tropical and sub-tropical soft-sediment biotopes (e.g. Price et al., 1987; Warwick and Ruswahyuni, 1987; Dittmann, 2000; Mackie et al., 2005; Cheung et al., 2008; Shin et al., 2008; Mair et al., 2009; Borja and Tunberg, 2011; Magni et al., 2015; Chan et al., 2016; Feebarani et al., 2016), there is still a bias towards temperate regions in the literature. Also, in tropical and subtropical regions, most of the studies addressing benthic onshore-offshore diversity patterns are focused on coral reef habitats (e.g. Harriott et al., 1994; Adjeroud, 1997; Cleary et al., 2005; Becking et al., 2006; Smith et
al., 2008; Polónia et al., 2015), whereas studies addressing changes in soft-sediment communities are still limited (Ellis et al., 2017).

Species richness, calculated as the number of species per sampling unit (i.e. alpha-diversity) or using different indices (the most common being Shannon-Wiener and Margalef species richness), is one of the classic attributes characterizing a community. Hence they have been consistently used, both in terrestrial and marine habitats, as a fundamental descriptor in ecological and conservation studies. However, those variables do not provide a comprehensive understanding of changes in the biodiversity patterns, as they do not account for changes in species composition. In the 1960’s, Whittaker introduced the concept of beta-diversity (Whittaker, 1960).

Beta-diversity can be analysed from different angles, from changes in the community composition along environmental gradients (spatial) or temporal scales, or simply as a ratio between gamma- (regional diversity, i.e. the total number of taxa found in a certain region) and alpha-diversity (Whittaker, 1960; Jost, 2007). Changes in beta-diversity can provide the scaling factor that allows the prediction of changes in gamma-diversity from measured changes in alpha-diversity (Socolar et al., 2016). One of the main strengths of beta-diversity measures is that they allows for a quantification of the degree of differentiation of the biological communities within a region (Tuomisto, 2010). Depending on the framework selected, beta-diversity can be, for example, partitioned into turnover (or replacement) and nestedness (or richness difference) components (Baselga, 2010; Legendre, 2014; Baselga and Leprieur, 2015).

The different types of partition suggest different mechanisms structure species assemblages (Loiseau et al., 2017). Turnover (or replacement) occurs when species are substituted along an environmental gradient, or in time, due to environmental sorting, spatial or historical constraints (or a combination of them) (Qian et al., 2005). Also, due to a gain or loss of species, one community may include a larger number of species than another (richness difference). This could be a result of the high number of different niches available or dispersal capacity within a region (Legendre, 2014). Nestedness is a special case of richness difference when the species at a site are a strict subset of the species at a richer site (Baselga, 2012). The comparative analysis of these components is essential to link community patterns (dissimilarities) to
ecological processes (Baselga, 2010a; Loiseau et al., 2017), such as species environmental tolerances, dispersal limitation, and selective extinction (Ulrich et al., 2009). These inputs can be extremely valuable not only for the management and conservation of marine areas (Baselga, 2010; Loiseau et al., 2017; Socolar et al., 2016) but also in the assessment of impacts resulting from anthropogenic disturbances (Bevilacqua et al., 2012; Piló et al., 2015; Borja et al., 2016). Therefore, the integration of beta-diversity components in marine biodiversity studies has recently increased (Koleff et al., 2003; Becking et al., 2006; Soininen et al., 2007; Bevilacqua et al., 2012; Dias et al., 2015; Piló et al., 2015; Boyé et al., 2017; Loiseau et al., 2017).

The present study analysed the spatial patterns of diversity and richness, abundance, and community structure of soft-sediment macrobenthic assemblages of the central-eastern Red Sea region, across an inshore/offshore environmental gradient. Our general goal was to identify the main environmental drivers shaping the distribution of those assemblages inhabiting habitats typified by high-salinity (>37), high seawater temperature (21-32ºC), and oligotrophic conditions that are not only characteristics of the Red Sea but also of most tropical regions. Given these extreme conditions, we hypothesize that soft-sediment benthic assemblages are characterized by high levels of alpha-diversity as reported for other marine habitats (e.g. coral reefs; Roberts et al., 2002; DiBattista et al., 2016) but low density, which will further decrease with depth (Joydas and Damodaran, 2009; Lubinevsky et al., 2017). Based on a combination of univariate and multivariate methods, we also aim to identify and characterize the main biotopes, i.e. seabed areas associated with different faunal assemblages that may occur across the depth gradient. We hypothesize that differences in the macrobenthic assemblages associated with the identified biotopes result from different processes (biotic and abiotic) acting on those assemblages, which will be reflected in beta-diversity patterns.
2.3. Material and Methods

2.3.1. Study area

The Red Sea is a unique ecosystem surrounded by deserts that extends for 2270 km. It is a semi-enclosed sea (280 km wide) connected to the Mediterranean Sea through the Suez Canal and to the Indian Ocean through the Strait of Bab el-Mandab. Precipitation levels in the whole region are low; there are no permanent rivers, and seawater temperature usually ranges from 21-28°C in the north to 26-32°C in the south. The high evaporation rates (~2 m yr⁻¹) makes it one of the most saline and warm seas in the world (Edwards, 1987; Sofianos and Johns, 2002), with average salinity in the south reaching 37 and in the north 42 (Sofianos and Johns, 2002).

High levels of biological diversity have been reported, with some extended endemism (Wehe and Fiege, 2002; DiBattista et al., 2016). For example, 15% of crabs are endemic (Guinot, 1966); 635 species of polychaetes, 211 echinoderms, and 1071 shallow fish species have been recorded; of which 12.6%, 8.1%, and 12.9%, respectively, were suggested to be endemic (DiBattista et al., 2016). The present study was undertaken in the eastern central Red Sea in an inshore and an open water coastal area (Fig. 2.1). The inshore area, a coastal lagoon (Al Qadimah lagoon, 22°22'39.3" N 39°07'47.2"E) and nearby offshore areas (22° 11'922" N 38° 55'774" E) are surrounded by mangroves (Avicennia marina) with some patches of seagrasses. The seagrass Cymodocea rotundata dominates in shallow areas down to 0.50m depth. Smaller patches of Cymodocea serrulata and a few specimens of Halophila stipulacea can also be found. Another seagrass species, Enhalus acoroides forms monospecific stands deeper than 0.50 m extending down to almost 2 m depth. The nearby coastal area comprises several coral reef systems from the nearshore to the offshore surrounded by extensive soft-sediments.
2.3.2. Sampling strategy

For the purpose of this study, samples were collected in two random surveys in winter 2014 (January and February-March) in both areas (the Al Qadimah lagoon and the coastal marine area). Benthic macrofaunal samples were collected at various depths in the nearshore and offshore waters. Macrobenthic assemblages in the lagoon were sampled in seagrass meadows (1-1.5m depths; two stations, stations S), and in the channel (10m depth, 1 station, station CH) that connects the lagoon to the coastal area. In the coastal area, ten stations were established toward the offshore (approximately along three transects perpendicular to the coast), from 25m down to a depth of 90m (stations TH, Table 1). Conductivity, Temperature, and Depth (CTD) casts were carried out at each station, recording conductivity, temperature, depth, and oxygen. All the samples were taken using a 0.1m$^2$ Van Veen grab (two replicates at each site) and sieved through a 1mm mesh screen. Samples were preserved in 96% ethanol. Sediment subsamples were taken for grain-size analysis and organic matter content. Oxidation/reduction potential of the top surface layer of sediment (~2-3cm)
was measured inside the grab using a SenTix PtR electrode (WTW GmbH, Weilheim, Germany).

2.3.3. Sample processing

The sediment particle size analysis was performed by wet separation of the silt and clay fractions from the sandy fractions (Carvalho et al., 2012). Each replicate of sediment samples was sieved through 63μm mesh (silt and clay fraction), and the retained fraction was dried at 80°C for 24-48h. Then, the dried sandy and gravel sample was sieved by using a column of sieves (63, 125, 250, 500, 1000, and 2000 μm mesh) to separate the sandy fractions (63-1000 μm) and the gravel (>2000μm). Organic matter content was determined by loss on ignition (LOI). Sediments were dried at 60°C for 24-48h and then placed in a muffle furnace at 450 °C for 4 h (Heiri et al., 2001). The LOI is then calculated using the following equation:

\[ \text{LOI} = \left( \frac{W_i - W_f}{W_i} \right) \times 100 \]

Where: LOI = Organic Matter content (%), Wi = Initial weight of the dried sediment subsample; Wf = Final weight after ignition.

Organisms from each sample were sorted, identified using a stereomicroscope, and counted. Organisms were identified to the highest taxonomic separation possible, usually species level. Unfortunately, taxonomic guides for the soft-sediment fauna of the Red Sea are almost non-existent and the literature available is almost exclusively from the northern tip of the Red Sea. Consequently, this impediment resulted in an inability to identify all the organisms to the genus or species level. Therefore, some were identified to the family level only. Intensive expert taxonomic revisions are still needed to confirm these identifications, and several species will be most likely new to science (both as records for the region and undescribed) given the lack of studies in soft-sediment ecology along most of the extension of the Red Sea.
2.3.4. Data analyses

The small-scale temporal variability in the communities was assessed for all sites that had two sampling events (sites S1, S2, CH, TH1, TH3, TH7, and TH8). The changes in community structure and composition over space and time (within a season) was assessed using permutational multivariate analysis of variance (PERMANOVA, function “adonis2” from the R package “vegan”) on the Bray-Curtis dissimilarity matrix with “site” and “sampling event” as factors (site (7) x sampling event (2) factorial design). Spatial and temporal changes (and their interaction) in the total abundance were assessed using analysis of variance (ANOVA) following the design used for the PERMANOVA. Due to the lack of statistical significance for the factor “sampling event” and to increase the power of the analyses, this factor was not further considered in the characterization of macrobenthic communities.

Inter-station relationships were investigated by classification and ordination techniques (cluster and non-metric multidimensional scaling, nMDS, respectively), based on the Bray–Curtis similarity coefficient applied to untransformed abundance data. These analyses aimed to identify benthic biotopes. To characterize the macrobenthic assemblages in terms of taxa composition associated with each biotope, the “Indicator species analysis” (ISA) (Dufrêne and Legendre, 1997) was conducted. This analysis is based on the calculation of an indicator value (Indval, ranging from 0 to 1) that reflects the frequency of occurrence and the abundance of each taxon in defined groups (in the present case the ones identified by the multivariate analysis). Rather than focusing on common taxa with high indicator values, ISA allows for the examination of common and rare taxa within a community (De Cáceres and Legendre, 2009). The ‘IndVal’ value was calculated using the R package ‘indicspecies’, specifying the combination of site groups and its equalization (“IndVal.g”) (De Cáceres et al., 2010). The indicator values were used to select the most characteristic taxa of each one of the biotopes. Monte Carlo simulations were used to test for the significance of the indicator values (999 randomized runs). Taxa represented by one or two individuals were not considered in the analysis. Each biotope was also characterized in terms of univariate descriptors of biodiversity, i.e. abundance (N), the total number of species (S), Hurlbert’s expected number of species...
(ES_{50}), Shannon-Wiener diversity index (H'), Pielou's Evenness (J'), and trophic functions. Hurlbert's rarefaction index determines the expected number of species (ES) in a randomly selected subset of individuals, e.g. 50 (ES_{50}), as used in this section (Hurlbert, 1971) and it is especially relevant when there are differences in the sampling effort. Rarity of species was analysed by calculating the number of singletons and doubletons (i.e. taxa represented by one or two organisms or found on one or two replicate only). Due to low abundance values and high diversity observed, analyses were based on the abundance data of two replicates.

The variation in the diversity patterns of benthic assemblages (beta diversity) was assessed using multivariate methods. Beta diversity was originally described for looking at changes in the species composition between two or more habitats (Koleff et al., 2003; Baselga, 2012). The beta diversity of the study area was estimated using the Sørensen dissimilarity as this metric has all the necessary properties to assess beta diversity (Legendre and De Cáceres, 2013) and it is widely used. The Sørensen dissimilarity is the one counterpart of the Sørensen similarity, which is the binary form of the Steinhaus similarity (alias Bray-Curtis similarity) (Legendre and Legendre, 2012). Therefore, there is a direct relation between Sørensen dissimilarity and the Bray-Curtis dissimilarity (Legendre and Legendre, 2012), used for assessing community patterns and for the identification of the benthic biotopes. The relative contribution of each sampling unit to the beta diversity, i.e. the “Local Contribution to Beta Diversity (LCBD)” (Legendre and De Cáceres, 2013) was also calculated using the Sørensen dissimilarity. The Sørensen dissimilarity index ($\beta_{sor}$) is formulated as:

$$\beta_{sor} = \frac{(b + c)}{2a + b + c}$$

Where:

- $a$ is the number of species common to both sites.
- $b$ is the number of species that occur in the first site but not in the second.
- $c$ is the number of species that occur in the second site but not in the first.
To assess which ecological processes determine beta-diversity patterns (in terms of species composition), beta diversity ($\beta_{sor}$) was partitioned into replacement ($\text{Repl}$) and richness difference ($\text{Rich}$), according to Legendre (2014; Podani family), using the following equation:

$$\beta_{sor} = \beta_{\text{Repl}} + \beta_{\text{Rich}}$$

Where:

$$\beta_{\text{Repl}} = \frac{2 \cdot \min(b, c)}{2a + b + c}$$

$$\beta_{\text{Rich}} = \frac{|b - c|}{2a + b + c}$$

Where:

- $a$ is the number of species common to both sites.
- $b$ is the number of species that occur in the first site but not in the second.
- $c$ is the number of species that occur in the second site but not in the first.

Alternatively, beta diversity ($\beta_{sor}$) was partitioned into turnover ($\beta_{\text{Turnover}}$) and nestedness components ($\beta_{\text{Nestedness}}$) following Baselga (2012) and (Baselga and Leprieur, 2015).

$$\beta_{sor} = \beta_{\text{Turnover}} + \beta_{\text{Nestedness}}$$

Where:

$$\beta_{\text{Turnover}} = \frac{\min(b, c)}{a + \min(b, c)}$$

$$\beta_{\text{Nestedness}} = \frac{\max(a, b) - \min(b, c)}{2a + b + c} \times \frac{a}{a + \min(b, c)}$$

The partition of the beta diversity components was done using the function “beta.div.comp” from R library “adespatial”, using presence/absence data.

Distance-based redundancy analysis was applied to estimate the contribution of each environmental parameter to the variation in the macrobenthic structure by analysing marginal test (was applied to show the significance of each variable individually to the model) and sequential test (was applied to show the subset of explanatory variables
that significantly contribute to explain the biological patterns). The analysis included depth, grain size fractions, oxidation-reduction potential, and LOI (%). The direction and the magnitude of the relationships between environmental variables and biological assemblages were visually presented in a dbRDA biplot.

The open source statistical software R was used for all the statistical analysis (R Core Team, 2017). The R package “vegan” was used for all multivariate analysis (nMDS, dbRDA) and also for alpha diversity calculations (Oksanen et al., 2017). For the calculation of the indicator value (IndVal) the package “indicspecies” was used (De Cáceres and Legendre 2009). For the calculations of the beta diversity indices and the local contribution for beta diversity (LCBD), the authors used the package “adespatial” (Dray et al., 2017).

2.4. Results

2.4.1. Small-scale temporal variability in macrobenthic patterns

The subset of samples collected on two different dates in winter 2014 showed spatial but no short-term variability in the composition and structure of the assemblages (PERMANOVA, Pseudo-F=1.14, p=0.273). The same pattern emerged when the total abundance of the sample was compared, significant differences between sites (ANOVA, F=3.25, p=0.032) but not for sampling dates (ANOVA; F=0.58, p=0.458) or the interaction between the two factors (ANOVA, F=0.65, p=0.688).

2.4.2. Spatial variability in macrobenthic patterns

A total of 240 taxa and 1237 organisms identified; 99 were identified to the species level, 106 to the genus, 24 to family, and 11 to higher taxonomic levels. Overall, the organisms were spanned over 120 families, and 12 phyla. A complete list of taxa is provided as supplementary material. Rare species, i.e. singletons and doubletons were extremely abundant contributing to 80% of the total number of taxa. Annelida (almost exclusively Polychaeta) was not only the most abundant group contributing to 36.7% of the total abundance, but the one with the highest number of taxa (97). Sipuncula (263 ind., 5 taxa), Arthropoda (210 ind., 63 taxa), Mollusca (155 ind., 48 taxa), and Echinodermata (117 ind., 18 taxa) were also well represented. Sipuncula
and Mollusca were more abundant in seagrass meadows, Crustacea and Echinodermata showed the opposite pattern.

The multivariate analysis highlighted a clear onshore-offshore gradient of change in the structure of macrobenthic assemblages, in addition to a depth gradient. Three main biotopes were identified: i) the shallow water seagrass meadows; ii) the nearshore shelf, including the lagoon channel (CH, TH1, TH2, TH5, TH7, TH8; TH10); and iii) the offshore shelf (TH3, TH4, TH6, TH9) (Fig. 2.2). The seagrass biotope, located at very shallow bottoms (1.7±0.6 m), was characterized by the highest density (602.5±90.3 ind.m⁻²) and the lowest evenness compared to the other biotopes (0.67 versus 0.91 and 0.95 for nearshore and offshore biotopes, respectively; Fig. 2.3; Table 2.1). The nearshore biotope comprising stations located between 10 and 34 m depths (average depth of 24.1±9.4 m) was associated with the highest diversity. A peak of species richness, expected number of taxa, Shannon-Wiener diversity, and singletons + doubletons were observed in this biotope (Table 2.1). A clear pattern of decreasing abundance and increasing evenness with depth was observed (Fig. 2.3; Table 2.1). The offshore biotope showed an average density of 109±17.6 ind.m⁻² (Fig. 2.3; Table 2.1). The number of exclusive taxa followed the trend registered for the total number of taxa, with a peak detected nearshore (121). Across the three biotopes, the percentage of exclusive taxa ranged from 39% (seagrass meadow) to 68% (nearshore) of the total number of taxa found in each biotope (Table 2.1). In all three assemblages, a high percentage of the exclusive taxa were singletons (76% to 83%).

The density in the studied area was overall low and decreased logarithmically with depth (y = -14.24ln(x) + 71.342 R² = 0.68) (Fig. 2.4 A) and LOI (y = -0.689ln(x) + 4.621 R² = 0.38). Species richness peaked at intermediate depths and showed no relationship with LOI. It was, however, exponentially related with abundance (y = 5.478ln(x) - 4.5576 R² = 0.59) (Fig. 2.4 B).
Fig. 2.2. Ordination (non-metric multivariate dimensional analysis) and classification diagrams of the sampling locations (average data per location and sampling event) based on the Bray–Curtis similarity. The same labels refer to two different sampling events. S = seagrass, NS = nearshore, O = offshore.
Table 2.1. Biological and environmental characterization of the identified macrobenthic assemblages according to the multivariate analysis. Sediment Oxidation-Reduction potential (Eh), organic matter (LOI), and Hurlbert’s expected number of species (ES (50)). S, seagrass; NS, nearshore; O, offshore.

<table>
<thead>
<tr>
<th></th>
<th>SG</th>
<th>NS</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of stations</td>
<td>2</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>No. samples</td>
<td>8</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Total area sampled (m²)</td>
<td>0.8</td>
<td>2.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>1.7±0.6</td>
<td>24.1±9.4</td>
<td>74.3±15.1</td>
</tr>
<tr>
<td>Depth (m) range</td>
<td>1.0-2.4</td>
<td>10-34</td>
<td>57.5-86</td>
</tr>
<tr>
<td>Water column</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity</td>
<td>40.53±0.429</td>
<td>39.64±0.25</td>
<td>39.43±0.11</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.33±1.78</td>
<td>25.75±0.92</td>
<td>25.44±0.16</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>4.23±1.37</td>
<td>5.95±1.46</td>
<td>6.41±1.38</td>
</tr>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silt and Clay (%)</td>
<td>57.15±22.03</td>
<td>47.61±29.47</td>
<td>41.45±24.46</td>
</tr>
<tr>
<td>Fine sand (%)</td>
<td>16.40±13.68</td>
<td>22.95±13.61</td>
<td>21.06±5.61</td>
</tr>
<tr>
<td>Medium sand (%)</td>
<td>17.14±8.98</td>
<td>19.65±16.32</td>
<td>25.70±19.89</td>
</tr>
<tr>
<td>Coarse sand (%)</td>
<td>4.85±2.75</td>
<td>5.25±8.59</td>
<td>5.94±2.11</td>
</tr>
<tr>
<td>Gravel (%)</td>
<td>4.43±3.63</td>
<td>4.55±5.07</td>
<td>5.86±2.11</td>
</tr>
<tr>
<td>Eh (mV)</td>
<td>40.44±7.02</td>
<td>50.58±9.06</td>
<td>53.25±12.42</td>
</tr>
<tr>
<td>LOI (%)</td>
<td>5.20±2.17</td>
<td>2.50±0.97</td>
<td>1.69±0.66</td>
</tr>
<tr>
<td>Biological variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total S</td>
<td>74</td>
<td>178</td>
<td>62</td>
</tr>
<tr>
<td>No. exclusive taxa</td>
<td>36</td>
<td>121</td>
<td>24</td>
</tr>
<tr>
<td>Total shared taxa</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shared taxa S, N</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shared taxa S, O</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shared taxa NS, O</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singletons</td>
<td>29</td>
<td>92</td>
<td>20</td>
</tr>
<tr>
<td>Doubleton</td>
<td>10</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Total no. ind.</td>
<td>482</td>
<td>646</td>
<td>109</td>
</tr>
<tr>
<td>S (taxa x 0.2m⁻²)</td>
<td>25.8±7.53</td>
<td>30.1±3.32</td>
<td>16.0±2.21</td>
</tr>
<tr>
<td>Density (ind.x m⁻³)</td>
<td>602.5±90.33</td>
<td>293.6±35.60</td>
<td>109.0±17.56</td>
</tr>
<tr>
<td>H' (bits x ind⁻¹)</td>
<td>3.14±0.60</td>
<td>4.4±0.16</td>
<td>3.8±0.19</td>
</tr>
<tr>
<td>J'</td>
<td>0.67±0.07</td>
<td>0.91±0.01</td>
<td>0.95±0.02</td>
</tr>
<tr>
<td>ES₅₀</td>
<td>15.8±3.35</td>
<td>25.1±1.85</td>
<td>16.0±2.21</td>
</tr>
</tbody>
</table>
Fig. 2.3. Density (ind. × m$^{-2}$), mean number of taxa (no. of taxa× 0.1m$^{-2}$), Margalef diversity, Shannon–Wiener diversity (bits × ind.$^{-1}$), and Pielou’s evenness ($J'$) of macrobenthic assemblages across the identified biotopes. S = seagrass, NS = nearshore, O = offshore.
Fig. 2.4. Relationship between (A) number of individuals and depth and (B) number of taxa and number of individuals.
The overall beta diversity (Sørensen $\beta_{sor}$) is high (0.43; maximum of 1). The Local Contribution to Beta Diversity (LCBD) values ranged between 0.042 and 0.060 and no significant differences were detected in the relative contribution of each sample ($P>0.05$ after Holm correction for 19 simultaneous tests). The partition of the beta-diversity (Fig. 2.5) shows that differences in species composition are mainly due to replacement (Podani family, 69.7% of the variation) and not to richness difference. What is more, using the alternative framework (Baselga family), the turnover component is also more important than the nestedness component (93.0% of the variation). The replacement component was significantly different between seagrass and coastal biotopes ($P<0.05$, PERMANOVA), whereas for turnover, significant differences were detected among all the biotopes. Overall, the seagrass community presented the lowest values. However, samples in each assemblage also presented high variation in composition (Fig. 2.5). Nevertheless, in each assemblage, the variation in species composition is mainly due to gain and loss of species (replacement or turnover components). Regarding the trophic function, facultative detritivores/suspension feeders were clearly dominant in the seagrass meadow, whereas carnivores increased in the coastal biotopes (Fig. 2.6).
Fig. 2.5. Triangular plots of the relationships among the 190 pairs of sites for the Al Qadimah lagoon macrobenthos data. Each point represents a pair of sites. Its position is determined by a triplet of values from the Similarity ($S = 1 - D$, where $D = Sørensen$ dissimilarity), Replacement (or Turnover), and RichDiff (or Nestedness) matrices; each triplet sums to 1. Graphs are shown for the indices of the (a) Podani and (b) Baselga families. Pairs of sites among (left) and within (right) assemblages are separated into different graphs. The large central dots in each graph is the centroid of the points for each assemblage and the lines extending to each axis represent the median values of $S = seagrass$, NS = nearshore, O = offshore.
2.4.3. Species-habitat associations

Only two species were shared between seagrass and the offshore biotopes, whereas nearshore assemblages shared 21 taxa with either of the other assemblages (Table 2.1). Less than 7% of the taxa were observed across the whole depth gradient (15 taxa; those that have been identified at the genus or species level were the polychaetes *Drilonereis* sp., *Eunice* sp., *Glycera* sp., *Goniada* sp., *Gymnonereis* sp., *Lumbrineris* sp., *Lysidice unicornis*, *Notomastus* sp., and cf. *Protocirrineris* sp., the sipunculid *Apionsoma (Apionsoma) misakianum*, and the brittle star *Amphioplus laevis*).

Clear changes in the species distribution were detected across the biotopes identified in the present study. The sipunculid *Phascolion (Phascolion) strombus strombus*, the bivalves *Afrocardium richardi*, *Cardiolumina semperiana*, and *Barbatia foliata*, the gastropods *Cerithium scabridum*, and *Ancilla* sp.3, and the polychaetes Capitellidae n.id., *Serpulidae* n.id., and *Sigambra* sp., and the brittle star *Amphioplus cyrtacanthus* were associated with the seagrass biotope. All but Capitellidae n.id., *Serpullidae* n.id., *Ancilla* sp.3, and *Phascolion (Phascolion) strombus strombus* were exclusive of this biotope (Fig. 2.7).
Crustaceans and echinoderms were particularly well represented in the nearshore biotope. According to ISA, the crustaceans *Macrophthalmus graeffei*, along with the sea urchins *Schizaster gibberulus*, and *Metalia persica*, as well as the brittle star *Amphiodia duplicate*, were significantly associated with the nearshore biotope (Fig. 2.7). The hermit crab *Diogenes* spp., the amphipods *Ampelisca* spp., and the decapod *Leptochela aculeocaudata* were also preferentially distributed between 10 and 34m depth. The decapod *Trypaea kowalevski* was exclusive of the offshore biotope. Although not exclusive, the IndVal also identified *Driloneris* sp. as significantly associated with the deepest bottoms.

### 2.4.4. Relationship between biological patterns and environmental variables

Marginal and sequential tests in distance-based redundancy analysis (dbRDA) showed that depth, redox potential, LOI, medium sand and fine particles were significant for explaining the biological patterns (Fig. 2.8). The reduced model explained 48.2% of the total variation (F=2.02, p<0.001) and the first dbRDA axes (F_{CAP1}=3.75, p<0.001; F_{CAP2}=2.50, p<0.01) explained 51.6% of the fitted variation. The CAP analysis confirmed the separation of the three biotopes with seagrass communities being associated with higher LOI and the coastal biotopes being associated with higher redox and oxygen in the water. Depth and medium sand increased toward the offshore, while the percentage of fine particles was a determinant driver for the nearshore communities (Fig. 2.8).
Fig. 2.7. Macrobenthic distribution patterns of abundance in the three biotopes identified in the multivariate analysis (seagrass, nearshore, and offshore). The abundance pattern is shown for the taxa identified by IndVal as characteristic of the groups identified by the classification analysis (green) and, additionally, also for the most abundant taxa (more than 14 individuals; red). Patterns are shown per site and sampling period. The annotated values represent the percentage of the total abundance of the taxon in each biotope.
2.5. Discussion

2.5.1. General patterns

Soft-sediment macrobenthic communities in the Red Sea changed across the onshore/offshore gradient, and three biotopes with distinct assemblages have been identified: seagrass meadows, nearshore and offshore. The biotopes were water-depth related but the macrobenthic communities are likely to be driven by a combination of factors (e.g. vegetation, grain-particle size variables, LOI) that may
change with depth rather than to the hydrostatic pressure alone. Molluscs were mainly associated with the seagrass meadows, whereas crustaceans and echinoderms increased their contribution to the overall abundance in the open coastal waters. This is in line with previous studies in the region based on molecular data (Pearman et al., 2016).

Average density in the offshore biotope was approximately 5 times lower than in the seagrass meadows. Low abundance values have been also reported for the molluscan and polychaete assemblages of the Seychelles Plateau (11-62m), in the Indian Ocean (Mackie et al., 2005). Overall, our density values are low compared to findings observed in other coastal areas in temperate regions (e.g. Ellingsen, 2002; Dolbeth et al., 2007), and even at similar latitudes (Jamaica, 240 ind.m⁻²; Wade, 1972). They are, however, similar to values reported by Shin and Thompson (1982) for Hong Kong. Evenness values (>0.9) were very high in the open water biotopes as species: abundance ratios were elevated due to the community being characterised by a plethora of low abundance species. However, in light of the expected increase in anthropogenic contamination from ongoing development, a decrease in alpha-diversity and evenness of soft bottom communities is expected to occur (Johnston and Roberts, 2009).

The logarithmical decrease of abundance with water depth observed in the present investigation is in agreement with a previously reported study in the southeastern Mediterranean Sea (Levantine Basin), the most oligotrophic region of the Mediterranean Sea (Lubinevsky et al., 2017). The depressed abundance of macrobenthic communities with depth may result from lower availability of food (Karakassis and Eleftheriou, 1997; Roy et al., 1998), as the flux of organic material to the sea bottom is more limited (Karakassis and Eleftheriou, 1997). This is particularly challenging in the Red Sea, as well as across other the sub-tropical and tropical regions where relatively high levels of oligotrophy do not seem to be able to support large populations of benthic organisms (Thiel et al., 1987; Raitos et al., 2013). Even for the Mediterranean Sea, oligotrophy has been linked to the decrease of benthic abundance and biomass (Pusceddu et al., 2010; Baldrighi et al., 2014). The role of food resources regulating benthic populations both in size and reproductive output has been
demonstrated by several manipulative laboratory and field experiments (Marsh and Tenore, 1990; Snelgrove et al., 2001; Currie and Small, 2005). Oligotrophy, combined with the extremely high seawater temperature in the Red Sea, which will induce higher metabolic rates and consequently higher energy demands (Karakassis and Eleftheriou, 1997), are most likely the reasons for the low densities observed particularly in deeper stations. From the trophic point of view, a shift from detritus-feeding/suspension-feeding (mainly polychaetes, sipunculids and bivalves) in the seagrass biotope to carnivory in the offshore biotope emerged from our dataset, in line with lower levels of organic matter observed offshore.

2.5.2. Biodiversity patterns across the biotopes

Seagrass beds harboured the most distinctive assemblages as observed elsewhere (Schreider et al., 2003; Cacabelos et al., 2010). Several studies have highlighted the significant role of habitat-forming species, such as seagrasses, on macrobenthic patterns (Coles and McCain, 1990; Boström et al., 2006; Vonk et al., 2008). Macrobenthic assemblages associated with the seagrass biotope were not the most diverse but were the most abundant (603 ind.m$^{-2}$). The higher abundance of invertebrates within seagrass meadows compare to nearby unvegetated habitats has been previously reported (Carvalho et al., 2006b; Short et al., 2007; Duarte et al., 2008; Vonk et al., 2008; Włodarska-Kowalczuk et al., 2014). This pattern results from the effect of the seagrass canopy that leads to the decrease of hydrodynamics (Koch and Gust, 1999; Koch et al., 2007; Herkül and Kotta, 2009). The architecture of seagrass meadows combined with the low hydrodynamics will increase the amount of organic particles settling compared to nearby unvegetated areas (Davis et al., 2001; Adam Gartner et al., 2010). Further, it will enhance the populations of suspension feeders (Cerrato et al., 2004; Wall et al., 2008; Sokołowski et al., 2015). This pattern has been attributed to a combined effect of a higher availability of ecological niches with protection from predators (Wilson et al., 1987; Davis et al., 2001; Gartner et al., 2010), as well as to the quantity and quality of food sources (Bologna and Heck., 1999; Sirota and Hovel, 2006; Włodarska-Kowalczuk et al., 2014; Sokołowski et al., 2015). The current study suggests that these meadows have a fundamental role providing the suitable conditions for filter feeders. Indeed, the bivalves *Barbatia foliata* and
Cardiolucina semperiana were exclusive of this biotope that is associated with higher chlorophyll a concentrations compared to the offshore.

The more organically enriched sediments observed in this biotope seems also suitable for the colonization of detritivores. Of interest, the polychaetes of the family Capitellidae, known as opportunistetic organisms (r-selected), were exclusively associated with the seagrass biotope. Despite the relatively low abundance, it seems that the organic matter accumulated in the seagrass meadows is sufficient to allow for the colonization and successful establishment of the population of such opportunistic taxa. The lagoon is surrounded by extensive mangroves (Avicennia marina) that may export organic material to the nearby subtidal areas, with the potential for net accumulation. Seagrass beds in tropical regions have also been recognized as pivotal in retaining nitrogen in usually nutrient-poor areas (Vonk and Stapel, 2008). The high levels of abundance and diversity observed in the seagrass areas may reflect a positive response of the macrobenthic populations to the increased food availability derived from fresh or degraded products of mangrove leaf litter (Sullivan and Moncreiff, 1990; Robertson, 1991). Elsewhere, the role of mangroves providing organic material that is usable by macrobenthos has been widely debated, and it may depend namely on the region the studies are undertaken (temperate versus tropical areas) (Ellis et al., 2004; Alfaro, 2006). Whereas some studies on tropical areas suggest nearby mangroves provide a rich source of labile organic matter that seems to be the main source of plant detritus for benthic communities (Boto and Bunt, 1981), some stable isotope studies suggested that mangrove litter does not play a significant contribution to many levels in food webs in tropical and subtropical coastal system, including the secondary consumers of benthic habitats (Loneragan et al., 1997; Bouillon et al., 2002, 2004). Unfortunately, there are no studies available in the region so no further conclusions can be drawn.

Overall, the three biotopes identified in the present study were associated with high beta-diversity. The high number of biotope-specific taxa, mostly comprised of singletons, indicated that assemblages were extremely patchy in nature and contributed to the differences in the macrobenthic communities. This is further supported by the high values of LCBD for all samples. The high levels of variability in
tropical soft sediment habitats associated with coral reef systems is in agreement with previously reported results for the Indian Ocean (Semprucci et al., 2013; Navarro-Barranco and Guerra-García, 2016). Marine colonization patterns are strongly influenced by the surrounding habitats (Takada et al., 2014). In tropical regions, the coexistence of sedimentary (including coral rubble) and reef biotopes is frequent, broadening the local pool of potential colonizers. Seafloor heterogeneity associated with this mosaic of habitats and the resulting variability in the measured environmental parameters even within small spatial scales provide a wide range of potential ecological niches promoting high variation in species occupancy patterns (Barton et al., 2013). Indeed, environmental variability even at relatively small spatial scales has a positive effect on both species composition and abundance (Loiseau et al., 2017).

It is worth noticing that high beta diversity at local scales can be also due to low sampling effort relative to the study area (Steinbauer et al., 2012; Barton et al., 2013). In the present study, a relatively large number of samples (20) were collected in a very small geographic area (nearshore biotope). In the seagrass meadow the number was smaller but the area is also comparatively reduced. Overall, we consider the sampling effort adequate, but we do recognize that in regards to the offshore biotope additional sampling effort would be advisable. However, to minimize potential bias caused by the sampling effort, for the beta diversity analysis, each sample unit consists of merging two Van Veen grab samples (0.2m$^2$ sample-unit area) thus increasing the spatial resolution and reducing the variation in species occupancy.

Overall, shallow water macrobenthic patterns in sedimentary biotopes in the Red Sea probably result from a complex interaction of habitat characteristics (e.g. topographical features), environmental conditions, species interactions, and historical events. Altogether, they contribute to high levels of alpha- and beta-diversity (even at small spatial scales). The partitioning of beta-diversity and the dominance of turnover (or replacement) over richness (or nestedness) suggests that the observed community patterns may result from environmental filtering, which has been suggested as a major driver in areas of high abiotic stress (Sommer et al., 2014). The seagrass biotope has relatively higher similarity and lower replacement (or turnover) compared to the
coastal biotopes. This may be due to the higher organic matter (LOI) and lower concentrations of oxygen, which will reduce the set of species that can use the niches available (the low richness difference indicates a lower diversity of niches) and promote the dominance of some organisms, as observed for example for sipunculids.

2.5.3. Final remarks

Considering the current rates of habitat changes in coastal areas, the specificity of a considerable amount of taxa to a set of environmental parameters characteristic of a habitat makes the loss of biodiversity a constant threat. With the loss of marine habitats, biodiversity will decrease (Waycott et al., 2009), which will affect the functioning of coastal marine ecosystems (Worm et al., 2006; Saunders et al., 2014). Areas such as the Red Sea, where baseline knowledge on benthic diversity is lacking, and national monitoring plans are non-existent, are vulnerable to biodiversity loss that may not be fully recorded or understood given scientists are still discovering new species (e.g., Ravara and Carvalho, 2017) and fully assessing biodiversity patterns in the Red Sea region. Being one of the few studies undertaken in this subtropical sea, the present study makes a relevant contribution to the ecological and environmental drivers of the soft-sediment assemblages in oligotrophic and warm waters that have been considerably understudied. Further studies on habitat-dependent associations for an effective conservation of marine populations are needed worldwide (Schneider and Mann, 1991; Cacabelos et al., 2010). They are particularly needed for soft-sedimentary biotopes in sub-tropical and tropical areas, especially in the study area where research efforts have been extremely low compared to that on coral reef biotopes.

Without a clear understanding on how the composition of species and their abundance change along spatial scales and across environmental gradients, one cannot predict how communities will respond to environmental change, limiting management and conservation actions (Sommer et al., 2014). In the Red Sea, studies addressing soft-sediment macrobenthic communities are still in their infancy and as such knowledge is very limited. Therefore, more studies are needed across the whole basin to have a more solid understanding of these systems. Further investigations
addressing beta-diversity patterns are also needed as anthropogenic impacts do not necessarily result in beta-diversity loss (Socolar et al., 2016). While the value of integrating beta-diversity measures into management and conservation plans is clear, caution is needed when interpreting the patterns (Socolar et al., 2016). However, based on the current results, and the contribution of turnover (or replacement) to beta-diversity patterns, conservation measures should prioritize a network of different areas (not necessarily the richest ones) comprising a range of different biotopes to better protect biodiversity in the region (Baselga, 2010).
2.6. References


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Chapter 3

3. Composition, uniqueness and connectivity among habitats in a tropical coastal lagoon

Photo credits: Zahra Alsaffar

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3.1. Abstract

Tropical habitats and their associated environmental characteristics play a critical role in shaping macroinvertebrate communities. Considering patterns of diversity over space and time with the biotic and abiotic factors that control and shape those patterns is crucial for conservation efforts. However, these are still poorly understood in sub-tropical and tropical regions. The present study applied a combination of uni- and multivariate techniques to test whether patterns of biodiversity, composition, and structure of macrobenthic assemblages change across different yet typical lagoon habitats (two mangrove sites; two seagrass meadows with varying levels of vegetation cover; and an unvegetated subtidal area) between seasons and years. In total, 4771 invertebrates were identified belonging to 272 taxa. We observed that macrobenthic lagoon assemblages are diverse, heterogeneous and that the most evident biological pattern was spatial rather than temporally related. To investigate whether macrofaunal patterns within the lagoon habitats (mangrove, seagrass, and unvegetated area) changed through the time, we analysed each habitat separately. The results showed high seasonal and inter-annual variability in the macrofaunal pattern among habitats. However, the seagrass beds that are characterized by variable vegetation cover, through time, showed comparatively higher stability - with the lowest values of inter-annual variability and a high number of resident taxa. These results support the essential roles of seagrass habitat complexity in promoting diversity and density of macrobenthic assemblages. Despite the structural and functional importance of seagrass beds documented in this study, the results also highlighted the small-scale heterogeneity of tropical habitats that may serve as biodiversity repositories. Comprehensive approaches at the “seascape” level are required for better future ecosystem management and to maintain the connectivity patterns particularly along the coast of the Red Sea, currently facing rapid urbanization and industrial development. Also, considering the high temporal variability (seasonal and inter-annual) of tropical shallow-water habitats, monitoring and management plans must include temporal scales into account.

Keywords: Inter-annual variability, spatial variability, macrobenthic communities, tropical habitats, seascape approach, Red Sea
3.2. Introduction

Coastal lagoons are important transition systems providing essential socio-economic goods and services (e.g., shore protection, fisheries, carbon sequestration; Harley et al., 2006; Waycott et al., 2009; Camacho-Valdez et al., 2013). Coastal lagoons harbour well-adapted and sometimes unique assemblages of species, which play a vital role directly supporting local populations. These ecosystems are naturally stressed on daily to annual-time scales (Carvalho et al., 2005; Como and Magni, 2009; Kennish and Paerl, 2010; Pereira et al., 2010; Tagliapietra et al., 2016) and display high environmental variability (e.g. temperature, salinity, primary productivity, nutrients, dissolved oxygen). Such variability is reflected in the biological patterns that alter in response to the new environmental conditions. Lagoon ecosystems are also being increasingly affected by human disturbances that can compromise their ecological and socio-economic values (Tagliapietra et al., 1998; Magni et al., 2004; Como et al., 2007; Como and Magni, 2009; Newton et al., 2014).

Subtropical and tropical coastal lagoons encompass a range of essential soft-substrate habitats, such as mangroves, seagrasses and unvegetated bottoms that are associated with different environmental conditions and biological assemblages (Nagelkerken, 2009; Pusceddu et al., 2014; Navarro-Barranco and Guerra-García, 2016; Alsaffar et al., 2019). Spatial distribution patterns and connectivity in subtropical and tropical habitats have been mainly assessed for fishes and other mobile marine fauna (Nagelkerken and Van Der Velde, 2003; Mumby et al., 2004; Gratwicke and Speight, 2005; Mumby, 2006; Dorenbosch et al., 2007; Berkström et al., 2012; McMahon et al., 2012; Unsworth et al., 2008). Whereas studies describing and comparing macrobenthic distribution patterns and the strength of connectivity linkages across different shallow-water tropical lagoon habitats are particularly limited compared to temperate systems (e.g. Sheridan, 1997; Lee, 2008; Kathiresan and Alikunhi, 2011; Navarro-Barranco and Guerra-García, 2016; Skilleter et al., 2017). Another gap in knowledge is the role of temporal variability in driving macrobenthic patterns (see: Paiva, 2001; Taddei and Frouin, 2005; Bigot et al., 2006; Pech et al., 2007; Lamptey and Armah, 2008; Magni et al., 2015; Belal et al., 2016).
Even though in tropical regions seasonal changes are usually less marked than in temperate regions (O'Reilly, 2006), variability in benthic patterns exists (Posey et al., 1998; Rosa and Bemvenuti, 2006; Lamptey and Armah, 2008).

Investigating temporal variability patterns is essential to obtain a deeper knowledge of the dynamics and processes regulating lagoon communities. Indeed, considering the current scenario of global climate change, it is critical to better understand their patterns of change and particularly how they respond to changes in temperature and other key environmental drivers (Harley et al., 2006; Norderhaug et al., 2015). Temporal variation patterns in the abundance and composition of macrofaunal invertebrates have been intensively studied in temperate coastal ecosystems in relation to environmental variables (Ysebaert and Herman, 2002; Biles et al., 2003; Giberto et al., 2004; Shojaei et al., 2016). Temporal variability in temperature and food availability, for example, can influence recruitment events with consequences for the structure, distribution, and abundance of the community (Desroy and Retière, 2001; Reiss and Kröncke, 2005; Van Hoey et al., 2007). Similarly, sediment composition, organic matter, and vegetation cover, which may vary in time, are also main drivers of observed ecological patterns. However, as previously highlighted, most of those studies have been conducted in temperate regions and, more recently in polar habitats (e.g. Wlodarska-Kowalczyk and Pearson, 2004; Wlodarska-Kowalczuk et al., 2005; Mincks and Smith, 2007; Glover et al., 2008; Pawłowska et al., 2011). Comparatively, less attention has been dedicated to sub-tropical and tropical areas (e.g. Rueda et al., 2001; Guzmán-Alvis et al., 2006; Hernández-Guevara et al., 2008; Kanaya et al., 2011). This is even more striking in regards to the assessment of inter-annual variability but see (McCarthy et al., 2000; Nicolaidou et al., 2006; Magni et al., 2015).

The typical habitats in tropical coastal lagoons (such as mangrove, seagrass meadows and unvegetated bottoms) differ in environmental conditions, resulting not only from their location along the depth profile but also their structural complexity. Thus it is important to investigate macrobenthic distribution patterns both spatially and temporally across those habitats. Spatial differences in the community will provide information regarding the ecological requirements of species. For example, species able to colonize multiple habitats will most likely be less sensitive to environmental changes, whereas those more directly associated with a specific habitat may be less tolerant. In general, harsher environmental
conditions are observed in the intertidal area, dominated by mangrove trees, with conditions being attenuated with increasing depth, a pattern that is associated with a consistent increase in species richness and abundance (Kristensen et al., 2008; Dissanayake and Chandrasekara, 2014). Indeed, mangrove habitats are characterized as unfavorable environments influenced by high salinity, high fluctuation of temperature, desiccation, and poor soil condition (depleted oxygen) (Amarasinghe, 2018). On the other hand, if undisturbed, seagrass habitats provide comparatively more stable environmental conditions through time (Short and Wyllie-Echeverria, 1996; Mateo et al., 1997; Reusch et al., 1999) as well as protection from predators (Leopardas et al., 2014).

Assuming the existence of this environmental gradient towards the intertidal area (i.e. mangrove habitats), we hypothesise i) a decrease in species richness (i.e. the total number of species) and in the number of exclusive species from subtidal to intertidal areas as the environmental gradient due to a progressive exclusion of less resistant species. We also hypothesize that ii) shallow water seagrass meadows will harbour higher number of species particularly compared with unvegetated bottoms, as a result of habitat complexity, protection from predators and food availability (Jackson et al., 2002; Fredriksen et al., 2010; Barnes and Barnes, 2012). Likewise, we hypothesize iii) that temporal changes will be less evident in subtidal (vegetated and unvegetated) than intertidal habitats (Daniel and Robertson, 1990; Amarasinghe, 2018) and that within subtidal areas seagrasses will support more stable communities through time.

3.3. Material and Methods

3.3.1. Study area and sampling design

The present study was carried out in the Al Qadimah lagoon (22° 22’ 39.3″ N, 39° 07′ 47.2″ E) located in the central region of the Saudi Arabian Red Sea (Fig. 3.1 A). This shallow lagoon (average depth 2.19 m) has an approximate area of 14 km² and is not impacted by direct anthropogenic disturbances typical of other coastal lagoons (e.g. freshwater or sewage discharges, fisheries, habitat destruction from coastal development). It is, however, situated between two urbanized areas, which are in development (King Abdullah University of Science and Technology, 7000 inhabitants; King Abdullah Economic City, currently 5000 inhabitants but it is expected to reach 50,000 by 2020 and it will become a major tourist destination in
the near future) but that are not directly connected with the lagoon. Hence, it offers a rare opportunity to study the natural roles of environmental drivers in shaping macrobenthic communities inhabiting such critical systems.

Scattered along the extent of its margins, well-developed mangrove stands of *Avicennia marina* are observed. The bottom of the lagoon, particularly in the inner areas is characterized by more or less fragmented seagrass meadows. To depths of approximately 50cm, *Cymodocea rotundata* is the dominant species with smaller patches of *Cymodocea serrulata* also being present. Below this depth, seagrass meadows are mainly characterized by monospecific stands of *Enhalus acoroides* down to 2 m depth. Towards the sea, unvegetated bottoms with either sponges mixed with coral rubble or sand progressively replace seagrass meadows.

In the tropics (and in the Red Sea), there are two marked seasons (Fig. 3.1 B), winter (November-April) and summer (May-October). In order to investigate inter-annual and seasonal changes in macrobenthic patterns, samples were collected in two different dates in winter (January; March) and summer (June; September) of 2014 and 2015.

Five permanent soft-sediment habitats typical of tropical coastal lagoons were selected: 1) upper mangrove area (M1); 2) deeper mangrove area (M2); 3) shallow seagrass meadow (S1, mix meadows of *Cymodocea serrulata* interspaced with *Cymodocea rotundata*; relatively high cover all year round); 4) deeper seagrass meadow (S2, monospecific stands of *Enhalus acoroides* with high variability in the vegetation cover throughout the study period); and 5) unvegetated soft-sediments (Fig. 3.1 A). The unvegetated sandy substrate was located between 8 and 10 m depth. Due to the widespread distribution of seagrasses, mangroves and in order to minimize the direct influence of those habitats on the colonization patterns of unvegetated areas, the site was located at the entrance of the lagoon.
Fig. 3.1. Map showing the locations of the habitats in the lagoon (A); annual variability in sea surface temperature is shown in plot (B).
3.3.2. Sampling strategy

At each habitat and sampling period, conductivity, temperature, and depth (CTD) casts were carried out with a multiparameter probe (OCEAN SEVEN 316 Plus and 305 Plus). The CTD casts also recorded oxygen saturation in the water column. Water samples for the analysis of chlorophyll \( a \) (chl \( a \)) were collected using a Niskin bottle at each station (2 L per station). Sediment samples were collected using a 0.1 m\(^2\) Van Veen grab in the seagrass meadows and the unvegetated area (subtidal stations), whereas in the mangrove habitats (intertidal), samples were collected using hand corers (3 x 10 cm i.d. making one replicate; total area per replicate ~0.024 m\(^2\)). In 2014, two replicates at each site and sampling date were taken for the study of macrobenthic communities, with additional samples being collected for the study of environmental variables (grain particle size distributions and organic matter content). In 2015, the same approach was followed but collecting three replicates for macrobenthic communities. Macrobenthic samples were sieved through 1 mm mesh screens and preserved in 96% ethanol.

3.3.3. Laboratory analyses

In order to estimate the primary production in the sampling area, the concentration of chlorophyll \( a \) was quantified by fluorescence using the EPA method 445.0 (Arar and Collins 1997). Water samples (500 ml) were collected using a Niskin bottle and filtered using GF/F filters as soon as possible. The filters were preserved at -80°C until extraction of the pigments. 10ml of 90% acetone were used for each extract and left for 24h in cold and dark conditions to minimize degradation. The procedure was undertaken in low light conditions as to minimize degradation. A Turner Trilogy® fluorometer (Turner Designs) was used to quantify the chlorophyll \( a \) content using an acidic module. The degradation of the chlorophyll \( a \) to phaeophytin was accomplished by acidifying the sample with 60\( \mu \)l of 0.1 N HCl.

Sediment samples were sorted after all the vegetation associated with sediment was removed. Organisms were whenever possible identified to the species level. Vegetation biomass (seagrass leaves, roots, and mangrove material) was quantified per replicate.

Grain particle-size distribution was quantified after initial wet sieving of the samples (63 \( \mu \)m mesh) to separate the silt and clay fraction from sandy fractions and gravel. The retained...
fractions were dried at 80°C for 24-48h. The dried sandy and gravel sample was then mechanically sieved by using a column of sieves to separate the sandy fractions and the gravel as follows: <63μm, silt-clay; 63-125μm, fine sand; 250-500μm, medium sand; 1000-2000μm, coarse sand; > 2000μm, gravel.

The organic content of the sediments was determined by loss on ignition (LOI). Sediments were dried for 24-48h at 60°C and then the samples were placed in the muffle furnace at 450°C for 4h. After cooling in a desiccator for 30min, samples were weighed and the LOI was calculated using the following equation (Heiri et al., 2001):

$$\text{LOI} = \frac{(W_i - W_f)}{W_i} \times 100$$

Where: LOI = Organic Matter content (%), $W_i$ = Initial weight of the dried sediment subsample; $W_f$ = Final weight after ignition.

3.3.4. Data analyses

3.3.4.1. General patterns

Macrobenthic patterns were analysed through a combination of univariate and multivariate techniques. Several univariate metrics were calculated including the total number of taxa ($S$, species richness), density (ind. x m$^{-2}$), and Shannon-Wiener ($H'$). Considering the different sampling methods, and the dependency of species richness on sample size (Chao, 1987), estimates of species diversity were also calculated and compared with $S$. The nonparametric species richness estimators used: Chao 1, jacknife 1 order and Bootstrap all follow an asymptotic approach to estimate the number of undetected species richness. These estimators are commonly used in ecological studies because they are simple, intuitive, relatively easy to use and perform reasonably well (Gotelli and Colwell, 2001). In order to have a balanced number of replicates, the analyses were conducted for two replicates, with those collected in 2015 being randomly selected.

The biased corrected form of Chao1 estimator (Chao, 1987; Chiu et al., 2014) uses the number of singletons and doubletons to estimate the lower bound of species richness. The first order jacknife estimator (Smith and van Belle, 1984) assumes that the number of species that are missed equals the ones that were seen once (singletons). The Bootstrap estimator is based
on the assumption that if the same data are resampled with replacement the number of missing species after resampling will be similar to those missed originally (Smith and van Belle, 1984). All estimators were calculated using the open source software R (R Core Team, 2018) using function “specpool” from “vegan” package (Oksanen et al., 2018). Abundance data were used for the calculations of all estimators.

To visualize multivariate patterns of abundance in macrobenthic communities within the seascape, non-metric multidimensional scaling (nMDS) was applied based on the Bray-Curtis dissimilarities matrix. Given the differences among habitats for some dominant species, when comparing habitats (i.e. full dataset), Bray-Curtis dissimilarities matrices were calculated using untransformed abundance data. Separate nMDS plots were generated for each one of the sites for a better visualization of the temporal variability. These analyses were also based on untransformed data. Within each sites, significant variability in the multivariate patterns over time was analysed initially according to a three-factor design (Year; Season; Date, nested within Season) using Permutational Multivariate Analyses of Variance (PERMANOVA). As the factor “Date” was found not significant, and to increase the power of the analyses, a two-factor PERMANOVA was applied. Whenever significant differences in the interaction term were detected (i.e. Year x Season), pair-wise comparisons tests were conducted.

3.3.4.2. Connectedness within the seascape and stability patterns over time

A preliminary investigation of the patterns of variability across the seascape was carried out to identify generalist versus specialist taxa, i.e. those that span across multiple habitats versus those that are particularly associated with a specific habitat, respectively. We aimed to characterize the main differences in the community patterns in terms of shared and exclusive species that could determine the cause of the connectivity across the lagoon. This analyses was conducted based on the whole dataset, disregarding the seasonal and annual changes, as our main question was related to the constancy of spatial changes in different habitats.

Finally, we analysed the frequency of occurrence of species in each habitat during the study period. Species were classified based on Habitat Preference Trait as follows: i) resident, present in over 85% of the sampling dates (i.e. eight events); ii) frequent, observed between 50% and 85% of the dates; iii) occasional, presence registered in between 25% and 50% of
the sampling occasions; iv) rare, observed in less than or equal to 25% of the sampling dates; v) seasonal, only observed in one season but in both years.

Community stability was also examined over the sampling period within each habitat based on the indices Bray-Curtis (community structure) and Jaccard (presence/absence; composition). Within each habitat, variability between all pairwise comparisons among terms of interest (e.g. within and between seasons; within and between years) was analysed. We established that low levels of similarity are related to high variability in the macrobenthic communities over time, whereas high similarity is indicative of more stable communities.

3.3.4.3. Relationships between environmental variables and assemblage structure

Distance-based redundancy analysis (dbRDA) was used to assess the relationship between each environmental variable and the variation in the community structure (given by the direction and length of vectors for each variable). The variables used for the analyses were salinity, temperature, depth, grain size fractions, organic matter content (% LOI), and chl a. Three of the variables were transformed to reduce skewness, namely the fines and coarse sand fractions of the sediment (square root) and organic matter content (natural log). Marginal tests are used to show the significance of each variable individually to the model and sequential tests show the subset of explanatory variables that significantly contribute to explain the biological patterns.

3.4. Results

3.4.1. Macrobenthic community composition: general characterization and connectivity among habitats

A total of 4771 invertebrates were identified, including 272 operational taxonomic units (OTUs) distributed among 11 phyla, 16 classes, 40 orders, and 80 families. Annelida dominated both in abundance and number of taxa, contributing to, respectively, 51.0% and 42.0% of the total values. Sipuncula (15.0%), Arthropoda (13.0%), Mollusca (12.0%), and Echinodermata (7.0%) also contributed to the overall density. Regarding the number of species, Arthropoda (28.0%) and Mollusca (18.0%) were, along with Annelida, the most speciose phyla.
At the species level, the sipunculid *Phascolion (Phascolion) strombus strombus* (12.2% of the total abundance) was the most abundant species, followed by *Simplisetia erythraeensis* (5.8%), *Eunice indica* (4.4%), *Metaprotella africana* (3.3%), *Ceratocephale* sp (3.3%), *Barbatia foliata* (2.7%), *Aonides* sp. (2.7%), *Lumbrineris* sp.1 (2.7%), *Lysidice unicornis* (2.6%) and *Paphies elongata* (2.4%). Most of these taxa were found in at least four of the studied sites, except for *Metaprotella africana* (exclusive to S1) and *Barbatia foliata*, exclusive to seagrass habitats (S1 and S2). All the remaining taxa contributed to less than 2% of the total abundance.

The high variability patterns in the seagrass biomass along the study period (Fig. 3.2), was reflected in the biological changes. When analysing the full dataset and regardless the diversity metric considered, S2 consistently presented the highest number of taxa (155, observed; 184.8-219.7, estimated), whereas the opposite pattern was overall found at the M2 site. Density was also higher at S2 (801.9 ind.m$^{-2}$) and lowest at the unvegetated area (388.8 ind.m$^{-2}$) (Table 3.1).

![Fig. 3.2. Biomass of seagrass plants along the study period (2014-2015) in both seagrass stations.](image-url)
Table 3.1. Total number of Operational Taxonomic Units (OTUs), estimated number of taxa based on Chao, Jacknife (1st order) and Bootstrap, and average density (ind. m\(^{-2}\)) per habitat. M1 and M2, mangrove; S1 and S2, seagrass. Highest value per metric is presented in bold.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>No. OTUs</th>
<th>Chao</th>
<th>Jacknife</th>
<th>Bootstrap</th>
<th>Av. Density (ind. m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>65</td>
<td>113.0 ± 23.80</td>
<td>95.0 ± 12.92</td>
<td>78.0 ± 6.94</td>
<td>634.4</td>
</tr>
<tr>
<td>M2</td>
<td>38</td>
<td>151.9 ± 79.76</td>
<td>63.3 ± 8.51</td>
<td>48.1 ± 3.81</td>
<td>434.4</td>
</tr>
<tr>
<td>S1</td>
<td>119</td>
<td>177.8 ± 21.07</td>
<td>171.5 ± 18.13</td>
<td>142.5 ± 9.68</td>
<td>722.5</td>
</tr>
<tr>
<td>S2</td>
<td>155</td>
<td>212.2 ± 18.19</td>
<td>219.7 ± 18.29</td>
<td>184.8 ± 9.36</td>
<td><strong>801.9</strong></td>
</tr>
<tr>
<td>Unvegetated</td>
<td>121</td>
<td>156.2 ± 13.81</td>
<td>163.2 ± 14.94</td>
<td>140.9 ± 8.05</td>
<td>388.8</td>
</tr>
</tbody>
</table>

To visualize the patterns of variability within the lagoon seascape, a non-MDS based on the Bray-Curtis dissimilarity index was plotted (Fig. 3.3 A). Patterns show that the macrobenthic community was structured by habitat with little overlap of samples from the different habitats. The environmental data that were collected and used for the analysis partially explains the multivariate variability of the biological data with the two main dbRDA axes explaining more than half of the constrained variability but only 19.1% of the total variability of the biological communities. However, the dbRDA plot reinforces a clear separation of the communities inhabiting mangrove areas, S1, and the unvegetated habitat, whereas S2 presents affinities with either S1 or mangrove stations depending on the sampling period (Fig. 3.3 B). Samples from the unvegetated habitat were associated with depth and percentages of mid and fine sand. Seagrass habitats (particularly S1) were separated based on the higher silt and clay (fine particles) content, whereas mangrove habitats presented a slightly higher percentage of coarse sand. Considering that the nature of the biotope itself seems to determine the composition and structure of macrobenthic communities, to increase the resolution when investigating the temporal variability, analyses was undertaken on the habitats separately.
Fig. 3.3. Multivariate analysis of the community data. A) Ordination (non-metric multivariate dimensional analysis) and classification diagram of the sampling habitats based on the Bray-Curtis dissimilarity on non-transformed data. B) Distance-based redundancy analysis (dbRDA) plot of based on a set of environmental variables; salinity, temperature, depth, grain size fractions (coarse sand, medium sand, fine sand, fines), organic matter: LOI (%) and chlorophyll a on biological data from lagoon habitats; M1 and M2, mangrove; S1 and S2, seagrass and unvegetated area (Unv). The points represented the sampling events (winter1, winter2, summer1, and summer2) for 2014 and 2015. Coarse sand and fines data are square root transformed and LOI log$_e$ transformed. Length and direction of vectors indicate the strength and direction of the relationship.
3.4.2. Alpha-diversity: temporal variability within habitats

In terms of average number of OTUs per sampling date, subtidal habitats were in general richer than the intertidal mangrove areas (Fig. 3.4 A), with M2 showing a consistently depressed number of taxa across all sampling dates. Subtidal habitats were not only richer but also displayed higher variability through time. Density was overall less variable among habitats except for the seagrass communities (Fig. 3.4 B). M2 also presented the lowest Shannon-Wiener values whereas, in general, higher values were determined for S2 or the unvegetated habitat (Fig. 3.4 C).

Fig. 3.4. Alpha-diversity metrics per habitat and over time. A, number of OTUs, B density and C Shannon-Wiener diversity. M1 and M2, mangrove; S1 and S2, seagrass and unvegetated area (Unv).
3.4.3. Consistency of multivariate patterns within habitat types over time

Biological similarity within each habitat was markedly low, ranging from 14% (M2) to 25% (S1) (Table 3.2). Both habitats also showed a higher dominance with only four and six species contributing to over 62% of the habitat’s abundance, respectively. In the remaining habitats, a minimum of 13 taxa was needed to reach the same level of abundance (Table 3.2). Except for S1, where none of the dominant taxa was a polychaete, this group dominated all the other habitats. S1 was dominated by a sipunculid (*Phascolion (Phascolion) strombus strombus*), two bivalves (*Barbatia foliata* and *Cardiolucina semperiana*), one amphipod (*Metaprotella africana*) and two echinoderms (*Aquilonastra burtoni* and *Amphioplus cyrtacanthus*).

Temporal variation in the structure of macrobenthic assemblages within each habitat examined on the basis of the Bray-Curtis and Jaccard resemblance measures indicated different patterns depending on the habitat in analyses. Major differences were not detected between metrics and therefore only plots for Bray-Curtis matrices are presented (Fig. 3.5).
Fig. 3.5. Non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity matrices based on untransformed data, for temporal variation in the structure of macrobenthic assemblages within each habitat. M1 and M2, mangrove; S1 and S2, seagrass and unvegetated area (Unv).
PERMANOVA results confirmed different temporal trajectories in the analysed habitats (Table 3.3). Both resemblance metrics applied to M1 and S1 datasets showed a significant interaction of the main factors (Year x Season). The pair-wise tests indicated for M1 a significant inter-annual difference both in winter and summer. For S1, inter-annual differences were only detected in winter. With regard to seasonal differences, S1 presented significant variability in both years (except in the composition – Jaccard - for 2015) but in M1 differences were only detected in 2014 (Table 3.3).

Macrobenthic communities at M2 and S2 showed significant inter-annual variability (except for S2 with presence/absence) (Table 3.3). Finally, the unvegetated area showed significant and independent seasonal and inter-annual variability (Table 3.3).
Table 3.2. Cumulative percentage of the taxa (Cum%) contributing to more than 60% of each habitat’s total abundance. For each taxa, it is provided the taxonomic group (Tax. Group) and the Habitat Preference Trait (HPT). Pol, Polychaeta; SIP, Sipuncula; BIV, Bivalvia; DEC, Decapoda; NEM, Nemertea; AMP, Amphipoda; Cum, Cumacea; OPH, Ophiuroidea; AST, Asteroidea; CEPH, Cephalochordata; ECH, Echinoidea; SCA, Scaphopoda; GAS, Gastropoda. RES, resident; FRE, frequent; OCCA, occasional; SEASONAL and RARE. See text for further explanations.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Tax. Group</th>
<th>Cum%</th>
<th>HPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 – Overall similarity 18%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceratocephale sp.</td>
<td>POL</td>
<td>8.9</td>
<td>OCCA</td>
</tr>
<tr>
<td>Phascolion (Phascolion) strombus strombus</td>
<td>SIP</td>
<td>16.7</td>
<td>FRE</td>
</tr>
<tr>
<td>Protodorvillea sp.</td>
<td>POL</td>
<td>24.6</td>
<td>RARE</td>
</tr>
<tr>
<td>Eunice indica</td>
<td>POL</td>
<td>30.5</td>
<td>FRE</td>
</tr>
<tr>
<td>Diogenes costatus</td>
<td>DEC</td>
<td>35.3</td>
<td>FRE</td>
</tr>
<tr>
<td>Lysidice unicornis</td>
<td>POL</td>
<td>38.8</td>
<td>OCCA</td>
</tr>
<tr>
<td>Simplicetia erythraeensis</td>
<td>POL</td>
<td>42.4</td>
<td>OCCA</td>
</tr>
<tr>
<td>Aspidosiphon sp.</td>
<td>SIP</td>
<td>45.5</td>
<td>RARE</td>
</tr>
<tr>
<td>Syllis hylebergi</td>
<td>POL</td>
<td>48.7</td>
<td>OCCA</td>
</tr>
<tr>
<td>Marphysa macintoshi</td>
<td>POL</td>
<td>51.7</td>
<td>RES</td>
</tr>
<tr>
<td>Scoleotoma sp.</td>
<td>POL</td>
<td>54.5</td>
<td>OCCA</td>
</tr>
<tr>
<td>Thalamita poissonii</td>
<td>DEC</td>
<td>57.2</td>
<td>OCCA</td>
</tr>
<tr>
<td>Linopherus sp.</td>
<td>POL</td>
<td>59.6</td>
<td>RARE</td>
</tr>
<tr>
<td>Nemertea</td>
<td>NEM</td>
<td>62.0</td>
<td>OCCA</td>
</tr>
<tr>
<td>M2 - Overall similarity 14%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simplicetia erythraeensis</td>
<td>POL</td>
<td>32.1</td>
<td>FRE</td>
</tr>
<tr>
<td>Paphies elongata</td>
<td>BIV</td>
<td>48.8</td>
<td>RARE</td>
</tr>
<tr>
<td>Ceratocephale sp.</td>
<td>POL</td>
<td>58.0</td>
<td>OCCA</td>
</tr>
<tr>
<td>Marphysa adenensis</td>
<td>POL</td>
<td>63.1</td>
<td>OCCA</td>
</tr>
<tr>
<td>S1 - Overall similarity 25%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phascolion (Phascolion) strombus strombus</td>
<td>SIP</td>
<td>28.3</td>
<td>RES</td>
</tr>
<tr>
<td>Metaprotella africana</td>
<td>AMP</td>
<td>42.1</td>
<td>RARE</td>
</tr>
<tr>
<td>Barbatia foliata</td>
<td>BIV</td>
<td>49.6</td>
<td>RES</td>
</tr>
<tr>
<td>Aquilonastra burtoni</td>
<td>AST</td>
<td>56.5</td>
<td>RES</td>
</tr>
<tr>
<td>Amphioplus cyrtacanthus</td>
<td>OPH</td>
<td>59.9</td>
<td>FRE</td>
</tr>
<tr>
<td>Cardiolucina semperiana</td>
<td>BIV</td>
<td>62.2</td>
<td>RES</td>
</tr>
<tr>
<td>S2 - Overall similarity 19%</td>
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</tr>
<tr>
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<td>12.1</td>
<td>RES</td>
</tr>
<tr>
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<td>POL</td>
<td>20.0</td>
<td>RES</td>
</tr>
<tr>
<td>Lumbrineris sp1</td>
<td>POL</td>
<td>27.1</td>
<td>RES</td>
</tr>
<tr>
<td>Aonides sp.</td>
<td>POL</td>
<td>33.5</td>
<td>OCCA</td>
</tr>
<tr>
<td>Lysidice unicornis</td>
<td>POL</td>
<td>39.1</td>
<td>FRE</td>
</tr>
<tr>
<td>Amphioplus hastatus</td>
<td>OPH</td>
<td>43.7</td>
<td>RES</td>
</tr>
<tr>
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<td>46.9</td>
<td>OCCA</td>
</tr>
<tr>
<td>Paradoneis lyra</td>
<td>POL</td>
<td>49.9</td>
<td>OCCA</td>
</tr>
<tr>
<td>Euclymene spp.</td>
<td>POL</td>
<td>52.8</td>
<td>FRE</td>
</tr>
<tr>
<td>Notomastus sp.</td>
<td>POL</td>
<td>55.3</td>
<td>FRE</td>
</tr>
<tr>
<td>Cardiolucina semperiana</td>
<td>BIV</td>
<td>57.7</td>
<td>FRE</td>
</tr>
<tr>
<td>Pseudosympodomma persicum</td>
<td>CUM</td>
<td>59.8</td>
<td>FRE</td>
</tr>
<tr>
<td>Goniada multidentata</td>
<td>POL</td>
<td>61.6</td>
<td>RES</td>
</tr>
<tr>
<td>Unvegetated - Overall similarity 16%</td>
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<td></td>
</tr>
<tr>
<td>Eunice indica</td>
<td>POL</td>
<td>5.5</td>
<td>FRE</td>
</tr>
<tr>
<td>Aonides sp.</td>
<td>POL</td>
<td>10.0</td>
<td>SEASONAL</td>
</tr>
<tr>
<td>Species</td>
<td>Habitat</td>
<td>Percentage</td>
<td>Category</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>Lumbrineris sp2</td>
<td>POL</td>
<td>13.7</td>
<td>OCCA</td>
</tr>
<tr>
<td>Ampelisca brevicornis</td>
<td>AMP</td>
<td>17.2</td>
<td>FRE</td>
</tr>
<tr>
<td>Ancilla sp3</td>
<td>GAS</td>
<td>20.3</td>
<td>OCCA</td>
</tr>
<tr>
<td>Glycine bonhourei</td>
<td>POL</td>
<td>23.3</td>
<td>OCCA</td>
</tr>
<tr>
<td>Schizaster gibberulus</td>
<td>ECH</td>
<td>26.2</td>
<td>FRE</td>
</tr>
<tr>
<td>Diplocirrus sp.</td>
<td>POL</td>
<td>28.9</td>
<td>SEASONAL</td>
</tr>
<tr>
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<td>SIP</td>
<td>31.7</td>
<td>OCCA</td>
</tr>
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<td>NEM</td>
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<td>RARE</td>
</tr>
<tr>
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<td>DEC</td>
<td>38.6</td>
<td>OCCA</td>
</tr>
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<td>Morphysea adenensis</td>
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<td>OCCA</td>
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<td>Magelona cincta</td>
<td>POL</td>
<td>49.0</td>
<td>OCCA</td>
</tr>
<tr>
<td>Macrophthalmus graeffei</td>
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</tr>
<tr>
<td>Amphiodia duplicata</td>
<td>OPH</td>
<td>52.4</td>
<td>OCCA</td>
</tr>
<tr>
<td>Antalis rossati</td>
<td>SCA</td>
<td>54.0</td>
<td>OCCA</td>
</tr>
<tr>
<td>Dentalium bisexangulatum</td>
<td>SCA</td>
<td>55.6</td>
<td>OCCA</td>
</tr>
<tr>
<td>Branchiostoma lanceolatum</td>
<td>CEPH</td>
<td>57.1</td>
<td>OCCA</td>
</tr>
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<td>Chaetozone setosa</td>
<td>POL</td>
<td>58.4</td>
<td>OCCA</td>
</tr>
<tr>
<td>Euclymene spp</td>
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<td>59.6</td>
<td>OCCA</td>
</tr>
<tr>
<td>Streblosoma persica</td>
<td>POL</td>
<td>60.9</td>
<td>RARE</td>
</tr>
</tbody>
</table>

**Fig. 3.6.** The illustration shows the highest percentage of resident taxa in the two seagrass habitats (S1 and S2) in terms of the number of individuals, compared to mangrove and unvegetated areas.
Table 3.3. Two-way PERMANOVA model and pair-wise tests based on Bray-Curtis and Jaccard matrices within habitats among seasons and year (Year and Season interaction; Yr x Se). Significant values are shown in bold in the main test. M1 and M2, mangrove; S1 and S2, seagrass and unvegetated area (Unv).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Source</th>
<th>df</th>
<th>Bray-Curtis</th>
<th>Jaccard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yr</td>
<td>1</td>
<td>M1</td>
<td></td>
<td>14075</td>
<td>6.5403</td>
</tr>
<tr>
<td>Se</td>
<td>1</td>
<td></td>
<td></td>
<td>799.6</td>
<td>3.7171</td>
</tr>
<tr>
<td>Yr x Se</td>
<td>1</td>
<td>M1</td>
<td></td>
<td>3967.2</td>
<td>1.8434</td>
</tr>
<tr>
<td>Res</td>
<td>12</td>
<td></td>
<td></td>
<td>2152</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
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<td></td>
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<td>M2</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>3050.7</td>
<td>0.85902</td>
</tr>
<tr>
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<tr>
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<td></td>
<td></td>
<td>3551.4</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yr</td>
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<td>S1</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Yr x Se</td>
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<tr>
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<td></td>
<td></td>
<td>2347.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yr</td>
<td>1</td>
<td>S2</td>
<td></td>
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<td></td>
<td>Unv</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>Unv</td>
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<tr>
<td>Res</td>
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<td></td>
<td></td>
<td>2725.5</td>
<td></td>
</tr>
</tbody>
</table>

**Pair-wise tests**

*Term 'Yr x Se' for pairs of levels of factor 'Year'*

Within level 'Su' of factor 'Season'

<table>
<thead>
<tr>
<th>M1</th>
<th>Groups</th>
<th>t</th>
<th>P(perm)</th>
<th>T</th>
<th>P(perm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014, 2015</td>
<td>2.2928</td>
<td>0.026</td>
<td>2.0907</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Within level 'W' of factor 'Season'

| M1            | 2014, 2015 | 1.7421 | 0.028   | 1.5051 | 0.038   |

| S1            | 2014, 2015 | 2.0553 | 0.023   | 1.5675 | 0.022   |

*Term 'Yr x Se' for pairs of levels of factor 'Year'*

Within level '2014' of factor 'Year'

| M1            | Su, W   | 2.1788 | 0.03    | 1.9213 | 0.029   |

Within level '2015' of factor 'Year'

| S1            | Su, W   | 1.2833 | 0.031   | 1.4205 | 0.037   |

| S1            | Su, W   | 1.3824 | 0.029   |        |         |
3.4.4. Habitat preference

Only eight taxa (3% the total number of taxa) spanned across the five habitats. Most of them were polychaetes (*Capitellethus* sp., *Drillonereis* sp., *Euclymene* spp., *Lumbrineris* sp.1, *Lysidice unicornis*, *Notomastus* spp.). Nemertea (und.) and the sipunculid *Phascolion* (*Phascolion*) *strombus strombus* were also observed across the five sites. *Simplisetia erythraeensis* was absent from the unvegetated site. There were 62 taxa shared between intertidal and subtidal sites, and only 18 exclusive species to the mangrove habitats (as a whole), representing 6.6% of the of the gamma diversity of all the samples (2.2%, M2; 4.4%, M1). On the other hand, subtidal habitats showed a rather consistent percentage of exclusive species, ranging from 29.4% in S1, 32.3% in S2 and 33.8% in the unvegetated areas (S1: 12.8%; S2: 18.4%; Unvegetated: 15.1% of the gamma diversity, i.e. the total number of taxa observed in the lagoon).

Both seagrass habitats showed a higher percentage of resident species compared to mangrove and unvegetated areas (Table 3.2). In terms of the number of individuals, those taxa contributed to 45.0% and 34.0% for S1 and S2, respectively, of the site’s total abundance (Fig. 3.6). S2 showed a more balanced distribution of the four habitat preference traits analysed and relatively stable numbers throughout the study period (Table 3.2). Regardless of the habitat, occasional species accounted for more than 12.6% of the total number of species.

3.5. Discussion

This study investigated the distribution patterns of macrobenthic communities inhabiting adjacent shallow-water habitats in a tropical coastal lagoon with particular focus on how they are connected and how communities within each habitat vary over time. Despite its small area, the Al Qadimah lagoon, similar to other sub-tropical and tropical lagoons, encompasses a wide range of habitats including both hard (not addressed here) and soft-substrates. Within the latter, changes in the vegetation cover result in a mosaic of habitats with different sedimentary properties that will determine the structure of local macrobenthic communities (Sokołowski et al., 2015). Indeed, with regard to the soft-substrates, a clear zonation of the benthic patterns was detected, driven by habitat-related factors acting at varying spatial scales. The present results also provide new insights into the temporal variability (seasonal
and inter-annual) of different lagoon shallow-water habitats in a tropical seascape, information that is currently limited.

3.5.1. Uniqueness of lagoon habitats within the seascape

A clear pattern of habitat-dependent association was observed with the different habitats harbouring distinct macrobenthic assemblages both in terms of composition (presence/absence) and structure (Bray-Curtis dissimilarity). The high spatial variability of macrofaunal patterns within the lagoon habitats is most likely linked to the heterogeneity of the seascape and to the high contribution of rare species to the overall abundance. Recent studies showed that biological variability is driven by the relative contribution of rare and common species, where rare species playing a major role in the temporal patterns due to their vulnerability to fluctuations with changes in environmental (e.g. Ellingsen et al., 2007; Benedetti-Cecchi et al., 2008).

Not surprisingly, most of the taxa were observed in the subtidal habitats, with 70% of the total number of species being exclusive of those habitats. Seagrass habitats showed the highest number of taxa in most of the sampling periods, which agrees with previous studies (Edgar, 1990; Nakamura and Sano, 2005; Fredriksen et al., 2010; Wlodarska-Kowalczuk et al., 2014; Sokolowski et al., 2015) but variability was high and significant differences within the subtidal area were not detected. Among the factors that have been indicated as favouring diverse communities, is the structural complexity provided by the canopy and the developed rhizome and root systems that contribute to sediment stability (Hendriks et al., 2008; Herkül and Kotta, 2009; Leopardas et al., 2014). In areas such as the tropics, the canopy can play an even more determinant role providing shadow that can attenuate the effects of temperature (Tagliapietra et al., 2016) that in the study region can reach over 32°C in the summer. However, the present results showed that the S1 habitat, which has overall the highest and the most stable vegetation cover, does not support the highest number of species or density. Indeed, S2, which displayed higher variability in the cover during the study period, contained the highest number of taxa and density of individuals. This observation suggests that dense seagrass meadows are not always the most favourable habitats for several invertebrates, even though this result may be site-dependent (Schneider and Mann, 1991; Barberá-Cebrián et al., 2002; González-Ortiz et al., 2014; McCloskey and Unsworth, 2015). For example, dense
vegetation can physically obstruct the movement of burrowing large macroinvertebrates (Ringold, 1979; Sokołowski et al., 2015), or despite the increased aeration within the sediment due to the developed root system (Mateo et al., 2006), the decomposition of the increased amounts of organic matter will require higher oxygen consumption demands and result in anoxic regions and accumulation of toxic products (Santschi et al., 1990; Hyland et al., 2005). Therefore, vegetated areas with low cover might harbour comparatively higher species numbers as a result of species avoiding toxic anoxic conditions in densely covered areas (Pihl et al., 1999). Also, S2 harboured the highest number of exclusive species (50; 32.3% of the site’s total number of species), in contrast with M2 (6 exclusive species, 15.8% of the site’s total number of species). The same pattern was noticed when we looked at species diversity as a whole, mangrove habitats showed the lowest number of species compared to nearby seagrass and unvegetated substrates. Once more, this is in agreement with previous studies (Dittmann, 2001; Alfaro, 2006). The same pattern though does not hold true for abundance (Lee, 2008). Within mangrove habitats species encounter harsh physical environmental conditions (e.g. high salinity, hypoxia, desiccation, high concentration of toxins) and in general nitrogen limitation (C/N ratio often >100) due to a low nutritional value of the main source of organic matter, i.e. leaf litter (Lee, 2008). Mangroves in the Red Sea, however, have been found to be more carbon limited compared to other locations (Almahasheer et al., 2017). However, as a result of these conditions, and despite the structural complexity offered by these habitats, populations of a few tolerant / opportunistic species will dominate the macrobenthic communities (Ingole et al., 2009; Lee, 2008). Indeed, high dominance levels were particularly evident in the deepest mangrove area (M2) where only four species, the polychaetes Simplicetta erythraeensis, Ceratocephale sp. and Marphysa adenensis, along with the bivalve Paphies elongata contributed to over 60% of the total abundance. In the shallowest mangrove area, on the other hand, despite the dominance of polychaetes, the sipunculid (Phascolion (Phascolion) strombus strombus) and some decapods (Diogenes costatus and Thalamita poissonii) were also dominant. Decapods are critical players for the ecosystem functioning of these habitats by processing leaf litter and oxygenating sediment through their burrows (Geist et al., 2012; Fusi et al., 2015) and therefore their dominance in the habitat is not surprising.
3.5.2. Connectedness and stability at the scale of the seascape

It is worth mentioning that even though nearby seagrass meadows differ in cover and depth location, harbouring distinct faunal communities (sharing 35.0% of total number of species). Higher similarities (=higher connectivity) were detected among subtidal habitats than between those and mangroves (intertidal habitats). Nevertheless, 62 taxa, representing 22.8% of the gamma diversity, were shared between intertidal and subtidal habitats, suggesting that several species may utilize contrasting yet adjacent habitats within the lagoon seascape. Despite the fact that the overlap of species across the five habitats is lower (eight taxa; 2.9% of the total number of taxa) than previously reported (Pante et al., 2006; Ludovisi et al., 2013), the present study confirms the connectivity between intertidal and subtidal areas and the need for integrated management measures. The results obtained may suggest that the area is not highly hydrodynamic but information on the hydrographic patterns is not available. The effect of tides can result in displacement of specimens through water movement (Norkko et al., 2001) and depending on the their height can also expose organisms to desiccation for variable periods of time, with several factors influencing mangrove species distribution may limited the distribution of most of the species toward the intertidal area. Specially, when analysed together, mangrove habitats contributed to 6.6% (M1, 4.4%; M2, 2.2%) of the gamma diversity, contrasting with the unvegetated subtidal area and the seagrass meadows that supported, respectively, 15.1% and 31.3% (S1, 12.9%; S2, 18.4%).

Mangrove forests are known to produce relatively large amounts of organic matter through the conversion of leaf litter into detritus (Kristensen et al., 2008) that are later exported to nearby habitats (Gong et al., 1980; Camilleri, 1992; Demopoulos et al., 2008). Therefore, the proximity of the mangrove stands to shallow water seagrass meadows will most likely contribute to the higher biodiversity and, particularly, higher density observed within seagrasses. The populations of suspension-feeders, such as Barbatia foliata, which was dominant in the seagrass meadow (S1), supports the idea of higher availability of organic suspended particulate matter derived from nearby mangrove canopies and this higher availability will also support more resident organisms (Alfaro, 2006; Sokołowski et al., 2015). Indeed, and despite the high temporal variability observed in all habitats, highlighted by the dissimilarity indices, seagrass habitats showed a comparatively higher stability/persistence, with the lowest values of inter-annual variability, similar to previous studies in temperate
areas (Mistri, 2002; Tagliapietra et al., 2016). These habitats also supported the highest number of resident species (i.e. those present in over 85% of the sampling periods). At the lagoon entrance, the exclusive presence of *Schizaster gibberulus*, a sea urchin previously associated with the nearshore coastal biotope in the region (Alsaffar et al., 2019), suggests that the unvegetated area may be located along a corridor connecting offshore and lagoon communities, with patterns likely dependent on the hydrodynamic processes (Irlandi et al., 1995). Its position between the lagoon and the open coastal water may also explain the high number of species observed (121), with a large proportion being exclusively associated with this habitat (33.9%).

The present findings reinforce the need for an integrated understanding of shallow-water habitats from a seascape perspective, in opposition to a fragmented analyses of the isolated habitats (Lundberg and Moberg, 2003; Berkström et al., 2012; Tano et al., 2017). Whereas the latter may be relevant when looking at particular species, the contribution of each habitat to the dynamics of the whole macrobenthic assemblages is relevant and should not be disregarded by managers when aiming for marine biodiversity conservation. Indeed, in tropical regions, seagrass beds and mangroves have been reported as key nursery areas for several reef fishes such as parrotfishes (Labridae, Scarini), grunts (Haemulidae) and snappers (Lutjanidae) (Nagelkerken et al., 2000; Lugendo et al., 2005; Gullström et al., 2008; Berkström et al., 2013) that rely on the macrobenthos as food resources. Large-scale migrations (over 30 km) by juvenile snappers, between inshore nursery habitats and reefs in the central Red Sea have been reported (McMahon et al., 2012). Also, mangrove forests have been linked to enhanced biomass and biodiversity of coral reef fishes (Mumby et al., 2004; Lugendo et al., 2005; Mumby and Hastings, 2008; Berkström et al., 2012; Saenger et al., 2013). Sustained connectivity of the habitats may enhance the resilience of coral populations to recover after disturbance (Mumby and Hastings, 2008). Therefore, disturbing the corridors connecting coral reefs with other inshore habitats may even have consequences for reef conservation at a local scale.
3.6. Conclusions

Overall, the present study confirmed a decreasing gradient in the total number of species and number of exclusive species towards the mangrove habitats, which supports our initial hypothesis; decrease in species richness and in the number of exclusive species as the environmental gradient increased towards the intertidal area. It also supports the roles of seagrass habitat complexity in promoting diversity and density of organisms. Nevertheless, high and stable seagrass cover does not necessarily result in the highest biodiversity levels. But the presence of these plants plays an essential role in the biodiversity of coastal lagoons. Seagrass habitats in contrast to mangrove forests and the unvegetated area show lower inter-annual variability and higher number of resident species, suggesting more stable communities.

Current findings highlight habitat-structured patterns and persistent patchiness evidenced by a limited overlapping species (dominance of habitat specialists over generalists) within the seascape. This is particularly relevant considering the proximity of the analysed habitats but may result from the low dominance levels compared to temperate regions (Mistri, 2002; Ludovisi et al., 2013). Therefore, populations are maintained at small sizes probably due to sub-optimal environmental conditions, which in addition to the limited connectivity may pose serious risks to the long-term survival of these species in case of disturbance (Ludovisi et al., 2013). Nevertheless, 22.8% of the gamma diversity was represented by taxa spanning between subtidal and intertidal habitats. Hence, holistic, i.e. interconnected seascape management approaches, rather than those focusing on single habitats should be prioritized to protect biodiversity and fisheries (McMahon et al., 2012; Perry et al., 2017; Whitfield, 2017).

Substantial intra-habitat and temporal variability in macrobenthic communities observed in the present study may be related to the wide range of habitats available in tropical coastal lagoons (coralligenous hard bottoms and sponge aggregations, mixed macroalgae and seagrass meadows), as well as its heterogeneous distribution in the area. Altogether, they contribute to a diverse pool of colonizers, with settlement patterns being dependent on the nature of the substrate that larvae or juveniles encounter, biological interactions, and variability in environmental conditions. Local factors related to small-scale environmental
heterogeneity rather than water quality seem to play a key role determining spatial patterns of variability (Mistri, 2002; Thrush et al., 2006; Zilli and Marchese, 2011; Ludovisi et al., 2013).

The strong temporal and spatial variability observed within the seascape preclude scaling patterns in a particular habitat to the whole ecosystem (Rooney et al., 2006). Likewise, due to the high seasonal and inter-annual variability observed, monitoring programs should be designed to allow us to distinguish multiple temporal scales in future. Collecting data different sampling periods is critical to make sure we capture the natural variability of the system and, therefore, not making conclusions based on limited information.
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Chapter 4

4. The role of seagrass vegetation and local environmental conditions in shaping bacterial and macrobenthic communities in the Red Sea

Photo credits: Georgina Short

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4.1. Abstract

This research investigates the influence of seagrass canopy on the biodiversity patterns of bacterial and macroinvertebrate benthic communities in tropical soft sediments of the Red Sea. Changes in abundance, number of taxa and assemblage structure were analysed among bottoms of different seagrass densities (low density, SLD; high density, SHD), as well as seagrasses interspaced with macroalgae (SA) and bare sediments (unvegetated). The four treatments were established at three different locations and used as proxies of fragmentation. Approximately, 25% of the gamma diversity was shared among all treatments or locations for macrofauna, whereas for bacteria this percentage dropped to less than half. The highest percentage of exclusive taxa (18% of the gamma diversity) was observed in the SHD treatment. Here we report high variability in biological responses driven by a combination of the presence of vegetation and environmental conditions that do not conform to the paradigm of increased biodiversity associated with seagrasses. Sustaining the connectivity among these heterogeneous bottoms appears to be essential for regional biodiversity. This study addresses a current scientific gap related to the biodiversity contributions of vegetated and unvegetated habitats for tropical regions, where a general lack of foundational knowledge limits the ability to predict future changes particularly in areas of intense coastal development.

Keywords: seagrasses, bacterial and macroinvertebrate communities, unvegetated habitats, ecosystem functions and services
4.2. Introduction

In tropical shallow waters, seagrass beds, mangroves and coral reefs are often linked through ecological, biological, chemical and physical processes (Birkeland and Grosenbaugh, 1985; Moberg and Rönnbäck, 2003; Gladstone, 2009; Berkström et al., 2012). These interconnected habitats form what is often referred to as “the tropical seascape” (Ogden, 1988). The tropical seascape contributes to a variety of ecosystem goods and services that are essential for human well-being (Nagelkerken et al., 2000; Moberg and Rönnbäck, 2003). They also play a critical role in shoreline protection and fisheries, and the integrity of the habitats at the seascape scale is vital to the sustained delivery of those goods and services (Grober-Dunsmore et al., 2009).

Among the typical triad of tropical key habitats, seagrass meadows are considered one of the most productive and valuable habitats in the marine ecosystem (Hemminga and Duarte, 2000; Short et al., 2007). Their presence increases habitat complexity providing a large range of ecological niches (Knowles and Bell, 1998; Connolly and Hindell, 2006; Horinouchi, 2007) and, consequently, high biodiversity. By reducing the velocity of the currents and wave action, they promote the deposition of organic matter and fine particles (Gacia et al., 2002; Hendriks et al., 2008). Both factors may contribute to a comparatively larger number of species or abundance being found within seagrass beds which can contribute to higher prey availability for economically important fish species compared to nearby unvegetated areas. These habitats also provide shelter from predators and juvenile nursery grounds. Additionally, for many flagship species such as dugongs, manatees and sea turtles there is a direct dependence on these habitats for food (Hemminga and Duarte, 2000; Beck et al., 2001; Heck et al., 2003; Boström et al., 2006; Unsworth and Cullen, 2010). Seagrasses, therefore, represent priority habitats for conservation (Leopardas et al., 2014). Nevertheless, they have been severely affected worldwide, with an estimated loss in cover of 110 km² yr⁻¹ since 1980 (Waycott et al., 2009).

While seagrass meadows can be naturally fragmented by processes such as extreme weather events, waves and currents (Short and Wyllie-Echeverria, 1996; Short et al., 2011), anthropogenic pressures such as coastal construction, eutrophication, dredging and infilling, trawling, sewage pollution and recreational activities (Hastings et al., 1995; Duarte, 2002; Ruiz
and Romero, 2001; Orth et al., 2006; Short et al., 2011) have greatly increased their fragmentation. Fragmentation in seagrass cover is likely to impact faunal assemblages (Frost et al., 1999; Hovel, 2003; Hovel and Lipcius, 2002), with exclusive species being usually the most vulnerable to habitat change (Thomas and Mallorie, 1985). Considering the functional connections that exist across the tropical seascape, impacts of fragmentation will have the potential to spread beyond the directly impacted local area and could alter patterns of ecological connectivity between coastal habitats (Fahrig, 2003; Fischer and Lindenmayer, 2007; Berkström et al., 2012). Ultimately, loss and fragmentation of seagrass habitat will likely disturb connectivity and linkages within food webs, affecting ecosystem resilience and functioning (Jackson et al., 2001; Link, 2002; Duffy, 2006; Berkström et al., 2012).

Few studies have, however, addressed the impacts of seagrass fragmentation on the structure and functioning of the biological community in tropical and subtropical habitats (Peterson et al., 2001; Leopardas et al., 2014; Sweatman et al., 2017). Furthermore, studies on the effects of seagrass cover usually focus on a single biological component, mainly fish (Williams et al., 2016) or macroinvertebrates (Frost et al., 1999), and to a lesser extent a combination of both components (Bell et al., 2001; McCloskey and Unsworth, 2015). While the majority of studies have focused on fish and macroinvertebrate communities, the contribution of microbial assemblages has received little attention to date. However, microbial communities in seagrass meadows are associated with a variety of key functions including nitrogen fixation (Welsh, 2000), nutrient recycling (Alongi, 1994) and decomposition of organic matter (Harrison, 1989). Indeed, much of the primary production in seagrass meadows is unavailable to animal consumers and becomes detritus (Mateo and Romero 1997). However, the high metabolism and production of bacteria provide a vital link in supporting the rich benthic food webs (Danovaro, 1996; Mateo and Romero, 1997). The bacterial production, ultimately, supports a wide diversity of macrofauna due primarily to their role in nutrient cycling. Of note, macrofauna also play a significant role in nutrient cycling (Lohrer et al., 2004). Macroinvertebrates including crustaceans, gastropods, bivalves, polychaetes and echinoderms have been implicated in: i) structuring the benthic environment through processes such as bioturbation and burrow construction (Rosenberg, 2001; Austen et al., 2002; Lohrer et al., 2004; Fanjul et al., 2011); ii) aeration of the sediment (Forster and Graf, 1995; Snelgrove, 1998); and iii) acting as food sources for higher trophic levels including many ecological and commercially important fish species (Hyndes and Lavery, 2005). Despite the
clear linkages between these two fundamental components of seagrass biotopes, they are rarely analysed together.

This study aims to investigate the potential changes resulting from seagrass fragmentation on tropical soft sediment communities. It represents one of the few studies combining the responses of two essential biological components of the benthic system: macroinvertebrates and bacteria. It also contributes to understanding the ecological role of these important habitat types in tropical systems for which there is more limited knowledge. Increased coastal development in the Red Sea may affect the distribution and abundance of seagrass-associated species as well as the supporting ecosystem functions and services. This study represents a first step in the Red Sea towards understanding the consequences of habitat fragmentation, which is critical to preserving biodiversity and ecosystem functioning as a whole. We use different densities of seagrass meadows as a proxy for fragmentation (high density, low density, seagrass and algae and bare sediment (unvegetated). We characterized and then compared benthic communities across the four treatments in three subtidal locations. The two main questions addressed in this study included: i) Does benthic community structure (macroinvertebrates and bacteria) or biodiversity change across different treatments (unvegetated, mixed meadows of seagrass and algae as well as seagrass patches with different percentage cover)? ii) Are patterns consistent across different locations? iii) What are the main drivers of the observed patterns?

4.3. Material and Methods

4.3.1 Study site

The present study was conducted at the Al Qadimah lagoon (22° 22′ 39.3″ N 39°, 07′ 47.2″ E; approximate area of 13.9 km²), located on the central part of the western coast of Saudi Arabian Red Sea (18°-25°N), where the highest seagrass diversity is observed (Jones, et al., 1987). The lagoon is dominated by shallow bottoms (average depth 2.19 m) with a maximum depth of approximately 10 m in the channel that connects the lagoon to the open sea. This lagoon does not experience direct anthropogenic disturbances typical of other coastal lagoons (e.g. sewage discharges, fisheries practices, habitat destruction for coastal development). Riverine inputs are also non-existent. However, it is located between two urbanized areas developed over the last two decades (KAUST, King Abdullah University of
Science and Technology, 7000 inhabitants; KAEC, King Abdullah Economic City, <5000 inhabitants but it is expected to reach 50,000 by 2020 and it is expected to become a major tourist destination in the future). Despite the lack of direct anthropogenic influences, recent studies (Ruiz-Compean et al., 2017) quantified moderate levels of metals in the seagrass meadows that could be associated with atmospheric contamination from industrial complexes located approximately 50 km north.

The bottom of the lagoon, particularly in the inner sheltered areas, is covered by seagrass meadows often consisting of at least two species. The areas are mostly dominated by *Cymodocea rotundata, Cymodocea serrulata* and *Enhalus acoroides*, sometimes with macroalgae interspersed. In general, multispecific meadows are dominant. Therefore, the selection of seagrass areas was not limited by the species composition. Previous studies in tropical areas did not find an effect of the vegetation type on densities, species richness or even composition of epi- and infaunal communities (Leopardas et al., 2014).

4.3.2. Sampling design and strategy

The survey was conducted in naturally fragmented seagrass beds in March 2017. Considering the lack of knowledge on the hydrodynamics of the lagoon, three locations were randomly selected (A, B, C; Fig. 4.1), to assess whether the location of the meadows would affect the response of microbial and macrobenthic communities to habitat fragmentation. All sampling sites were established within depth range 1.6 - 2.8 m.

In each location, four different treatments were established. These are representative of what is commonly observed in the lagoon but also of different alternative states associated with seagrass fragmentation. The treatments were established based on the seagrass beds density: unvegetated (UnV) with estimated percentage cover of 0 - 5%; seagrass low-density (SLD) with estimated percentage cover of 10 - 25%; high-density (SHD) with estimated percentage cover of 50 - 75%; and seagrass and macroalgae (SA) mixed meadows. In the field, quadrats (50 x 50 cm) were placed at random three times within the treatment in order to obtain the percentage of seagrass and macroalgae cover (%) and to measure shoot density (m²).
A combination of sediment and water samples was collected at each location and treatment, following a block sampling design with two factors: Location (fixed, three levels: A, B and C) and Treatment (fixed, four levels: UnV, SA, SLD, SHD).

**Water samples.** At each sampling site, CTD casts were carried out for conductivity, temperature, and depth (CTD) with a multiparameter probe (OCEAN SEVEN 316Plus 316 Plus and 305 Plus CTD). Divers, using 1 L bottles, collected samples a few centimeters above the bottom while avoiding sediment disturbance. The collected samples were kept on ice while being transported to the laboratory and later analysed for nutrients and chlorophyll \(\alpha\) (chl \(\alpha\)). For nutrients, 50 mL of water was filtered through 0.22 \(\mu\)m membranes and the filters were frozen at \(-20^\circ\text{C}\) until analyses. For chl \(\alpha\), 1 L water samples were filtered through 47mm GF/F filters that were later wrapped in aluminium foil and preserved at \(-80^\circ\text{C}\) until extraction of the pigments.
Sediment samples. In each location and treatment, three replicate core samples were taken (12 cm internal diameter, 20 cm depth) for the study of macroinvertebrates. Each sample was composed of three cores (total area of 0.03 m²), 36 replicates in total were collected. In order to avoid edge effects typical of seagrass meadows (Bologna and Heck, 2002), samples were whenever possible taken from the innermost area of each treatment. In the field, sediment cores were washed over a 1 mm sieve, and samples were preserved in ethanol (96%).

For bacterial analyses, 5 g of sediment was taken in duplicates. These 5 g samples were placed in RNA later, and frozen until further laboratory sampling.

Additional sediment cores were collected to quantify associated environmental variables, specifically, total organic carbon (TOC), grain-particle size, and nutrients in pore-water (NO₃⁻, NO₂⁻, PO₄³⁻, and SiO₂).

4.3.3. Laboratory analyses

Water samples. Nutrients (NO₃⁻, NO₂⁻, PO₄³⁻, and SiO₂) were quantified using a Continuous Flow Analyzer (SEAL AutoAnalyser 3 with XY2/3 Sampler). To quantify nutrients in pore water samples, sediment was centrifuged for 30 minutes at 3000 rpm to separate the pore water from the solid fraction. Water was filtered using the same filters used for the overlying water and analysed following the same protocol. Chl a quantification was undertaken using the Acetone (10 mL at 90%) extraction method. Briefly, samples were left in acetone for 24h in low light conditions to minimize degradation. A Turner Trilogy® fluorometer (Turner Designs) was used to quantify the chl a content using an acidic module. The degradation of the chl a to phaeophytin was accomplished by acidifying the sample with 60 µL of 0.1N HCl.

Sediment samples. These samples were dried at 60 °C until constant weight prior to their homogenization in an automatic mill grinder (model PM200 from Retsch) where the samples were milled for 4 min at 420 rpm. Afterward, the fine powder was collected and prepared for analyses. Total carbon and nitrogen were determined for the powder samples. Inorganic carbon and nitrogen fractions were also calculated after loss on ignition (450 °C for 3 h). Analysis was performed in a CHNS-O “Thermo Scientific” organic elemental analyzer (model Flash 2000), with soil standard 2G NCS as reference material. Particulate and sediment organic carbon (POC and TOC, respectively) and nitrogen (PON and TON, respectively) were
calculated by the difference between the total and inorganic fractions. For trace element analysis (Al, Ba, Cd, Co, Cr, Cu, Fe, Pb, Mg, Mn, Ni, Se, V, and Zn), samples were dried at 40 °C and 0.2 g was analysed by flame atomic absorption spectrophotometer AAS (Perkin Elmer, Model 2380A Spectrophotometer) following the EPA 2007 methods, as detailed by (Ruiz-Compean et al., 2017). Particle size analysis was conducted by wet separation of the silt and clay fractions from the sandy fractions. Replicates of sediment samples were sieved through a 63 μm mesh (silt and clay fraction), and the retained sediment fraction was dried at 80 °C for 24–48 h. The dried sample material was sieved by using a column of sieves. Fractions were classified as: gravel (>2 mm), very coarse sand (>1 mm and <2 mm), coarse sand (>500 μm and <1 mm), medium sand (>250 μm and <500 μm), fine sand (>125 μm and <250 μm), very fine sand (>63 μm and <125 μm), and silt-clay (<63 μm).

**Macrofauna.** Samples were sorted and organisms identified to the highest taxonomic separation level and counted. The lack of taxonomic knowledge for most of the soft-sediment invertebrates in the region, together with the preservation of samples in ethanol precluded the identification of organisms to the same taxonomic level. Seagrass material (roots and leaves) was separated and biomass measured as dried weight.

**Bacteria.** Samples were processed using the PowerMax Soil DNA isolation kit (Qiagen) as per the manufacturer’s instructions with the exception of step 3 where the bead beating was replaced by the addition of 0.4 mg ml⁻¹ of Proteinase K and an overnight incubation in a shaking incubator at 56 °C (Leray and Knowlton, 2015). The bacterial component was amplified with PCR by targeting the v3 and v4 regions of the 16S rRNA gene using the primers and PCR conditions described in Klindworth et al. (2013). A negative PCR control was run at the same time as the sample PCR’s. The triplicate PCR products from each sample were pooled, cleaned and normalized using SequelPrep Normalization plates (ThermoFisher Scientific). After cleaning and normalization, Illumina tags were added via a second round of PCR amplification using the Illumina 16S metagenomic sequencing library preparation protocol and, subsequently cleaned and normalized using the SequelPrep Normalization plates (ThermoFisher Scientific). An Illumina MiSeq sequencing platform (v3 chemistry) at the King Abdullah University of Science and Technology (KAUST) Bioscience Core Laboratory (BCL)
was used to generate the sequences (2 x 300 bp). Raw reads were deposited at the NCBI Short Read Archive.

4.3.4. Data visualization and statistical analyses

*Environmental data.* Principal Components Analysis (PCA) was used to create an ordination plot of the samples based on the environmental variables that were measured. Prior to the analysis, the correlation between variables was assessed (Pearson R). Highly correlated variables were excluded, which primarily related to the concentration of metals and grain size. The concentrations of the trace elements Ba (highly correlated to most others), Cd, Cu, and Pb were used. Regarding grain size, gravel (>2000 µm), very coarse sand (1000-2000 µm), silts and clays (fines; <63 µm) were used in the analysis. Most of the variables that were selected for the analysis showed a normal distribution of the data and, therefore, were not transformed. Data were normalized prior to the analysis as the different variables have different scales.

*Macrofauna.* Several univariate metrics were calculated including density (ind. m⁻²), and the average number of taxa (i.e. #taxa x 0.03m²). Two-way ANOVAS were conducted for the factors *Treatment* and *Location* after checking for normality and homogeneity of the data. Community structure was investigated by ordination techniques (Principal Coordinate Analysis), based on the Bray–Curtis dissimilarity coefficient applied to untransformed abundance data. Permutational multivariate analysis of variance (PERMANOVA), for the two-factors in analysis (i.e. Treatment and Location) was applied to assess for significant differences in the associated fauna. When significant differences were detected, pair-wise tests were applied. Distance-based redundancy analysis (dbRDA) was also applied in order to understand the relationship between each environmental variable and benthic community structure (given by the direction and length of vectors for each variable). Due to differences in the environmental variables, normalization of the environmental data were carried out before the analyses. In addition to the environmental data analysed in the PCA (see previous section), seagrass biomass (Biomass \textsubscript{SG}) and shoot density (Density \textsubscript{SG}) were also included in the analysis. This subset of variables was still too large in comparison to the number of biological samples. Therefore, some environmental variables were further excluded based on the PCA results, we looked at those whose vectors had the same direction and picked those
with highest contribution to the separation of the samples. The significance of each variable retained in the model was assessed using marginal and sequential tests. All analyses were undertaken in R (R Core Team, 2018); with following packages: PCA, “FactoMineR” (Lê et al., 2008); PCoA, “ecodist” (Goslee and Urban, 2007); dbRDA, “vegan” (Oksanen et al., 2018); “VennDiagram” (Chen, 2018), except for the PERMANOVA that was conducted using Primer v7.

A forward step-wise regression model using the BIC exit criterion was used to determine the environmental parameters that explained benthic taxa differences between treatments. The taxa identified as important contributors for the biological patterns based on SIMPER analysis (Primer 7) were analysed using step-wise regression. Regression models that account for a large number of potentially relevant variables may lead to over-fitting and poor prediction performance. Hence, environmental variables excluding those that were highly correlated were carried forward in the regression. Linear regression models were then applied to identify the magnitude and direction effect of the important environmental variables identified from the stepwise regression that significantly contributed to benthic abundance data.

*Bacteria.* The “dada2” package (Callahan et al., 2016) was used to process the raw sequencing reads within R (R Core Team, 2018). Briefly, the raw reads were truncated based on quality and then filtered with a maxEE of 2 for forward reads and 6 for reverse. Error rates were calculated for the filtered reads and subsequently paired ends were merged with a maximum mismatch of 1 and a minimum overlap of 10. The function removeQimeraDenovo was used to remove chimeras and taxonomy was assigned to each representative sequence against the Silva v128 database (Pruesse et al., 2007) within the “dada2” package resulting in a table of operational taxonomic units (OTUs) against sample, using the “phylseq” package (McMurdie and Holmes, 2013). Any sequences assigned as eukaryotes were subsequently removed bioinformatically. After the creation of the OTUs table, the same statistical analytical routines were undertaken as described for the macrofauna. Multivariate analysis was based on the log-transformed data.
4.4. Results

4.4.1. Environmental variability among locations and treatments

The first two dimensions (Axis 1: Fines, Cu, Ba, gravel and vcsand; Axis 2: PO$_4^{3-}$, SiO$_4^{3-}$, and NO$_3^-$, Chl a also depth, NO$_2^-$, and NO$_3^-$) of the PCA analysis explain about 55.3% of the variability in the environmental data (Fig. 4.2). The PCA diagram shows a general separation of the unvegetated samples (UnV, lower right quadrant) from the rest of the samples. Samples from location C mainly cluster in the left quadrants.

Fig 4.2. Principal Components Analysis (PCA) ordination plot of environmental variables, sediment grain size fractions [Gravel, >2000µm], very coarse sand (VCSand, 1000-2000µm) and silts and clays (Fines, <63µm)]; chlorophyll a (Chl a); seawater and pore water nutrients (SiO$_4^{3-}$, NO$_2^-$, PO$_4^{3-}$, NO$_3^-$; pw denote those in pore water); trace element concentrations ([Ba], [Cd], [Cu] and [Pb]); total organic carbon (TOC); temperature (SST); and depth (Depth).
The variables that make the most contribution to explaining differences between samples were related to the grain size (fines opposed to gravel and very coarse sand) as well as some metals (Ba, Cd, Cu; correlated to fines) and depth. These variables seem to explain why vegetated samples from Location C are different from locations A and B. Within locations A and B, some nutrients in the porewater (silicates, phosphates and nitrates), TOC and chl a present higher values in vegetated treatments compared with the unvegetated ones.

4.4.2. Major patterns in benthic diversity and structure

A total of 78 taxa and 920 individuals were identified belonging to 58 families. The most abundant phyla were Annelida (39%), Mollusca (25%), Sipuncula (22%), and Arthropoda (9%), followed by Echinodermata (4%). Total abundance ranged from 156 (unvegetated) to 323 individuals in SLD. For the bacteria there were 13896 OTUs representing 202 families. The dominant phyla in the bacterial community were Proteobacteria (65.6%), Chloroflexi (7.6%), Acidobacteria (6.1%) and Bacteroidetes (4.1%).

Overall, benthic macrofaunal density and number of taxa did not vary significantly among treatments but there was a significant effect of Location. Both metrics were significantly higher in location C, compared to locations A and B (Fig. 4.3 A, B). For bacteria, the number of OTUs was generally higher in locations A and B in comparison to location C (Fig. 4.3 C). The observed number of taxa showed, however, a significant interaction between Location and Treatment (F=3.102, p=0.04). Post-hoc tests did not show any significant differences within locations or between locations A and B. When observed, significant differences were only detected between locations C and A or B particularly for the treatments UnV, SA and SLD.
Fig. 4.3. Alpha-diversity metrics for macrofaunal (A, B) and bacterial communities across treatments and locations. Unvegetated (UnV); seagrass and macroalgae (SA) mixed meadows; seagrass low-density (SLD); seagrass high-density (SHD). The dots referred to the average on the plots.
A total of 18 taxa representing 23% of the gamma diversity (i.e. all taxa identified) were shared among treatments across the three locations. Among the shared taxa, the most abundant was the sipunculid *Phascolion strombus strombus* (20.2% of the total abundance). Other main contributors to the total abundance were the opportunistic polychaetes Capitellidae und. (7.8%), bivalves *Barbatia foliata* (7.8%) and *Cardiolucina semperiana* (6.1%), as well as the polychaetes Eunicidae (6%). SHD showed the highest percentage of exclusive taxa (18% of gamma-diversity), twice as much as the unvegetated treatment and 3.5 times more than the mixed meadow (Fig. 4.4). Regarding factor *Location*, 20 taxa were present at the three locations, with location C showing the highest percentage of exclusive taxa (i.e. 24; 31% of gamma diversity) (Fig. 4.4).

![Venn diagram illustrating the numbers of shared and exclusive taxa of macrofauna (top) and bacteria (bottom) regarding the two factors in analysis, i.e. treatment (on the left) and location (on the right). Unvegetated (UnV); seagrass and macroalgae (SA) mixed meadows; seagrass low-density (SLD); seagrass high-density (SHD).](image)

A total of 1460 OTUs were shared amongst all treatments, representing 10.5% of the gamma diversity (yet accounted for 78.7% of the relative abundance). The majority of the abundance in these shared OTUs was classified as belonging to the families Desulfobacteraceae and Syntrophobacteraceae. In contrast to that observed for macrofauna, the SA treatment showed the highest number of exclusive taxa (19.6%). Also, in contrast with macrofaunal
patterns, location C showed the lowest percentage of exclusive taxa (8%) with the highest percentage being observed in location B (35%).

The principal component analysis (PCoA) showed no clear pattern in the structure of macrofauna assemblages among the treatments with vegetation (i.e. SA, SLD, and SHD) (Fig. 4.5). However, samples from the unvegetated treatment tend to group separately from other treatments (SLD, SHD, and SA). PERMANOVA analysis showed significant and independent differences in the structure of macrofauna both for *Treatment* (Pseudo-F=3.1521, p=0.001) and *Location* (Pseudo-F=2.5591, p=0.001) factors (Table 4.1.). Pairwise tests showed that assemblages inhabiting unvegetated areas were significantly different from those associated with vegetation (UnV≠SA=SLD=SHD). Analysis also showed that location C was significantly different from A and B (P<0.01).

![Fig. 4.5. Principal coordinates analysis (PCoA) of macrofauna and bacterial communities based on Bray-Curtis dissimilarity matrices (bacteria dataset was log transformed). Samples are coloured by treatment type, whereas locations (A, B and C) are represented by symbols. Unvegetated (UnV); seagrass and macroalgae (SA) mixed meadows; seagrass low-density (SLD); seagrass high-density (SHD).](image-url)
Multivariate patterns of bacterial communities showed that communities associated with location C were separated along the first axis from those sampled in locations A and B. Samples from the unvegetated habitats were also mainly separated from the remaining treatments on the lower half of the plot (Fig. 4.5). PERMANOVA analysis identified a significant interaction between Treatment and Location (Pseudo-F=1.291, p<0.05). Pair-wise comparisons showed significant differences between locations B and C for the SHD treatment. Also, unvegetated assemblages in location C were significantly different from those associated with the high density (SHD) (Table 4.1).

Table 4.1. Two-way PERMANOVA results and pair-wise tests based on Bray-Curtis dissimilarity. Unvegetated (UnV); seagrass and macroalgae (SA) mixed meadows; seagrass low-density (SLD); seagrass high-density (SHD). A, B and C denote locations. . Significant values are shown in bold.

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<th>Pseudo-F</th>
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Pair-wise comparisons

**Term 'Tr'**

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<tr>
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<td><strong>0.001</strong></td>
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<td>UnV, SLD</td>
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<td>UnV, SA</td>
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**Term 'Location'**

Within level 'C' of factor 'Location'

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<td>B, C</td>
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**Term 'Tr x Lo'**

Within level 'SHD' of factor 'Treatment'

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Within level 'UnV, SHD' of factor 'Treatment'

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<td>UnV, SHD</td>
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4.4.3. Environmental drivers of benthic communities

For the macrofauna, the dbRDA (Fig 4.6) indicated that depth, seagrass biomass and the concentration of Ba were the environmental variables significantly driving the community composition. The first two axes explained approximately 30% of the total variation. For the bacteria, along with those variables significant for the macrofauna, seagrass density and TOC were also significant. However, less of the community variation was explained (16.6%) compared to the macrofauna. The general pattern observed in the PCoA analysis for both communities is also observed here, i.e. the separation of the unvegetated samples from the remaining sites. In both biplots, the discrimination between these two communities is mainly due to depth, TOC and shoot density. Grain size, seagrass biomass, as well as nutrients appear to be more important in explaining the variability in the bacterial communities than macrofaunal patterns. In the macrofauna biplot, two SLD samples from location C with very high biomass of seagrass root system correlate with the grain size differences in this location (higher coarse sand and gravel contents).

![Distance based redundancy analysis (dbRDA) of macrofaunal and bacterial samples based on Bray-Curtis distance matrices. The dbRDA axes describe the percentage of the fitted or total variation explained by each axis. Samples are colored by treatment type, whereas locations (A, B and C) are represented by symbols. Unvegetated (UnV); seagrass and macroalgae (SA) mixed meadows; seagrass low-density (SLD); seagrass high-density (SHD).](image-url)
4.4.4. Response of taxa to habitat drivers

Taxa selected for regression modeling showed species-specific responses to a combination of seagrass properties, temperature, depth, salinity and grain-size. Taxa that were associated with seagrass habitats, and more specifically that were positively associated with higher seagrass biomass, included the polychaete worms Eunicidae, Terebellidae, Capitellidae and Syllidae (Table 4.2). The brittlestar, *Amphiura* sp., was also positively associated with seagrass beds (notably seagrass shoot density). Conversely, the bivalve *Cardiolucina semperiana* was negatively associated with higher seagrass biomass (Table 4.2). Eunicidae and Cardiolucina semperiana were also associated with higher TOC which is not surprising given TOC was slightly elevated in the seagrass beds which often trap and retain organic matter. A number of macrofaunal taxa were found to be sensitive to increasing metal concentrations in the sediment, with slightly higher concentrations of metals recorded at the unvegetated sites which were also associated with a higher percentage of fines (or <63 µm silt). For example, the polychaetes Capitellidae, Terebellidae, Eunicidae and Syllidae were all negatively associated with increasing Ba, Cd and Pb levels (Table 4.2). For the bacterial communities, genera associated with higher seagrass biomass and shoot densities included *Pelobacter, Maricurvus, Tistlia, M2PT2-76* and *Candidatus Omnitrophus* (see Table 4.2).
Table 4.2. Main environmental parameters identified from regression-based analysis associated with changes in the macrofauna and bacteria and whether the parameter acted to increase (+) or decrease (-) abundance.

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<th>Parameter</th>
<th>D (m)</th>
<th>SST (°C)</th>
<th>S</th>
<th>SG dens.</th>
<th>SG biom.</th>
<th>TOC</th>
<th>Fines (%)</th>
<th>Vcsand (%)</th>
<th>Gravel (%)</th>
<th>NO2 [μM]</th>
<th>PO4 [μM]</th>
<th>NO3 [μM]</th>
<th>Ba [mg/kg]</th>
<th>Cd [mg/kg]</th>
<th>Pb [mg/kg]</th>
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4.5. Discussion:

4.5.1. Vegetation and environmental conditions drive benthic patterns

This study provides evidence of the role of seagrass vegetation and local scale environmental processes in influencing benthic community patterns. We observed that the presence/absence of vegetation along with changes in local scale sedimentary features (notably grain size, organics and metals) influenced the observed patterns in benthic communities, particularly for macroinvertebrates. Seagrass biomass had an overall positive effect while fine mud (>63 μm fraction) and metals had a primarily negative effect on the abundance of dominant benthic taxa. The high-density treatment supported the highest number of exclusive taxa (18% of the gamma diversity), twice as much as the observed in unvegetated areas and more than three times the exclusive taxa recorded in mixed meadows. These results highlight the role of vegetation (presence rather than species composition or density) and most likely its secondary effects on local hydrodynamics, food availability and quality, as well as protection from predators. We did not find an unequivocal evidence of a positive correlation between seagrass canopy and the diversity, density and community structure of either bacterial and macrobenthic communities, as responses were site-specific. Furthermore, we did not find significant differences between seagrass and mixed meadows (seagrass and algae). Considering that approximately 25% of invertebrate taxa were shared among treatment or locations, and that high-density treatments supported the highest number of exclusive taxa, sustaining ecological connectivity across the seascape is thus critical in maintaining the biodiversity at the regional scale.

There has been a considerable amount of literature produced on the effects of seagrass canopy on associated assemblages, particularly in temperate regions (Lobelle et al., 2013; Ávila et al., 2015; Barnes and Hendy, 2015; York et al., 2018). Less research has been, however, directed at the investigation of differences in benthic assemblages between vegetated (seagrass or seagrass and algae) and unvegetated substrates within coastal lagoons (Magni et al., 2017), and particularly in sub-tropical and tropical regions. Seagrass meadows have traditionally been considered as diversity hotspots (Thomsen et al., 2018). However, researchers worldwide have reported a broad range of ecological responses of benthic fauna to seagrass canopy that do not support the paradigm of increased diversity and abundance in
seagrass beds compared to unvegetated sediments (Attrill et al., 2000; Lee et al., 2001; Nakamura and Sano, 2005; Barrio Froján et al., 2009; Barnes and Barnes, 2014). Indeed, in the present study, we found evidence for the role of vegetation on benthic communities but responses were inconsistent denoting the influence of local scale processes reflective of differences in the environmental characteristics. For example, the combination of characteristics such as the nature of the meadow (i.e. multispecific versus monospecific; species composition; Hovel, 2003), shoot density and biomass (Webster et al., 1998), local hydrodynamics and environmental conditions (Bell et al., 2001), size of the meadow (Bowden et al., 2001), location of sample collection (i.e. “edge-effect”)/ environmental variables within the meadow (Turner et al., 1999; Tanner, 2005), and seasonality (Włodarska-Kowalczuk et al., 2014) create specific environmental and biological conditions at the local scale that might change the direction and magnitude of the observed responses. The variability in the patterns across the three locations does not allow us to disentangle the relative contribution of each factor in explaining the benthic patterns. For example, in location C, higher similarities were noted among different treatments within this location (e.g. unvegetated versus vegetated), than between the same treatments in different locations (e.g. unvegetated location A versus unvegetated location C). This was particularly evident for bacterial communities and has been previously reported for invertebrates (Barnes and Barnes, 2012).

4.5.2. Diversity patterns across locations

Our results, therefore, do not conform with previous studies reporting a significant and positive effect of seagrass cover on faunal density and diversity due to, for example, a combined effect of protection from predators, increased structural complexity and ecological niches availability (McCloskey and Unsworth, 2015), genetic diversity (Reusch et al., 2005), or even food availability (Castel et al., 1989). Here, we provide further evidence revoking that theory (Barrio Froján et al., 2009; Barnes and Barnes, 2014) that is likely not to be true in bacterial communities either. There are several plausible reasons that can contribute to the high variability in biological patterns. One of them is the high temporal dynamics of the seagrass canopy. As demonstrated for temperate regions, significant positive effects of vegetation on diversity and biomass of macrobenthic communities may only be detected when seagrass cover (and biomass) reaches its maximum (Włodarska-Kowalczuk et al., 2014).
Therefore, a sound understanding of the seasonal dynamics of seagrass meadows and the annual cycles of the plants appears critical for a thorough assessment of the effect of seagrass cover on shaping marine communities.

A third potential explanation is the focus of the present study in sediment samples and the associated infaunal communities. Indeed, the method used for collecting the samples (hand corer) is more appropriate for infaunal organisms. Macroinvertebrates living in sediments have been suggested to be less affected by fragmentation compared to large motile fauna that usually display predominantly epibenthic behaviour and are more associated with the aboveground biomass. Indeed, Frost et al. (1999) suggested that infaunal species are less likely to show species-specific responses resulting in small shifts in community composition rather than clear changes in density as remarked for epifauna. However, for bacteria, a contrasting result has been found. When comparing bacterial communities associated with *Zostera marina* meadows in sediments, root and leaves from different locations (inside patch, edge, outside) Ettinger et al. (2017) found significant changes across the locations in sediments only, attributing differences in part to the availability of organic matter. However, inconsistency in the ecological patterns associated with the presence of seagrasses appears to be a trend observed for bacteria. Holmer et al. (2004) using a stable isotope approach showed that the isotopic signals in bacteria inhabiting unvegetated patches was similar to that of adjacent seagrass meadows indicating that seagrass detritus may still contribute substantially to available organic matter even in unvegetated patches. This emphasizes the relevance of taking a functional analysis approach rather than looking at the structural aspects of the community alone. Thus, if seagrass derived organic matter was a valuable contributor even in unvegetated areas it could explain the lack of differentiation between treatments in the present study.

Regression model results suggest that changes in the overall community composition between sampled plots of varying cover can be attributed to the individual habitat preferences of selected species. This has been highlighted for some invertebrates, such as mysids. Researchers have found a positive effect of fragmentation in the density and species richness when compared to homogenous bottoms (Barberá-Cebrián Cebrina et al., 2002). Dense seagrass meadows do not necessarily support high diversity and abundance. The broad
range of ecological traits observed in macroinvertebrates may contribute to the inconsistency in patterns that have been reported in the literature regarding the relationship between canopy and associated communities. For example, large tube-dwelling and burrowing species can be affected by high shoot density (and root-rhizome mat) that will limit their capacity to penetrate in the sediment (Ringold, 1979). However, complex and well-developed rhizome structures may not affect small polychaetes, also tube-dwellers, which do not require big areas in the sediment to build their tubes (Leopardas et al., 2014). Seagrass bed cover can also limit the movement of mobile organisms (Barnes and Barnes, 2014) and ultimately affect their ability to escape from predators. In the present study, shoot biomass was most often associated with increases in the abundance of some macrobenthic taxa, which traits can be favoured by the conditions promoted by the presence of the seagrasses. Based on the regression analysis, we observed that certain taxa were preferentially associated with seagrass habitats. Taxa that were positively associated with higher seagrass biomass included the polychaete worms Eunicidae and Syllidae (mobile, carnivores) as well as Terebellidae and Capitellidae (sessile or of limited mobility, suspension feeders/detritivores). The increase in the functional trait suspension feeders/detritivores in areas of higher seagrass is not surprising as flow rates within seagrass meadows have been demonstrated to be reduced resulting in higher rates of deposition of organic matter providing a food source for these organisms (Ricart et al., 2015; Samper-Villarreal et al., 2016; Serra et al., 2018). The analysis of the exclusive species associated with each treatment, also showed a prevalence of gammarid amphipods associated with high density of seagrasses. These are preferential prey items for fish (Choat and Kingett, 1982; Nakamura et al., 2003) and higher abundance of these crustaceans has previously been reported for seagrass beds when compared to unvegetated sediments possibly due to the protection that seagrass beds provide (Eklöf et al., 2005; Nakamura and Sano, 2005). The bacterial genus *Tistlia*, known for nitrogen fixation, was also associated with higher seagrass and may possibly be in a symbiotic/mutualistic relationship with the rhizome. In contrast, the bivalve *Cardiolucina semperiana* was negatively associated with both seagrass biomass and TOC possibly due to seagrass roots limiting bivalve density. As highlighted previously, future research is needed to better understand how different traits will respond to varying conditions and then to evaluate the causes of observed trends. However, regarding macrofauna and to a lesser extent bacteria, the lack of taxonomical resolution for a large proportion of the OTUs hinders such kind of analysis.
4.5.3. Implications for conservation and management

The present findings emphasize the dynamic nature and complex interplay of ecological and environmental processes driving benthic communities in tropical coastal lagoons with heterogeneous bottoms. Variables found to be important in predicting the response of benthic communities were related to processes working across multiple scales. Overall, our study suggests that sustaining existing mixed habitats at the lagoon seascape level, including those without vegetation, is critical for conservation and management (Barnes and Hamylton, 2016).

Indeed, habitats surrounding mature patches are not “ecological deserts” (Haila, 2002). For example, studies of birds and ground arthropods in terrestrial habitats have shown that the majority of species can persist in modified habitats (Haila, 2002 and references therein). However, between 5-10% of species are exclusively restricted to mature habitats (Haila, 2002 and references therein) and in order to conserve these species, conservation strategies should prioritise connectivity between habitats to maintain natural migration of species (Mumby, 2006). Conservation measures should thus include both vegetation and bare habitats, which will also allow for the evaluation of potential changes associated with anthropogenic and natural impacts given the dynamic nature of the ecological linkages across the seascape (Barnes, 2013; Barnes and Hamylton, 2016).

Our results, suggest that existing habitats should be preserved. If restoration efforts are required they should focus on preserving or restoring as much habitat as possible, regardless of its configuration (Trzcinski et al., 1999). Heterogeneity of habitats appears to be determinant for maintaining biodiversity. Managers cannot ignore that restoration programs are usually costly and of limited success (Bayraktarov et al., 2016), therefore, priority should be placed into mitigation of impacts potentially affecting seagrass health. Indeed, regardless the efforts, it is unlikely to restore systems to pre-distributed states but managers and scientists can still aim to achieve their “remaining natural potential” (Elliott et al., 2016).

Globally, increasing anthropogenic impacts such as overexploitation, physical disturbances, nutrient enrichment, pollution and increased turbidity, growth of invasive species, overgrazing and climate change are threatening seagrasses (Orth et al., 2006). Responsible
stewardship should tackle prevalent local pressures to promote the favourable environmental conditions for seagrass growth, which in turn will result in meadows, which are more resilient against global pressures (Waycott et al., 2009).
4.6. References


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Chapter 5

5. Discussion

5.1. Summary of the main contributions to advancing the research field

The main findings of this thesis provide novel and critical knowledge for a better understanding of the biodiversity patterns of soft-sediment biotopes in tropical areas. Among the main advances in the field, here we summarize the most relevant:

i) To our knowledge, this is the only study in the main basin of the Red Sea addressing lagoon to offshore gradient patterns in soft-sediment benthic communities, and one of only a few in tropical regions. The results provide relevant information on the ecological and environmental drivers of the soft-sediment assemblages in the context of high temperature and salinity (chapter 2).

ii) It is also the first study investigating mechanisms/processes of change using beta diversity analysis across the same lagoon to offshore gradients. This study may catalyze others investigating cross shelf beta diversity patterns that may be relevant for future conservation and management plans (chapter 2).

iii) Results also highlighted the small-scale heterogeneity of tropical lagoon habitats (mangroves, seagrass and unvegetated biotopes) that may serve as biodiversity repositories at the seascape level. Space, more than time, played a critical role in driving soft-sediment macrobenthic communities in tropical shallow-waters, stressing the importance of the co-existence of the habitats for biodiversity (chapter 3).

iv) Given the structural and functional importance of seagrass beds, documented worldwide and confirmed in our studies, we conducted for the first investigation of the role of seagrass fragmentation on the abundance and diversity of macroinvertebrates and bacterial communities in the central Red Sea (chapter 4). We found evidence for the role of vegetation on benthic communities but responses were inconsistent denoting the influence of local scale processes driven by environmental characteristics. Presence of vegetation rather than species composition or density and most likely the associated secondary effects on local hydrodynamics, food availability and quality, as well as protection from predators
are determinant for benthic community structure. Approximately 25% of the invertebrate taxa were shared among treatments or locations suggesting that sustaining ecological connectivity across the seascape is critical for regional biodiversity patterns.

v) This thesis, overall, provides baseline data that can be used in future to conservation and management plans in area like Red Sea experiencing intense and fast coastal development that is not supported by sound baseline knowledge regarding macrobenthos or other components of the marine system. This will be beneficial considering the ongoing expansion of nearby urban areas and future urban development. In particular, we provide an inventory of taxa to be used as a reference for the region. We are currently working with taxonomists confirming the identification of some of the most representative taxa as well as some potential new species/records for the regions. Specimens will be barcoded, and DNA sequences will be publicly available in the BOLD (http://www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov) DNA databases. This data will support future monitoring of the Red Sea based on molecular tools. Also, under the current scenario of global warming, we believe information from warm areas like the Red Sea may be crucial for a better understanding of potential changes arising from the increase in seawater temperature.

5.2. From Central Saudi Arabian Red Sea to broader latitudinal patterns

This thesis is part of a large research program (Saudi Aramco / KAUST Center for Marine Environmental Observations - SAKMEO) aiming to establish a baseline characterization of the marine biodiversity along the Red Sea. Soft-sediment macrobenthic communities were for the first time assessed at the scale of the Red Sea basin. The results of the present thesis, along with the data generated for the Red Sea within SAKMEO, could serve as the foundation for long-term monitoring efforts of these communities in Saudi Arabia. Conducting high-resolution studies at both spatial and temporal scales along the 18° of latitude of the Red Sea was unfeasible. Therefore, current findings, although spatially limited provide critical knowledge on the variability of macrobenthic communities across multiple biotopes and temporal scales, serving as a foundation for future investigations in other regions.
Due to the existing natural gradients in salinity, temperature, nutrients, and productivity in the Red Sea, analysing the changes in biodiversity patterns in multiple regions centrally important. This understanding will allow us to investigate the relative contribution of the major regional processes and environmental settings in relation to basin-scale biodiversity patterns. Processes that operate across multiple spatial scales (e.g. oceanographic processes) are known to shape the dynamics of benthic communities. Such processes can influence the regional species pool that will then affect patterns at local levels (Chaudhary et al., 2016; Gallon et al., 2017). Considering the singularities of different habitats and potential discrepancies in the responses of organisms associated with them, scientists have highlighted the need to incorporate multiple habitats and multiples scales of sampling (Snelgrove et al., 2014) if we aim to preserve the socio-economic values of the marine ecosystem (Fig. 5.1).

![Image](image_url)

**Fig. 5.1.** The challenge of scaling up from small experiments to the ecosystem: the biodiversity-ecosystem functioning approach (from Snelgrove et al., 2014).

### 5.3. Application of biotic indices for Red Sea soft-bottom macrobenthic communities

Over the last two decades, a considerable body of literature has been dedicated to developing, testing and validating several indices to assess the ecological quality within estuarine and coastal environments. Among those indices, AMBI, M-AMBI, BIBI, BOPA and BENTIX have been extensively applied (Borja et al., 2000; Gesteira and Dauvin, 2000; Simboura and Zenetos, 2002; Borja et al., 2004; Borja, 2006; Borja et al., 2008; Pinto et al.,...
The development of these indices resulted as a response of the scientific community to summarize the complex biological information in order to better communicate results with politicians and managers, namely regarding the development of environmental laws and directives. Decision makers often request simple responses from the scientific community, however, this is not always possible given the high complexity and stochasticity observed in the marine environment. For example, in one of the most commonly used indices, M-AMBI (Muxika et al., 2007), the characterization of the environmental status is based on the classification of species into five different ecological groups based on their sensitivity or tolerance to disturbance (organic input) (Table 5.1). Species though, may have divergent responses depending on both the disturbance pressure and region. Indeed, several authors have claimed that the use of biotic indices should be adjusted to different geographical regions (Dauvin and Ruelle, 2009; Borja et al., 2014; Uusitalo et al., 2016). In the Red Sea, ecological indices still need to be tested and validated to allow for the establishment of target boundaries for both the ecological indicators and environmental parameters before they can be integrated into monitoring studies. The results obtained in this thesis (low dominance values, high species to abundance ratios), however, suggest that due to the peculiar environmental characteristics of the Red Sea and the limited information in terms of behaviour for most of the species, the currently available indices might be inadequate and alternative indices may be needed. Therefore, although the application of biological indices with values linked to a straightforward classification is very appealing, they cannot always be directly applied. These indices need to be applied with care following the right criteria as they may provide misleading interpretations and ecological meaning possibly lost (Borja et al., 2008; Uusitalo et al., 2016). Future efforts to achieve a better understanding of the responses of benthic communities are critical, as well as validation and calibration of indices across the whole region.
Table 5.1. Biotic index values of M-AMBI and corresponding classifications of ecological status. Modified form Pinto et al. (2009).

<table>
<thead>
<tr>
<th>Biotic index</th>
<th>Ecological Group</th>
<th>Site Pollution Classification</th>
<th>Ecological Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.82</td>
<td>I</td>
<td>Unpolluted</td>
<td>High</td>
</tr>
<tr>
<td>0.62–0.82</td>
<td>III</td>
<td>Slightly Polluted</td>
<td>Good</td>
</tr>
<tr>
<td>0.41–0.61</td>
<td>IV-V</td>
<td>Moderately Polluted</td>
<td>Fair</td>
</tr>
<tr>
<td>0.20–0.40</td>
<td>V</td>
<td>Heavily Polluted</td>
<td>Poor</td>
</tr>
<tr>
<td>&lt;0.20</td>
<td>Azoic</td>
<td>Extremely Polluted</td>
<td>Bad</td>
</tr>
</tbody>
</table>

5.4. Major challenges for monitoring programs in Saudi Arabia targeting soft-sediments

5.4.1. Taxonomic expert knowledge in the Red Sea

One of the major challenges for the development of comprehensive biodiversity inventories is the time required for sampling and processing samples. The Saudi Arabian Red Sea covers an extension of over 2000 km² and presents strong latitudinal gradients in temperature, salinity and nutrients that affect the distribution of marine species (Pearman et al., 2017). The Red Sea is characterized by high biological diversity, as has been reported for coral reefs (Berumen et al., 2013) and recently confirmed for soft sediments (SAKMEO, unpublished data), with many rare species that are difficult to sample and identify using traditional methodologies. The traditional methodologies used in this thesis are based on the morphological identification of marine species. Morphology-based taxonomic identification is an essential step for biodiversity studies, but the high diversity recorded in the Red Sea requires intensive and expert taxonomic revisions to confirm the identifications, which has led to the discovery of new species (Ravara and Carvalho, 2017). However, it also results in delays in processing samples and generating results, as well as in the difficulties to identify species to low taxonomic levels. Therefore, even though taxonomic studies are critical, the challenge to identify and describe new species due the limited availability of taxonomists is creating a bottleneck using traditional morphological approaches (Costello et al., 2010). In the Red Sea, although taxonomic studies started many years ago (Hottinger, 1977; Banner and Banner, 1981; Wehe and Fiege, 2002; Wehe, 2007), most of the attention has been dedicated to coral reefs (Verseveldt, 1969; Benayahu and Loya, 1977; Vine and Bailey-Brock, 1984). Despite the existence of an inventory of polychaete species for the region (Wehe and Fiege,
taxonomic keys for invertebrates inhabiting soft sediments are limited, particularly compared to other regions, complicating the studies based on traditional taxonomy.

Over the last decade, several researchers have claimed the need to incorporate other aspects of diversity in environmental assessment studies (Norling et al., 2007; Van Hoey et al., 2010). Considering that benthic species play essential roles in ecosystem processes, species functional attributes (e.g. longevity, body size, feeding mechanisms, mobility, position in sediment and reproduction) can more effectively contribute to a better understanding of the impacts on the functioning of ecosystems than species richness or abundance alone (Bremner et al., 2003; Lavesque et al., 2009). Indeed, alpha-diversity measures alone appear to be insufficient to detect changes in soft-sediment communities with several disturbance pressures, unlike multivariate techniques which target changes in composition (Bevilacqua et al., 2012; Piló et al., 2015). However, changes in functional traits may occur as a response to natural variability (storms, sand movements, changes in general environmental conditions, etc.) or human pressures (van der Linden et al., 2012). Without a thorough understanding of the patterns of change of local communities to both natural and anthropogenic stressors, using functional trait analysis as a tool will not be feasible. In areas such as the Red Sea, where large-scale soft-sediment biodiversity assessments are still in their infancy, analyzing changes in functional diversity is still a major challenge. In this thesis, a proxy to functional diversity was assessed regarding feeding habits or trophic behaviour. The results showed that detritivores/suspension feeders were clearly dominant in the seagrass meadow, whereas carnivores increased in the coastal biotopes (nearshore and offshore). This was related to the food availability and variability in each biotope. We cannot forget that trophic changes provide a very limited picture of functional diversity, and that additional research is needed in the region (both from a taxonomic perspective and analysing responses of communities to multiple environmental gradients) before this promising tool can be fully implemented.

5.4.2. Timely and more affordable methods

Currently, environmental assessment of the marine environment is often limited in the taxonomic groups it assesses. Including multiple biological components (e.g. microbial communities, meiofauna, macroinvertebrates, fish) of the ecosystem will provide a more comprehensive picture of the ecological changes due to single or multiple pressures
(Radulovici et al., 2010). One possibility to address this is to further utilize molecular-based techniques (e.g. eDNA, genetic fingerprinting), a trend which has been increasing over the last decade. Indeed, in the near future, molecular-based biodiversity assessments might provide a cost-effective and complementary tool to marine monitoring (Aylagas et al., 2014, 2018). In fact, molecular tools will allow the inclusion of bacterial communities into environmental impact studies. These communities might be useful indicators detecting early changes in sediment condition due to short generation times (Drummond et al., 2015; Caruso et al., 2016). Also, they are critical players in the ecosystem functioning with a variety of essential ecological functions including nitrogen fixation, nutrient recycling, and decomposition of organic matter. Thanks to the advances in high-throughput sequencing technologies, bacterial communities have been recently incorporated into biological indices (Aylagas et al., 2018) and, with further improvements, the response of bacterial communities to stressors may be incorporated into decision-making processes in the near future. Molecular approaches may be particularly relevant if standardized protocols are applied, especially to perform assessments over large spatial scales and within short periods of time. However, substantial improvements are still required before we can incorporate the results from molecular techniques into the decision-making process. For instance, incomplete reference databases lead to the inability to assign taxonomy to some of the sequences obtained during environmental studies, which means that changes in composition and functional diversity cannot be properly investigated (Taberlet et al., 2012). Thus, complementary efforts between traditional methodologies and DNA-based analysis in order to increase the representation of species within DNA reference databases are an urgent requirement to be improved (Borja et al., 2007; Aylagas et al., 2018). This will enable more sequences from environmental metabarcoding studies to be assigned a taxonomic classification (Pearman et al., 2016). Although molecular-based indices are very promising, current indices are often based on abundance measures, which are currently unable to be determined using metabarcoding approaches due to unequal amplification efficacy across taxa and the detection of different life history stages (e.g. larvae and adults) (Dowle et al., 2016). While abundance is often uncorrelated with the abundance of sequences there is a correlation with specimen biomass (Elbrecht and Leese, 2017; Aylagas et al., 2018), thus providing information on the structure of the community.
Well-designed experimental studies to evaluate the response of communities to single and multiple stressors ideally combining traditional and molecular approaches is another research avenue that scientists need to pursue. These approaches will allow for a comparison between the responses of currently known indicators of change in the marine environment with the new ones emerging from molecular approaches. This can be further linked to ecosystem functioning especially with Quantitative Real-Time Polymerase Chain Reaction (qPCR) that can target specific functional genes of relevance assessed in the monitoring programs (Birrer et al., 2017). In addition, such a comparative approach can be extremely useful for a thorough knowledge of functional traits of species and how they change in response to environmental variability, so that this critical information can later be included in management and conservation decisions (Fig 5.2).

Implementing trait-based approaches with traditional species-based methods will help to the understanding of the rapid changes occurring in the functioning of the ecosystem. Several challenges limit the broad application of these approaches, particularly in less well-studied areas (Degen, 2018): 1) Creating a global professional network for research; 2) standardizing terms and methodology to be used; 3) Constructing and crosslinking trait databases such as, The Marine Life Information Network (MarLIN); 4) Performing trait-function experiments; 5) Applying traits to models; and (6) advising decision makers and stakeholders through transfer of knowledge between scientists and policy makers (Borja et al., 2017).
Fig. 5.2. Proposed roadmap as regards to the successful use of the application of trait-based approaches in addition to traditional species-based methods (Degen et al., 2018).
5.5. Marine spatial planning: towards the sustainable development of the Red Sea

Previous studies have shown that the Red Sea and Gulf of Aden environments and resources are generally in a healthy state, probably due to limited development in many parts of the region (Gladstone et al., 1999). However, recent studies have illustrated some signs of degradation of Red Sea habitats as a result of urban development and other human activities along the entire latitudinal gradients (Ali et al., 2011; El-Sorogy et al., 2012; Gladstone et al., 2013). If not well managed, the anticipated pressure on the Red Sea environment resulting from Vision 2030 will intensify the ongoing degradation of natural resources.

The current expansion of economic activities in the kingdom related to the use of the sea (e.g. tourism, aquaculture), might result in potential conflicts between different users that can be minimized with effective management. The Red Sea natural resources are crucial to the Kingdom of Saudi Arabia and their conservation will result in long-term benefits to the Kingdom, as well as to other countries in the region. However, in Saudi Arabia, a national strategy for prioritizing the multiple uses of the Red Sea is still emerging and has been limited by a focus on individual maritime sectoral policies (Al-Bisher et al., 2012; Meaden et al., 2016). Therefore, the application of marine spatial planning (MSP) to ensure the wellbeing of local populations while supporting economic growth is timely. MSP has been described by Ehler and Douvere (2009) as “a public process of analysing and allocating the spatial and temporal distribution of human activities in marine areas to achieve ecological, economic, and social objectives that are usually specified through a political process”. MSP will promote socioeconomic activities (e.g. industrial and recreational activities) without compromising the health of the marine ecosystems or the cultural value of their biodiversity. In this way, it has the potential to sustain the delivery valuable goods and services for current and incoming generations (Martin et al., 2016; Meaden et al., 2016). There are several marine sector activities that are likely to experience growth, for which if there are no coordinated management efforts from the government, may affect the marine environment and in turn also affect the productivity of the sector itself. For example, the quality of the environment is critical for aquaculture, however impacts resulting from these activities can be detrimental to other sectors such as tourism (e.g. aquaculture may result in nutrient enrichment that affects coral reefs both changing hard corals to the dominance of algae and even the fish
communities and this will influence tourists satisfaction). Therefore, before allocating an activity to a certain area, it is critical to plan the location carefully to avoid future conflicts among users (Costanza et al., 1997; Meaden et al., 2016). Sustained scientific research will help in developing essential knowledge to safeguard biological and ecological values that will benefit national and regional economies in the future (Martin et al., 2016). Even with MSP, it will not be possible to expand multiple activities in the Red Sea without leaving a human footprint. To mitigate the impact associated with those activities, coordinated and immediate action from the government is required including the design and implementation of Marine Protected Areas (MPAs).

The relatively semi-enclosed and oligotrophic nature of the Red Sea with minimal physical barriers between countries requires special attention from governments when planning to expand the Blue economy. Indeed, intensive pressure in one country may lead to trans-bordering effects that can result in diplomatic conflicts (Carvalho et al., 2019). Therefore, along with developing sound management plans, strict enforcement of regulations on national and regional levels will need to be applied (Carvalho et al., 2019). Collaborative efforts among the countries bordering the Red Sea and Gulfs of Aqaba and Aden are critical. Most of these countries (Saudi Arabia, Egypt, Jordan, Somalia, Sudan and Yemen) are involved in the Regional Organization for the Conservation of the Environment of the Red Sea and the Gulf of Aden (PERSGA). This organization provides guidance to governments toward the sustainable use of coastal and marine resources. For example, based on PERSGA’s effort (PERSGA/GEF, 2004) several marine protected areas (MPAs) have been proposed along the Red Sea and the Gulf of Aden (Fig 5.3). However, there most of the MPAs proposed remain to be implemented and among those that have been implemented only a few are managed properly, due to lack of enforcement (Gladstone, 2000).
Several initiatives, such as the Strategic Action Program (SAP), have introduced monitoring programs with an aim of recommending a system of nature protectorates (PERSGA, 2010). The Kingdom signed an agreement in 2001 with the Convention on Biological Diversity (CBD) to protect natural ecosystems and the associated biodiversity. Several Saudi Authorities, such as the National Commission for Wildlife Conservation and Development (NCWCD) and the Meteorology and Environmental Protection Administration (MEPA), dedicated some efforts to the establishment and development of protected nature areas in the kingdom (Abuzinada, 2003). Indeed, terrestrial protected areas have been established to protect habitats of key biological importance and provide concrete economic benefits to the local people [https://www.swa.gov.sa/en/#](https://www.swa.gov.sa/en/#). However, the protection of marine habitats in the kingdom is
lagging behind that of terrestrial counterparts and this needs to be rectified in light of the expected expansion of marine activities and mega-projects already announced by the Government (i.e. NEOM and Red Sea project).

5.6. Implications for further research

The current PhD research findings suggested that future environmental studies to be comprehensive and allow for meaningful conclusions will need to integrate multiple biotopes as they harbour relevant and unique subsets of organisms. The variety of habitat types (mangrove, seagrass and unvegetated) and their connectivity are crucial for sustaining biodiversity (Fig 5.4).

![Image](image_url)

Fig. 5.4. Illustration main of biological patterns observed. Biodiversity patterns were primarily driven by habitat characteristics along the inshore/offshore gradient. Symbols used in the illustration: courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

The environmental conditions associated with those distinct habitats and the associated ecological niches are also fundamental in structuring macrobenthic communities. For successful development of conservation policies in the region, sound knowledge of species distribution, size and biomass, functional attributes, habitat configuration, as well as connectivity, is critical. However, within each biotope, temporal variability becomes more relevant with different patterns in the composition and structure of macrobenthic communities emerging, depending on the habitat analysed. This suggests that when designing conservation policies, managers cannot ignore the variability across temporal scales (Bunn and Davies, 2000; Popp et al., 2000; Borja et al., 2014). Red Sea marine science
will be an area for national growth. The Red Sea is an interesting area for scientists to conduct research and there are several ideas have been proposed for future research such as:

- Evaluating the effects of human activities (e.g. aquaculture, water production through desalination, oil drilling) in the marine environment based on the responses of benthic communities.
- Further studies on habitat-dependent associations for an effective conservation of the Red Sea are also needed.
- Using soft-bottom macrobenthic communities to assess the quality status in Red Sea coastal areas under different levels of anthropogenic pressures aiming to evaluate the most responsive indicators for the ecological quality assessment.
- Assess the ecological status of marine systems over the years in response to main coastal development projects.

5.7. Communication, Ocean Literacy and Education are keys for a sustainable future

To ensure marine environmental quality and maintain the biodiversity and the sustained delivery of goods and services, a number of integrated actions will be required (Fig 5.5). Our role as scientists should not be restricted to conducting research and writing papers. We should find ways to transfer our knowledge to decision makers and to allow for a better implementation of measures but also support environmental education and literacy. This is particularly critical in Saudi Arabia where environmental awareness is still in its infancy. There is a need to understand coastal demands from local to regional and ultimately global contexts (Gladstone et al., 1999; Elliott, 2011, 2013). Sufficient and efficient communication between local communities, fishermen, governments, decision-makers, scientists from a wide range of disciplines, and multiple research institutes will contribute to the development of the marine management actions (Gladstone, 2000; Freedman, 2015; Meaden et al., 2016; Visbeck, 2018; Carvalho et al., 2019). Scientists need to work with local communities, and involve them through outreach activities, such as beach clean-ups. Governments also need to boost environmental education programs across the kingdom to increase public awareness about the importance of the ocean and how the marine environment influences our wellbeing. Investing in young people is vital to the future of the kingdom; schools and universities should provide the opportunity for involving young students in environmental research (Gladstone,
Education is a powerful tool, and can positively affect people’s behavior, motivating them to change some daily actions that can contribute to the protection of our seas (Gladstone et al., 2003; Visbeck, 2018).

Fig. 5.5. A conceptual model of multiple integrated actions; these actions are keys for the environmental quality and the sustainable future. Modified from Freedman (2015).
5.8. References


APPENDICES

**Supplementary material (SM-1),** List of macrofauna taxa (The list is related to Chapter 2)

**Supplementary material (SM-2),** other contribution arising during my PhD period