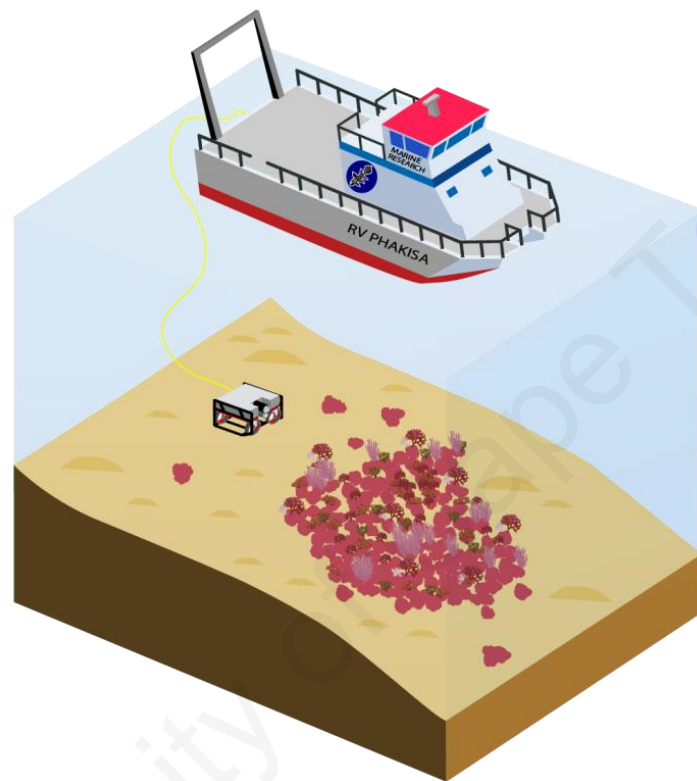


Patterns and potential environmental drivers of mesophotic communities of the warm temperate shelf of the Amathole Region, South Africa.

by

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Declaration

I hereby declare that “Patterns and drivers of mesophotic communities of the warm temperate shelf of the Amathole Region, South Africa” is my own work and that all the sources I have used or quoted have been indicated and acknowledged by means of complete reference.

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Abstract

Foundational biodiversity research has seen a recent shift from the collection of epibenthic data using destructive methods to less destructive methods that include visual surveys- using underwater camera platforms to explore the seabed. South African mesophotic ecosystems are under-sampled compared to both their shallower and deeper counterparts. The Amathole offshore region, considered as a transition zone between the Agulhas and Natal ecoregions, is a historically unexplored region of the South African coastline. This thesis aimed to define and describe the benthic communities and identify the processes driving their distribution on the temperate shelf in the Amathole offshore region, using a Remotely Operated Vehicle (ROV). This study piloted the application of the Australian developed CATAMI classification scheme to annotate images collected by ROV in South Africa. Data were collected on the ACEP: Imida Frontiers project on board the RV Phakisa during January and May 2017 off the Kei River, Amathole offshore region. This survey combined 14 sites comprising 215 images from remotely operated vehicle imagery and nine environmental variables from 30 to 100 m water depth. Multivariate analyses (multidimensional scaling and a cluster dendrogram) of image data produced nine benthic communities. Similarly, multivariate analyses (principal component analyses, distance-based linear model and distance-based redundancy analysis and constrained binary divisive clustering analysis) of the environmental data revealed that substratum type and correlates of depth to be the main variables likely responsible for the observed biodiversity patterns. Additionally, the LINKTREE analysis revealed a depth break at 74 m which established the boundary between the upper and lower mesophotic zone in this region. Rhodolith bed communities were discovered in the upper mesophotic and are a welcomed novel ecosystem type for South African benthic ecologists. The upper mesophotic zone was also characterised by communities of dense brittle star aggregations and reefs dominated by macroalgae. The lower mesophotic zone was characterised by animal forests consisting of communities dominated by sponge gardens with diverse growth forms and dense stands of canopy forming gorgonians. This thesis provides recommendations for future research and guidelines for future ROV field sampling. It also highlights the need for greater sampling effort by ROV on unconsolidated substratum and at depths greater than 74 m. The use of morphospecies in image classification to define macrobenthic communities on an unexplored continental shelf was effective despite limited knowledge of species. Similarly, the environmental variables that structure these temperate shelf communities were identified. Information from this study contributed to the foundational biodiversity information needed to inform marine spatial planning and spatial management efforts for the newly proclaimed Amathole Offshore Marine Protected Area and the greater Amathole offshore region.

Chapter 1 Introduction

The distribution of marine benthos is structured by a complex interplay between abiotic and biotic variables. Our understanding of these interactions are particularly limited in offshore regions, as deep water epifauna and epiflora visual surveys are challenging and generally require remotely operated camera platforms. Although these remote camera platforms are expensive to acquire, they can be deployed in hazardous seas while their depth and duration capabilities far exceed that of SCUBA aided surveys (Mallet and Pelletier 2014). Besides capturing biological data, these camera platforms can also be outfitted with numerous instruments to measure the physical environment allowing benthic ecologists to explore pertinent ecological questions. As access to remote camera technology increases, research efforts have focused on exploring and describing deeper subtidal ecosystems (Turner et al. 2017). Since research efforts deeper than SCUBA diving capabilities have increased, benthic ecologists have recognised depths deeper than the shallow subtidal as the mesophotic zone which is an ecologically distinct bathymetric range characterised by light-dependent algal and invertebrate communities (Kahng et al. 2010). The mesophotic zone worldwide is relatively unexplored and most countries are still in the exploratory phase, therefore, descriptive studies mapping the distribution and structure of mesophotic ecosystems have been the focus in this zone (Turner et al. 2017, Cerrano et al. 2019).

1.1.1 Mesophotic Ecosystems

Mesophotic ecosystems are light-dependent communities that typically occur between ~30 – 150 m depth (Lesser et al. 2009, Kahng et al. 2010). Benthic ecologists recognise that mesophotic ecosystems can be distinguished by their geography, namely tropical and temperate mesophotic ecosystems (Cerrano et al. 2019). Polar mesophotic ecosystems are also recognised as unique but limited research at these latitudes mean literature is scarce (Cerrano et al. 2019). The mesophotic zone can be subdivided into the upper and lower mesophotic zones, because upper mesophotic communities resemble their shallow-subtidal counterparts (Slattery et al. 2011). The upper mesophotic is generally considered to be located between 30 - 60 m (Turner et al. 2017). The transition zone between the upper and lower mesophotic zone varies geographically and is also dependent on whether fish or benthos are sampled (Lesser et al. 2019). The lower limit of the mesophotic zone spans a much wider bathymetric range, which includes 120 m in the Mediterranean but it had been suggested that the limit can extend to 150 m and as deep as 300 m (Cerrano et al. 2019) depending on water clarity (Kahng et al. 2010). The mesophotic zone and animal forests have only recently been recognised in South Africa (Samaai et al. 2020).

Animal forests are components of the mesophotic zone and are characterised by communities of habitat forming heterotrophic epifauna which dominate the seabed where benthic primary producers

are absent (Rossi et al. 2017). The main components of animal forests are sponges, cnidarians, bryozoans, ascidians, polychaetes and bilvalves (Rossi et al. 2017). Marine animal forests have the potential to be considered Ecologically and Biologically Significant Areas (EBSA) or Vulnerable Marine Ecosystems (VME) because many organisms of animal forests are habitat forming, fragile and slow growing (Rossi et al. 2017, Samaai et al. 2020). This makes animal forests susceptible to anthropogenic impacts such as trawling and warrants their protection (Samaai et al. 2020, Soares et al. 2020) . Therefore, their global distribution and ecological processes needs to be understood. To focus research in the mesophotic zone where animal forests occur, Turner et al. (2019) proposed a set of biological to ecological through to conservation and policy related questions to focus and facilitate research in the mesophotic zone.

1.1.2 Biodiversity patterns of Mesophotic Ecosystems

Overall, mesophotic coral ecosystems and temperate mesophotic ecosystems have similar community patterns but differ in biodiversity structure. Tropical communities, known as mesophotic coral ecosystems, are often dominated by zooxanthellate scleractinians and include algae, sponges and fish assemblages (Kahng et al. 2010, Loya et al. 2016). An extensive body of literature (Hinderstein et al. 2010, Bridge et al. 2011a, Slattery et al. 2011, Kahng et al. 2012, 2017, Cordeiro et al. 2015) on the distribution and ecology of mesophotic coral ecosystems exists relative to the limited knowledge of their temperate counterparts (Ponti et al. 2018, Cerrano et al. 2019, Enrichetti et al. 2019). Temperate Mesophotic Ecosystems (TME) are communities dominated by habitat-forming macroalgae and a high diversity of filter-feeding assemblages (Ballesteros 2006) also known as animal forests (Kahng et al. 2017) of which South Africa boasts numerous records (Samaai et al. 2020). The major differences between these two ecosystem types are the dominant organisms that form complex 3-dimensional habitats for other organisms. Both ecosystem types can produce distinct biogenic reefs (Cerrano et al. 2019). Zooxanthids generally build reefs in tropical regions whereas; crustose coralline algae produce coralligenous concretions in dim light in temperate regions in the mesophotic zone (Ballesteros 2006). These differences are due in large part to the difference in climate and other physical factors that control the community patterns, and are often identified using standardised benthic image classification schemes. Image classification schemes are generally specific to a particular country, region (CATAMI, Althaus et al. 2015), institution or research group and depending on the level of information available. These image classification schemes are essentially on catalogues of reference images with associated unique morphospecies or Operational Taxonomic Units (OTU) to identify organisms from marine imagery e.g. Deep Reef Benthos of Bermuda (Stefanoudis et al. 2018)

Various image annotation software packages exist to classify benthic imagery and a table comparing different software packages was produced in Gomes-Pereira et al. (2016). Bio-Image Indexing and

Graphical Labelling Environment (BIIGLE, Schoening et al. 2009) is the most popular web-based image processing software whereas, Image Processing and Analysis in Java (ImageJ, <https://imagej.nih.gov/ij/>) and Coral Point Count with Excel extensions (CPCe, Kohler and Gill 2006) are the most popular desktop based image processing applications (Gomes-Pereira et al. 2016). Software package preference is attributed to different factors including but not limited to cost, research group preference, research community support, local expertise using the software and user-interface (Gomes-Pereira et al. 2016). Benthic ecologists in South Africa make use of a number of image annotation softwares (Table 1.1). CPC-e is used to annotate images of coral communities collected by divers on shallow reef visual surveys (Joshua et al. 2018) and mesophotic reefs using ROV (Heyns et al. 2016). EventMeasure (www.seagis.com.au) has been used to annotate video for Baited Remote Underwater Vehicle (BRUVs) to survey fish communities (Bernard 2012). Only recently has TransectMeasure (www.seagis.com.au) been used to annotate images from towed camera platforms (van der Heever pers comm). The benthic ecology community in South Africa is small and the number of researchers studying underwater imagery is even smaller. These researchers are also exploring the use of different sampling equipment, survey methods and testing the application of different image annotation software packages and classification schemes because South Africa does not have national image classification scheme or annotation software for benthic biota. These are among the reasons why preference towards software packages and image classification schemes differ amongst research groups and institutions in South Africa.

Table 1.1 List of annotation software packages used to classify benthic imagery in South Africa. Details about the camera platforms used, institution and available literature are provided

Image annotation software	Camera platform	Institution	Target taxa	Literature
TransectMeasure	Ski-Monkey towed camera	SAEON	Invertebrates	(van der Heever pers comm)
EventMeasure	BRUV	SAIAB	Demersal fish	(Bernard 2012)
Coral Point Count with Excel extensions (CPC-e)	ROV	Various	Invertebrates	(Heyns et al. 2016)
(CATAMI)	ROV	UCT	Invertebrates	None

CATAMI is a hierarchical marine image classification scheme developed in Australia and its strengths lie in its hierarchical structure which combines broad level taxonomy of morphology to identify marine

fauna and flora (Althaus et al. 2015). This is particularly useful for mesophotic ecosystems because they are often unexplored and species level knowledge of the benthos is limited. Moreover, it also allows for biodiversity pattern comparisons at a regional scale. Several studies in Australia and Tasmania have used this classification scheme to classify and/or annotate images collected from underwater cameras (Bewley et al. 2015, Lawrence et al. 2015, Monk et al. 2016, Perkins et al. 2016, James et al. 2017, Marzloff et al. 2018). South Africa does not have a standardized classification scheme for analysing marine images because the use of underwater imagery to study marine fauna, especially benthic invertebrates, has only gained popularity in recent years (Franken 2015, Heyns et al. 2016, Makwela et al. 2016, von der Meden et al. 2017). Instead of developing a novel image classification scheme, the CATAMI classification scheme was explored in this study because it was compatible with CPCe; open-source image annotation software specifically designed for benthic imagery.

1.1.3 Environmental controls of Mesophotic Ecosystems

Macrobenthos in the mesophotic zone are primarily driven by light (Lesser et al. 2009). Other environmental variables that drive community patterns include topography (Bridge et al. 2011a), temperature (Kahng et al. 2012), sedimentation, nutrients (Heyns-Veale et al. 2019), particulate organic matter (Heyns et al. 2016), dissolved organic carbon (Ribes et al. 1999), water movement (Ballesteros 2006), salinity (Ballesteros 2006) and depth (Bridge et al. 2011a). Environmental variable importance differs geographically and can even vary drastically at the microhabitat scale such as caves, cracks, crevices and steep facades of reefs (Ballesteros 2006).

Light quantity and quality directly influences the bathymetric range, cover and structure of light-dependent corals and red macroalgae in mesophotic coral and temperate mesophotic ecosystems, respectively (Lesser et al. 2009, Kahng et al. 2014). These communities need sufficient light to produce their complex frameworks. Under- or overexposure to levels of irradiance inhibits growth and in extreme cases can cause die-offs. The lower limit of surface irradiance of coralligenous communities is 0.05% surface irradiance (Ballesteros 2006) whereas; the lower limit for light-dependent coral communities are 0.5% in optically clear tropical waters (Dubinsky and Falkowski 2011). Light quantity (amount of light) is more important than light quality (spectrum of light) to algae, especially red macroalgae (Luning 1981) in the mesophotic zone, because they produce organic compounds called phycobiliproteins to absorb green and blue wavelengths of the visible light spectrum (Ballesteros 2006), which are generally not absorbed by other macroalgae in low light conditions.

Variations in topography can directly influence light availability to light-dependent benthic organisms by casting productivity shadows (Bridge et al. 2011a). Irradiance levels within the mesophotic zone

could possibly resemble abyssal light conditions, almost 0 % irradiance, in certain microhabitats such as deep inside caves and small holes within reefs (Ballesteros 2006). Similarly, reduced irradiance and low light conditions on steep reef walls can even completely exclude photosynthetic organisms and favour heterotrophs (Bridge et al. 2011a).

Water temperature is important in regulating the bathymetric distribution and physiology of benthic organisms (Ballesteros 2006). The thermal zonation of some assemblages of the mesophotic zone can also be attributed to highly stratified water. This is particularly evident in mostly stenothermal organisms in lower mesophotic zone that never migrate to warmer shallow waters (Ballesteros 2006, Bridge et al. 2011a). The growth of tropical corals may also be limited by temperatures less than 18°C and temperature fluctuations caused by internal waves (Wolanski et al. 2004). The thermal tolerances of temperate organisms are often slightly colder and wider ranging than compared to their tropical counterparts (Ballesteros 2006, Cerrano et al. 2019). Temperate mesophotic ecosystems in the Mediterranean Sea experience temperatures ranging between 10-23°C which promotes the development of coralligenous communities (Ballesteros 2006). Fluvial effluent may not necessarily alter water temperature of subtidal coralligenous ecosystems but its different chemical composition and physical properties could affect the growth of coralligenous ecosystems.

Rivers transport vast quantities of freshwater which may alter the water chemistry and hydrography of the adjacent marine environment (Moura et al. 2016); in particular salinity levels and other constituents such as sediment, nutrients, particulate organic matter and dissolved organic carbon (Heyns et al. 2016). Reduced salinity from rivers not only aids in water stratification, it may also facilitate the bathymetric restriction of stenohaline organisms (Ballesteros 2006). Biogenic reefs are generally absent adjacent to large rivers in tropical and subtropical latitudes because hyposalinity, reduced pH, reduced light penetration and sedimentation inhibit reef formation (Goldberg 2006, Moura et al. 2016). Large rivers such as the Amazon River can form a biogeographic break of many benthic organisms (Moura et al. 2016). Fluvial derived nutrients are important for subtropical ecosystems, but form a small contribution to the trophic budget of filter feeder invertebrate communities in subtidal assemblages (Porter et al. 2014). Fluvial derived particulate organic matter may form as much as 33% of trophic budget for these filter feeders (Porter et al. 2014). Heavier fluvial sediments settle first, thereafter finer sediments settle which can form muddy habitats. Depending on the degree of sedimentation, fluvial sediments can form an integral portion of biogenic reef formation. This is particularly important in high energy environments where accumulated sediments, particulate organic matter and nutrients are re-suspended into the water column.

Water movement facilitates the transport of nutrients and particulate matter which can influence the benthic environment. Nutrients derived from upwelling and internal waves may also promote the growth of macroalgal species in the lower mesophotic zone (Leichter et al. 2008) and heterotrophic coral species in the upper mesophotic zone (Leichter and Genovese 2006). Conversely, internal waves often cause the extreme temperature and water chemistry fluctuations which can inhibit the growth of corals (Lesser et al. 2009). Another process related to water movement is wave energy which is strongly correlated to depth (James et al. 2001). Prolonged high wave energy and sweeping currents may act as erosional forces which facilitates the generation of biogenic sands from calcareous biotas through wave abrasion (James et al. 2001).

Research into the complex interplay between environmental variables and biotic communities in mesophotic ecosystems is relatively novel and predominantly from tropical regions (Lesser et al. 2009, Bridge et al. 2012, Turner et al. 2017). Temperate mesophotic and polar ecosystems have not been studied as extensively as mesophotic coral ecosystems (Turner et al. 2017, Cerrano et al. 2019). The majority of the mesophotic research in temperate regions comes from the Mediterranean and temperate North America, with relatively fewer studies being conducted in the Southern Hemisphere and are localised in the Great Barrier Reef, Australia (Bridge et al. 2011b, 2011a, 2012, Kahng et al. 2017, Turner et al. 2017, Figure 1.1). Africa has the lowest research output from mesophotic ecosystems with South Africa being the only country to have published research from the mesophotic ecosystem (Götz et al. 2009, Heyns et al. 2016, Heyns-Veale et al. 2016, Makwela et al. 2016). The paucity of mesophotic research, particularly in temperate regions, in South Africa is more a function of lagging data processing as opposed to limited underwater camera availability and capability. Funding may also limit the scope of underwater visual surveys. Moreover, the eastern parts of the South African coastline is data poor because of limited sampling opportunities and precarious seas.



Figure 1.1 Locations and number of research outputs in mesophotic ecosystems before 2017 (Turner et al. 2017). Blue dot indicates the location and number of research outputs in South Africa

1.1.4 Study area – Amathole region

The Amathole Region is a district of the Eastern Cape province surrounding the city of East London. This region is comprised of marine protected areas known as the Amathole Inshore and Offshore marine protected area complex. It forms a cluster of protected areas spanning from the nearshore zone down a depth of 2200 m (DEFF 2019). The marine protected areas are further subdivided into restricted and controlled zones, allowing recreational line-fishing and SCUBA diving within the latter zone. The study sites cover areas within the Kei Offshore Controlled and Restricted Zones.

The climate is warm temperate with year-round rainfall. This rain feeds a number of river systems that transport vast quantities of freshwater and solutes into the adjacent marine space. One major river system, the Great Kei River catchment receives a mean annual raining of 1 064.1 Mill m³/a (Van Niekerk and Turpie 2012). This fluvial input into the marine system influences the seabed geology (Kineke et al. 1996, Nittrouer and DeMaster 1996), hydrography (Geyer et al. 1996) and ecology of the nearshore and offshore environment such as off the Amazon River mouth (Moura et al. 2016, Omachi et al. 2019).

The seabed topography in this region is described as narrow and tapered (Green et al. 2020). The continental shelf is narrower than on the West Coast of South Africa but relatively wider than on the East Coast of South Africa (Figure 1.2), with paleo-shorelines observed at 105 m and 60 m deep (Green et al. 2020). The shelf is characterised by a sandy inner shelf, whereas the middle shelf is characterised by a mosaic of rhodolith beds, gravel, and bedrock outcrops forming high relief reefs; the outer shelf is characterised by bedrock covered in a veneer of coarse sand (Green et al. 2020). The continental shelf is moderately sloped and the shelf edge is steep. The shelf profiles, along with the major freshwater input from the Kei River potentially shape the biological communities in this offshore region.

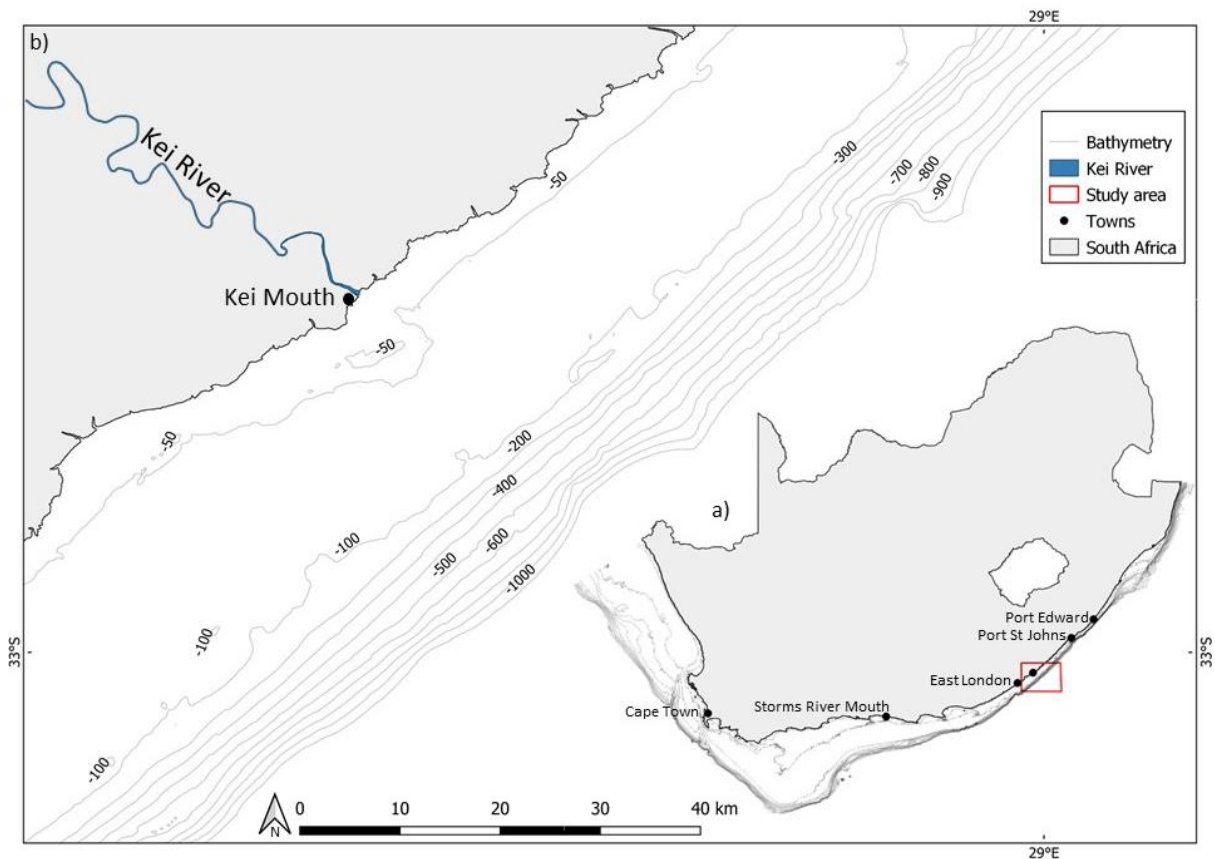


Figure 1.2 Bathymetric map of a) South Africa depicting the wide continental shelf on the west and narrower shelf on the South-Eastern and Eastern part of South Africa (DeWet and Compton 2021), (b) shows the bathymetry of the East London shelf. Each bathymetric line represents a 100 m depth interval

The physical oceanography in the Amathole Region is not well understood. This region is historically one of the least sampled regions along the South African coast (Lamont pers comm). While regional processes between Cape Town and Cape St Francis are known, the regional hydrography between Cape St Francis to Port Shepstone have only been investigated in recent years (Russo et al. 2019). The effect of sea surface temperature on primary productivity in the euphotic zone was investigated on the south eastern coast of South Africa (Barlow et al. 2010). Two stations were in the greater Amathole region whereas; only a single station was off the Kei River. Sea surface temperature and the depth of the euphotic zone at a single location were the only oceanographic measurements on record in this region before this project was undertaken. Similarly, few studies, off the Storms River Mouth in the Tsitsikamma MPA (Heyns et al. 2016, 2019) and between Port St Johns and Port Edward in the Pondoland MPA (Celliers et al. 2007) have been conducted in the warm-temperate environment adjacent to the Amathole region. These are the only other studies that provide knowledge of the benthic communities and their associated environmental drivers of temperate mesophotic ecosystems in South Africa.

1.1.5 Thesis aims and overview

The aim of this thesis was to define and describe the benthic invertebrate communities and investigate the potential environmental drivers influencing the structure and distribution of these communities on the warm-temperate river-influenced shelf off the Kei River in the Amathole region of the Eastern Cape coast. This research used ROV and underwater camera imagery to investigate an area that was historically understudied and establish foundational biodiversity information of the then proposed Amathole Offshore MPA. This collection of foundational biodiversity information is essential for informing regional and local management plans for this MPA and others like it.

Chapter 2 aimed to define and explore the benthic invertebrate communities off the Kei River for the first time. Images collected by remotely operated vehicle (ROV) were annotated and analysed using the CATAMI classification scheme. Multivariate analyses were used to explore the similarities between sites and identify distinguishing morphospecies that shape these communities. The results from this chapter provided the data required for Chapter 3.

Chapter 3 related environmental variables to the benthic communities determined in Chapter 2 using multivariate analyses. Thereafter, the findings were discussed and compared to river-influenced and mesophotic ecosystems elsewhere.

Chapter 4 interrogated the findings of Chapters 2 and 3, raised knowledge gaps, and further discussed visual surveys and made general recommendations for this method of data collection and research in similar systems in South Africa and elsewhere.

Chapter 2 The description of benthic mesophotic communities from a temperate river influenced shelf off the Kei River

2.1 Abstract

Mesophotic ecosystems are poorly understood worldwide; particularly in temperate regions. In South Africa, few studies have analysed ROV imagery of benthic assemblages in this depth range. This chapter aimed to explore and describe the benthic communities off the Kei River, in the Amathole Region, South Africa. ROV imagery was collected from transects that covered a number of substratum types on the narrow continental shelf between 30 and 100 m. Replicate images (205) were collected from 18 sites off the Kei River and were annotated using the CATAMI classification scheme. Multivariate analyses were performed on benthic morphospecies abundance data to investigate patterns in benthic communities. Site clustering was well defined using cluster dendrogram and multidimensional scaling yielding nine distinct communities. Between 30 – 74 m, photosynthetic taxa (red macro- and coralline algae) dominated communities on reef, mixed reef and coarse sand. Filter feeding taxa (sponges and gorgonians) dominated deeper areas (74 – 100 m) on mixed reef, and the varied sponge morphology of such deeper communities suggests variable current speed environment. The discovery of dense rhodolith aggregations was a first record in South Africa. Similarly, echinoderm aggregations, namely the feather star and brittle star aggregations were also noteworthy. Greater sampling effort of offshore benthic ecosystems, particularly off rivers, should be prioritised and the discoveries of several species aggregations made here highlight the paucity of shelf ecosystem research at mesophotic depths in South Africa.

2.2 Introduction

Temperate mesophotic ecosystems (TME) comprise light-dependent benthic communities dominated by red macroalgae between ~30 – 150 m depth. This zone supports distinct assemblages adapted to low light and with some species not found in the shallow subtidal < 30 m. The coralline macroalgae in this zone form biogenic reefs by creating carbonate accretions. These biogenic reef habitats are well documented in the Mediterranean (Cerrano et al. 2010, 2015, 2019, Bo et al. 2014, Ponti et al. 2018) and parts of temperate Australia (James et al. 2017). Biogenic reefs are not the only biogenic habitats found at these depths, rhodolith aggregations are also formed by free-living non-geniculate coralline algae accretions on sand environments. Like temperate biogenic reefs, rhodolith aggregations are important producers of calcium carbonate and support distinct benthic communities (Cerrano et al. 2019). These photic communities are generally restricted to the upper mesophotic zone, which is defined by the depth limit of benthic primary production, and can range between 50 – 75 m depth depending on light penetration and turbidity. Upper mesophotic communities are comprised of corals, molluscs and are dominated by red macroalgae. Below the upper mesophotic is the lower mesophotic zone. This zone extends down to anywhere between 150 – 200 m (Cerrano et al. 2019). The lower mesophotic zone is characterised and distinguished by obligate heterotrophs and supports a number of sponge, bryozoan and coral taxa (Lesser et al. 2019). Still, little is known about the community ecology and structure of temperate mesophotic communities (Harris et al. 2021), especially in the southern hemisphere where capacity and resources to sample this depth zone are limited.

All visual surveys conducted in the mesophotic zone utilise remote camera platforms to survey benthic communities and have advanced our understanding of mesophotic research globally. This research effort however, has a reef and coral taxa bias. Few studies focus on or include non-reef habitats or other invertebrate taxa. Even more so, most mesophotic research in South Africa has been focused on reef habitats with very few including unconsolidated (sand, gravel and mud) shelf habitats (Celliers et al. 2007, Samaai et al. 2010, Franken 2015, Heyns et al. 2016, Makwela et al. 2016). Given the scarcity of foundational biodiversity information (species occurrence and distribution records) of temperate mesophotic ecosystems and in the greater Amathole region (Heyns-Veale et al. 2016, 2019), this study aims to explore and describe the benthic communities in the warm temperate river-influenced mesophotic zone off the Kei River mouth, South Africa. The objectives are two-fold. Firstly, to utilize the CATAMI classification scheme and CPCe on oblique ROV imagery to determine communities; and secondly to identify distinguishing and characteristic morphospecies for benthic communities off the Kei River.

2.3 Materials and Methods

2.3.1 Study Area

This study investigated the river-influenced shelf off the Kei River mouth between Morgans Bay and Qholorha Mouth, in the Amatole region, Eastern Cape (Figure 2.1). The study area was assumed to be within the Kei River's influence due to the proximity to the Kei River mouth. Observations extended from 30 m to 100 m. The continental shelf is terraced off the Kei River mouth and a fluvial fan produced by the Kei River is the main submarine feature on the shelf and extends over the inner shelf and onto the middle shelf (DeWet and Compton 2021). Substratum type ranges from consolidated through mixed to unconsolidated substrata across the shelf. This area was selected for two reasons: (i) limited biodiversity information was available for the offshore region due to limited sampling opportunities, and (ii) to provide foundational biodiversity knowledge for the Amathole Offshore Marine Protected Area when it was in its proposal stage.

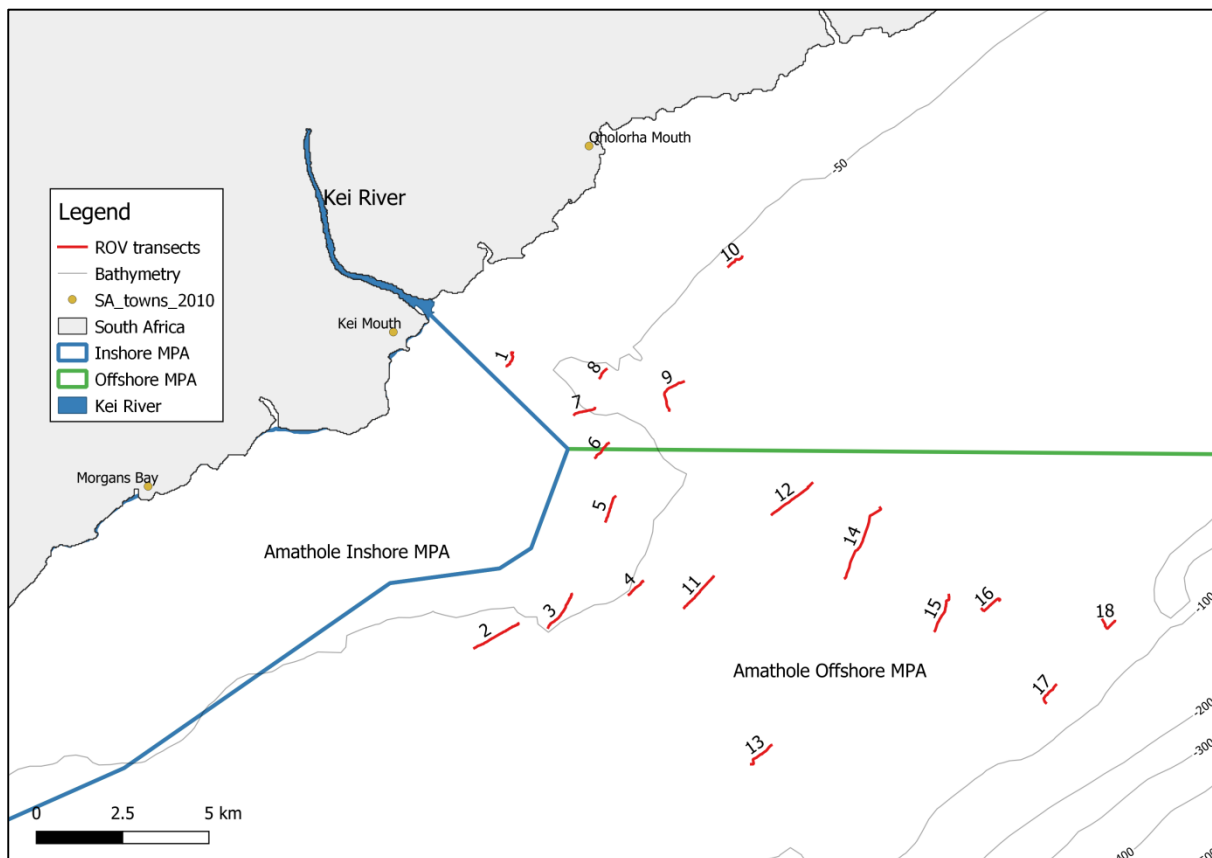


Figure 2.1 Location of the visual transects undertaken during January/February 2017 and May 2017 off the Kei River mouth. The red lines depict 18 remotely operated vehicle transects over the river influenced continental shelf off the Kei River mouth. The coastal Amathole MPA is outlined in blue, whereas the Amathole Offshore MPA boundary is shown in green

Table 2.1 Remotely Operated Vehicle transects, the number of samples and images conducted during January and May 2017 on the river-influenced shelf off the Kei River mouth, South Africa. Transect start location represented by latitude and longitude

Transect ID	Number of cells/ samples	Number of images	Latitude	Longitude	Depth range (m)
1	3	15	-32.6925	28.40542	29-34
2	5	25	-32.75719	28.40996	53-73
3	2	10	-32.75086	28.422013	44-47
4	1	5	-32.74824	28.44058	49-50
5	1	5	-32.7255	28.43449	46-49
6	3	15	-32.71231	28.43166	47-49
7	2	10	-32.70509	28.42183	43-44
8	1	5	-32.75095	28.42202	44-49
9	3	15	-32.69636	28.45122	58-68
10	1	5	-32.6669	28.46317	54-56
11	3	15	-32.74447	28.46091	59-69
12	3	15	-32.72108	28.48633	52-75
13	1	5	-32.79003	28.47448	91-93
14	5	25	-32.72908	28.50238	75-87
15	3	15	-32.75118	28.52118	87-90
16	1	5	-32.75342	28.53358	86-91
17	2	10	-32.78094	28.54629	87-90
18	1	5	-32.75829	28.56022	96-97

2.3.2 Survey protocol

A total of 18 underwater video transects were completed in the study area with the use of a Remotely Operated Vehicle (ROV) during January and May 2017 as part of the ACEP Imida Frontiers project survey (Table 2.1, Figure 2.1). The transect locations were determined from a database of known reef positions and multibeam interpretation (Appendix 1). These transects span across the continental shelf off the Kei River mouth, Eastern Cape ranging between 30 m and 100 m deep and were evenly distributed along the depth gradient. Transects were between 30 min to 1 hour long with an average travel speed of 0.5 knots. Transect lengths varied between 1 km and 1.5 km long and deviated from a straight line when bottom and/or surface currents were variable or too strong to optimally pilot the ROV.

The camera platform, a Sub Sea Imaging 1Cam HD Camera, outfitted onto a SeaEye Falcon 12177 ROV was used for the study. The ROV was connected to the vessel by means of two tethers (Figure 2.2a). One tether, a 300 m long fibre optic cable, was the interface connection between the ROV and the surface unit control on the vessel. This tether was also equipped with polystyrene floats spaced 1 m apart to limit the risk of entanglement. The second tether, a high tensile strength steel cable attached to an A-frame and hydraulic winch was used to aid deployment and retrieval of the ROV. Furthermore, the two connections acted as fail safes in the event that either connection was to break. Moreover, a 300 kg weight, positioned 50 m from the ROV, aided in the descent to the seabed in strong currents and to position the ROV directly underneath the vessel. A deviation of up to 50 m was assumed due to relative position of the weight to the ROV (Figure 2.2b).

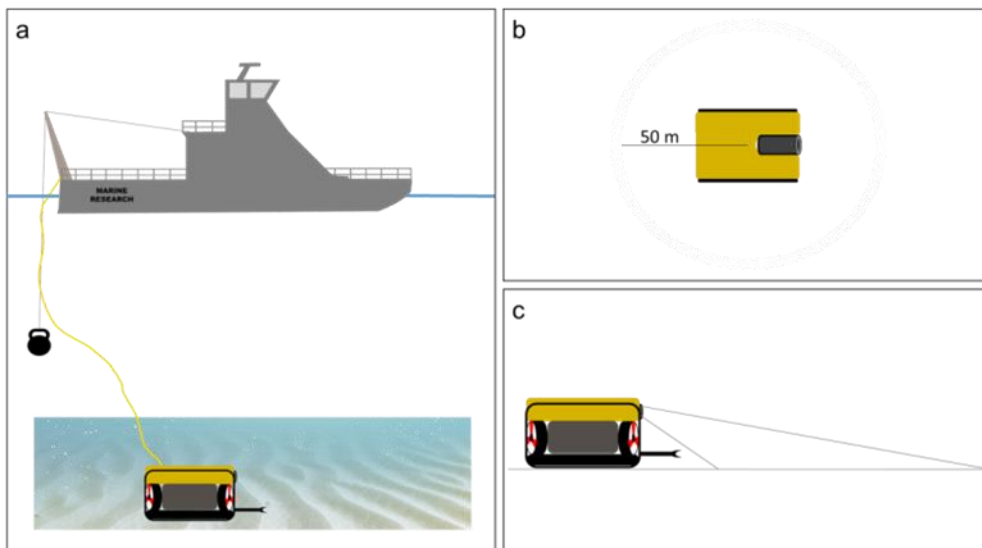


Figure 2.2 ROV survey protocol for the Imida Frontiers project off the Kei River mouth. (a) The fibre optic cable and high tensile strength steel cable connections and weight relative to the ROV, (b) the ≤ 50 m position deviation of the ROV relative to the vessel and (c) the camera orientation of the ROV.

2.3.3 Data processing

Firstly, in QGIS (version 3.4.2, QGIS Development Team, 2018), a 0.25 km^2 (0.075 nmi^2) grid layer was superimposed over the study area in order to split the area into sampling units (henceforth referred to as cells) and to mitigate spatial autocorrelation (Figure 2.3). The GPS track of the vessel was used as a proxy for the ROV's position underwater because the ROV was not equipped with a GPS. Therefore, the GPS track of the vessel was superimposed onto the gridded study area. From this, it was possible to synchronise the vessels GPS with the ROV footage enabling geo-referenced frame grabs to be extracted from the video footage in each cell.

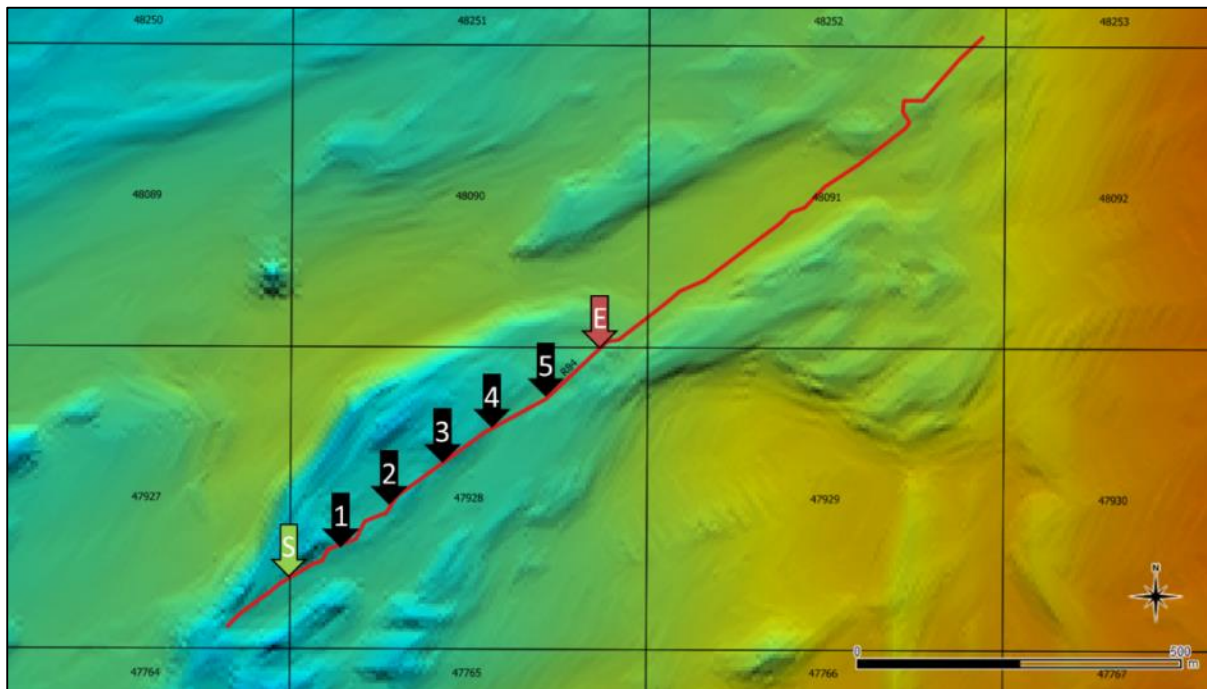


Figure 2.3 Illustration of location of replicate images (1-5) analysed for a grid cell (sample) along an ROV track (red line). Five images were extracted from each cell at a $\frac{1}{4}$ mile grid cell scale. The red line represents the track from one ROV survey (R84).

Secondly, each transect covered one or more cells and consisted of five images per cell. These images were extracted from the video footage at predefined GPS points, which were determined by temporally dividing the transect segment in each cell into seven GPS points (Figure 2.3). A minimum time criteria of 3 min was used to exclude cells with insufficient seabed coverage. The cell boundary points were discarded, and a single image was taken from the video footage at each of the remaining GPS points resulting in five images per cell (Figure 2.3).

Thirdly, this study made use of abundance-annotation classification to identify the visible biota instead of using percentage cover, this is due to the fact that the ROV images could not be scaled in this study. To ascertain the appropriate number of annotations to be used per image, a single sample (5 images) from the main substratum types identified *a posteriori* were classified for produce morphospecies accumulation curves. The accumulation curves were generated for five predefined substratum types in Primer version 6 (Clarke et al. 2014), using bootstrap as the non-parametric estimator. The slopes of the curves were used to determine the appropriate number of points per image.

Fourthly, whole image and abundance-annotation classification were used in conjunction with the CATAMI classification scheme (Althaus et al. 2015) to classify the ROV images. Whole image and abundance-annotation classification were used to classify substratum but only the product from the

former classification type was used in the analysis. Whole image classification was performed for substratum type, i.e., visually examining and recording the dominant habitat across a sample (five images) as opposed to using the annotations to consistently record the dominant substratum type across images. Substratum was also image-defined due to the absence of sediment samples to pair with images from the study area. In addition to substratum types inherent in CATAMI; a mixed reef category was added for the purpose of this study. The classification of substratum and visible biota based on CATAMI was also exhaustive in order to extract the greatest detail possible from the images for this image identification approach.

Finally, abundance-annotation was used to classify macrobenthic morphospecies according to the CATAMI classification scheme (Althaus et al. 2015). This classification scheme has a number of tiers for visible biota. The highest tier is phylum level followed by major growth form type whereas the final tier(s) are descriptive morphology. A CATAMI code file was uploaded into the image annotation program Coral Point Count (CPCe, Kohler and Gill 2006). This program was used to annotate the images by superimposing a fixed number of randomly assigned points onto the images (Figure 2.4). Morphospecies were used due to the lack of species level information in this region and to prevent species misidentification of the macrobenthos. The latter is especially true for sponges because this phylum can display a high degree of morphological plasticity within a single species. Sponges may also encrust other organisms such as fan shaped octacorals. In the event an annotation landed on an encrusted octacoral, the annotation was assigned to the octacoral because the sponge would not occupy the water column in the octacorals absence. This should not underestimate encrusting sponge abundance because its encrusting growth form lends to high detectability. In the event one or more annotations landed on the same individual or colonial unit, only the first annotation was used to avoid pseudoreplication. The other annotations were assigned as "same individual" and were not included in the analysis. The use of morphospecies grossly underestimates species diversity because it may encompass several species but it appropriately captures functional morphospecies groups which are often used in ecological studies.

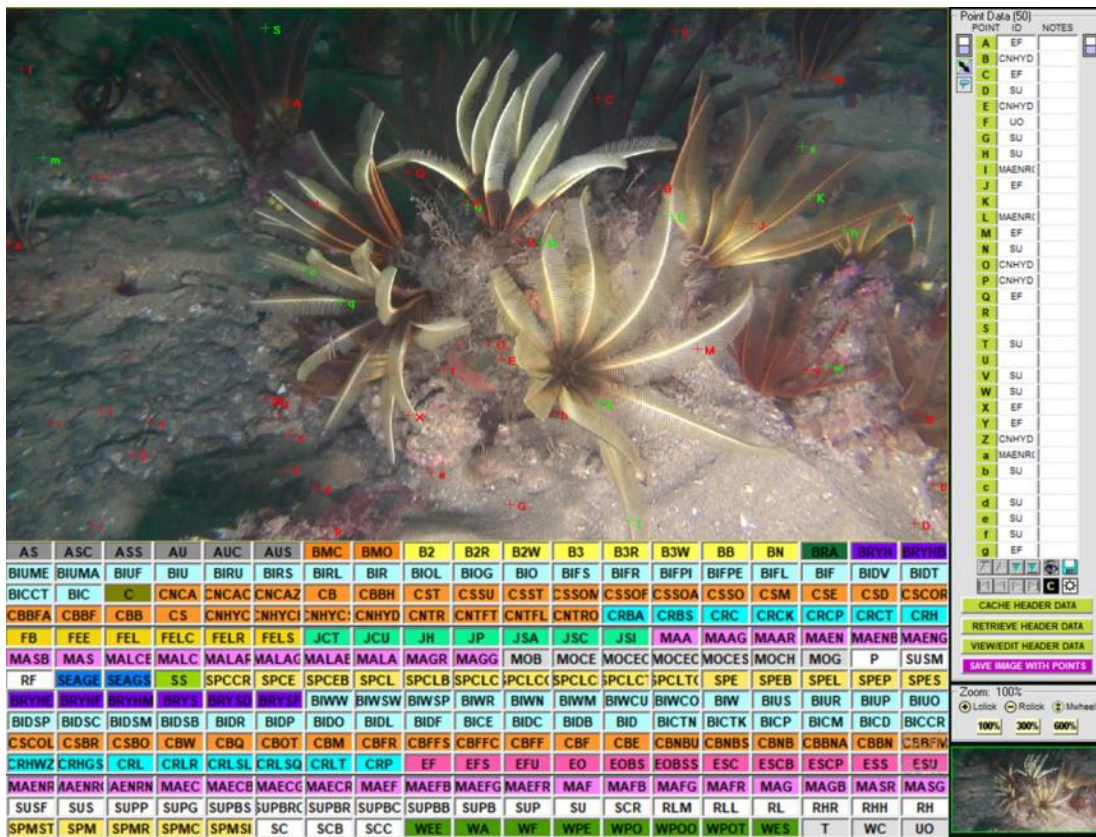


Figure 2.4 Example of an annotated oblique remotely operated vehicle image in Coral Point Count using the CATAMI classification scheme with 50 randomly placed annotations.

2.3.4 Numerical analysis

Large, dominant and conspicuous organisms are easier to see and identify, and may obscure the view of smaller and inconspicuous morphospecies such as molluscs (bivalves, gastropods and cephalous). Such morphospecies may have been under-sampled because they may be covered in turf algae and epibionts, or hidden from sight in holes, cracks and crevices. The morphospecies abundance data were therefore, square-root transformed using Primer version 6 (Clarke et al. 2014) to down-weight the presence of abundant morphospecies. Fourth-root transformation was tested but a square-root transformation was chosen instead, because the latter underestimated the number of significant groups.

Association based multivariate analyses were used to assess biota count data collected from 43 cells using PRIMER version 6 + PERMANOVA add-in (Clarke et al. 2014). The Bray-Curtis similarity measure was performed on transformed data to generate a resemblance matrix and community patterns were explored using Cluster analysis, based of group average linkage method. A similarity profile routine (SIMPROF, Clarke et al. 2008) was used to define the biological communities. Multi-dimensional scaling (MDS) was performed to graphically depict the relationship between samples and to define macrobenthic communities (Field et al. 1982). Lastly, Similarity Percentages (SIMPER) analysis was

applied to the square-root transformed abundance data to detect which morphospecies contributed the most to the similarity within and between communities. From this, characteristic and distinguishing morphospecies for each community were identified.

2.4 Results

2.4.1 Dataset description

This study surveyed 43 cells from 18 underwater transects off the Kei River mouth, resulting in a total of 205 images. Of these, 13 cells from 6 transects were recorded on coarse sand deeper than 60 m, whereas 30 cells from 12 transects were recorded on reef and rhodolith beds shallower than 60 m deep. Macroalgal morphospecies accounted for the majority of annotations that landed on biota and accounted for 46 % of the biota annotations, whereas sponges, corals and echinoderms collectively accounted for 34 % (Figure 2.5). Photosynthetic morphospecies dominated the consolidated substratum (reef and rhodolith) shallower than 60 m. Sponges were present at all depths and on all substratum types, excluding sand. Furthermore, sponge morphology type was more rich in deeper areas (> 60 m) compared to shallower areas (< 60 m). Branching octocorals were only common at approximately 90 m depth. In total, 60 morphospecies were recorded off the Kei River mouth according to the CATAMI classification scheme (Appendix 2). Ascidians and bryozoans represented a very small percentage of annotations. Unidentified organisms contributed 19 % of the total biota identifications but were not included in the multivariate analysis because this category consisted of several morphospecies which could not be assigned a morphospecies category, not even at the highest tier of the CATAMI scheme such as bryozoans, coral or sponges.

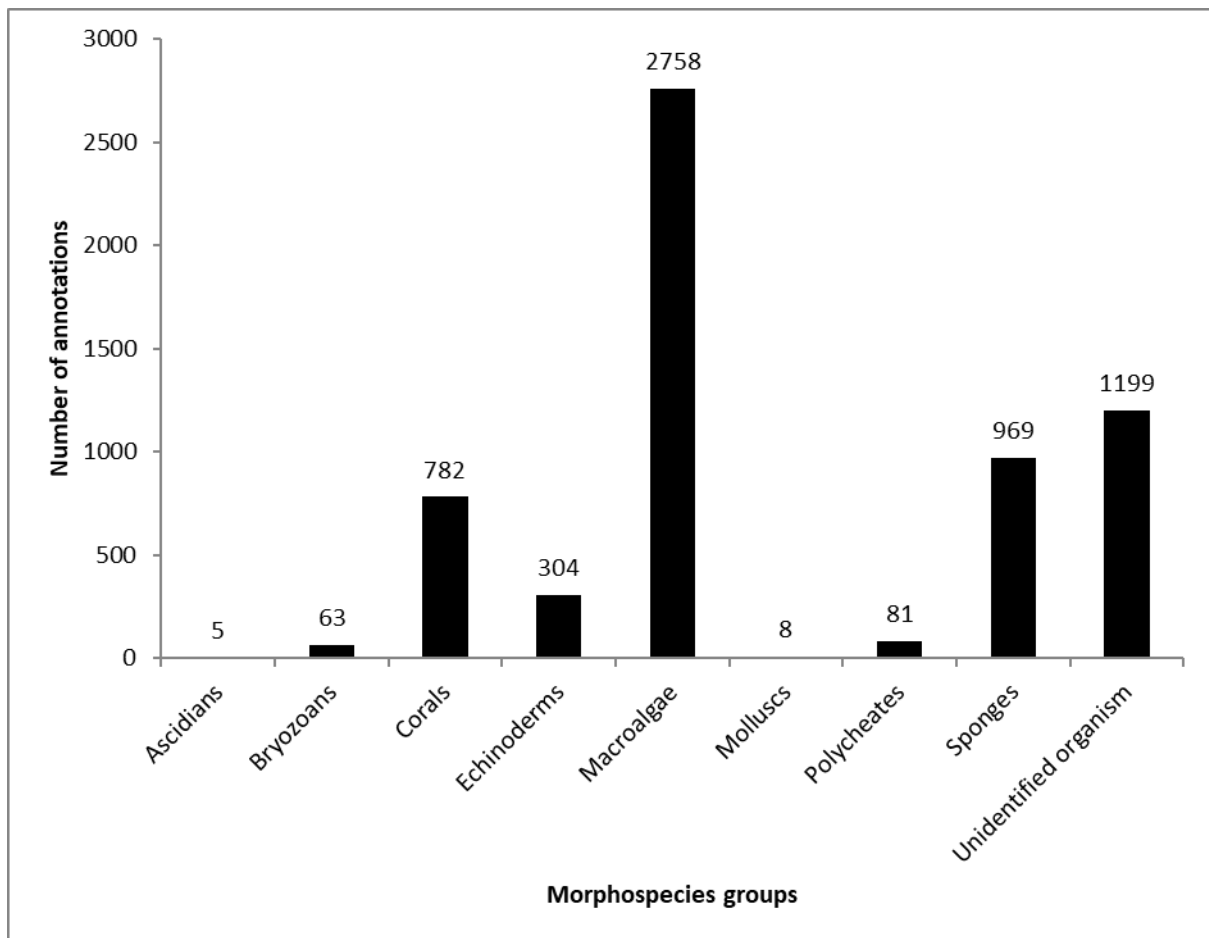


Figure 2.5 Annotation contributions of each morphospecies group for 205 ROV images from the ACEP: Imida Frontiers project. Organisms were placed into the unidentified organism category when it was not possible to assign a morphospecies identification.

Accumulation curves suggested that 250 annotations from five images was a sufficient number of data points to fully sample morphospecies diversity on all substrate types, except sand (Figure 2.6). The slope for sand substratum suggested that 250 annotations were insufficient; however, this steep slope of occurrence is caused by the isolated geography of the small sand patches adjacent to consolidated substratum types which harbour a greater number of epifauna. Therefore, limited inference can be drawn from the sand community as morphospecies richness may be influenced by nearby reef “islands”.

Mean morphospecies richness was lowest close to the river mouth (< 50 m) and generally increased across the shelf, with a peak on the middle shelf (< 50 – 90 m, Figure 2.7). Mean morphospecies richness was greatest on reef and mixed reef substrata, and lowest on unconsolidated substrata (Figure 2.8).

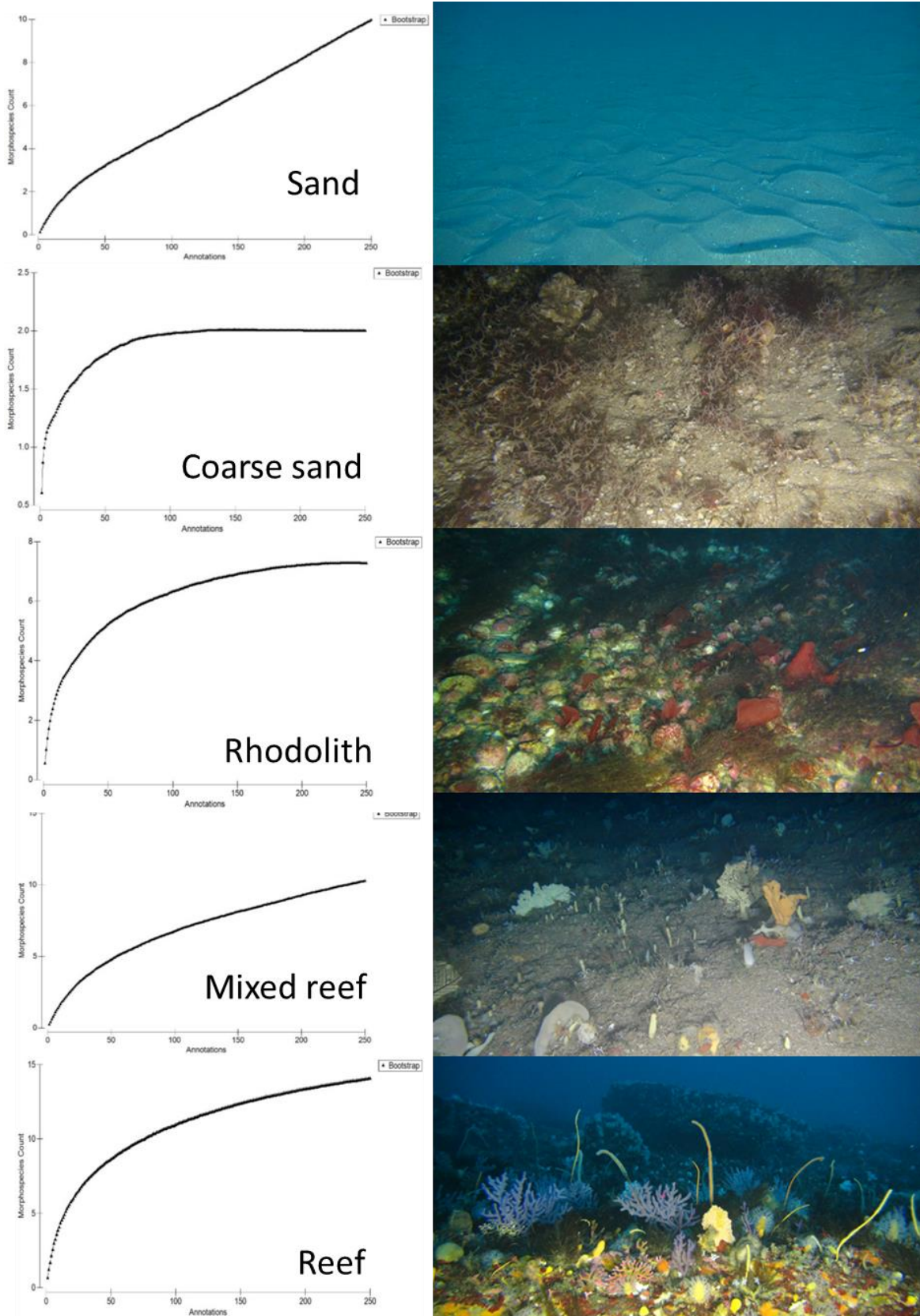


Figure 2.6 Morphospecies accumulation curves (bootstrap measure) of one random site from each substratum type and exemplar photographs of five substratum types on the temperate shelf off the Kei River mouth, South Africa

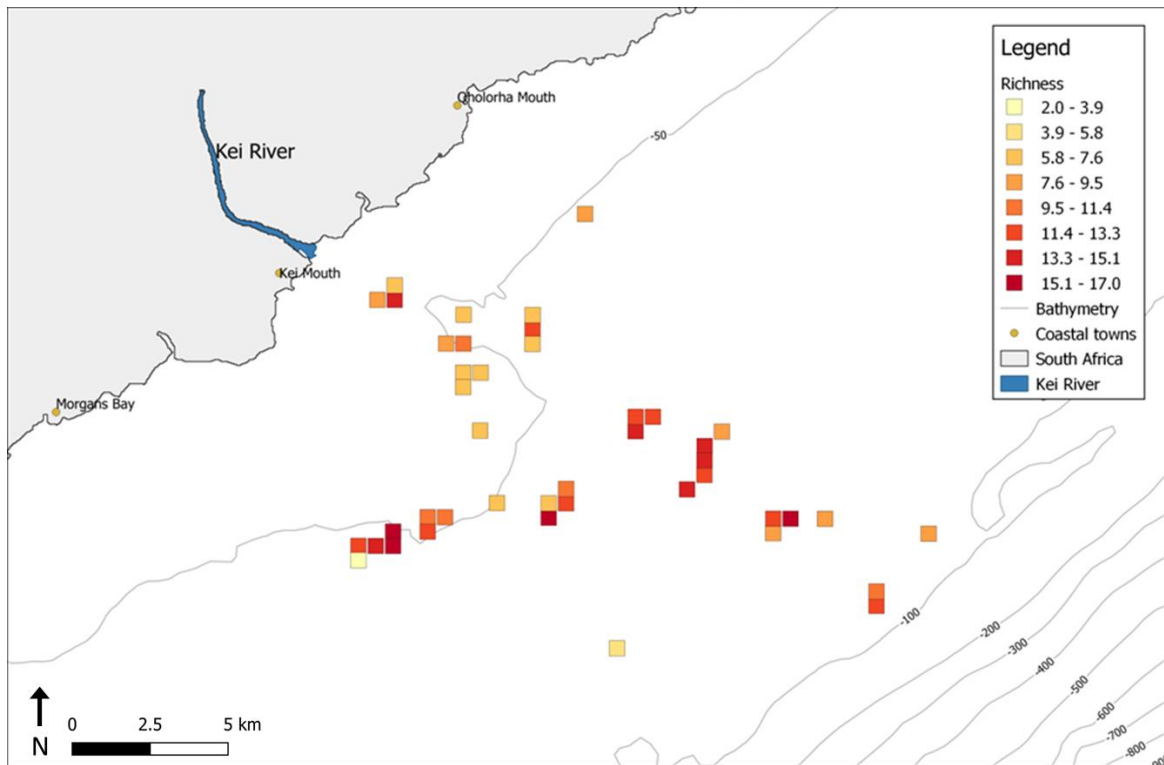


Figure 2.7 Mean morphospecies richness of each sample on the shelf off the Kei River mouth. Yellow to orange squares indicate the magnitude of the morphospecies richness per sample

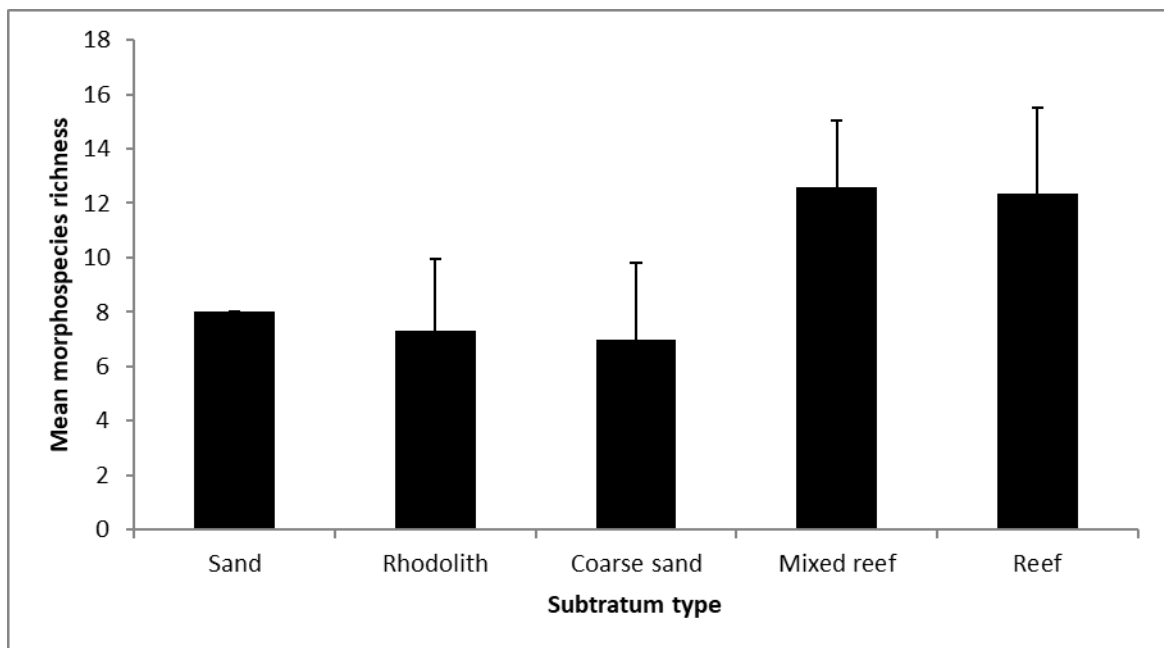


Figure 2.8 Mean morphospecies richness across five substratum types on the shelf off the Kei River Mouth

2.4.2 Community analysis

Defining macrobenthic communities

Nine distinct benthic epifauna communities (A – I), and an outlier (X) were identified from the cluster dendrogram (Figures 2.9a) and the MDS ordination (Figure 2.9b), using association-based multivariate analyses (SIMPROF, $p < 0.001$). The dendrogram displays three broad cluster groupings (Figures 2.9a), consisting of communities A, B-G and H-I, respectively. The first two groupings (A and B – G) comprise the upper mesophotic communities whereas, the third grouping (H – I) make up the lower mesophotic zone (Figure 2.9a). The upper and lower mesophotic clusters only shared 28% similarity.

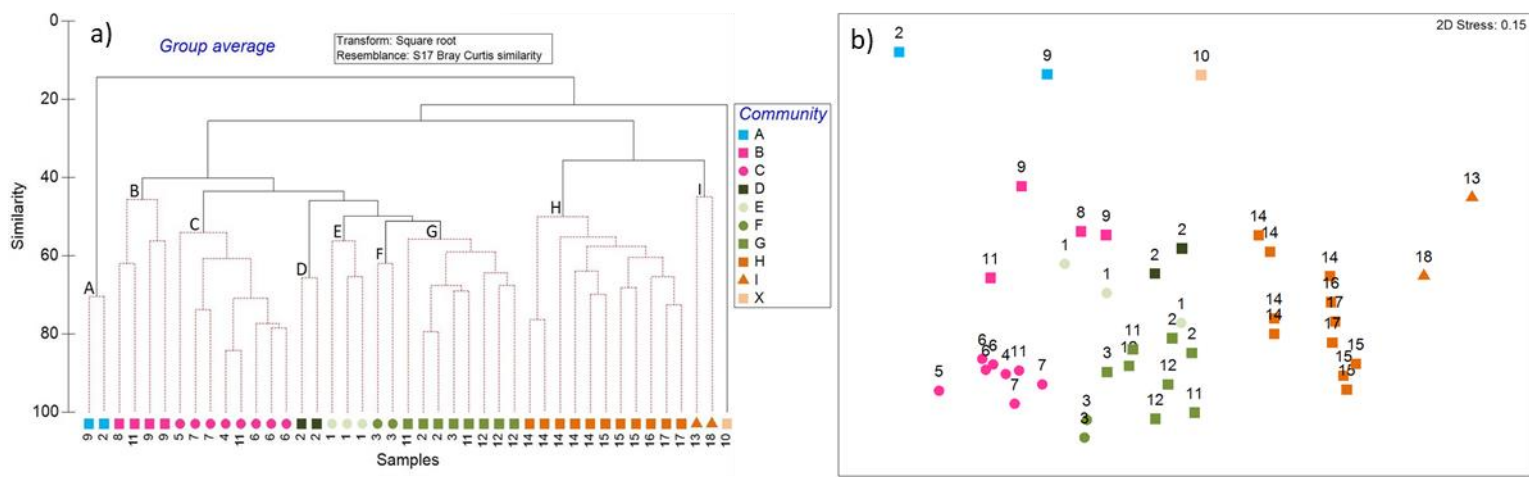


Figure 2.9 a) Dendrogram for hierarchical clustering based on group-average linking of Bray-Curtis similarities calculated on square-root transformed abundance data of 43 samples recorded on the shelf off the Kei River mouth. SIMPROF routine was used to define clusters. Numbers indicate sites. Shapes denote position on continental shelf (Square = inner shelf, circle = middle shelf and triangle = outer shelf). b) Multidimensional scaling based on Bray Curtis similarity matrix using epifauna abundances formed nine distinct communities from 43 samples recorded on the river-influenced shelf off the Kei River mouth (2D stress = 0.15). Numbers indicate sites. Shapes denote position on continental shelf (Square = inner shelf, circle = middle shelf and triangle = outer shelf)

Describing macrobenthic communities

Space and/or distance played a role in determining community similarity between sites (Figure 2.9b). The upper mesophotic communities occurred on the inner and middle shelf (30 – 74 m) whereas, the lower mesophotic communities occurred on the middle and outer shelf (74 – 100 m). Community A displayed the greatest community similarity (70.37 %), whereas, Community I displayed the lowest (44.93 %). Community I was also the most distinct; with dissimilarity between 64 - 97 % among the groups. Communities C and I were the least similar (97.29 % dissimilar). The greatest similarity was between Communities F and G (48.66 % dissimilarity). Characteristic and distinguishing morphospecies were compared between the inner, middle and outer shelf.

Table 2.2 Bray-Curtis dissimilarity (%) between benthic communities based on the SIMPER analysis. Dark grey shading indicates highest similarities between communities

Community	A	B	C	D	E	F	G	H
B	68.53	-						
C	84.24	57.68	-					
D	83.61	61.65	67.71	-				
E	80.17	55.44	61.70	53.36	-			
F	87.04	67.61	50.84	61.97	53.80	-		
G	84.74	61.22	53.24	52.49	49.28	48.66	-	
H	93.76	76.09	85.43	65.82	66.66	75.42	58.85	-
I	94.26	92.07	97.29	80.46	84.97	92.86	80.44	64.34

Several characteristic morphospecies were shared among communities (Table 2.3). Macroalgae characterised the upper mesophotic communities on the inner and middle shelf, whereas, sponges characterised lower mesophotic communities on the middle and outer shelf (Figure 2.11). Macroalgae was the most common morphospecies and was shared between seven communities (A – F). Hydroids were common in four communities (B, C, E and G), encrusting coralline algae in three (B, C and H) and sponges in three communities (F – H). Characteristic morphospecies were distinct for four of the communities. These included brittle stars for Community A, bryozoans and arborescent octacorals for Community D, coarse branching red macroalgae for Community F and massive sponges for Community I. Community X had less than two samples and therefore the SIMPER analysis could not be performed on this group. This community however, was characterised by feather stars.

Distinguishing morphospecies overlapped between three communities (Table 2.3). Communities B, D, E shared erect fine branching red algae as a distinguishing morphospecies. Similarly, Community C and E shared hydroids as distinguishing morphospecies. The red macroalgae was also the most common distinguishing morphospecies. Several distinguishing morphospecies were also distinct. These included brittle stars for Community A, bryozoans and arborescent octacorals for Community D, coarse branching red macroalgae for Community F, encrusting sponges for community G, erect sponges for community H and massive sponges for Community I. Some of these characteristic and distinguishing morphospecies also only occurred in specific depth zones, with photosynthetic taxa more common at shallower sites and filter feeders such as sponges more common at deeper depths.

Table 2.3 Similarity percentage analyses SIMPER for communities based on square-root-transformed abundance data, displaying the top three characteristic morphospecies groups. Distinguishing morphospecies is indicated by an asterisk (*). n = the number of cells per community. Sim/SD is undefined for communities with samples ≤ 2

A) Community A					
Average similarity: 70.37 %					
n = 2					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Brittle stars*	10.36	54.23	Undefined	77.06	77.06
Fine branching red macroalgae	2.82	16.14	Undefined	22.94	100.00
B) Community B					
Average similarity: 50.10 %					
n = 4					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Hydroids	4.81	14.30	2.15	28.54	28.54
Fine branching red macroalgae*	3.57	12.24	5.82	24.44	52.97
Encrusting coralline algae	4.05	10.25	2.49	20.45	73.42
C) Community C					
Average similarity: 64.38 %					
n = 8					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fine branching red macroalgae*	9.61	30.95	3.59	48.07	48.07
Encrusting coralline algae*	5.17	16.41	3.68	25.49	73.56
Hydroids	1.66	5.52	7.16	8.58	82.14
D) Community D					
Average similarity: 65.66 %					
n = 2					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bryozoans	5.00	14.94	Undefined	22.75	22.75
Fine branching red macroalgae*	4.47	13.29	Undefined	20.24	42.99
Arborescent octacorals	3.07	8.62	Undefined	13.13	56.12
E) Community E					
Average similarity: 59.26 %					
n = 3					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fine branching red macroalgae*	5.55	18.49	7.64	31.19	31.19
Hydroids	4.17	13.48	7.07	22.74	53.94
Gorgonians	3.92	11.34	4.44	19.14	73.07
F) Community F					
Average similarity: 61.98 %					
n = 2					
Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fine branching red macroalgae	8.92	19.77	Undefined	31.89	31.89
Coarse branching red macroalgae*	5.49	10.28	Undefined	16.58	48.47
Encrusting sponges	3.56	9.75	Undefined	15.73	64.20

G) Community G

Average similarity: 61.24 %

n = 8

Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Fine branching red macroalgae*	7.92	17.88	3.68	29.20	29.20
Encrusting sponges	3.95	9.49	5.27	15.49	44.69
Hydroids	2.89	5.44	1.99	8.88	53.57

H) Community H

Average similarity: 56.59 %

n = 11

Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Erect sponges*	4.07	13.56	6.42	23.96	23.96
Encrusting sponges	4.31	11.43	1.86	20.19	44.15
Hydroids	2.69	7.09	2.69	12.52	56.67

I) Community I

Average similarity: 44.93 %

n = 2

Morphospecies	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Massive sponges*	1.73	15.12	Undefined	33.66	33.66
Erect sponges	2.12	12.35	Undefined	27.48	61.14
Gorgonians	1.00	8.73	Undefined	19.43	80.57

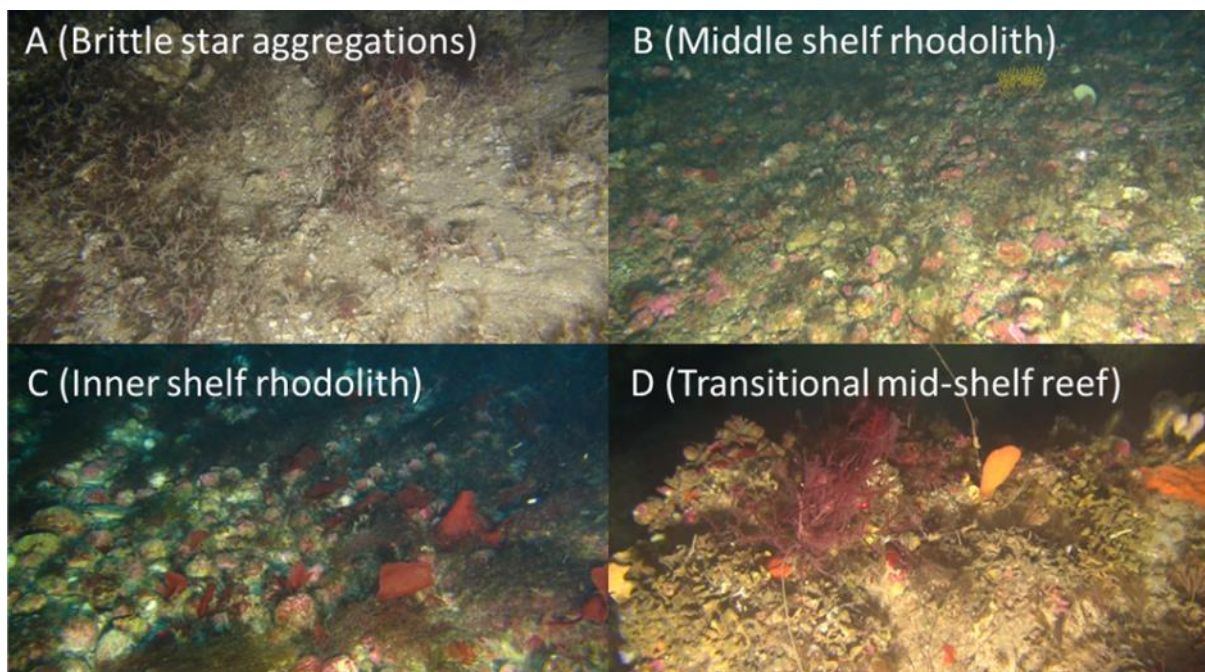


Figure 2.10 Exemplar images of communities A-D off the Kei River mouth. Descriptive community names are provided in brackets

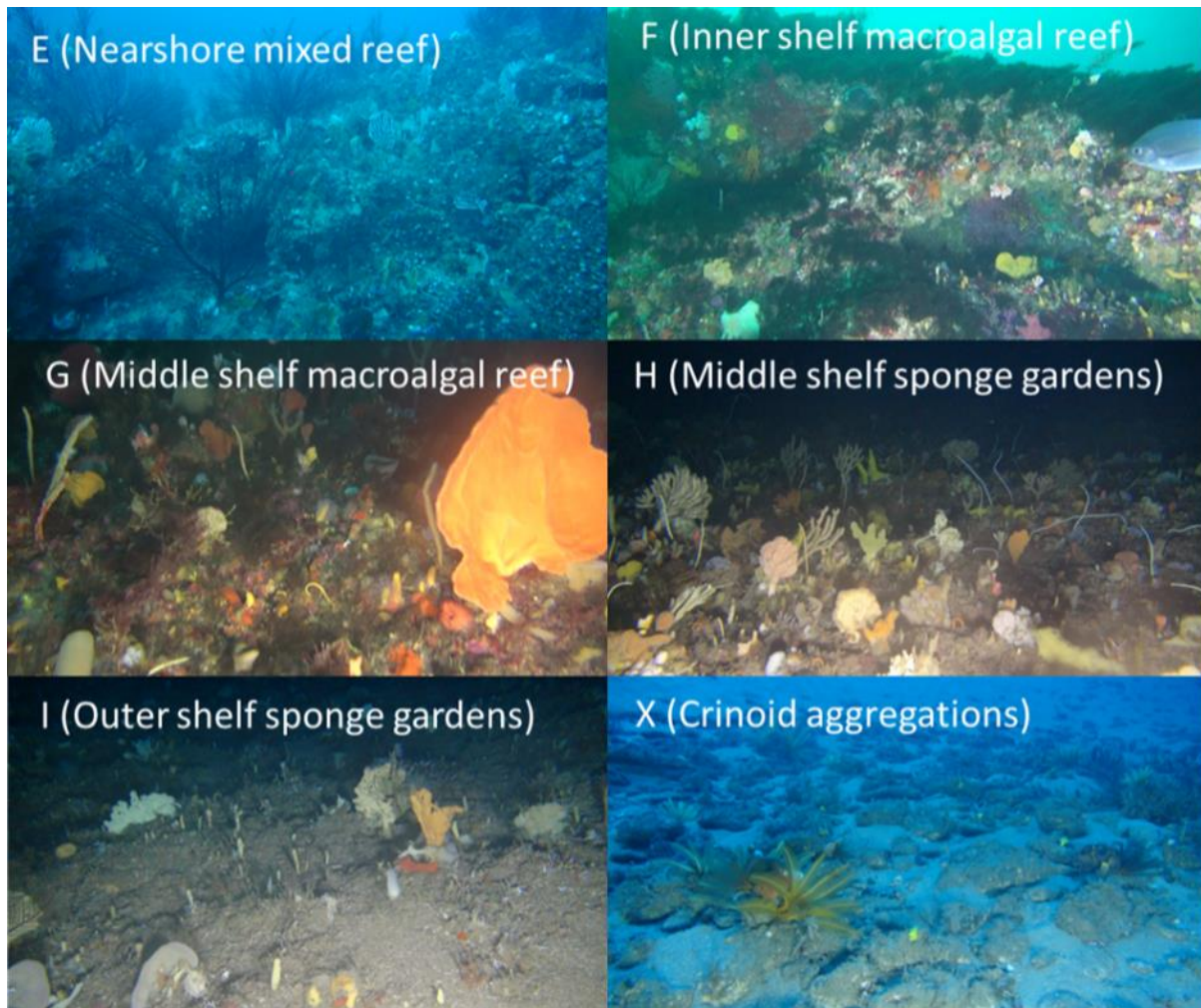


Figure 2.11 Exemplar images of communities E-I and the outlier (X) off the Kei River mouth. Descriptive community names are provided in brackets

Upper and lower mesophotic community description

The macrobenthos off the Kei River are easily separated into shallow or deep communities. Therefore, these communities can be separated into upper and lower mesophotic communities based on the premise that upper mesophotic communities are generally dominated by photosynthetic organisms whereas they are absent in lower mesophotic communities. Communities A – G and X in the upper mesophotic zone whereas Communities H and I in the lower mesophotic zone (Figure 2.11). This means seven macrobenthic communities occupy the upper mesophotic zone and two communities occupy the lower mesophotic zone off the Kei River. Moreover, the upper mesophotic community contained 30 samples whereas; the lower mesophotic only had 13 samples. The lower mesophotic samples were also restricted to a narrow depth range with 61 % of those samples collected between 90 – 100 m. The upper mesophotic samples however, were collected over a wider bathymetric range (30 – 70 m).

Morphospecies richness was similar in the upper (10.20 ± 3.83) and the lower (11.46 ± 3.33) mesophotic zones. Moreover, Communities F from the upper mesophotic and Community H from the lower mesophotic had the greatest morphospecies richness, whereas Communities B and I had the least number of morphospecies from their respective zones (Figure 2.13).

The upper mesophotic is made up of distinct macroalgal forests (E, F and G), brittle star (A), feather star (X) and rhodolith aggregations (C, Figure 2.12). The macroalgal forests were found on the reefs shallower than 65 m. The brittle star communities were found on coarse sand at 59 and 74 m. Feather star aggregations were only found at 55 m on a sand starved paleodune outcrop. Rhodolith aggregations are fields of habitat-forming free-living balls of non-geniculate coralline algae. The rhodoliths were only found between 30 – 65 m on fine to coarse sand and in between reefs. The lower mesophotic is made up of sponge and gorgonian aggregations (H and I, Figure 2.12). These aggregations were common on bedrock covered in coarse sand deeper than 75 m.

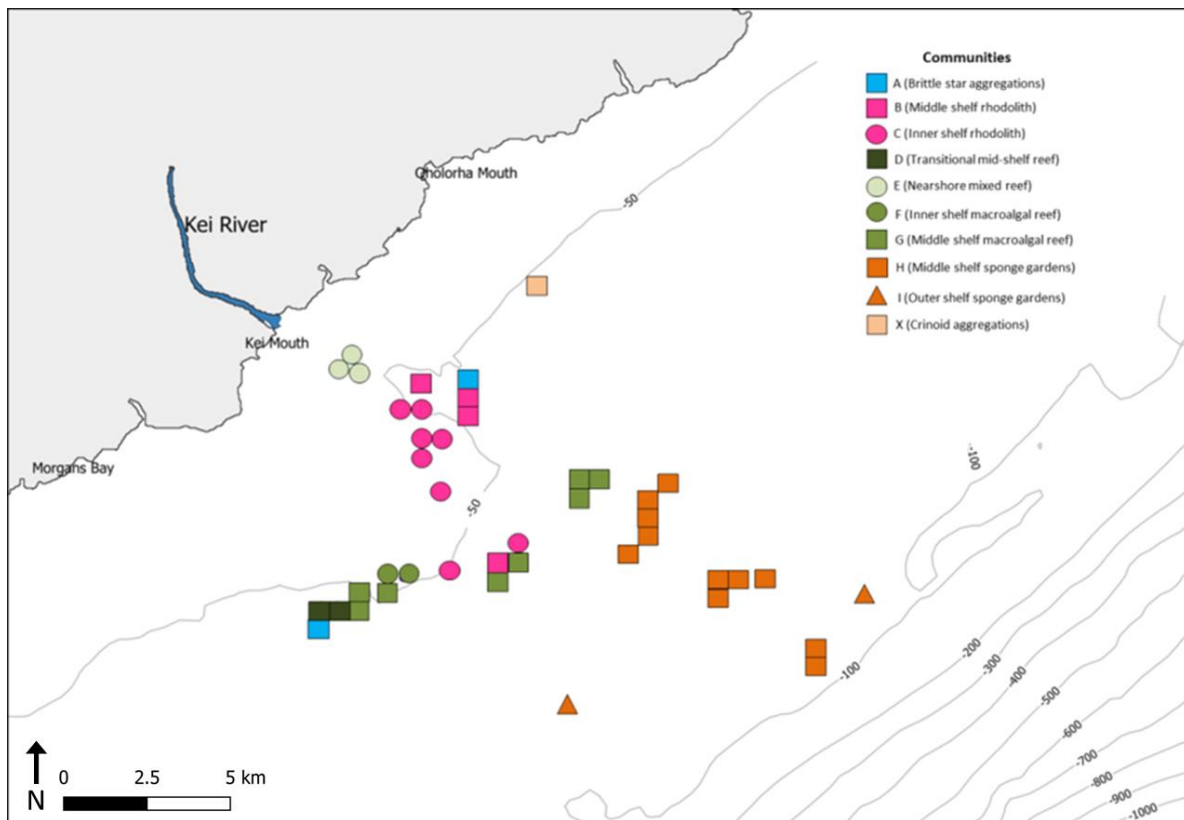


Figure 2.12 Map of macrobenthic communities on the temperate river-influenced shelf off the Kei River mouth, South Africa

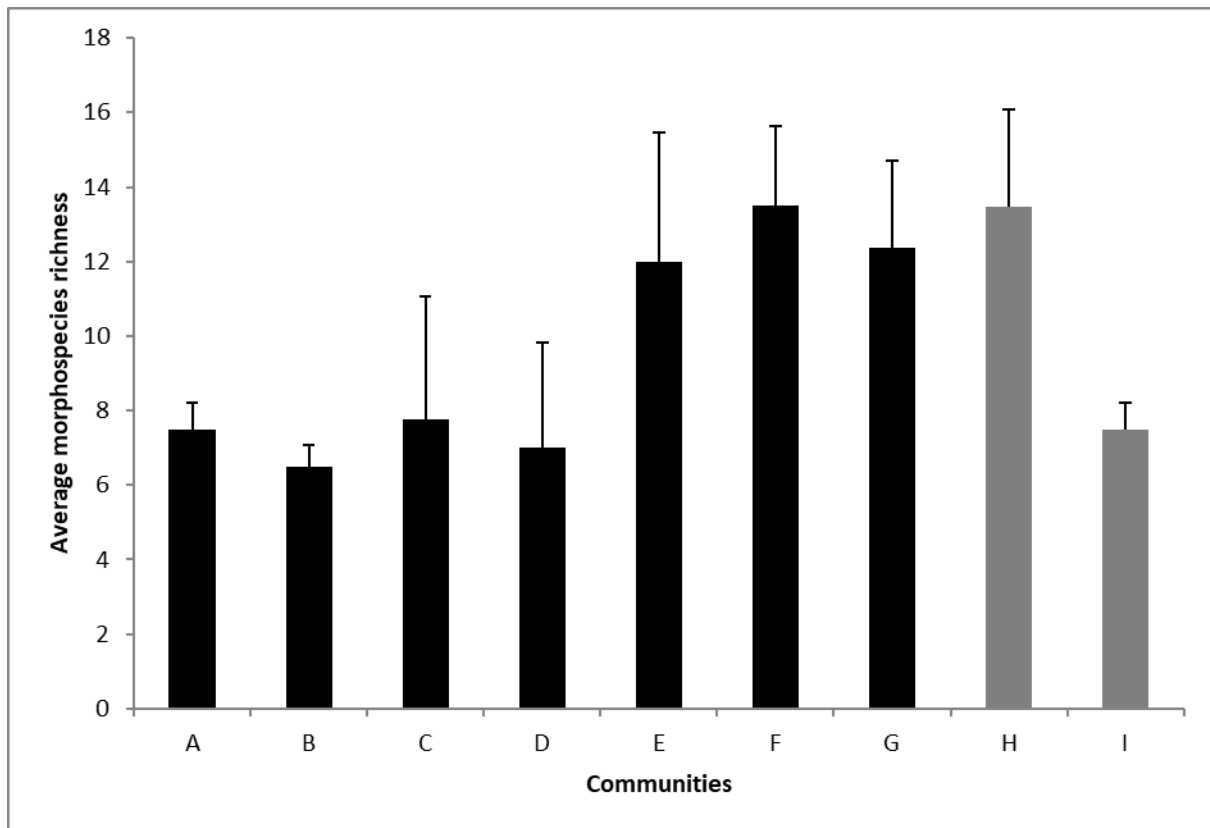


Figure 2.13 Morphospecies richness of macrobenthic communities on the shelf off the Kei River Mouth. Upper mesophotic communities are indicated by black bars whereas; lower mesophotic communities are indicated in grey bars. Numbers indicate richness

2.5 Discussion

Temperate mesophotic ecosystems are complex with many different habitats but our understanding of them is limited (Cerrano et al. 2019). Benthic invertebrate research has tended to separate the seabed by habitat because traditional sampling gear tend to be somewhat selective to study specific habitats, study designs or sampling gear restrictions. These restrictions include but are not limited to grabs, trawl and dredge for unconsolidated seabeds. Reefs are generally not sampled with these gear types but with camera platforms such as ROVs. This again often creates an unintentional selection bias towards sampling a specific substratum type – reefs. This should not be the case because ROVs are capable of sampling all substratum types and should allow the comparison across habitats. Non-reef (sand, gravel and mud) habitats and their associated epifauna in temperate and tropical mesophotic research are often overlooked, especially in ROV surveys which tend to target reefs with an emphasis on corals (Kahng et al. 2017, Cerrano et al. 2019). These non-reef habitats may occupy the space directly adjacent to reefs and may share similar species or may be completely distinct from reefs. Even more so, river-influenced mesophotic ecosystems may be distinct from mesophotic ecosystems found elsewhere, like in Brazil (Moura et al. 2016), but the paucity of comparable examples limit this dialogue. To overcome this, I provide the first description defining the characteristics of a South African river-influenced mesophotic ecosystem across several habitats.

The temperate shelf off the Kei River mouth supported nine distinct benthic communities between 30 – 100 m. Seven communities (A – G) were found within the upper mesophotic zone (30 – 75 m), whereas, two communities (H – I) were found in the lower mesophotic zone (75 – 90 m). Upper mesophotic communities were dominated by photosynthetic macroalgae, whereas, lower mesophotic communities were dominated by filter and suspension feeders. Erect fine branching red algae and coralline algae dominated the upper mesophotic, whereas the lower mesophotic was dominated by sponges and gorgonians. Sponges distinguished all lower mesophotic communities. The most noteworthy association is that of the dense brittle star aggregations on the fringes of and adjacent to the deeper parts of the rhodolith beds. Another association was the dense isolated aggregations of feather stars on patches of consolidated substratum in the sandy habitat where the outlier Community X was found. Feather stars in Community X and Bryozoans in Community D were characteristic and distinguishing morphospecies. These taxa were absent in other communities.

2.5.1 Upper Mesophotic Communities

Community A was characterised and defined by dense aggregations of brittle stars. Brittle star aggregations have been observed on various substratum types and depths in the subtidal (Turon et al. 2000) and their ecology is well studied (Broom 1975, Turon et al. 2000, Muths et al. 2009, Dauvin et al. 2013). Dense aggregations of monospecific brittle stars are known to be permanent features of the

seabed with seasonal variation in density and spatial extent (Dauvin et al. 2013). In the bay of Seine, in France, dense aggregations of *Ophiothrix fragalis* dominate sand and coarse sand habitats (Dauvin et al. 2013), similar to that found here. In the North Eastern Atlantic however, these dense aggregations mainly occupy pebbles with much lower densities on rhodoliths and rock (Muths et al. 2009). Brittle stars are also known to aggregate on habitat forming sponge species (Turon et al. 2000). They noted that juveniles favoured settlement on certain sponge species to facilitate feeding and suggested that they had the ability to select preferred sponge morphologies. Brittle star aggregations are known to occur from the shallow subtidal to the bathyal zone (Fujita and Ohta 1989, Muths et al. 2009). In the Bay of Sein dense patches mainly occur along the 30 m isobaths but are known to occur slightly deeper too (36 – 37 m). In the North Eastern Atlantic however, dense aggregations occupy a wider depth range (11 – 54 m, Muths et al. 2009). In this study, dense aggregations were only found in two potentially isolated patches of scattered rhodolith-sponge bottoms and on coarse sand with scattered dead rhodoliths at 48 m and 74 m (Appendix 3). These were mainly adjacent to or on the fringes of living rhodolith aggregations (Community C). Interestingly, the aggregations were not observed on coarse sediment deeper than 75 m. Moreover, the echinoderm aggregation at 48 m was composed of brittle stars, coralline algae, macroalgae, encrusting sponges and feather stars whereas, the latter groups were absent from the potentially monospecific brittle star aggregations at 75 m. This community was defined from only two samples and therefore additional samples are required to provide a more robust understanding of its occurrence and distribution in this region.

Community B was most likely a transition community between communities A and C. This community can be considered a marginal rhodolith and/or biogenic community due to the high abundance of fine branching red macroalgae which characterise communities A and C. Similarly, this community had a high number of dead rhodoliths which were also observed in Community A. Given that these rhodoliths were dead, any annotations that landed on them were assigned a substrate category instead of encrusting coralline algae. If the rhodoliths were alive, Communities A, B and C would likely have been more closely related, perhaps even lumped into the same major cluster grouping or Community A would possibly be dissolved into Community B due to the dense cover of brittle stars (Appendix 3).

Community C was characterised and distinguished by erect fine branching red algae and the rhodolith forming encrusting coralline algae. This rhodolith bed is a welcomed novel ecosystem type to South African marine scientists (Adams et al. 2020). The structure of rhodolith beds are well documented in the Mediterranean Sea (Basso 1998, Basso et al. 2009, 2017, Sañé et al. 2016, Rindi et al. 2019), South America (Amado-Filho et al. 2007, Bahia et al. 2010, Vale et al. 2018), New Zealand (Nelson et al. 2015)

and Southern Australia (Goldberg 2006). Rhodolith beds often boast diverse assemblages of coralline algae and upright macroalgae (Joher et al. 2012, 2015, 2016, Nelson et al. 2015) and invertebrates such as crustaceans, polychaetes and molluscs (Moura et al. 2016). In this study, however, small species were not detectable and morphologically similar species could not be distinguished due to the use of visual sampling methods. Only two upright macroalgal and a single coralline algae morphospecies were therefore observed. These morphospecies were also indistinguishable from the upright macroalgae and coralline algae found on the surrounding consolidated substratum (reefs and rocks). A recent study however identified three genera (*Lithophyllum*, *Lithothamnion* and a non-descript genus) of rhodolith forming coralline algae from dredge samples collected here, which formed extensive aggregations on the seabed and also occupied inter-reef areas. (Adams et al. 2020).

Communities D, E and F were all distinct and formed tight cluster groupings. Community D was most likely transitional mesophotic reef community based on community composition (Lesser et al. 2019), because although macroalgae was present in this community bryozoans and arborescent octacorals formed a greater proportion of the community similarity. The shallowest, and also the community closest to the Kei river mouth, was Community E. This community occurred at what is globally considered the transition depth of the shallow and upper mesophotic zone and therefore resemble a community composition similar to shallow-subtidal reefs (Shoham and Benayahu 2017). Gorgonian and hydroid contribution in this community outweighs the proportion of heterotrophs in Other communities in the upper mesophotic zone. The absence of octacorals at the sites where community F was found contradicts the findings of Shoham and Benayahu (2017) which states that octacoral richness increases with increasing depth in the upper mesophotic zone. Community F may be an exception because octacoral richness and abundance increased with increasing depth elsewhere on the shelf (e.g. Community I). Although Community E and F share similar characteristic morphospecies (Table 2.4), the difference in abundance and the presence of coarse branching red macroalgae distinguished these communities. All three communities were reef communities dominated and characterised by macroalgae but were at most 45-50 % similar to each other. Although Communities A – G were characterised and distinguished by macroalgae, filter feeders may constitute a greater proportion to the observed biota within the mesophotic zone. Macroalgae-dominated reefs have yet to receive targeted research. Of the limited research that exists on temperate mesophotic reefs, a single study mentions macroalgae as dominant features of temperate reefs in the upper mesophotic zone in South Africa (Heyns et al. 2016). Likewise, macroalgae dominates the upper mesophotic depths in the Mediterranean sea (Ponti et al. 2018, Rindi et al. 2019). However, sponges formed the bulk of the biomass between depths 30 – 100 m in parts of temperate Australia with no mention of macroalgae cover throughout the depth range (Keesing et al. 2012, James et al. 2017).

The depth related patterns of photosynthetic communities described in this study mirror that of photosynthetic communities off the Storms River mouth in the Tsitsikamma Marine Protected Area (MPA) (Heyns et al. 2016). Although they only targeted reef habitats as opposed to several habitat types as in the current study; they noted ascidians as major component of shallow reefs (12 – 22 m), but almost absent from deep reefs (45 – 75 m). Despite the aforementioned study not surveying depths 12 – 45 m, the presence of ascidians could possibly extend into this depth range if substrata for attachment is available. However, in the current study ascidians did not contribute a significant percentage to the composition of communities throughout the 70 m depth range. This may be attributed to three reasons; namely ascidians were (i) possibly more difficult to detect (small morphologies and abundances), (ii) misidentified as sponges since these organisms display similar morphologies or (iii) ascidians are simply not a common feature of the communities off the Kei River. These reasons can easily be investigated with detailed comparisons once dredge samples from this area are examined and morphologically distinct ascidians are identified. In addition, they also noted that macroalgae was absent from reefs at deeper than 45 m. In the current study, however, macroalgae was only replaced by suspension and filter feeders on reefs at depths deeper than 75 m.

Echinoderm aggregations are common off the Kei River mouth. Community X was characterised and distinguished by dense aggregations of feather stars. This site however, only consisted of a single sample. This sample was taken on rippled fine sand flats with a ridge of consolidated sediment possibly from a paleo-dune (Green et al. 2020). The sand flats had little to no visible biota but the ridge supported the feather star aggregations as it may facilitate their suspension feeding behaviour. This also explains the steep curve of the accumulation curve for this single sand site (Figure 2.6) because consolidated substratum provides attachment for benthos and therefore supports more benthic organisms. This sample (54 – 56 m depth) was also most closely related to the shallow (48 m) brittle star aggregation from Community A (Table 2.3). This similarity between Communities A and X was attributed to the sparse aggregations of feather stars present at the shallow sample from Community A. Additional ROV samples and comparison of dredge samples in and around the echinoderm sites off the Kei River should explain the relatedness between these aggregations

2.5.2 Lower Mesophotic Communities

Community H and I could possibly be a single community which displayed high variability in sponge morphology. The characteristic and distinguishing morphospecies for the lower mesophotic were sponges. The coarse sand assemblages defined in this study exhibit similarities to those found off the Amazon River, with filter-feeding assemblages dominating areas of strong current, high turbidity, high light attenuation (Bo et al. 2012, Schönberg and Fromont 2012). Similarly, upright octocorals (sea fans and sea whips) were observed and particularly massive sponges dominated the coarse sediment

communities off the Kei River as seen off the Amazon River (Moura et al. 2016). Sponge gardens, dense aggregation of sponges, are a common feature of the lower mesophotic zone (Lesser et al. 2018, 2019, Bell et al. 2019, Enrichetti et al. 2019, Harris et al. 2021) and compete with octacorals for food and space (Slattery and Lesser 2021). Sponges may display plasticity depending on the range of environmental conditions (Idan et al. 2018, 2020). Fine resolution analysis of glass sponge gardens on the Canadian coast revealed that sea whips were common in sponge beds at a mean depth 79 ± 9 m and ranging between 59 – 102 m (Chu and Leys 2010). Similar patterns were observed in this study. Chu and Leys (2010) however, used a fine resolution grid-point survey method and noted that crustaceans and fish were more abundant in the presence of sponge beds than the surrounding area. That survey method deliberately aimed to quantify sponge coverage and associated organisms whereas the current study's survey protocol was purely exploratory and had multiple objectives to verify the geology and all faunal and floral groups.

Again, samples from sites 13 and 18 from Community I may belong to the well-defined Community H due to spatial proximity. Over half of the images found in these samples had no visible biota. Elevated sediment accumulation, covering low-lying biota, may attribute to the distinction of this community as observed in shallower (<30 m) river influenced benthic communities in the Pondoland region (Celliers et al. 2007). Alternatively, these could in fact be distinct communities. Firstly, Community H occupied the 75 – 91 m depth range, whereas Community I occurred between 91 – 97 m and possibly deeper. Secondly, encrusting and massive sponge morphologies (I) are known to prefer high current areas whereas upright growth forms (H) prefer low current areas (Heyns et al. 2016, Moura et al. 2016).

Filter-feeders, specifically sponges, dominated areas deeper than 75 m, but were present at all depths. In this study, erect sponges were mainly classed in two groups according to the CATAMI classification scheme: simple erect or branching sponges. This group is known to be morphologically plastic and generally requires microscopy to discern between species (Samaai et al. 2010). Therefore, the similarity observed across sites is likely partly attributable to an inability to identify colonies to species level and it is unlikely that the encrusting or erect sponges observed in the upper and lower mesophotic are of the same taxa. Similarly, this applies to encrusting coralline algae that were observed in the rhodolith and reef community. Findings off the Storms River in the Tsitsikamma suggested that ascidians were almost absent from reefs below 25 m (Heyns et al. 2016). A similar pattern of low abundance or absence of ascidians throughout the depth range (30 – 100 m) was found off the Kei River, particularly in the lower mesophotic zone. In addition, bryozoans did not contribute a significant percentage to the lower mesophotic community similarity as was found in Tsitsikamma.

This may mainly be attributed to detection issues, which may have influenced the ability to discern particular morphologies and discern difference between assemblages.

2.5.3 Limitations and recommendations

All underwater visual surveys have inherent design constraints; from equipment depth limitations to information resolution captured by underwater imagery – the list is long. Still, these obstacles do not deter benthic ecologists from extracting foundational biodiversity information and biological patterns from underwater video and images. The constraints of the survey design, the sampling method and the CATAMI classification scheme relating to the current study are discussed below, and recommendations are made for future ROV exploratory and targeted surveys.

Underwater camera surveys generally underestimate richness and overestimate abundance when compared to traditional macrofauna sampling such as benthic dredging (Williams et al. 2015). This is because camera data often consolidate several species into a single phototaxa often to avoid misidentifications whereas traditional sampling allows for species level identification. Furthermore, camera surveys usually favour large and conspicuous organisms because they are easier to identify whereas, dredge material often contain smaller and inconspicuous organisms that go undetected with camera surveys (Williams et al. 2015). In the current study, reefs were more species rich than compared to non-reef substratum. This was not unexpected. What was unexpected was that sand was more species rich than coarse sand. The latter substrata however, had greater abundance of the few morphospecies than compared to the morphospecies on fine sand.

Recommendations

I recommend the continued use of Coral Point Count (CPCe) for benthic invertebrate image annotation for ROV imagery regardless of camera orientation. Although CPCe is traditionally used for overhead imagery; this study however used oblique imagery and along with the CATAMI classification scheme demonstrated its ability to pick up community patterns. Moreover, the classification system was freely available and readily compatible as a code file for CPCe.

I also recommend the addition of a dedicated downward-facing or an additional stereo camera system mounted to the ROV to enhance the benthic research capabilities and possibly allow the comparison of imagery from drop and towed camera systems. Depending on the configuration of the downward-facing, it will not only facilitate the parallel sampling of fish and benthic invertebrates but also overcome the scaling issue that plagues forward facing/ oblique camera orientation. The addition of a stereo camera configuration, similar to that used on Baited Remote Underwater Vehicle, will allow for volumetric calculations of the macrobenthos. This may allow for the comparison benthic biomass estimations between different bathymetric zones and regions.

2.6 Conclusion

Nine benthic communities were defined using ROV imagery and the CATAMI classification scheme in the mesophotic zone off the Kei River in South Africa. Of these communities, the rhodolith aggregations and its associated biological community were identified as a novel habitat type in South African waters. Distinct zones of macrobenthic communities were observed in the mesophotic zone off the Kei River, with photosynthetic morphospecies dominating the seabed shallower than 60 m depth and filter-feeders dominant from ~60 m to 100 m. The use of ROV imagery as a survey tool coupled with the CATAMI classification scheme successfully identified benthic communities and their associated habitats off the Kei River. This study highlights the need for exploratory surveys to investigate all substratum types because some assemblages may go undetected if the focus is on consolidated or unconsolidated substratum only. Furthermore, the need for greater sampling efforts in unexplored regions, especially on potentially river-influenced shelves, is emphasized as they may host unique and previously undiscovered benthic ecosystems.

Chapter 3 Linking patterns in mesophotic benthic communities with environmental variables in a biogeographic transition zone

3.1 Abstract

Environmental drivers of community patterns in mesophotic ecosystems are not as well understood as their shallower counterparts. Benthic communities of the temperate shelf off the Kei River were defined and described in Chapter 2. This chapter therefore, aims to identify the key environmental variables responsible for driving the benthic community patterns observed offshore of the Kei River. Arrays of in situ and remote sampling methods were used to quantify nine environmental variables: depth, temperature, salinity, substratum, rugosity, latitude, aspect and relief. LINKTREE and Distance-based Linear Model (DistLM) analyses were performed to investigate possible relationships between the defined communities and the nine environmental variables. The Distance-based Linear Model showed that the combination of the aforementioned environmental variables explained 67 % of observed variation in benthic communities. Distance-based Redundancy Analysis (dbRDA) revealed that depth, substratum, temperature, latitude and salinity were the environmental variables that correlated with the observed benthic community patterns. Of these variables, the LINKTREE analysis identified depth and substratum as the best distinguishing environmental variables; a major depth break was evident at 75 m that separated communities into the upper and lower mesophotic zone whereas, substratum further separated communities within the upper mesophotic zone. This study showed that the drivers of community pattern determined by this study are consistent with those observed globally. Benthic community patterns of temperate mesophotic ecosystems could therefore be attributed to depth and substratum type derived from imagery. These variables are readily obtainable using most underwater camera platforms whilst advancing non-destructive and non-intrusive offshore benthic research.

3.2 Introduction

Identifying and understanding the environmental variables that drive benthic ecosystem function is important for benthic ecologists (Karenzi 2014) and underpins the conservation of ecosystem processes (Bridge et al. 2011a, Slattery et al. 2011, Loya et al. 2016, Turner et al. 2017, Sink et al. 2019). Yet, environmental processes structuring mesophotic ecosystems are not well understood despite this being identified as a key priority of mesophotic research (Turner et al. 2017). Several variables have been previously recognised as key drivers of the benthic environment in the mesophotic zone. Macrobenthos in the mesophotic zone are generally physically-controlled (Kahng et al. 2010, Turner et al. 2017, Cerrano et al. 2019), and are mostly determined by light quality and quantity (Lesser et al. 2009, Kahng et al. 2014). Other environmental variables that drive macrobenthic community patterns include topography (Bridge et al. 2011a), temperature (Kahng et al. 2012), sedimentation (Obura and McClanahan 1997, Golbuu et al. 2008, 2011, Polónia et al. 2015), nutrients (Rabalais et al. 1996, 2004, Gao and Wang 2008, Heyns-Veale et al. 2019), particulate organic matter (Heyns et al. 2016), dissolved organic carbon (Ribes et al. 1999), water movement (Goreu and Goreau 1973; Sheppard 1982, Ballesteros and Zabala 1993, Ballesteros 2006), salinity (Ballesteros 2006) and depth (Ballesteros and Zabala 1993). The complex interplay between these variables maintains and supports essential processes that determine the distribution and structure of offshore macrobenthos.

Along the subtropical KwaZulu-Natal shelf, several studies have investigated the effects of riverine inputs on the near shore and offshore environment (de Lecea et al. 2013, 2016, Porter et al. 2014), bearing in mind that this region has a particularly narrow shelf (DeWet and Compton 2021). Fluvial organic matter is an important component supporting the demersal food webs, and that fluvial input was the main biological driver of Natal Bight's benthic environment (de Lecea et al. 2016). Similarly, freshwater flow reduction through the development of freshwater infrastructure (dams, weirs etc.) was shown to reduce shelf-wide productivity to adjacent ecosystems (de Lecea et al. 2016, Scharler et al. 2016). In other studies, terrigenous sediments originating from the Thukela estuary were found to be important in forming and maintaining soft sediment habitats (MacKay et al. 2016) which are essential to the demersal fish communities and subsequently the linefish fishery in the region (Lamberth et al. 2008, Fennessy 2016). Fluvial-derived particulate organic matter is also more important for inshore filter feeders than for offshore filter feeders (Porter et al. 2014). The latter rely more heavily on marine-derived particulate organic matter as a primary food source (Porter et al. 2014). Although these studies identified key abiotic variables of river influenced ecosystems, they were all conducted in a subtropical region.

This chapter aimed to investigate the key environmental variables that influence the benthic communities described in the Chapter 2. The objectives of this chapter were two-fold. Firstly, to use multivariate analyses to explore patterns in potential drivers of macrobenthic communities, and secondly to identify the environmental variables that best explain the macrobenthic communities on a temperate shelf.

3.3 Materials and Methods

The descriptions of the study area and survey protocol were detailed in Chapter 2, sections 2.3.1 and 2.3.2, respectively. Described below are the methods used to derive the environmental data and the numerical analyses used to identify the environmental variables responsible for the community patterns detailed in section 2.4. Community analysis and species richness were detailed in Chapter 2.

3.3.1 Environmental data selection

Nine environmental variables including depth, temperature, salinity, rugosity index, latitude, relief, substratum, aspect and distance to mouth were included in the analyses. Distance to shore, shelf edge and mouth were strongly correlated to depth (Pearson's correlation $p > 0.9$, Appendix 4). The distance to river the mouth, shore and shelf edge distance metrics were measured in QGIS (version 3.4.2, QGIS Development Team, 2018) as the linear distance from the centroid of the cell to a central point at the Kei River mouth and perpendicular to the shoreline and shelf edge respectively. The latter variables were discarded but distance to river the mouth was retained in the analyses because this variable could potentially give insight into the effect of fluvial input from the Kei River mouth. Although latitude also strongly correlated with the distance metrics, it was retained in the analysis because this variable did not correlate as strongly with depth and could be useful to understand to influence of the river plume. This was recognised in the interpretation of the results. Not all environmental variables covered the entire study area. These incomplete data layers were manually calculated (Rugosity and aspect) or statistically interpolated by kriging (temperature and salinity). Multibeam bathymetry data were available but covered only 60% of the sample sites; therefore, rugosity index and aspect were calculated manually. Rugosity calculations followed the "tape and chain" rugosity calculation (Walbridge et al. 2018) because multibeam bathymetric data were not available for all cells. For each cell, rugosity was calculated as the ratios of the 3D substratum distance over the 2D linear transect distance within a cell (Figure 3.1). The 2D distance was calculated as the linear distance between consecutive GPS points. The 3D distance was calculated as the sum of the difference in depth between consecutive GPS points derived from the vessels single-beam echosounder. Aspect per cell was derived in QGIS (version 3.4.2, QGIS Development Team, 2018) as the cardinal direction (e.g. SW, NE, NW, etc.) of the transect orientation and converted to the corresponding azimuth angle (e.g. SW = 240°). In situ temperature and salinity were collected from a Star-oddi temperature logger attached

to the ROV and were statistically interpolated using kriging in QGIS (version 3.4.2, QGIS Development Team, 2018) to fill data gaps where these variables were not recorded. Average depth per cell was derived from the average depth of a GPS track across a cell/sample. Latitude of the cell was derived by calculating the centroid coordinate of each cell. Substratum and relief were recorded as ordinal categorical variables assigned to each image. These categories were assigned by simply noting the dominant substratum in each photo. Substratum were categorised as sand, rhodolith, coarse sand, mixed reef and reef. Relief was also a categorical variable which had three categories; flat, moderate and steep. These variables were collated to perform the multivariate analyses.

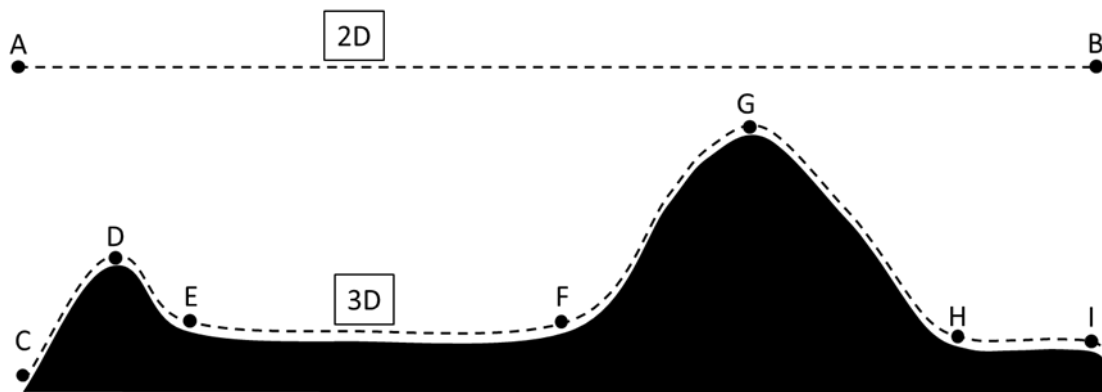


Figure 3.1 Diagram displaying "tape and chain technique" applied to single beam echosounder GPS points used to derive rugosity per sample unit (cell) (3D/2D). 2D distance was calculated as the linear distance between consecutive GPS points whereas the 3D distance was calculated as the sum of the difference in depth ($\Sigma=(C-D)+(D-E)+\dots$)

3.3.2 Numerical analyses

Univariate analyses involved producing box-and-whiskers plots for all environmental variables, excluding latitude and aspect, to explore the environmental data.

Macrobenthos abundance data were square-root transformed and a resemblance matrix was produced in PRIMER version 6 and the PERMANOVA+ add-on (Clarke et al. 2014). The environmental variables were normalised to produce Euclidian distances. A draftsman plot was produced to investigate correlation between the normalised variables and strongly correlated variables were subsequently removed. A principal component analysis (PCA) was performed on the normalised environmental variables and graphically represented to investigate relatedness between the variables. The community samples were superimposed onto the PCA plot to investigate the relationship between the environmental variables as they relate to the samples.

A distance-based linear model (DISTLM, PRIMER version 6 with PERMANOVA+ add-on, Clarke et al. 2014) was used to relate the (non-normalised) environmental variables to the macrobenthos resemblance matrix in the community analysis (Section 2.4 in Chapter 2) to assess the significance

levels of each variable. A distance based redundancy analysis (dbRDA) plot was produced to graphically represent the relationship between significant variables and the biotic communities. The parameters for the DISTLM were set to AIC for selection criterion and the selection procedure was set to “Best” to select the model with the best combination of environmental variables that explain the biotic community patterns.

The environmental variables that strongly correlate with the community resemblance were selected from the BIO-ENV analysis in PRIMER version 6 (Clarke et al. 2014). This subset of variables that best explain the community pattern were subjected to a constrained binary divisive clustering analysis (LINKTREE routine in PRIMER version 6, Clarke et al. 2014) on the same macrobenthos resemblance data. This analysis displays the ANOSIM R statistic and Bray-Curtis dissimilarity percentage at each binary split. LINKTREE parameters were set to limiting the minimum split size to three, limiting the minimum group size to 1 to maximise the number of clusters because multiple communities (Chapter 2) consisted of two or less samples, and limiting the ANOSIM R = 0.2 to maximise the variation between clusters (Clarke et al. 2014).

3.4 Results

3.4.1 Description of environmental variables

The descriptive statistics and box-and-whiskers plots were produced and explored for six of the nine environmental variables (Table 3.1) and five of nine variables (Figure 3.2). Mixed reef and flat relief were the most common substratum and relief type, respectively.

Table 3.1 Descriptive statistics of six environmental variables collected off the Kei River

	<i>Depth (m)</i>	<i>Temperature (°C)</i>	<i>Salinity (ppt)</i>	<i>Rugosity Index</i>	<i>Aspect</i>	<i>Distance to mouth (km)</i>
Mean	62.37	14.85	36.88	1.39	178.95	10.30
Standard Error	2.71	0.15	0.12	0.04	9.35	0.74
Median	60.02	14.75	37.31	1.32	202.50	9.42
Standard Deviation	17.74	0.97	0.77	0.24	61.32	4.85
Sample Variance	314.73	0.94	0.59	0.06	3759.59	23.57
Range	66.08	3.66	2.24	0.91	180.00	18.97
Minimum	30.24	12.75	35.48	1.08	45.00	1.89
Maximum	96.32	16.41	37.72	1.99	225.00	20.86

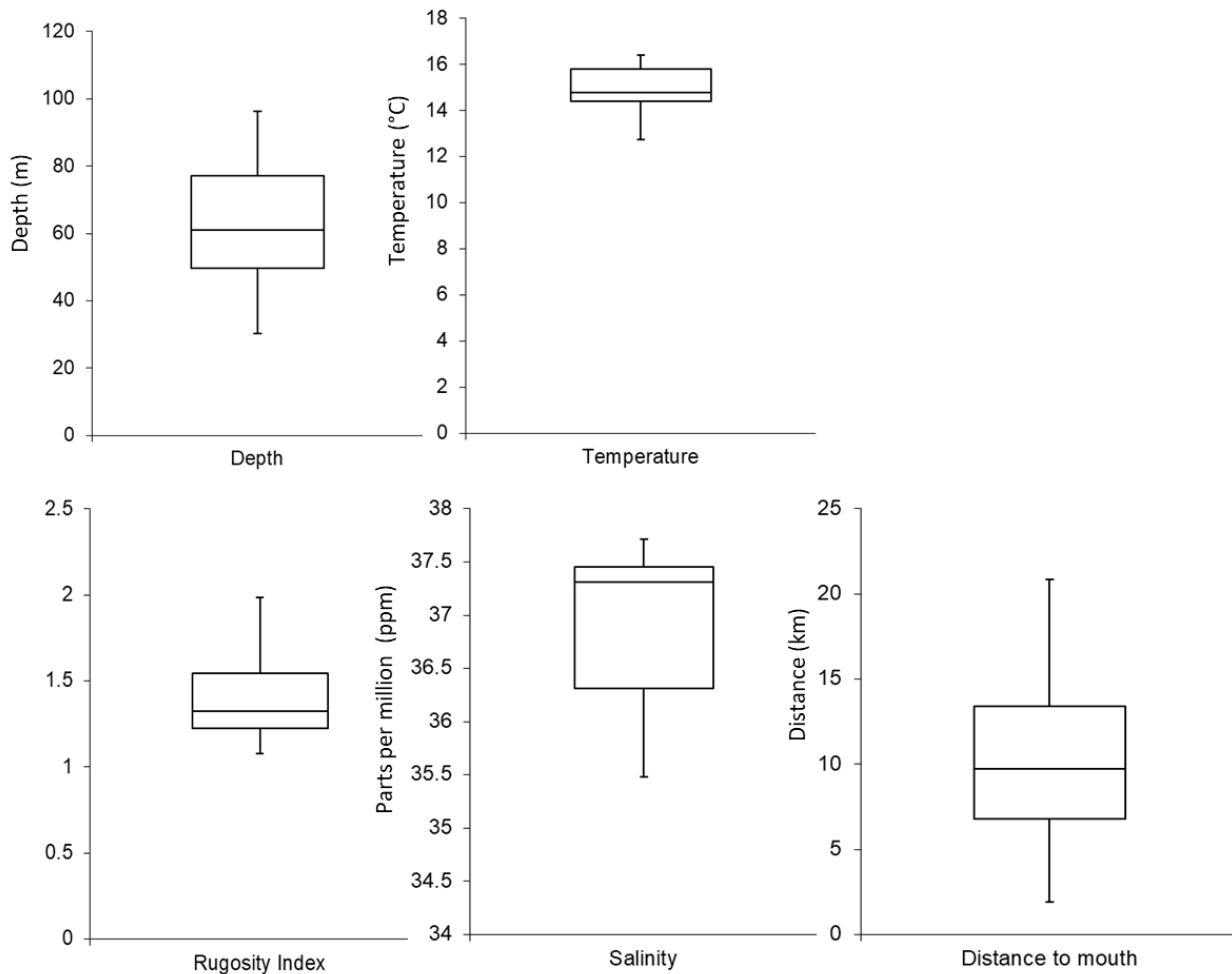


Figure 3.2 Box–and–whiskers plots for seven environmental variables from the continental shelf found off the Kei River mouth. Variables include (a) Depth in metres, (b) Temperature in °C, (c) Salinity in parts per million (ppm), (d) Rugosity index, and (e) Distance to river mouth in kilometres. The middle line inside the box indicate the median, upper and lower hinges indicate 75 and 25 % quartiles, whiskers indicate the minimum and maximum values of each variable

3.4.2 The influence of environmental variables in structuring macrobenthic communities on the shelf offshore of the Kei River mouth

A PCA of the six environmental variables showed that no single variable had a dominant influence on the community samples (Table 3.2, Figure 3.3). Samples from communities C and H were the most widely dispersed. The first two PC axes explained 71.8 % of the total variance. The addition of the third axis improves the total variance to 89.7 %. Distance to mouth and depth, had the highest positive influence on the PC1 axis, whereas, latitude had the highest negative influence (Table 3.2). Temperature and salinity dominated the PC2 axes (Table 3.2), while rugosity index had the highest influence on the PC3 axis.

Table 3.2 Results of the principal component analysis showing the first three ordination axes. The values of the most important variables for each axis are highlighted in bold

	Axis 1	Axis 2	Axis 3
Principal component			
Eigenvalues	2.71	1.59	1.08
Percentage (%)	45.2	26.5	17.9
Cummul. Perc. (%)	45.2	71.8	89.7
Variables			
Depth	-0.560	0.087	-0.155
Temperature	-0.175	-0.704	-0.129
Salinity	0.226	-0.658	0.189
Rugosity Index	-0.062	0.097	0.938
Latitude	-0.508	0.190	-0.204
Distance to mouth	0.585	0.136	-0.040

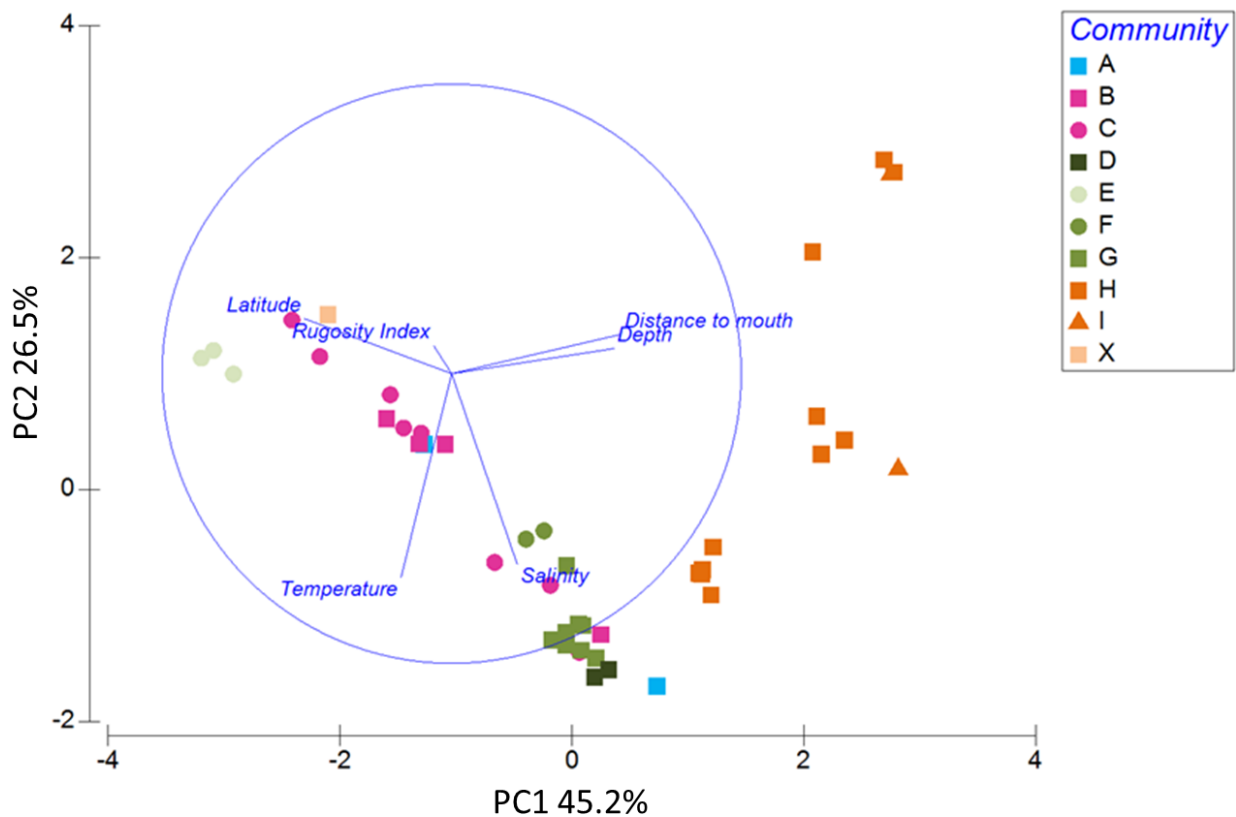


Figure 3.3 Principal Component Analysis (PCA) plot for all samples showing the first two ordination axes. Symbols indicate the community, as described in Chapter 2, from which each sample was derived

DISTLM analyses of macrobenthos morphospecies against the 9 environmental variables showed that depth, substratum, distance to mouth, salinity and latitude are the most important, and best combination of environmental variables, when explaining macrobenthic community composition on the continental shelf offshore of the Kei River mouth (AIC = 314.3, $R^2 = 0.5$) (Table 3.3, Figure 3.4). Marginal tests also revealed the significance of the nine environmental variables (Table 3.4). The five most important environmental variables (Table 3.3) combined explained 62 % of the total fitted variation. The first two dbRDA axes explained 81.8 % of the fitted model variance and 40.2 % of the total variation in the multivariate cloud. The first dbRDA axis was correlated most strongly with depth (0.84) which was mainly responsible for the variation among Communities H and I (Table 3.3). The dbRDA second axis negatively correlated with distance to mouth (-0.62) and substratum (-0.61) and distinguished Communities E, F and G from communities A, B and C (Table 3.3, Figure 3.4). Salinity (0.60) and latitude (-0.77), to a lesser extent, influence the distribution of communities C, E and F.

Table 3.3 Relationships between dbRDA coordinate axes and orthonormal X variables (multiple partial correlations). Important variables highlighted in bold

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4
Depth	0.845	0.472	0.208	-0.029
Salinity	-0.147	-0.187	0.604	-0.564
Latitude	0.137	-0.031	-0.203	-0.772
Substratum	0.394	-0.601	-0.538	-0.090
Distance to mouth	0.301	-0.616	0.511	0.276

Table 3.4 Results from DISTLM marginal tests, important variables highlighted in bold

Environmental variables	SS (trace)	Pseudo-F	P	Proportion
Depth	24050	13.759	0.001	0.251
Temperature	7904.9	3.691	0.005	0.083
Salinity	3220.3	1.428	0.178	0.034
Rugosity Index	1609	0.701	0.664	0.017
Latitude	10340	4.966	0.003	0.108
Relief	5312.4	2.409	0.037	0.056
Aspect	2692.1	1.187	0.251	0.028
Substratum	9057	4.285	0.002	0.095
Distance to mouth	23257	13.160	0.001	0.243

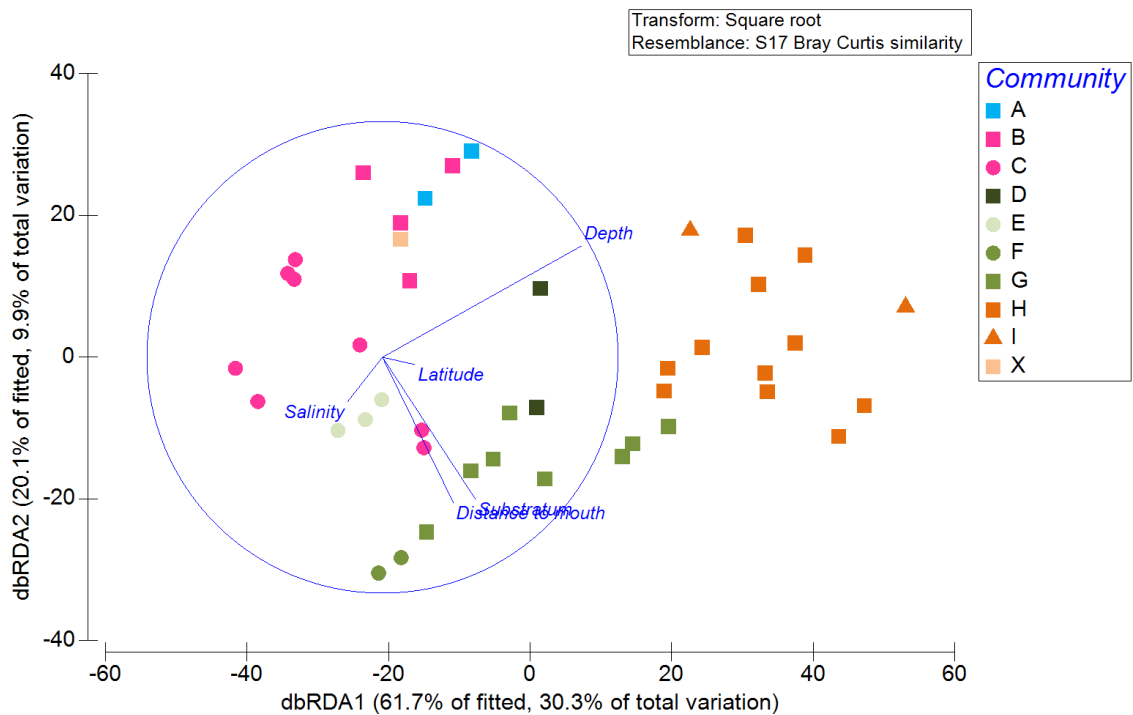


Figure 3.4 Distance-based redundancy analysis (dbRDA) based on the resemblance matrix using benthic biota abundance data with aspect, depth, relief, substratum and temperature and substratum as environmental variables

Depth and distance to mouth are strongly correlated and independent variables that have difference influence on the macrobenthos community structure (Figure 3.4). Communities H and I were strongly influenced by greater depth than compared to the rest of the communities which were also influenced by depth but to a lesser extent. Similarly, distance to the river mouth also has a greater influence on communities (F and G) further from the river mouth. Figure 3.4 also shows that distance to mouth and substratum influence the communities in the same way. This could mean that the former variable could possibly be used as a proxy for substratum because it appears that substratum changes from mostly mixed-reef to coarse sand with increasing depth. Communities F – I therefore, occurred on more consolidated substratum such as reef furthest from the Kei River mouth. Communities A – C, however, occurred on unconsolidated substratum closer to the Kei River mouth. Salinity may not have the same magnitude of influence on the communities as the aforementioned values but it has the opposite influence of depth. This could indicate the increase in bottom salinity with increasing depth because freshwater input from the Kei River reduces salinity at shallower depth and closer to the river mouth. Shallow communities (C and E) closer to the river mouth and at shallower depths experienced lower water salinity than compared to other communities.

The BIO-ENV analysis from the BEST routine suggested that depth, substratum and distance to mouth combined had the highest correlation to the macrobenthos resemblance ($\rho = 0.55$, $p < 0.001$, Table

3.5). These variables were used in the subsequent LINKTREE analysis to identify significant divisions in environmental variables related to community resemblance.

Table 3.5 BIO-ENV results showing the environmental variables that best explain the biological data

	Correlation	Variables				
1 st	0.550	Depth	Substratum	Distance to mouth		
2	0.531	Depth	Latitude	Substratum	Distance to mouth	
3	0.530	Depth	Temperature	Substratum	Distance to mouth	
4	0.529	Depth	Temperature	Latitude	Substratum	Distance to mouth
5	0.515	Depth	Latitude	Distance to mouth		
6	0.508	Depth	Temperature	Latitude	Substratum	
7	0.503	Depth	Substratum			
8	0.503	Depth	Rugosity Index	Substratum		
9	0.499	Depth	Latitude			
10	0.497	Depth	Temperature	Rugosity Index	Substratum	Distance to mouth

LINKTREE analysis using depth, substratum and distance to mouth as constraining variables produced 11 SIMPROF clusters (Figure 3.5). This dendrogram mirrors the cluster dendrogram groupings of the biotic community resemblance matrix (Figure 2.7a, Chapter 2) with almost all samples grouped according to biotic community clusters. The first binary division separates the samples at depths < 63.7 to > 73.7 m (Figure 3.5, node A of dendrogram). Samples at depths < 63.7 m represent the upper mesophotic communities, whereas, > 73.7 m represents the lower mesophotic communities. The second binary divisions separates the upper mesophotic communities by substratum type, namely, fine sand (< 1) and anything courser or more consolidated than fine sand (>2) (Figure 3.5, node B of dendrogram). The upper mesophotic communities are further separated by substratum type, namely, rhodolith substratum (<2), and reef and mixed reef (> 4) (Figure 3.5, node C of dendrogram).

All subsequent divisions are mostly attributed to depth and a few are distance to mouth. For samples with rhodolith substratum (< 2, left of node C), communities were distinct at depths < 49.6 to > 54.4 m (node D), with samples further separated by depths < 46.7 to > 47.9 (node E). Reefs closest to the river mouth are distinguished by a distance < 2.08 km (node F). Reefs furthest from the river mouth (> 2.31 km) are further separated by depth divisions < 66.3 to > 67.3 km (node G) and < 44.8 to > 48.4 m (node H). The latter node clearly separates biotic communities F and G. A noteworthy depth break at approximately 50 m is also highlighted multiple times (nodes D, E and H) within the dendrogram divisions. For the lower mesophotic (> 73.7 m), distance to river mouth separates a sample from Community I (< 9.72 km) from the clearly defined groups of Communities H and I (> 13.2 km). Lastly, the most notable division in the lower mesophotic communities is node J which separates biotic Communities H and I at depths < 89.6 m and > 90.3 m, respectively (Figure 3.5).

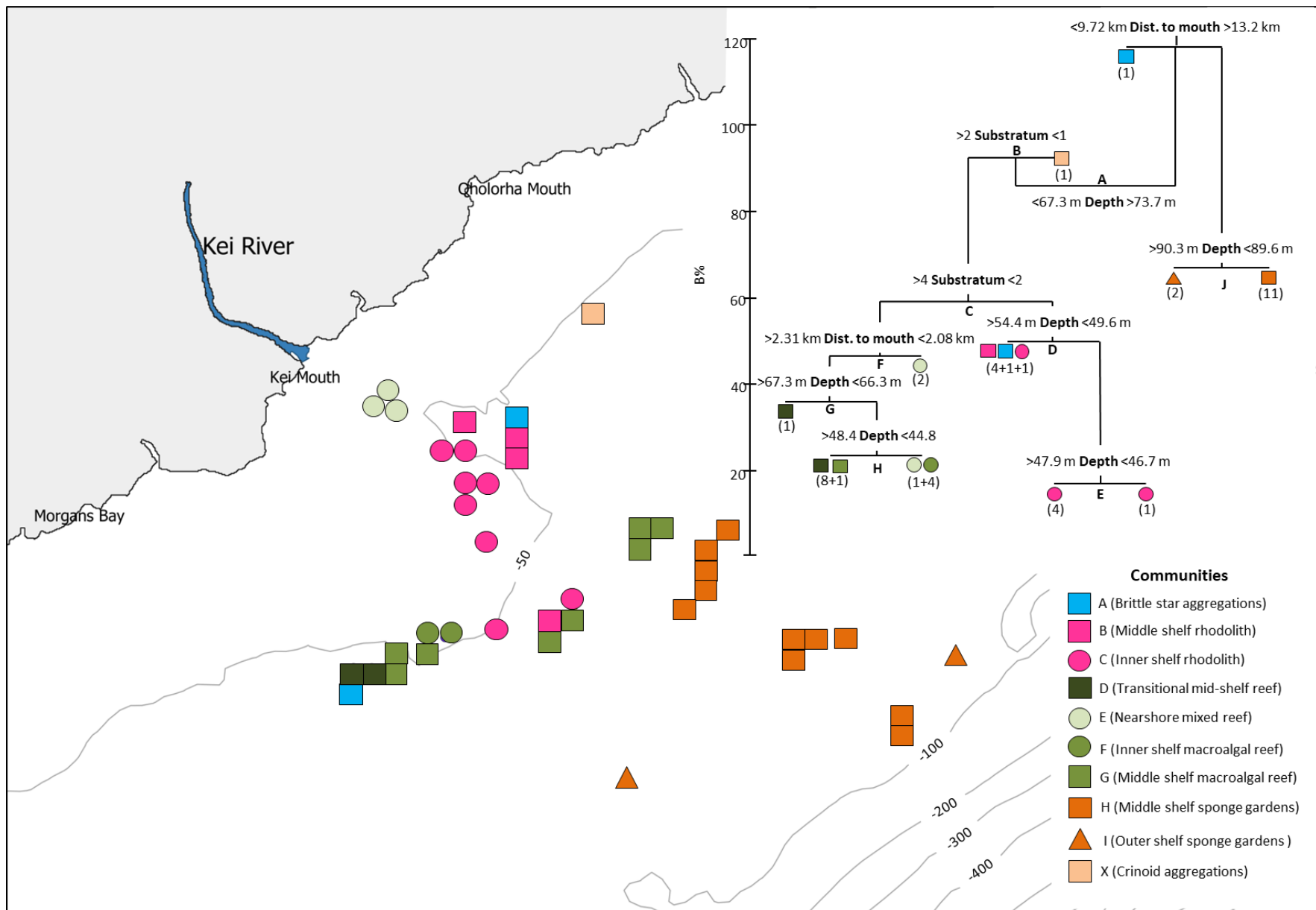


Figure 3.5 LINKTREE analysis plot (top right) showing the most appropriate breaks in environmental variables in relation to benthic communities off the Kei River mouth (left). Environmental variable thresholds are indicated chronologically (A-J). Map (left) showing macrobenthic communities. B% and R-statistic values in Appendix 5

3.5 Discussion

This study provides a broad assessment of the environmental variables that drive the distribution of macrobenthos on the temperate mesophotic shelf off the Kei River mouth. Multivariate analyses identified depth, salinity, latitude, substratum and distance to mouth as important variables, with depth, substratum and distance to mouth having the greatest influence on the community patterns. The latter variables also provide significant thresholds that divided communities (Figure 3.5). Importantly, the environmental variable sampling was not exhaustive and additional sampling of other environmental variables, especially correlates of depth, will complement this dataset and could reveal other aspects of the abiotic environment to be explored. Here I provide a conceptual model (Figure 3.6) for the temperate shelf off the Kei River mouth of the Amathole region which combines, geological bathymetric shelf data (DeWet and Compton 2021), environmental variable data and macrobenthic community data from this thesis to explain the distribution of the mesophotic macrobenthos off the Kei River mouth.

3.5.1 Environmental controls of temperate mesophotic shelf off the Kei River mouth

Depth had the greatest influence on the structure of communities in the lower mesophotic > 74 m, where light is limited (Lesser et al. 2009, 2019, Kahng et al. 2017, Turner et al. 2017, Cerrano et al. 2019). Depth further separated the lower mesophotic communities at 90 m and separated the shallower, upper mesophotic communities along the 50 m depth contour (Figure 3.6). The depth at 74 m depth break concurs with the transition depth of benthic invertebrates in the Australian region ($75.3 \text{ m} \pm 3.4 \text{ m}$) and in the Pacific region ($74.7 \text{ m} \pm 3.6 \text{ m}$) (Turner et al. 2017). The ecological depth breaks recorded at ~50 m somewhat coincides with 60 m depth break in mesophotic coral ecosystems (Lesser et al. 2019) and at 90 m, however, the latter is not discussed in the literature. These depth breaks coincide with the geological lower boundary of the inner shelf at 50 m (DeWet and Compton 2021) and the geological boundary between the middle and outer shelf at 90 m (DeWet and Compton 2021) off the Kei River. de Wet and Compton (2021) also notes the boundary of the nearshore sediment wedge, likely produced by the Kei River during the Cenozoic, at 70 m depth which for all intents and purposed will be used as to separate the middle shelf into shallow and deeper parts. This clearly places communities C, D, E and F on the inner shelf (< 50 m), communities B, E and G on the shallower-middle shelf (50 – 70 m), community H on the deeper-middle shelf (70 – 90 m) and community I on the outer shelf (90 – shelf break). Community E was restricted to depths shallower than 30 m deep, which is also the boundary of the inner and middle shelf. Since there were no other photographic transects at comparable depths, it is difficult to ascertain if community E resembles a shallow-subtidal community or an upper mesophotic reef community. Greater sampling effort is needed at this depth. Community A occurred only at the lower and upper limits of the middle shelf,

57 m and 75 m respectively. This is probably more a collective function of depth, substratum type and possibly another environmental variable that could explain the geographic patterns of this community. The division of the middle shelf may be confusing but it is not without merit. The East London middle shelf is generally wide but is particularly narrow off the Kei River due to the nearshore fluvial fan intruding onto the nearshore middle shelf (DeWet and Compton 2021). This means geologically that the inner and part of the middle shelf is indiscriminate down to 70 m depth. The narrow and steep middle shelf is therefore confined to between 70 – 90 m. The topography of the fluvial fan therefore strongly influences the structure of the benthic communities off the Kei River mouth and explains the bathymetric separations of the benthic communities. The ecological boundary at 50 m depth (Figure 3.5), which distinguishes inner from middle shelf reefs and rhodolith beds, coincides with the geological boundary of the inner and middle shelf off the Kei River. Similarly, the ecological boundary at 90 m depth (Figure 3.5), which separates sponge garden communities, also coincides with the geological boundary of the middle and outer shelf off the Kei River mouth. The ecological break at 74 m depth, that separates photosynthetic from filter-feeder communities, does not exactly align with the boundary of the nearshore sediment wedge at 70 m depth. The ecological break is however, supported by the sudden change in communities over a short distance (1.4 km) and a 20 m depth range (eastern most transect with cells from communities A, D and F, Figures 3.5 and 3.6)

Depth is often also used as a proxy for light and is known to stratify the vertical composition of benthic communities (Lesser and Slattery 2011). Depth also regulates the density and size of rhodoliths (Bahia et al. 2010, Oliveira et al. 2013). A narrow shelf with steep slopes facilitates the decrease in size and the increase in abundance of rhodoliths in Brazil (Bahia et al. 2010). Likewise, the East London shelf is narrow and has a steep slope (0.5 to 1.3 °) (Green et al. 2020, de Wet and Compton 2021) which may explain the difference between the rhodoliths deeper than and shallower than 50 m depth. Red macroalgae (encrusting and foliose morphologies) generally dominated the upper mesophotic, whereas, sponges and gorgonians dominated the lower mesophotic. Red algal species are known to thrive in low light conditions as found off the Amazon River in Brazil (Moura et al. 2016), Mediterranean Sea (Cerrano et al. 2019) and Australia (Ryan et al. 2007). Sponges, however, dominate the lower mesophotic depths (Lesser et al. 2009). Within the Kei River's plume the euphotic zone may be as shallow as 14 m deep due to the high suspended sediment load in the water column which resulted in lower primary production than adjacent areas (Barlow et al. 2010). Although primary production was low in the water column, red macroalgal assemblages dominated reefs and rhodolith beds in the upper mesophotic. This is possibly attributed to the increasing light attenuation with increasing depth (Lesser et al. 2009) which promotes the dominance of red algae as opposed other

algal taxa. Besides increased light attenuation, increased particulate organic matter accumulation at 60 m depth on the fronds and thalli of the macroalgae may further limit photosynthetic activity. The macrobenthic community composition therefore changed from being dominated by photosynthetic organisms (primary producers) to active filter and suspension feeders at depths deeper than 60 m (Heyns et al. 2016). This change in community composition at this depth coincidentally coincided with the change in substratum type. This change in substratum type from the inner shelf to the outer shelf may also be linked to wave abrasion which produces biogenic sediment eroded from surrounding sessile calcareous macrobenthos which over time covers the underlying bedrock (James et al. 2001). This phenomenon was evident from the middle and outer shelf habitats.

Substratum may equally be the environmental variable which best explains the variation in the benthic assemblages and is recognised as a key driver defining the distribution of macrobenthos in mesophotic depths (Bridge et al. 2011a, Heyns et al. 2016, MacKay et al. 2016). The shelf off the Kei River mouth is largely sand starved due to the depth of the Agulhas Current (Green et al. 2020), which can extend to 2400 m (Bryden et al. 2005). It is therefore, no coincidence that substratum types are comprised of coarse sand, high relief reef (bedrock outcrops) and rhodoliths shallower than 70 m, and a thin layer of coarse sand (of variable thicknesses) overlaying subdued reef in areas deeper than 70 m (Green et al. 2020). This is due to the extension of the fluvial fan over the inner shelf and parts of the middle shelf (DeWet and Compton 2021). Moreover, the shelf being starved of fine sand due to the Agulhas current is a common geological phenomenon along the eastern continental margin of South Africa (Green and MacKay 2016). It is unclear whether or not the sand settles and is later weathered away or if the sand settles in the nearshore environment or elsewhere on the East London shelf. Likewise, this coarser sediment found between 60 – 100 m in the current study matches description of the current-swept unconsolidated habitats on the KwaZulu-Natal shelf (Green and MacKay 2016). The current study showed that mixed reef substratum below 70 m provided attachment to support the sponges and gorgonians. Similarly, the substratum ranged from fine to coarse sediment and rhodolith between 30 and 67 m with a distinct ridge of bedrock outcrop with large patches of rhodolith. These rhodoliths constitute the greater rhodolith bed found in the region, which is also of geological importance (Adams et al. 2020, Green et al. 2020).

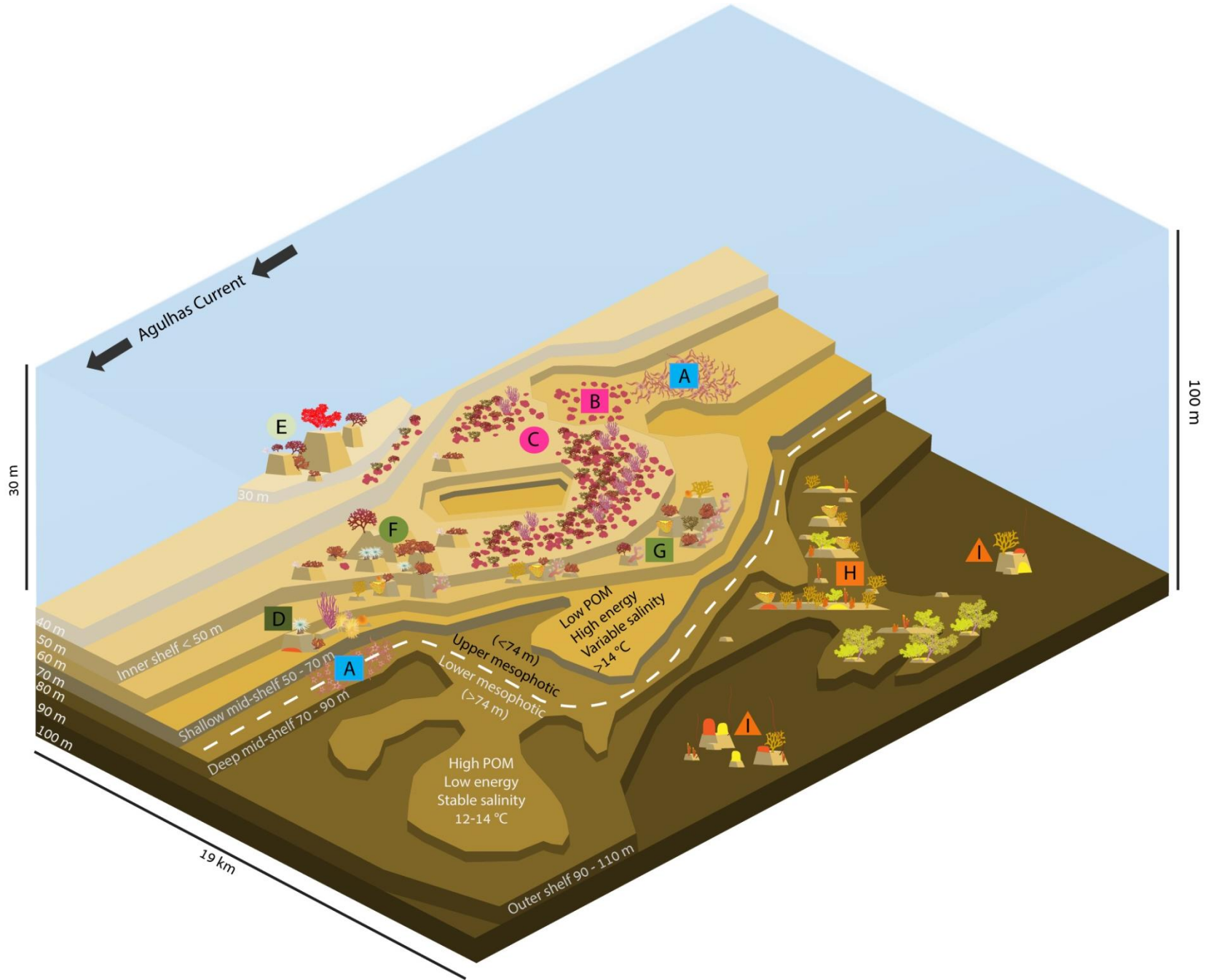


Figure 3.6 Conceptual model of the warm temperate shelf off the Kei River Mouth, South Africa. Shelf topography was interpreted from detailed bathymetric maps of the East London Shelf (de Wet 2013). Community positions reflect the real-world locations of ROV transects of the Kei River mouth, whereas, areas with no biota are unexplored. The white dotted line represents the depth break at 74 m which separates the study area into upper and lower mesophotic zones

Sediment and particulate organic matter accumulation increased with increasing depth off the Kei River (pers obs.). Fluvial sediment dynamics off the Kei River Mouth is poorly understood, however, the presence of certain organisms may be indicative of the degree of accumulation in the benthic environment. Heyns et al. (2016) found that deep reefs (45 – 75 m) in the Tsitsikamma MPA hosted upright, active filter feeding growth forms in the presence of elevated levels of settled particulate matter, whereas shallow reefs hosted mostly photosynthetic growth forms. The communities distinguished in the current study are similar in structure to that of the deep reefs in the Tsitsikamma MPA. Furthermore, sponges with large, cup-shaped and massive sponge morphologies like the sponge gardens found in the current study are adapted to sedimentation off the Amazon River (Moura et al. 2016). Surprisingly in the current study, the presence of the rhodolith bed between 30 – 65 m suggests that although suspended sediment in the water column was high, accumulation was low enough to prevent die offs through smothering; a phenomenon known to cause rhodolith bed die offs (Wilson et al. 2004). The biomass that constituted the thalli of the rhodoliths off the Kei River mouth were largely fossilised and comparatively smaller than the global average, and suggested it was likely due to ephemeral growth regimes induced by a dynamic river plume (Adams et al. 2020).

Communities within the river's plumes are usually filter-feeder dominated due to increased turbidity and lower light penetration, whereas, areas not within the plume are algal dominated in temperate settings because of greater light penetration (Celliers et al. 2007). Likewise, proximity to a river mouth does not necessarily indicate a food source of benthic filter feeders (Porter et al. 2014), but may facilitate the growth and dominance of macroalgae. The presence of dense rhodolith beds with foliose algal cover has been noted beneath the Amazon River's plume where these aggregations are able to mineralise calcium carbonate in low light conditions (Moura et al. 2016). Moura et al. (2016) also found that sponge abundance and diversity was greatest at levels of intermediary plume influence. Although the current study area was directly off the Kei River, algae dominated the upper mesophotic which may indicate that the limited light regime produced by Kei River's plume does not inhibit the photosynthetic processes of the red macroalgae. This fluvial input could have a net positive nutritive influence on benthic primary production in the otherwise oligotrophic waters of the East London shelf. Acquiring measurements of other environmental variables may support this claim and aid in understanding the water movement of the plume.

Other significant variables including temperature and salinity were also not suitable predictors of community pattern. They do, however, provide insight to the hydrodynamic environment experienced off the Kei River mouth which is unique along the same stretch of coastline (Russo et al. 2019). In the current study, warmer water (up to 24 °C) was generally found in the upper mesophotic depths

whereas colder water (14 °C) was found at lower mesophotic depths. Sudden fluctuations in bottom temperature between 24 – 14 °C were however, observed on consecutive days in the same area. This is mainly attributed to upwelling at the front of an anticyclonic eddy in this region (Russo et al. 2019). Deep mesophotic communities may be controlled by upwelling of cold, nutrient-rich water (Leichter and Genovese 2006). Although the upwelled water off the Kei River was cooler and nutrient-rich, it did not promote benthic primary production as in other sites at similar depths along the same stretch of coastline (pers obs.), nor did it promote pelagic primary production directly off the Kei River (Barlow et al. 2010). The discovery of rhodolith beds on the inner shelf could be linked to the periodic upwelling off the Kei River mouth which mimics the occurrence of rhodolith beds on the inner shelf subjected to similar upwelling cycles on the Greater Australian Bight (James et al. 2001). Essentially, the periodic upwelling is what provides the nutrients and sustains the macrophytes and rhodoliths off the Kei River.

The plume from the Kei River also reduces light penetration which resulted in considerably lower primary production in the water column (Barlow et al. 2010). This phenomenon coupled with diminished light, low wave energy and increased particulate matter accumulation may explain absence of rhodoliths deeper than 65 m. This hydrographic regime coupled with the seasonal freshwater inflow from the river may promote a dynamic and ephemeral growth regime evident in the growth of the rhodoliths found here (Adams et al. 2020).

The shelf topography off the Kei River mouth supports a unique suit of communities because the nearshore sediment wedge extends the boundary of the inner shelf (50 m) from 5km offshore to 14 km offshore (DeWet and Compton 2021). This could explain the distinction of communities at 50 m depth. These community separations could also be more related to the distance from the shore (also a proxy for the distance to mouth) rather than depth. The extended inner shelf widens the area for algal dominated communities and creates a more gradual gradient of the environmental parameters across this feature relative to the narrower inner shelf adjacent to the sediment wedge. This inner shelf extension also severely restricts the area of the middle shelf, particularly the 70 – 90 m depth range, creating a steep slope onto the outer shelf > 90 m. The steep slope on some areas of the shelf at 70 m may explain the numerous communities at or near the 70 m isobath. The shelf topography is also important in determining the community distributions of the deep communities on the coarse sand. This is evident in the location of communities H and I (Figure 3.5). Community H occurred over several kilometres, an indication that a narrow seaward platform created by the nearshore sediment wedge extends the 90 m isobath seaward. Coincidentally, majority of the samples collected deeper than 70 m were collected on this sedimentary platform. Samples from community I are parallel to the coast and are the same distance if not even closer to the shore than the deepest samples from

community H (Figure 3.5). This supports the idea that unique topography created by the sediment wedge is important in structuring macrobenthic community distribution off the Kei River mouth (Figure 3.5 and 3.6).

Aspect was not identified as an important variable structuring community pattern but it, along with current speed and distance to mouth, could explain the distribution of brittle star aggregations off the Kei River mouth. The aggregations were only found relatively close to the river mouth and only found deeper than 50 m on either sides of the sediment wedge. They were not found on or near the distal end of seaward wedge. These were suspension feeding brittle stars so depth and light should not influence their distribution. The position, direction and intensity of the prevailing current could explain the distribution of the brittle stars. The Agulhas Current runs parallel and close to the shore. This fast-moving current could facilitate feeding and larval dispersal and encourage the dense aggregation behaviour of the brittle stars to prevent from being swept away. In this case, current direction could be used as a proxy for aspect or vice versa.

The other environmental variables that have been highlighted as significant in structuring mesophotic ecosystems but were not measured in this study include slope, chlorophyll concentration, turbidity, light, sediment grain size, percentage settled particulate matter (Bridge et al. 2011a, Heyns et al. 2016). A calculation for rugosity index was included in the multivariate analysis in the current study and was found not to be a significant variable. Microtopography scale of rugosity may be more important in structuring the vertical zonation of mesophotic macrobenthos (Bridge et al. 2011a), than rugosity calculated at the scale in the current study. The most appropriate method to derive rugosity is from multibeam bathymetry but some transects covered areas without multibeam bathymetry and an improvised calculation was used (see section 3.3.1). The distance-based linear model (DistLM) performed better with the inclusion of rugosity. These calculations may not reflect the community patterns because the five images used to define communities covered a significantly smaller area of the seabed than the rugosity estimate for the entire cell. Similarly, the inclusion of aspect and relief improved the models performance even though they were subjectively assigned to each transect based on the vessels bearing for the former and image interpretation for the latter. Aspect seems to be the one environmental variable that is consistently not influential in structuring mesophotic communities (Bridge et al. 2011a).

3.6 Conclusion

When investigating the processes that shape the distribution and composition of benthic communities on a temperate mesophotic shelf, depth and substratum type were the two environmental variables that best explained the observed community patterns. Depth, however, is also known as a “super

parameter” and is often a proxy for other underlying processes that may be at play such as light, turbidity and pressure. The unique physical hydrography off the Kei River allows for persistence of a diverse temperate mesophotic ecosystem. The known distribution of mesophotic ecosystems are limited and the knowledge of the key environmental variables structuring these ecosystems are even scarcer. Therefore, dedicated instruments measuring the physical environment should accompany all underwater visual surveys, regardless of the camera platform. Environmental variables such as water clarity and productivity should also accompany all future visual surveys in the mesophotic zone (Bridge et al. 2011a). Incorporating numerous environmental variables alongside image data analyses will not only help us to better understand the ecology of all mesophotic ecosystems, it will also assist in determining which variables are important to the different mesophotic ecosystems worldwide.

Chapter 4 Synthesis

South Africa's offshore region, the continental shelf in particular, is understudied when compared to the inshore region (Griffiths et al. 2010). Continental shelf research in the mesophotic zone has been focused in tropical regions and particularly coral species, with limited research conducted in temperate settings and species other than corals (Turner et al. 2017, Cerrano et al. 2019). This research is also concentrated in developed countries where resources are not as limited as in developing countries. Of the developing countries in Africa, South Africa possibly has the greatest capacity and resources actively directed to offshore foundational research using underwater camera platforms. Despite costly ship time and elaborate data processing requirements, remotely operated vehicle surveys have produced new foundational biodiversity information for several areas in South Africa, namely the Alghard Banks (Gotz et al. 2010), Tsitsikamma (Heyns et al. 2016), KwaZulu-Natal (Franken 2015, Makwela 2017) and now the Amathole region- a historically under sampled and unexplored stretch of South African coast. This information has assisted South African benthic ecologists to better understand the benthic communities, their distribution and their drivers.

4.1 Mesophotic ecosystems

In response to the identified knowledge gaps, this thesis surveyed the temperate shelf within the Amathole Offshore Marine Protected Area Complex off the Kei River. This produced the first conceptual framework for river-influenced mesophotic ecosystems based on macrobenthic communities and environmental variables that could be used elsewhere (Chapter 3). It not only advanced the foundational biodiversity knowledge for the Amathole region, but it also led to the discovery of rhodolith aggregations in South Africa (Adams et al. 2020) and several dense echinoderm aggregations. The rhodoliths aggregations are a novel ecosystem for South African benthic ecologists. Their discovery raises several questions. Why have we not discovered rhodoliths elsewhere along the South African coast? Why have we not found rhodoliths in the intertidal zones in South Africa? Where else do rhodoliths occur along the South African coast and are they associated with rivers? What is the bathymetric distribution of rhodoliths in South Africa? Do rhodoliths support the life history stages of important commercial fish or invertebrate species? Essentially, how important are rhodoliths in South Africa, are they afforded adequate protection and what are the possible anthropogenic impacts on this ecosystem?

Community patterns and their associated environmental variables constitute biotopes (Costello 2009). Depth and substratum type were identified as responsible for the distinction of communities (Chapter 3). Light quality and intensity is a correlate of depth, which is therefore most likely responsible for the distinction between photosynthetic macroalgae and filter-feeder dominated

communities in the mesophotic zone (Ballesteros 2006). Substratum type most likely relates to the attachment sites for sessile organisms (Bridge et al. 2011a). Latitude, which strongly correlated to proximity to the river mouth, could be related to the degree of sedimentation and particulate organic matter accumulation (Celliers et al. 2007). Similarly, salinity also proved useful in understanding how fluvial input could affect benthic communities. More biologically relevant environmental variable measurements may provide a more developed understanding of river-influenced mesophotic communities and allow important variables of fluvial influence to be identified and measured.

The biologically significant depth break represents the transition from photosynthetic communities in the upper mesophotic to filter-feeder communities in the lower mesophotic zone. The communities identified in the current study are functional; therefore, improved species level information will undoubtedly increase community diversity and possibly enhance community separations. This is attributed to numerous morphologically similar species that were classified as a single morphospecies using the CATAMI classification scheme. The broad community pattern and distribution should hold. Improved species level information should verify this claim. Nonetheless, this distinction is biologically relevant and forms the foundation of the conceptual framework for a river-influenced mesophotic ecosystem in temperate South Africa.

The main findings of this thesis are (i) seven upper mesophotic and two lower mesophotic communities, (ii) a biologically significant depth break at 74 m separating the upper (photosynthetic) and lower (filter feeder) communities and (iii) depth, substratum and distance to river mouth as the main drivers for community pattern.

4.2 Implications of this study

This research supports the design and proclamation of the Amathole Offshore Marine Protected Area. It also contributes to the management of South Africa's marine biodiversity through providing a map of macrobenthic communities offshore of the Kei River mouth, which can be used by the regional management agency to create a management plan for the restricted and controlled zones within the Amathole Offshore Marine Protected Area. The ROV footage collected on the ACEP: Imida Frontiers project, along with expert opinion, was used to delineate the boundaries and assign zonation of the Amathole Offshore Marine Protected Area complex. The protected area network not only protects the sensitive and slow growing animal forests in the lower mesophotic zone, but affords protection to the newly discovered rhodolith and the reef ecosystems. These ecosystems host a number of endangered and critically endangered fish species, notably, the red steenbras and seventy-four (Sink et al. 2019). These sensitive and novel discoveries aided the proclamation of the Amathole Offshore Marine protected Area Complex.

The main objective of the Imida project was biodiversity exploration of the historically unexplored East London shelf offshore region with the area off the Kei River earmarked as an area of interest. In addition, ground truthing the mud banks off the Kei River (Sink pers comm) mapped in the 2011 marine habitat map (Sink et al. 2012) was also a priority. Several ROV transects showed that the mud grounds had either been replaced by sandy substratum or the mud grounds had shifted outside of the survey area. There was evidence that the strong currents could contribute to the movement of underwater mobile dunes to cover fossilised mud and uncover paleo-dunes. The absence of mud could also be attributed to reduced fluvial input (Sink et al. 2019). This evidence together with observations from the current study suggests that the physical environment off the Kei River is more dynamic than previously thought. Although mud habitats were not found in 2017, future exploratory surveys are necessary and will likely uncover evidence muddy bottoms off the Kei River. This hopeful statement is in response to organic matter found layered within the framework of rhodoliths collected off the Kei River (Adams et al. 2020). This may suggest that the rhodoliths are frequently covered in a mud when the river is in flood.

Unlike in the current study area, several other river-influenced ecosystems in South Africa are currently not afforded formal protection (Sink et al. 2019). The KZN Bight Mid shelf reef Complex, Orange Cone Inner Shelf Mud Reef Mosaic and the Orange Cone Muddy Mid shelf are also sensitive ecosystems and protection of these should be prioritised in the proclamation of the next round of marine protected areas. Furthermore, few marine ecosystems are dependent on the protection of another realm of ecosystems. These river-influenced ecosystems are vulnerable to impacts kilometres away from them. Drought, pollutants and reduced freshwater flow (abstraction, dams, ports and harbours) may disrupt the functional ecology of fluvial ecosystems. Land-sea connectivity is greatest in the nearshore environment and fluvial input amplifies and extends the connectivity and influence of the land into the offshore environment. The effects of reduced fluvial input on benthic invertebrates are not well studied but chemical signals and detritus from rivers are necessary for the development of many fish species and is a source of essential organic or inorganic carbon for benthic invertebrates.

4.3 Future research and knowledge gaps

Future research priorities were identified and classified into five themes. These included but are not limited to (i) image analysis, (ii) spatio-temporal environmental variable monitoring, and (iii) defining and delineating the mesophotic zone along the temperate shoreline, (iv) understanding discrete communities and their interactions with each other, and (v) mapping plume and fluvial fan dynamics to understand the intensity and extent of the influence of the Kei Rivers. Advancing these areas of study will help map and understand the seabed for better management. Focusing effort into these

themes will address some of the knowledge gaps identified in the mesophotic zone (Turner et al. 2019).

Underwater image analyses from remotely operated vehicles have been slow to produce publications in South Africa with only three publications since the acquisition of an ROV in 2011. This lack of publication is however, mainly related to the processing time because the main image analysis method is manual image annotation. Although this method is necessary, semi-automated and automated image annotation is widely used (Taylor et al. 2008, Gomes-Pereira et al. 2016, Mahmood et al. 2016, Mohamed et al. 2018). To date, very few if any marine scientists (technicians, post-graduate students and established scientists) in South Africa have had adequate training to use and teach autonomous image annotation through machine learning and artificial intelligence. Resources invested into building capacity will surely address the backlog of underwater imagery collected from underwater camera platforms and should enable research focus to shift beyond exploratory science from the backlogged image data. In addition, recent efforts to establish a standard image catalogue for in situ morphospecies (Howell et al. 2019) from local imagery will support a more standardised approach and improved analyses, particularly as taxonomic work advances in synergy.

The ROV used in this study is mainly used for exploratory surveys to address the lack of foundational biodiversity knowledge in the offshore environment (Griffiths et al. 2010, Sink et al. 2012). At present, exploratory surveys are prioritised because they are resource intensive (ship time, crew, mooring, skilled technician) and often have multiple research objectives from several disciplines that need to be addressed. For this thesis, the ROV video footage was outfitted with a single camera used to collect fish and invertebrate data. This meant that the imagery was collected at forward facing and/or oblique angles. Although this camera orientation allowed for adequate definition of community patterns and identification of morphospecies, a downward facing camera orientation is would be advantageous because it allows for the comparison of images collected by scuba (< 30 m), downward-facing towed cameras and Autonomous Underwater Vehicles (AUV). A downward facing camera will also increase the detection of organisms with smaller morphologies that are often obscured by their taller counterparts. In addition, standardisation of image area in conjunction with scaling of ROV imagery will allow finer scale investigation and produce richness and diversity estimates per unit area.

Remotely operated vehicle image surveys and analysis is not standardised. The main survey protocol used is the transect method and images have been analysed by overlaying a grid (Franken 2015, Heyns-Veale et al. 2016, Makwela 2017) or random annotation onto images as in the case of the current study. These methods have not been compared to determine which method is best practice in South Africa. Nevertheless, a standardised survey protocol and image analysis technique needs to

be established. This it will enable the comparison and incorporation of datasets at regional and national levels and benefit ecosystem classification in South Africa. All this new biodiversity information helps to form a baseline to base our understanding of offshore benthic ecosystems on.

Depth was identified as one of the main environmental variables driving community distributions. It is widely known as a super-parameter and is often a proxy for other key variables such as light. Other variables such as substratum, percentage particulate organic matter, rugosity, slope and chlorophyll are also known to be important drivers of mesophotic shelf communities (Bridge et al. 2011a, Heyns et al. 2016, Cerrano et al. 2019). Defining the spatial and temporal plume dynamics and the bi-annual upwelling cell off the Kei River will help us better understand the growth regimes of mesophotic macroalgal communities. Adams et al. (2020) found that the rhodoliths off the Kei River persist in a volatile environment with extreme temperature and salinity fluctuations. Although these rhodoliths persist, evidence suggest that increased pH, high temperatures (> 28°C) (Horta et al. 2016) or the combination of the two causes high mortality of the coralline algae (Horta et al. 2016). This persistence of the rhodoliths off the Kei River may be attributed to eurythermal macrobenthos and possibly the proximity to high relief reef – which may provide refugia for rhodolith forming coralline algae. The depth limits of the observed rhodolith bed needs further investigation as they seem to be resilient enough to persist between 30 – 65 m, but are found down to 90 m on the Kwa-Zulu Natal coast. Is this depth limit a matter of the limited ROV surveys or does settlement occur at a threshold that inhibits rhodolith formation and growth?

Inter community interactions and connectedness is not well understood on the continental shelf offshore of the Kei River mouth. This exploratory research has led to several pertinent questions that require investigation. Why do the brittle star communities occur closer to the shore on current-swept benthic habitats and adjacent to rhodolith aggregations? Are strong currents necessary for the existence of rhodoliths and brittle star aggregations? Do the animal forests in the lower mesophotic depend on the upper mesophotic for food during droughts when freshwater flow is reduced, or are the nutritional needs of the animal forests met by upwelling processes in the region? Are the macroalgal forests a permanent feature of the reefs or do they exhibit ephemeral growth regimes that mirror freshwater flow? Answering these questions will improve ecological understanding and help develop an effective benthic biodiversity management plan for the Amathole Offshore MPA.

The term mesophotic is not prominent in South African literature. It is a fairly new term used in benthic ecology and few studies that have been conducted at mesophotic depths with underwater camera platforms. The bulk of South Africa's mesophotic shelf knowledge has been collected using benthic sleds or dredges and grabs even though the term has not been associated with these sampling gears.

In South African benthic research, the common terms used to describe the bathymetric limits of the continental shelf are the inner-, middle- and outer shelf. These zones seem to place more emphasis on depth and the fair weather wave base, as opposed to light (Sink et al. 2012, 2019). These zones however, seem to coincide with the depth limits of the mesophotic zones. For example off the Kei River, the inner shelf (< 50 m) and middle shelf (50 – 70 m) form the upper mesophotic (30 – 74 m current study) and the outer shelf (70 – 110 m or shelf break) form the lower mesophotic (74 – 100 m current study). This does not mean that former zones should be renamed or discarded but to recognise that the mesophotic zone is an ecologically defined zone based on light. This also does not mean that all benthic ecologists studying epifauna, meiofauna or macrofauna between 30 – 150 m should report their study depth range as the mesophotic zone. The depth limits for TME in the Mediterranean Sea has been delineated as from the lower limit of the euphotic zone to the limit of the benthic primary productivity (Cerrano et al. 2019). As proposed in the previous chapter, South African benthic ecologists are encouraged to consider these guidelines when reporting on temperate mesophotic ecosystems especially because the continental shelf is highly variable in width, turbidity levels and in climate on the western, southern and eastern margins of the coastline.

4.4 Recommendations

Future ROV study designs should consider the following essential recommendations. i) The ROV should be equipped with 3 lasers at all times. This will enable scaling of the seabed and volumetric calculations of invertebrates, currently unavailable for South Africa's footage repository. ii) When performing transects, 45° angled photographs while the ROV is grounded on the sea floor should be taken of the seabed at 1 – 3 min intervals. This means high resolution photographs can be used instead of using stills extracted from video which has reduced quality. These oblique photographs may also allow for the comparison between ROV images and the vast tow-camera image repository. iii) In the absence of a GPS transponder (as in the case of this study), time and date, depth and other metadata should be displayed on all images and video. This will streamline the unnecessarily time consuming image geo-referencing process. Separate, purely exploratory surveys can be conducted without the metadata displayed on the imagery. These three recommendations should be non-negotiable.

Optional recommendations include the addition of a multibeam echosounder mounted on the ROV. Although costly, it will allow the integration of rugosity, slope and aspect at a site scale. Additional cameras to allow for stereo imagery or downward facing imagery and instruments to measure parameters such as bottom dissolved oxygen, carbon dioxide, pH, light and nutrients (organic carbon, calcium carbonate etc.). The final recommendation is the investment into capacity building for machine learning and artificial intelligence for semi-autonomous or automated video and image annotation. In the long term this will be an invaluable investment which will unlock vast quantities of

data currently sitting in image form and may allow more time to think about and answer novel ecological questions about South Africa's marine ecosystems instead of manually annotating the growing backlog underwater imagery.

This study highlighted limited taxonomic information and a ROV sampling bias as two key knowledge gaps. Foundational taxonomic investigations are needed to address the general lack of species level knowledge in this region. More remotely operated vehicle surveys should be performed on low relief and unconsolidated substratum types as an overwhelming bias exists towards higher relief reef substratum (Heyns et al. 2016, Makwela et al. 2016, Makwela 2017, Heyns-Veale et al. 2019). Although towed camera systems are generally and appropriately used to survey low relief unconsolidated habitats, I encourage the use of ROV surveys of unconsolidated seabeds adjacent to reefs. This produces a better representation of the seabed. Many, if not all future ROV surveys in South Africa will be exploratory and surveys will undoubtedly be multidisciplinary and aim to accomplish several research objectives simultaneously. Future surveys will uncover novel species, many of which could be endemic; this will encourage future research and the conservation of these unexplored and potentially fragile offshore ecosystems.

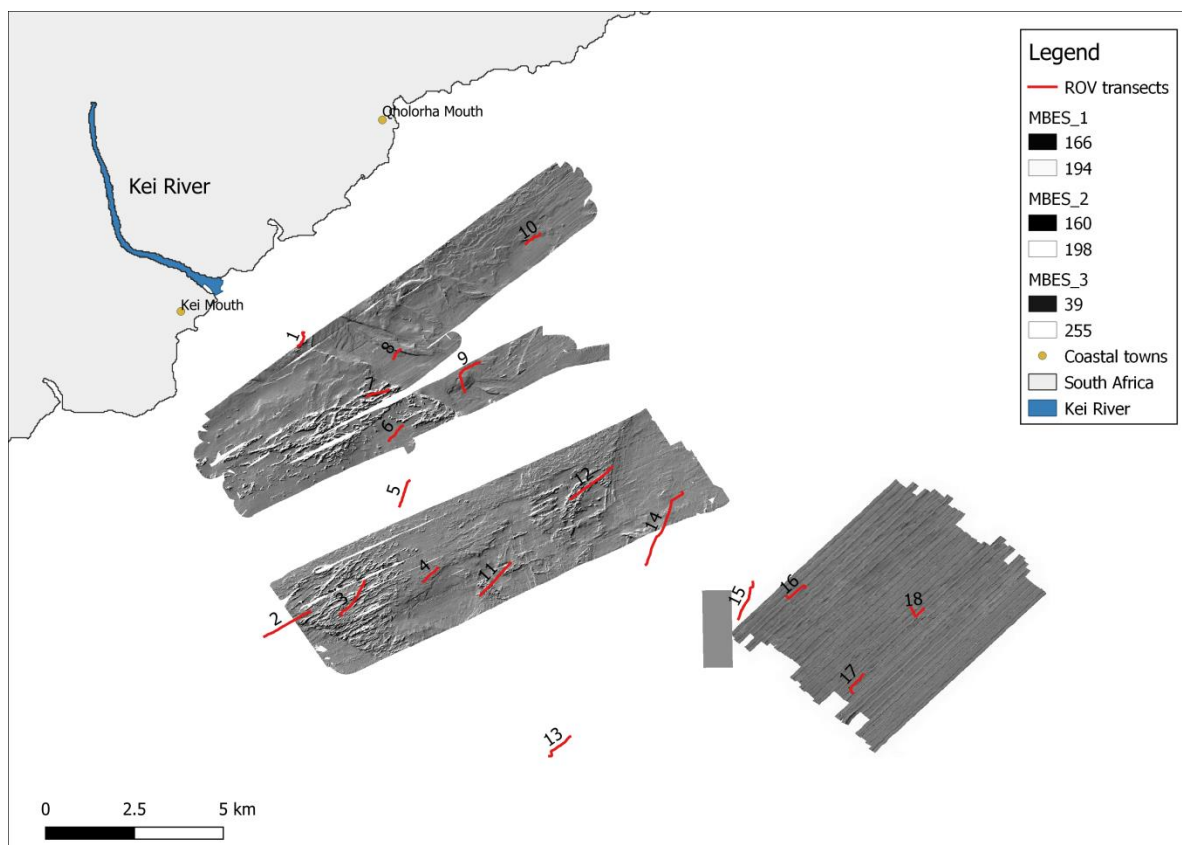
Recently, 24 questions paramount to the advancement of Mesophotic Ecosystem (ME) research worldwide were proposed (Turner et al. 2019). Although the inception of this thesis was conceived prior to the publication of Turner et al. (2019), it has addressed some of these questions proposed here and has advanced mesophotic ecosystem research in South Africa. The overall aim of this thesis has addressed multiple questions across several themes. Chapter 2 addressed question 1 "How are ME distributed globally?" in Theme 1 (Distribution). Chapter 3 addressed question 3 "What are the biological, (specifically) environmental, and geological factors controlling ME distribution, accretion, and function across space and time?" in Theme 2 (Environmental and Physical Processes) and question 7 "What are the variables that influence community structure and biodiversity across depths and regions?" and question 8 "How does biodiversity (taxonomic and genetic) change across depths and regions?" in Theme 3 (Biodiversity and Community Structure). Through answering aspects of these questions, this thesis has added to the knowledge base of South African and global ME research. The recent work by Samaai et al. (2020) has also highlighted knowledge gaps pertaining to animal forests, a major component of mesophotic ecosystems, will help identify priority areas research within this depth zone. It also highlights the research efforts from the last decade in South Africa

4.5 Conclusion

This thesis has provided baseline information of the macrobenthic communities and the environmental variables that structure them on a temperate river-influenced shelf on the Eastern Cape Coast. I found that the use of morphospecies, instead of taxonomic species, adequately discriminated macrobenthic communities from ROV imagery. Depth, substratum and distance to the river mouth were identified as the most important environmental variables driving community pattern in the mesophotic zone. This thesis also highlights the discovery of rhodolith aggregation; a novel ecosystem for benthic ecologists in South Africa. Other notable aggregations include echinoderms, particularly brittle stars and feather stars. In addition, this thesis produced the first conceptual framework of a river-influenced mesophotic ecosystem in South Africa. This framework and the statistical approaches used demonstrate the use of underwater imagery to extract foundational biodiversity and ecological information in data poor, remote and unexplored areas.

Appendices

Appendix 1 Map of multibeam echosounder bathymetry on the shelf off the Kei River mouth. Red lines depict ROV transects



Appendix 2 List of morphospecies identified on the temperate shelf off the Kei River, South Africa

CATAMI DISPLAY NAME	CPC CODES
Ascidians: Unstalked: Colonial	AUC
Ascidians: Unstalked: Solitary	AUS
Bacterial mats: Other	BMO
Bryozoa: Hard	BRYH
Bryozoa: Hard: Branching	BRYHB
Cnidaria: Corals	C
Cnidaria: Corals: Black & Octocorals	CB
Cnidaria: Corals: Black & Octocorals: Branching (3D): Fleshy: Arborescent	CBBFA

Cnidaria: Corals: Black & Octocorals: Branching (3D): Fleshy: Mushroom	CBBFM
Cnidaria: Corals: Black & Octocorals: Fan (2D)	CBF
Cnidaria: Corals: Black & Octocorals: Fan (2D): Fern-frond	CBFF
Cnidaria: Corals: Black & Octocorals: Fan (2D): Fern-frond: Complex	CBFFC
Cnidaria: Corals: Black & Octocorals: Fan (2D): Rigid	CBFR
Cnidaria: Corals: Black & Octocorals: Whip	CBW
Cnidaria: Corals: Stony corals: Bottlebrush	CSBO
Cnidaria: Corals: Stony corals: Solitary: Attached	CSSOA
Cnidaria: Hydrocorals: Branching	CNHYCB
Cnidaria: Hydroids	CNHYD
Crustacea: Prawns / shrimps / mysids	CRP
Echinoderms: Feather stars	EF
Echinoderms: Ophiuroids: Basket stars	EOBS
Echinoderms: Ophiuroids: Brittle / snake stars	EOBSS
Echinoderms: Sea stars	ESS
Macroalgae: Articulated calcareous	MAA
Macroalgae: Articulated calcareous: Red	MAAR
Macroalgae: Encrusting: Green	MAENG
Macroalgae: Encrusting: Red	MAENR
Macroalgae: Encrusting: Red: Calcareous	MAENRC
Macroalgae: Encrusting: Red: Non-calcareous	MEANRNC
Macroalgae: Erect coarse branching	MAEC
Macroalgae: Erect coarse branching: Red	MAECR
Macroalgae: Erect fine branching	MAEF
Macroalgae: Erect fine branching: Red	MAEFR
Macroalgae: Filamentous / filiform	MAF
Macroalgae: Filamentous / filiform: Red	MAFR
Macroalgae: Laminate: Red	MALAR
Macroalgae: Sheet-like / membraneous: Red	MASR
Molluscs: Bivalves	MOB
Molluscs: Gastropods	MOG
Sponges: Crusts: Encrusting	SPCE
Sponges: Cup-likes	SPCL

Sponges: Cup-likes: Cups	SPCLC
Sponges: Cup-likes: Cups: Incomplete cup / curled fan	SPCLCI
Sponges: Cup-likes: Cups: Tables / discs	SPCLCT
Sponges: Cup-likes: Tubes and chimneys	SPCLTC
Sponges: Erect forms	SPE
Sponges: Erect forms: Branching	SPEB
Sponges: Erect forms: Laminar	SPEL
Sponges: Erect forms: Palmate	SPEP
Sponges: Erect forms: Simple	SPES
Sponges: Massive forms	SPM
Sponges: Massive forms: Balls	SPMR
Sponges: Massive forms: Cryptic	SPMC
Worms: Polychaetes: Tube worms	WPOT

Appendix 3 Image of dead rhodoliths on coarse sediment and covered by a dense brittle stars aggregations at 58 m depth off the Kei River mouth



Appendix 4 Pearson's correlation worksheet of 10 environmental variables collected off the Kei River mouth between January and May 2017

	Depth	Temp'	Salinity	Rugosity Index	Latitude	Relief	Aspect	Substratum	Dist' to Shore	Dist' to mouth
Temperature	-0.31									
Salinity	0.24	0.50								
Rugosity Index	-0.19	-0.17	0.03							
Latitude	-0.60	0.05	-0.46	-0.05						
Relief	-0.36	0.27	0.20	0.47	-0.05					
Aspect	0.32	0.27	0.72	-0.10	-0.46	-0.08				
Substratum	-0.15	0.15	0.12	0.17	-0.20	0.54	-0.22			
Distance to Shore	0.90	-0.44	0.17	-0.11	-0.70	-0.22	0.25	0.03		
Distance to mouth	0.92	-0.39	0.19	-0.10	-0.73	-0.20	0.26	0.02	0.99	
Distance to shelf break	-0.89	0.39	-0.23	0.10	0.76	0.19	-0.30	-0.06	-1.00	-0.99

Appendix 5 SIMPROF test results for the LINKTREE analysis of communities off the Kei River mouth (R-statistic) and the average between-group rank dissimilarities from the original morphospecies resemblance matrix

Break	R-statistic	B%
A	0.63	87
B	0.84	95
C	0.45	53
D	0.37	51
E	1	20
F	0.44	38
G	0.37	34
H	0.3	29
I	1	127
J	0.88	64

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