COMMUNITY STRUCTURE AND TROPHIC ECOLOGY OF SHALLOW AND DEEP ROCKY REEFS IN A WELL-ESTABLISHED MARINE PROTECTED AREA

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ABSTRACT

The now formally adopted ecosystem approach to fisheries (EAF) considers not only commercially important species, but the entire ecosystem and the processes that support these species. A key component of EAF management is the implementation of no-take Marine Protected Areas (MPAs). Shallow water fish stocks are depleted and fishing effort is moving deeper and further offshore to keep up with demands. This situation calls for a detailed investigation of deep nearshore reefs to provide critical information relevant to policy uptake and management decisions regarding existing and new MPAs in terms of zonation and use. To address this need, the aim of this thesis was to investigate reefs that lie between 45 and 75 m and compare them in terms of community structure and function to the relatively well-studied shallow reefs that lie within SCUBA diving depth (<25 m). Ecological collections were made in the centre of a large and well-established MPA, Tsitsikamma National Park, to ensure that data represented non-anthropogenically impacted communities. Data were collected from two study sites; Rheeders Reef, (shallow reef) and Middlebank, a deep reef complex situated near the Storms River Mouth. The first step to address the aim of this study was to obtain baseline data on the distribution patterns of both the macrobenthic invertebrates and fish assemblages. Baseline data were obtained by underwater video methods and included the use of a remotely operated vehicle, baited remote underwater stereo-video systems (stereo-BRUVs) and traditional underwater camera equipment operated by SCUBA divers. To establish functional differences between the two study sites, fatty acid (FA) and stable isotope (SI) analyses were employed. These biomarker techniques provided insight into the importance of different sources of primary production, nutritional condition and species packing.

From 360 photoquadrats examined for macrobenthic invertebrate distribution patterns, 161 invertebrates were identified that demonstrated a clear changeover of species along the depth gradient. Species richness was highest on the shallow reef and decreased with an increase in depth. To understand how the measured environmental variables impacted the macrobenthic assemblage data a LINKTREE analysis was performed. LINKTREEs produce hierarchical cluster analysis based on the macrobenthic assemblage data and provide a threshold of environmental variables that correspond to each cluster. The outcome of the LINKTREE analysis indicated that the changeover of species resulted in four distinct clusters, each cluster associated with a particular set of environmental variables that fell within a depth range. On the shallowest sites, the high energy environment resulting from wave action and surge prevented the settlement of suspended particles. The high energy environment of the shallow reef selected for low-growing encrusting species. High

light intensities supported great abundances of benthic algae, and as light was lost with increasing depth, algal cover gradually diminished until it was completely absent on the deep reef. The reduced impact of surface wave action on the deep reef caused increased levels of settled suspended particles. The high levels of settled particles likely caused clogging of feeding parts of the encrusting species. Consequently, upright growth forms were more common in the lower energy environment of the deep reef.

A total of 48 fish species were identified from 51 stereo-BRUVs samples. Fish assemblages differed significantly between the shallow and deep reefs. The shallowest sites were characterised by many small and juvenile fish species that fed at lower trophic levels. The deep reef supported the majority of the large predatory fish that fed at higher trophic levels. Many species demonstrated depth-related ontogenetic shifts in habitat use, and as such the deep reef hosted the majority of the sexually mature individuals. The fish assemblages also demonstrated a strong association with the macrobenthic clusters identified as habitat types by the LINKTREE analysis.

The results from 201 FA and 191 SI samples provided information on specific feeding interactions, but more importantly shed some light on different processes that supported the shallow and deep reef communities. The shallow reef community was characterised by greater diversity of food sources, a pattern that could be explained by the presence of benthic algae and terrestrial inputs. Greater diversity of carbon sources at the bottom of the food web meant that a larger variety of species could be supported. Higher species richness increased the number of distinct taxa that performed similar functions, rendering the shallow reef more redundant and consequently more resilient to disturbance. In contrast, the deep reef demonstrated a food web supported mainly by pelagic production, which was more variable both over space and time. The deep reef was less redundant when compared to the shallow reef, as fewer species demonstrated similar trophic niches. These factors, in addition to the increased presence of sensitive calcareous macrobenthic species on the deep study site, rendered the deep reef more vulnerable to disturbance when compared to the shallow reef. Although the data presented here were from a single study area, the limitations typically associated with these inaccessible and challenging sampling environments made the dataset a significant contribution to the knowledge of reef ecosystems. The study addressed priority research questions for South Africa as identified during the National Biodiversity Assessment. The observable differences in structure, function and vulnerability point to the need for continued protection of our shallow reefs and offshore expansion of our MPA networks. Future research should determine if the patterns identified here are common throughout the Agulhas Ecoregion to provide managers with robust evidence for the extension our MPAs offshore.

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Typical reef community in Tsitsikamma

DECLARATIONS

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless otherwise stated.

1 GENERAL INTRODUCTION

1.1 PROBLEM IDENTIFICATION

Globally, of all the services and functions that ecological systems provide to human welfare (ecosystem services), 63% comes from the marine realm (Costanza et al. 1997). In South Africa, coastal ecosystems provide significantly to its Gross Domestic Product, which includes roughly R2.5 billion from our fisheries alone (Sink et al. 2012). However, the growing human population places increasing pressure on the goods and services that marine ecosystems provide. Consequently, coastal environments, and specifically subtidal rocky reefs, have been identified as one of the most impacted ecosystems in the world. This mounting pressure has resulted in the collapse of several fish stocks and loss of biodiversity through exploitation, habitat destruction and pollution (Worm et al. 2006). Globally, 80% of fish stocks are either fully exploited, overexploited or have collapsed (Mora et al. 2009). Within South Africa, 61% of marine resources are overexploited and several marine species are threatened (Sink et al. 2012).

Conventional fisheries management strategies are based on single- and multi-species stock assessments which are implemented by controlling the gear, catch or size limits (Claudet et al. 2006). However, there are several weaknesses associated with these practices that are related to unsuccessful implementation, underestimation of the severity of fish stock decline, creation of models built on deficient data, and unexpected cascading effects caused by the removal of large predatory fish (Pauly et al. 2002). More recently, fisheries management strategies have begun to incorporate the value of biodiversity in supporting marine resources. Increasingly, biodiversity has been shown to play a key role in supporting and maintaining ecosystem resilience (Micheli & Halpern 2005). Ecosystem resilience is a community's ability to resist, reverse or recover from disturbance, which indirectly ensures sustainable access to ecosystem services (Worm et al. 2006, 2009, Sink et al. 2012). As such, management now considers not only important target species, but the entire ecosystem and processes that support them. This concept is termed the 'ecosystem approach to fisheries' (EAF; Garcia et al. 2003). No-take MPAs are important tools in EAF management. All harvesting is prohibited within no-take MPAs, allowing recovery of depleted stocks and increasing

fisheries yields outside borders through spill-over of adult fish and larval export through dispersal (Kerwath et al. 2013).

1.2 REEF COMMUNITIES

In the marine component of the National Biodiversity Assessment for South Africa (Sink et al. 2012) the expansion and strengthening of MPA networks was identified as a priority action. The expansion of South Africa's MPA network is in line with global efforts and supported by commitments to several international conventions and agreements (Sowman et al. 2011). To extend our MPA network in a systematic and efficient manner, detailed species inventories and species distribution patterns are needed to help identify critical habitats that require protection. The fauna and flora of the subtidal communities along the South African coastline have been relatively well documented (Griffiths et al. 2010). This is especially true for the fish, but also for a number of invertebrate and algae taxa (Turpie et al. 2000, Awad et al. 2002, Griffiths et al. 2010). Nonetheless, substantial gaps in our knowledge about subtidal reefs exist (Karpov et al. 2006, Sink et al. 2006, Love & Schroeder 2007). The lack of knowledge about our subtidal reefs is due to the difficulty in obtaining data from hard-substrate habitats. Destructive sampling methods such as dredging and trawling, employed to analyse and describe soft bottom habitats, are unsuitable for sampling rocky reef habitats (Love et al. 2009). Methods such as controlled angling suffer from numerous biases, as they actively select for scavenger and aggressive predatory species and are size selective (Götz et al. 2009, Bennett et al. 2009). Additionally, controlled angling provides no information on the relationship between the benthos and its associated fish assemblages. Consequently, most of the knowledge on subtidal reefs is obtained by SCUBA diving surveys, which are limited by depth (<30 m) and time (decompression limits). Deep reefs have been identified as some of the most poorly described ecosystems in the world (Heemstra et al. 2006, Sink et al. 2006, Love & Schroeder 2007). This situation is of concern, as subtidal rocky substrate habitats deeper than 30 m support unique benthic communities and the bulk of the commercially important reef fish (Sink et al. 2006). While little is known about deep reefs, research on deep reefs does occur, usually by means of remotely operated vehicles (ROVs) or manned submersibles. However, such studies mainly focus on reefs deeper that 150 m (Stein et al. 2005, Sink et al. 2006, Colaço et al. 2013). This practice has resulted in considerable gaps in our understanding of the structure and function of intermediate (30 – 150 m) reef communities (Sink et al. 2006), which usually lie closer to the shore.

With the continued depletion of our shallow water fish stocks, fisheries effort is increasingly moving deeper and further offshore (Morato et al. 2006, Watson & Morato 2013). Due to the difficulty of

sampling this realm, the importance of deep nearshore reefs for both conservation of biodiversity and resource management has rarely been assessed in detail. To date, only one study conducted on subtropical reefs off the east coast of South Africa has addressed this lack of information regarding the nearshore deep reef communities (Sink et al. 2006). The study by Sink et al. (2006) provided important descriptive information about the community structure, but they were not able to relate the species distribution data to the local environmental conditions. It remains important to establish baselines of entire reef communities (macrobenthos and fish) and determine if the processes that support the functioning of relatively well-studied shallow reefs differ from those of deep nearshore reefs. The knowledge obtained from this research provide critical information that will guide biodiversity and fisheries management strategies, including MPA establishment, zonation and monitoring.

1.3 AIMS

In view of the current state of our marine resources, especially the lack of baseline information on the sub-tidal rocky reef community below 30 m, a comprehensive and holistic approach towards understanding the ecological functioning of this realm is clearly necessary. I utilised a combination of methods including underwater video and biomarker techniques to determine differences in species distribution patterns and trophic structure and function of shallow (12 – 25 m) and deep (45 – 75 m) reefs situated in a well-established no-take MPA. The use of several complementary state-of-the-art techniques to investigate ecosystem functioning of shallow, and, for the first time in South Africa, deep reefs will critically improve our understanding of how conservation and resources should be managed holistically and across all photic depth ranges.

1.4 APPROACH

With advances in technology, many of the obstacles associated with subtidal reef research in the past have become surmountable. The application of underwater video techniques such as baited remote underwater stereo-video systems (stereo-BRUVs) and ROVs now allows for quantitative surveys of reef fish and their habitats that are non-destructive and are not limited to SCUBA diving depths. Stereo-BRUVs provides a standardised, cost-effective, comprehensive and precise method to estimate fish abundance and length data (Harvey et al. 2002, Langlois et al. 2010, Bernard & Götz 2012). The stereo configuration (application of two cameras simultaneously) allows for very precise length measurements (Watson et al. 2005, Harvey et al. 2007, Langlois et al. 2010). Although no

method is without bias, the use of stereo-BRUVs are considered superior to more traditional methods such as controlled angling, underwater video census, trapping and research trawling. Stereo-BRUVs outperform these traditional methods due to low levels of data variability, high abundances of commercially and recreationally targeted species, high measures of species richness, and accurate population size structure information (Watson et al. 2005, 2010, Langlois et al. 2010, Langlois, Harvey, et al. 2012, Harvey et al. 2012). Furthermore, the application of stereo vision allows for the distance from the camera to the fish to be measured, thereby standardising the sampling area (Harvey et al. 2004). Nonetheless, irrespective of any bias that might be associated with stereo-BRUVs, reef fish communities surveyed during this study employed stereo-BRUVs over the entire depth range sampled. Thus, any bias associated with these systems would apply equally to all samples.

Similarly, conventional methods to study food web interactions and trophic structure are conducted mostly through stomach content analyses or examination of faecal pellets. Both methods are prone to biases associated with an underestimation of easily digestible dietary items, and often an over estimation of recently consumed foods, with both methods providing only snapshots of consumer diets (Kelly & Scheibling 2012). Besides the biases associated with conventional methods to study trophic ecology, these approaches are even more impractical for deep reef research. It becomes progressively more difficult to obtain stomach contents for specimens collected from deep habitats, as rapid changes in pressure result in animals expelling food when brought up from depth (Dodds et al. 2009). Thus, the use of indirect methods such as biomarker techniques (fatty acids and stable isotopes) is more suitable for this study, as these approaches provide time-integrated views of consumer diets and they are not affected by loss of samples due to expulsion of food.

As most coastal ecosystems are exposed to some degree of anthropogenic disturbance, a sliding and continually reduced expectation of how normal ecosystems should function exists (shifting baseline; Dayton *et al*. 1998). Through the protection of species within its boundaries, MPAs allow exploited populations to recover and restore the ecological integrity of a system. Thus, ecological benchmarks or baseline data collected from large, well established no-take MPAs would better reflect natural or pristine conditions (Shears & Babcock 2002) and improve the understanding and knowledge on which management policies are based. Consequently, considering that the collection of baseline data was one of the aims of this study, research was conducted in a well-established MPA.

1.5 THESIS OVERVIEW

To achieve the aims set out for this study, the first step was to identify a shallow and a deep reef within the borders, but preferably in the centre, of a large and well-established MPA. Chapter 2 introduces the study region and general methodology. Chapter 2 includes information on the marine ecoregions of South Africa, the hydrography and climatic conditions during the time of study, and a description of the basic strategy behind the selection of the study area and sampling approach.

To describe the community composition of the macrobenthos and fish assemblages, stereo-BRUVs, ROV and SCUBA diving were chosen as survey methods (Chapters 3 and 4). In Chapter 3, the distributional patterns of the macrobenthic community were assessed. Firstly, species richness and community composition were determined, and feeding guilds and indicator species were identified and compared between the reefs. Secondly, cluster analysis was conducted to determine if the macrobenthic species separated according to a depth gradient. Lastly, the environmental processes that structure the macrobenthos were considered.

In Chapter 4, the distributional patterns of the fish community were assessed. More specifically, habitats characterised by the macrobenthos in Chapter 3 were used to predict distributional patterns of fish species. Furthermore, depth related ontogenetic shifts of fishes were considered.

To determine if trophic structure and food web dynamics differed between the shallow and the deep reefs, fatty acid and stable isotope biomarker techniques were employed (Chapters 5 and 6). In Chapter 5, the trophodynamics of the shallow and deep reefs were assessed. Specifically, differences in sources of essential fatty acids were compared between the reefs. Furthermore, the processes that support the shallow and deep reef suspension-feeders were investigated by examining the contributions of terrestrial input and the importance of bacterial degradation of plankton during transit to depth.

Chapter 6 focuses on the broader trophic organisation of the reefs. Trophic levels, trophic diversity and trophic redundancy were compared between the reefs using community-based metrics and calculations of food chain length, average diversity and carbon source diversity.

Finally, Chapter 7 contains a synthesis of the core study findings, a critical evaluation of the research conducted, and suggestions for improvements and their implications for management.

2 STUDY REGION & GENERAL METHODOLOGY

2.1 STUDY AREA

2.1.1 SELECTION OF A SUITABLE STUDY AREA

The position of South Africa on the tip of the African continent has a major influence on the biodiversity of marine and terrestrial life (Gibbons 1999, Gibbons et al. 2010). In fact, South Africa's terrestrial realm has been recognised as the third most diverse in the world (Griffiths et al. 2010). In a recent review of the Census of Marine Life, South Africa ranked third in terms of number of marine species per unit area (Costello et al. 2010). The high biodiversity in South Africa's marine realm can be attributed to the presence of two very distinct ocean currents that run along the coast (Brown & Jarman 1978, Gibbons et al. 2010). The warm south-flowing Agulhas Current, a typical western boundary current, is deep, fast and narrow, and is characterised by low productivity and biomass, but high diversity (Lutjeharms 2006, Gibbons et al. 2010). The cold eastern-boundary Benguela Current is broad, slow and transports cold water northwards (Griffiths et al. 2010, Gibbons et al. 2010). The Benguela Current is characterised by seasonal wind-driven coastal upwelling resulting in low diversity, but high biomass and productivity (Gibbons et al. 2010). The prevalence of these two markedly different ocean currents results in a highly complex hydrographical environment (Bustamante & Branch 1996). As a consequence, the coast of South Africa is a transitional zone between the Indo-Pacific and Atlantic biomes, and is characterised by the presence of organisms representing both biomes and a high number of endemics (Teske et al. 2011).

South Africa's marine ecoregions are defined according to the combined effects of temperature, geology and biological interactions on species range and distribution (Figure 2.1; Turpie et al., 2000). Sea temperature is considered the most influential parameter for the broad-scale distributional patterns of both fish and invertebrate species (Brown & Jarman 1978, Turpie et al. 2000, Awad et al. 2002). The influence of sea temperature can be explained by the loss of tropical species as a result of their intolerance to rapid changes in oceanographic conditions (Turpie et al. 2000). Consequently, the South African coast is characterised by a progressive loss of species from the north-eastern

Mozambican border through the Delagoa and Subtropical Ecoregions to the westerly edge of the Agulhas Ecoregion near Cape Town (Gibbons 1999, Turpie et al. 2000, Awad et al. 2002, Griffiths et al. 2010). The entire South African West Coast demonstrates low species richness compared to the East Coast (Turpie et al. 2000, Gibbons et al. 2010). This general pattern holds true for fish and coral species. Concerning other invertebrate groups, only five out of 11 taxa follow this pattern. Most groups (octocorals, chitons, polycheates, amphipods, isopods and ascidians) demonstrate peaks in species richness on the South Coast (Agulhas Ecoregion; Turpie et al. 2000; Awad et al. 2002). Furthermore, with the highest number of endemic fish and invertebrate species, the Agulhas Ecoregion is the centre of endemism in South Africa (Turpie et al. 2000, Awad et al. 2002, Griffiths et al. 2010). Endemism peaks in the region of Port Elizabeth, coinciding with increasing distances from our political borders (Fig. 2.1; Turpie et al. 2000, Awad et al. 2002).

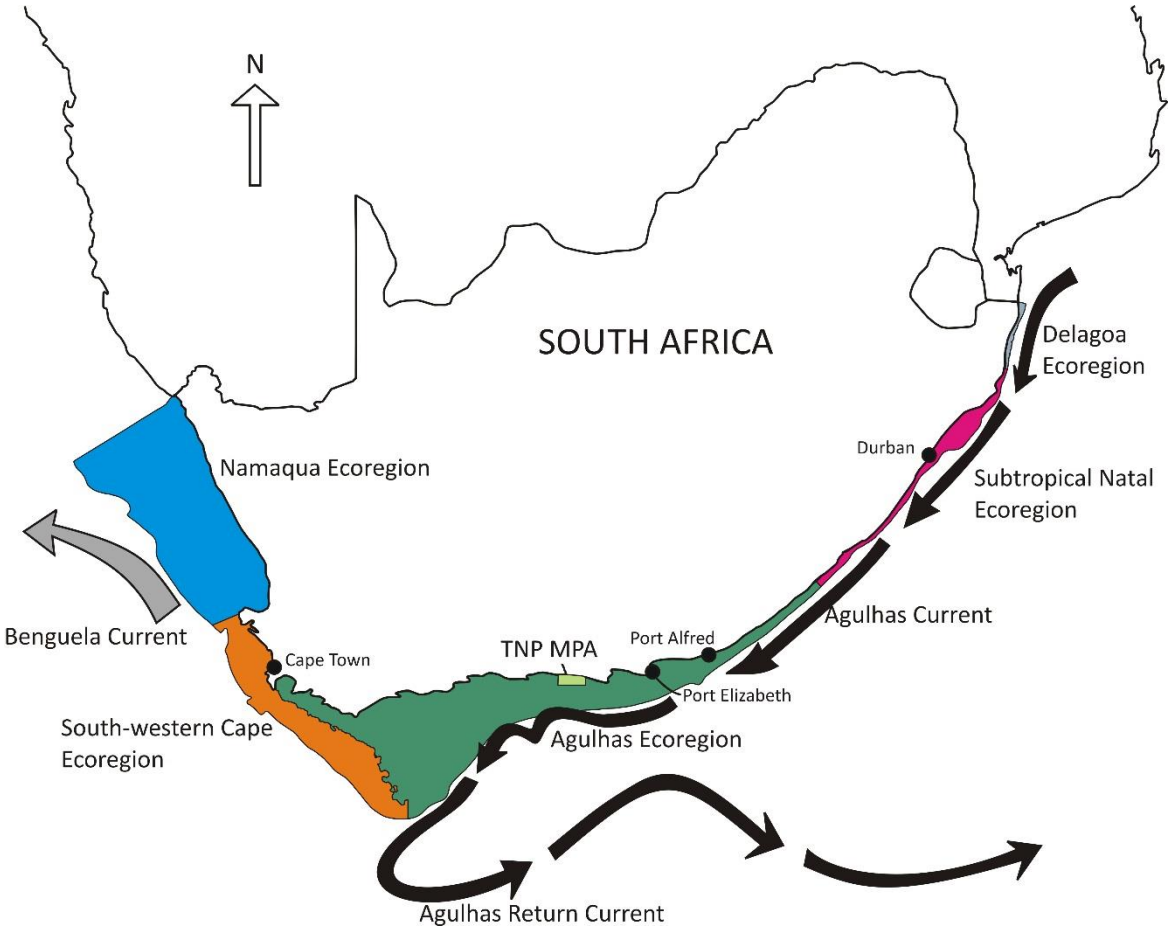


Figure 2.1. South Africa's five marine ecoregions. The position of the Tsitsikamma National Park Marine Protected Area situated in the centre of the Agulhas Ecoregion. Map modified from GIS maps provided by the South African National Biodiversity Institute. Black arrows represent the Agulhas Current and the grey arrow represent the Benguela Current [modified from Lutjeharms (2006)].

Although it is difficult to get a true representation of communities in pristine condition, the best alternative is to obtain information from communities that benefitted from protection, such as no-take marine protected areas (MPAs). Besides fisheries and conservation benefits associated with MPAs, there are several other benefits associated with complete protection from exploitation. One that is of concern here is the invaluable knowledge gained from an environment that is functioning in a pristine or near pristine state. Information gained from such reference sites can represent a baseline, giving scientists and managers a benchmark of ecosystem health and functioning for comparative studies.

The Tsitsikamma National Park (TNP) MPA is one of Africa's oldest (established in 1964) and largest no-take MPAs (Tilney et al. 1996), covering approximately 360 km^2 (Hanekom et al. 2012). It is situated almost exactly in the centre of the Agulhas Ecoregion (Figure 2.1). Due to the MPA's long history of protection, the fish stocks have largely recovered from previous exploitation (Buxton & Smale 1986, Cowley et al. 2002) and so TNP MPA is considered one of the best examples of a pre-exploited inshore temperate reef ecosystem (Bernard & Götz 2012). Consequently, the TNP MPA was selected as a suitable study area. The TNP MPA provides an opportunity to study rich and unexploited shallow and deep reefs, and due to its position (in the middle of the Agulhas Ecoregion), the data obtained from this study are most representative for this Ecoregion.

2.1.2 DESCRIPTION OF THE STUDY AREA

The TNP MPA straddles the Eastern and Western Cape Provinces of South Africa (Figure 2.2 A) and protects a 60km stretch of coastline between the East and West Groot Rivers (Hanekom et al. 2012). The MPA extends three nautical miles offshore to a depth of approximately 100m (Tilney et al. 1996). A short section between Groot River (west) and the Bloukrans River (east) at the western end of the MPA extends only 0.5 nautical miles offshore (Figure 2.2 B; Hanekom et al. 2012).

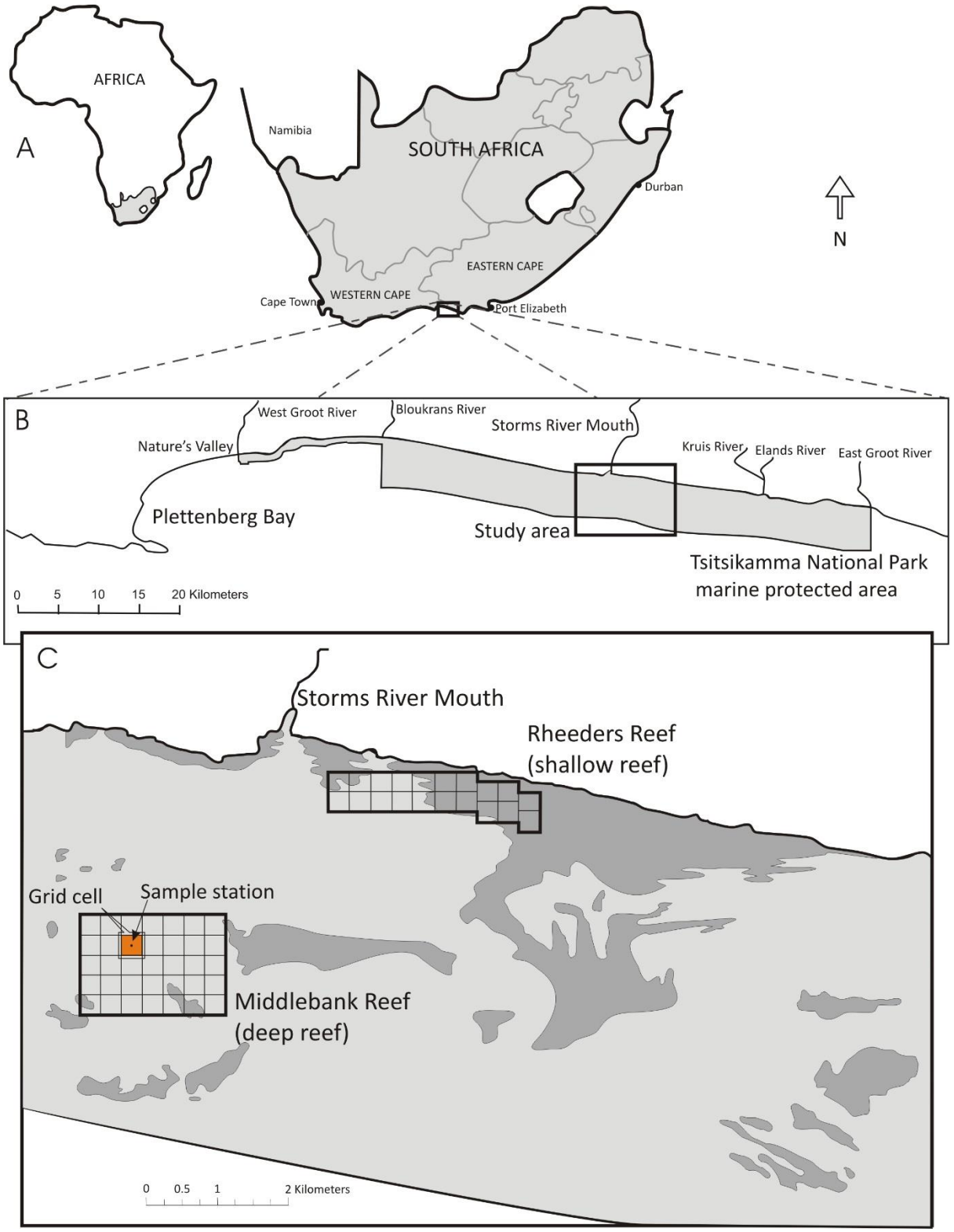


Figure 2.2. Location of the study area. Map of South Africa (A) indicating the position of the Tsitsikamma National Park Marine Protected Area, (B) the study area (Tsitsikamma), (C) the two study sites and a weather station situated in the middle of the park. The shallow reef is called Rheeders, and the deep reef is called Middlebank, and sample stations are the midpoints in the grid cells. The launch site and weather station are situated near the Storms River Mouth (dot). The two sections framed in the insert were mapped bathymetrically in more detail, see Figure 2.5. Original side-scan sonar data (dark grey) obtained by Flemming et al. 1983 (see Buxton, 1987).

The Tsitsikamma coast forms an escarpment that rises sharply to about 180 m above sea level (Toerien 1976), creating the sheer cliffs characteristic of this shoreline. The coastline consists of a number of headlands and associated bays (Bennett et al. 2009). Apart from the sandy beach at Nature's Valley, the entire shoreline consists of rocky cliffs exposed to strong wave action (Cowley et al. 2002, Hanekom 2011). These rocky ridges comprises steeply dipping quartzitic sandstone beds which lies parallel to the coastline along an east-west axis (Buxton 1987, Hanekom 2011). Subtidally, these beds form a series of parallel reef ridges separated by valleys filled with fine-grained sand (Buxton & Smale 1984).

2.1.2.1 COASTAL HYDROGRAPHY

The TNP MPA is situated on the eastern Agulhas Bank, a triangular shaped continental shelf, south of South Africa. The Agulhas Bank is approximately 800 km long and extends 250 km offshore at its apex (Hutchings 1994). The shelf drops steeply at the coast to a depth of 50 m, and then gradually increases to about 200 m towards the shelf edge (Hutchings 1994). The Agulhas Current, which closely follows the break of the shelf, plays a notable role in the oceanography and ocean circulation on the bank.

Once the Agulhas Current is formed, somewhere north of Durban, it flows closely along the steep and narrow continental shelf and demonstrates very little variation (Lutjeharms 2006; Figure 2.1). In the vicinity of Port Alfred, the shelf starts widening and the current's characteristics change dramatically (Figure 2.1). Here, the Agulhas Current causes kinematically driven shelf edge upwelling (Hutchings 1994, Lutjeharms 2006) which lifts cold, nutrient rich water onto the shelf, inducing a semi-permanent upwelling cell (Port Alfred Upwelling Cell; Hutchings 1994; Lutjeharms 2006). The upwelled water slowly moves westwards along the 100 m-isobath, spreading over the shelf (Lutjeharms 2006). At the same time, the warmer surface waters are continually fed by the warm Agulhas Current, resulting in a well-established thermocline that is most pronounced during the summer months (Schumann 1999).

Along the South Coast of South Africa, wind-driven upwelling cells originate at the prominent capes and headlands, then move offshore in a westward direction (Schumann et al. 1982, Schumann 1999, Lutjeharms 2006). This type of upwelling is caused by easterly winds, characteristic of the summer months in the region (Schumann et al. 1982, Tilney et al. 1996). Easterly winds cause deflection of surface water offshore due to the Coriolis effect and the resulting Ekman transport. Cold, dense nutrient rich bottom water moves upward to replace the water lost by Ekman transport (Schumann et al. 1982). Upwelling at Tsitsikamma is well documented (Schumann 1999, Hanekom et al. 2012)

and has marked effects on the mean sea temperature in the region. Besides the obvious effect of upwelling on sea temperatures, the seasonal stratification of the water column along with upwelling events are the driving forces of primary productivity in the region (Schumann et al. 1982) .

A well-established alongshore current known as the Tsitsikamma Current (Hancke 2010) has been the subject of several studies (Attwood et al. 2002, Roberts & van den Berg 2005, Roberts 2005). At the surface, the current flows strongest and can reach a maximum velocity of 115 $cm.s^{-1}$, but decreases with depth to 65 $cm.s^{-1}$ near the bottom at about 30 m (Roberts & van den Berg 2005). The surface current flows predominantly eastward (Roberts & van den Berg 2005). During well-established thermoclines in summer, surface and bottom currents can flow in opposite directions (Roberts & van den Berg 2005). Several factors influence the direction of both the surface and bottom currents including wind direction, coastal trapped waves, thermal stratification and frontal jets caused by upwelling (Tilney et al. 1996, Schumann 1999, Roberts & van den Berg 2005).

2.1.2.2 CLIMATIC CONDITIONS

Weather data for the study area was provided by the South African Weather Service from a weather station at Storms River Mouth. River flow data of three rivers in the TNP, the Elands, Bloukrans and Kruis (Figure 2.2 B), were obtained from the Department of Water Affairs.

A. WIND

Wind data for the period of this study indicated a clear north-easterly / south-westerly prevalence (Figure 2.3). This onshore-offshore component is due to the channelling of the land-sea breeze through the Storms River Gorge (Hancke 2010). Summer and winter months both had a high frequency of west-south-westerly winds with 31% and 20%, respectively (Figure 2.3 C, D). In summer, easterly winds were more prevalent (20% in summer compared to 5% in winter), a component responsible for upwelling in the region (Schumann 1999).

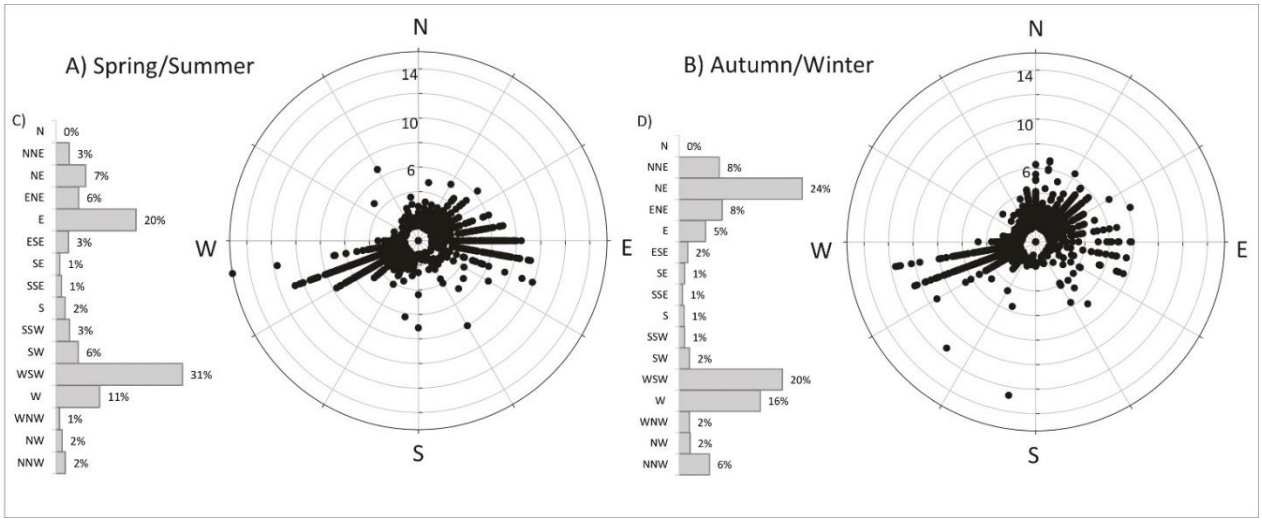


Figure 2.3. Wind speed and direction at the study area. Wind speed ($m.s^{-1}$) and direction during spring/summer (A) and autumn/winter (B) taken at 8:00, 14:00 and 20:00 from July 2010 to September 2012 at the Tsitsikamma weather station (7m above sea level), situated near the Storms River Mouth. Each point indicates a specific measurement taken at the different times. Bar graphs demonstrate the percentage contribution of the different wind directions in spring/summer (C) and autumn/winter (D). Data were provided by the South African Weather Service.

B. FRESHWATER RUN-OFF

July 2012 was marked with particularly heavy rainfall and associated increase in freshwater run-off (Figure 2.4). Collections of plankton samples intended for stable isotope and fatty acid analysis were done at times indicated by red arrows (Figure 2.4). Plankton samples were collected during normal run-off conditions and plankton stable isotopes can therefore be considered representative for the area.

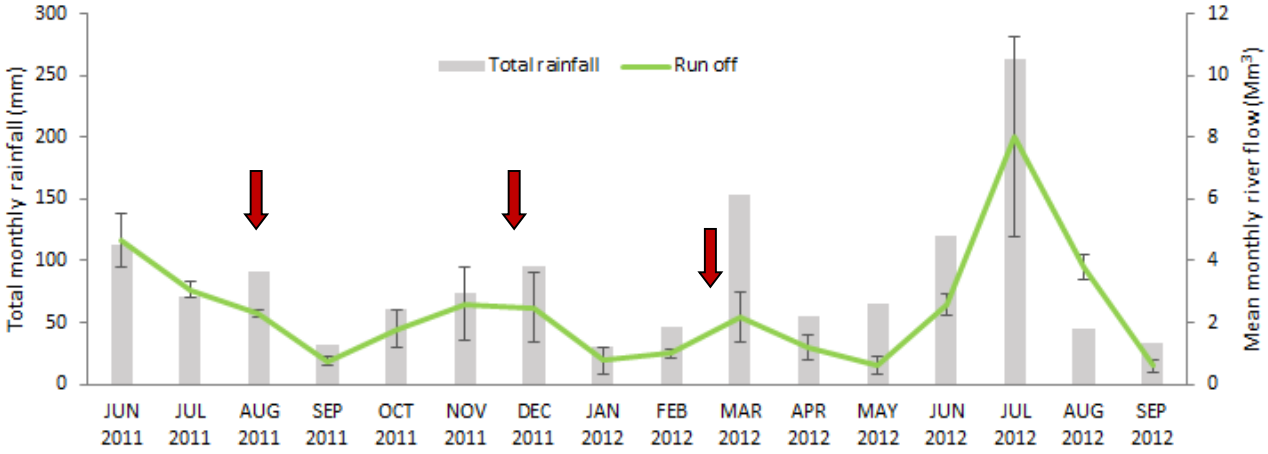


Figure 2.4. Fresh-water run-off into Tsitsikamma. Grey bars represent total monthly rainfall measured at the Storms River Mouth weather station for the duration of this study. The green line (error bars: standard deviations, n=3) represents the mean monthly river flow (mm^3) measured at three rivers (Elands, Bloukrans and Kruis rivers) in the Tsitsikamma National Park Marine Protected Area. Data were provided by the Department of Water Affairs and the South African Weather Service.

2.2 GENERAL METHODOLOGY

2.2.1 SELECTION OF STUDY SITES AND SAMPLE STATIONS

To obtain a characteristic representation of reef community structure and function at both shallow and deep reefs, several conditions had to be met by prospective study sites. Firstly, two spatially isolated reefs with suitable depth ranges (shallow: <30 m and deep: 45 – 75 m) had to be identified. Isolated reefs were selected to ensure minimal exchange of fish and invertebrate species, thereby ensuring low levels of spatial autocorrelation while providing high geographic proximity to keep reefs comparable in terms of environmental conditions. Secondly, these reefs needed to be large and non-fragmented, because small and isolated reef patches are often subject to highly variable recruitment and chance disturbances (Ault & Johnson 1998). Thirdly, to ensure that the data do in fact describe the function and structure of a community in an unexploited condition, sites needed to exclude edge effects and be positioned far from anthropogenic impacts (i.e. as far as possible from the MPA border). Finally, logistical considerations such as the availability of a nearby launch site, fuel costs and a safe site for mooring research vessels had to be taken into account.

The Storms River Mouth, situated in the centre of the TNP MPA (excludes edge effects) has a launch site close to the conservation offices, thereby ensuring frequent patrols (Tunley 2009; Figure 2.5). Moorings for smaller research vessels (up to 15m) had been installed in the Storms River Gorge as part of a preceding project. Research conducted in the TNP MPA (Bennett 2008, Bernard & Götz 2012) identified a large shallow (<30 m) reef just east of the launch site, named Rheeders Reef (Figure 2.5, A; shallow study site). From side-scan sonar data obtained by Flemming et al. (1983; see Buxton, 1987), Bernard (2012) identified a deep reef complex just south of Storms River Mouth. Footage obtained by a remotely operated vehicle (ROV) during 2010 confirmed the presence of a large reef named Middlebank Reef (Figure 2.5 B; deep study site).

The shallow reef was selected according to the depth range within which SCUBA divers can comfortably conduct research. The turbulent conditions on very shallow sites and dive limitations associated with decompression and time at the deep sites limited research to depths between 12 and 25 m. The deep reef started at 45 m, and was limited by the depth to which equipment could safely be deployed, and the availability of suitable reef within the MPA (75 m). Thus, from here-on the term ‘shallow reef’ refers to the warm-temperate rocky reefs in Tsitsikamma accessible by SCUBA divers and situated between 12 and 25 m (Figure 2.5 A). The term ‘deep reef’ refers to the near-shore deep reefs that lie between 45 and 75m (Figure 2.5 B).

Bennett (2008) recommended the use of 150 x 150 m grid-cells to avoid pseudoreplication and autocorrelation on shallow subtidal reefs (5-30 m). Considering the maximum depth of sampling in the current study (75m), GPS error and boat swing on anchor, 300 x 300 m grid-cells were chosen. To ensure independence of samples, these cell dimensions were standardised for both reefs in the study area. Sampling was conducted from the mid-point of each grid cell, termed stations (Figure 2.2 C). Only stations with medium to high profile (elevated topography) were selected, as determined from detailed bathymetric habitat maps (Figure 2.5). For instance, stations DR 3 and DR 7 were not included as the bathymetric maps indicated that these sites were either sandy or low profile (flat topography). Medium and high profile reefs were selected to avoid sampling stations that had recently been subjected to sedimentation and to minimise the chances of sampling sandy areas. After exclusion of the low profile stations, sample stations were selected randomly using a random number generator.

2.2.1.1 HABITAT MAPPING

The size, profile and depth range of the shallow and deep reefs were determined by producing detailed bathymetric maps (Figure 2.5). The map of the shallow reef was created with data obtained from Bennett (2008) and Bernard (2012), while the map of the deep reef was created with data collected during the present study. To produce a vertical profile, the points recorded from an echo-sounder linked to a Global Positioning System (GPS) receiver were interpolated using the software package Geographic Information System (GIS, ArcMap 10). Points were converted into a raster file with tension-splines (Götz 2005), and the raster data were interpolated to slope values (percentage slope) using the 3D Analyst tool in GIS.

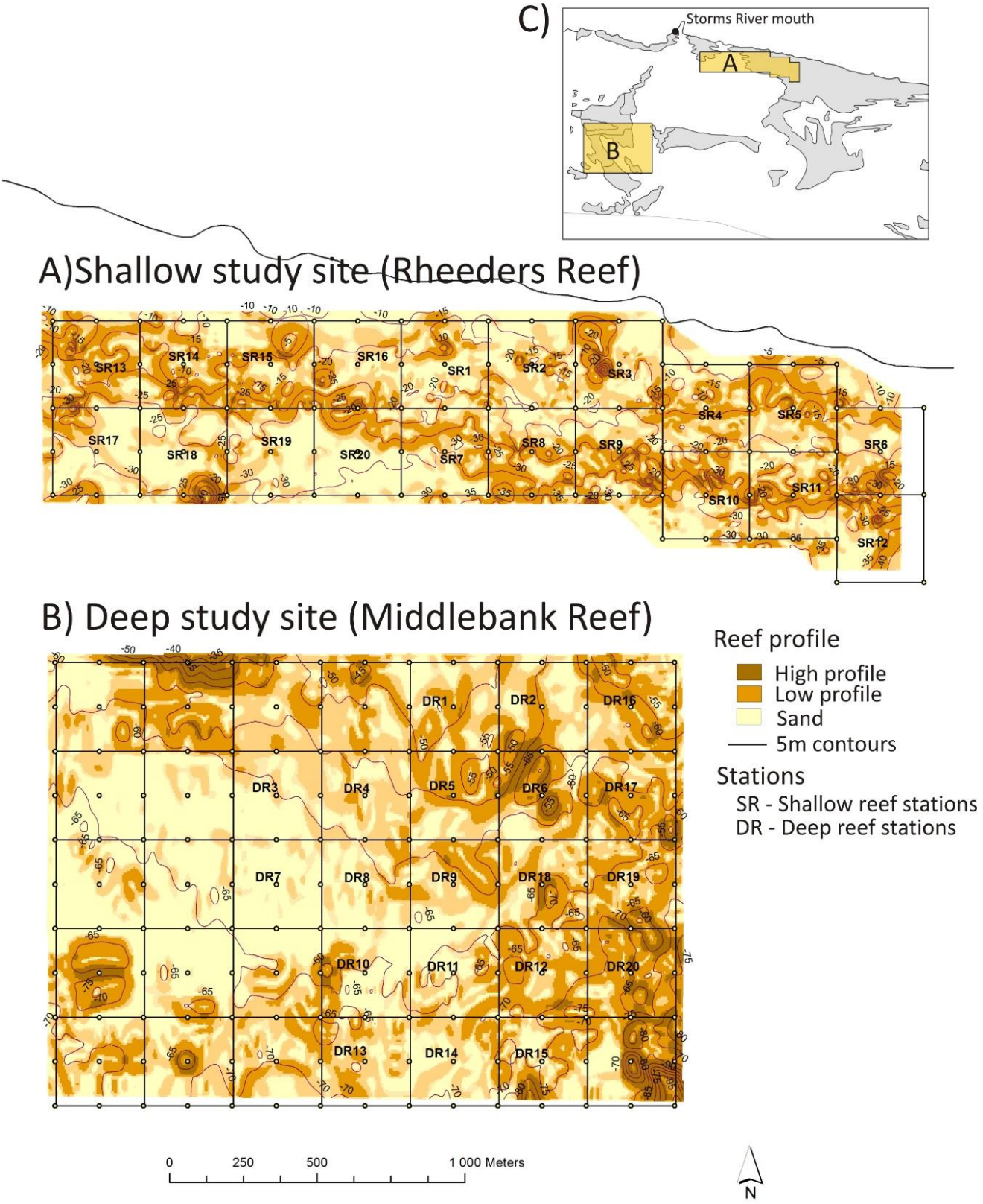


Figure 2.5. Maps of the two study sites. Detailed bathymetry map of the shallow (A) and deep (B) reefs indicating the 300 x 300 m grid-cells and the midpoints from which sampling was conducted (sample stations). Insert (C) indicates the position of the reefs in the study area in relation to each other and the launch site (black dot).

2.2.2 SAMPLING STRATEGY

Although slight differences in both the fish and macrobenthic community structure should occur over time, these differences were assumed to be negligible compared to differences related to the depth gradient. This assumption was necessary because bad sea conditions in combination with the use of specialised equipment that required trained teams limited the number of sampling methods that could be used during a sampling season. As such, sampling that involved the use of the remotely operated vehicle (ROV) and baited remote underwater stereo-video systems (stereo-BRUVs) occurred during different sampling seasons.

The sampling strategy can be divided into three components: (i) collections of physico-chemical parameters, (ii) fatty acid and stable isotope sample collections and, (iii) assessments of abundance and biomass of reef fish and the hard-substrate macrobenthos. Apart from the macrobenthic and fish tissue samples intended for fatty acid and stable isotope analyses, which were collected opportunistically, all other samples were collected from the midpoints of the grid cells (Figure 2.5). Oceanographic conditions of the study area were determined by measuring water temperature, dissolved oxygen, specific conductivity, salinity, chlorophyll-*a* and light intensity on three occasions (July 2011, November 2011 and February 2012).

Dominant fish and invertebrate species intended for fatty acid and stable isotope examinations were collected during February and March 2012 by a variety of techniques, namely SCUBA diving, spear fishing, trapping, angling and ROV. The logistics and costs associated with collecting samples with the ROV limited the collection of animals to one sampling event with this technique. Plankton samples intended for fatty acid and stable isotope analyses were collected in July and November 2011 and February 2012. Seasonal plankton samples were collected to account for the high variability observed in plankton communities due to changes in seasonal oceanographic conditions.

Photo and video techniques were employed to describe the fish and macrobenthic community composition and size structure. Photoquadrats of the hard-substrate macrobenthos were recorded by SCUBA divers from shallow reefs in July 2009 and February 2011. On the deep reef photoquadrats were obtained by ROV in July 2012. Methods for collecting shallow and deep photoquadrats differed because decompression limits and time constraints restricted SCUBA diving to the shallow reef. In contrast, control of the ROV due to strong surge on the shallow reef deemed the ROV uncontrollable for shallow photoquadrat collection. Fish community composition and biomass were assessed with stereo-BRUVs in February 2013 and 2014. An eight-meter, semi-rigid inflatable ski-boat fitted with a winch and davit was employed as a research platform for the majority of tasks. Activities involving the ROV were conducted from a 13-meter rigid ski-boat.

3 DEPTH RELATED DISTRIBUTION PATTERNS OF THE TSITSIKAMMA SUBTIDAL MACROBENTHOS

3.1 INTRODUCTION

Reef communities host unique assemblages of invertebrates, which in turn provide niche habitats for many commercially important fish species (Brouwer 2002, Griffiths & Wilke 2002, Brouwer & Griffiths 2004, Sink et al. 2006). Reef habitats are dominated by suspension-feeders that construct diverse, intricate, three-dimensional habitats, and thereby increase the complexity of the reef topography and ecosystem functioning through inter-specific facilitation (Gili & Coma 1998, Cardinale et al. 2002). This inter-specific hydrodynamic facilitation optimises particle capture of the suspension feeder community by altering near bed flow regimes (Cardinale et al. 2002). Furthermore, for most of the suspension-feeders, cost of foraging is nil, and for active filter-feeders, respiratory output is minimal (Riisgard et al. 1993, Gili & Coma 1998). Consequently, benthic suspension feeding communities are considered optimal foragers (Gili & Coma 1998). As such, these suspension feeding communities have major impacts on marine ecosystems through the regulation of primary production (Barange & Gili 1988, Coma et al. 1994) and are responsible for the bulk of the energy flow from pelagic to benthic systems (Gili & Coma 1998).

Despite their ecological importance, macrobenthic communities situated deeper than the conventional SCUBA diving depth limit (30 m) have received very little attention, both globally (Virgilio et al. 2006) and specifically in South Africa (Sink et al. 2006). Only recently have some aspects of the ecology of these deep nearshore reef communities been touched upon, with the bulk of the research occurring in tropical seas (Lesser et al. 2009, Bongaerts et al. 2010, Kahng et al. 2010, Sherman et al. 2010, Locker et al. 2010, Hinderstein et al. 2010). Research on such deeper tropical reefs has focused on the light dependent reef building zooxanthellate corals found between 50 – 120m, known as mesophotic coral reefs (Lesser et al. 2009). However, only a handful of studies have been conducted in the same bathymetric belt in more temperate seas, most of which were conducted in the Mediterranean (Rossi et al. 2008; Bo et al. 2008, 2009, 2011; Gori et al. 2011a, b; Gori et al. 2012). In South Africa, marine biodiversity has received considerable attention, especially

when compared to the rest of the African continent (Griffiths et al. 2010). Even so, and in line with the global focus, most of the research has been conducted in the intertidal zone and more rarely in the subtidal, accessible by SCUBA divers (<30 m), leaving the deep nearshore reefs largely unexplored. As a result, besides the absence of data from deep reefs, there are still substantial gaps in our knowledge about South African invertebrate taxa, even in the groups that have received most attention (Griffiths et al. 2010). Many macrobenthic species remain undescribed and often very little information exists on community structure and factors influencing species distribution (Sink et al. 2006, Griffiths et al. 2010).

Abiotic factors such as light, water movement, nutrient availability, sedimentation and temperature vary predictably with depth (Garrabou et al. 2002). Compared to deeper reef habitats, shallower reef environments are usually characterised by more extreme and variable conditions (Garrabou et al. 2002). These predictable changes in abiotic variables are important elements that influence the structure of macrobenthic reef communities (Bell 2001, Garrabou et al. 2002, Bell & Smith 2004).

3.1.1 STUDY AIM

To confirm if the predictable changes in abiotic factors influence macrobenthic assemblages, the shallow reef (<25 m) were compared with the deep nearshore reef (45 – 75 m) within the well-established Tsitsikamma National Park (TNP) Marine Protected Area (MPA). This chapter describes the differences between the shallow and deep reef macrobenthic assemblages, and explores the patterns and variables that explain the observed differences.

My specific objectives were to:

i) determine *a priori* if shallow and deep reefs have different macrobenthic assemblages in terms of:
   a. species richness,
   b. species composition,
   c. indicator species,
   d. guilds,
   e. population size structure,

ii) establish *a posteri* if the macrobenthic assemblages demonstrate depth related clustering,

iii) identify the environmental variables that correlate with the observed macrobenthic assemblage patterns.

3.2 MATERIALS & METHODS

3.2.1 STUDY AREA & SAMPLING STRATEGY

Research was conducted on the Middlebank and Rheeders Reef complexes situated close to the Storms River mouth in the TNP MPA. A full study site description can be found in Chapter 2, Section 2.1.2. Sampling was stratified between two study sites within the study area (Tsitsikamma) in the TNP MPA, corresponding to a shallow (Rheeders Reef) and a deep reef (Middlebank Reef). The macrobenthic assemblages were estimated from six sample stations in each reef, ensuring that all depth strata were targeted. Light intensity was measured at three sample stations per reef (Chapter 2, Figure 2.2 C).

3.2.1.1 LIGHT PROFILES

Changes in the photosynthetic active radiation (PAR; 400 – 700 nm) were measured by lowering a LICOR LI-193 Spherical Quantum Sensor three times at each station down onto the reef. Replications were conducted to account for changes in irradiance due to variability in cloud cover. The vertical profiles consisted of PAR measurements recorded at five-meter intervals in micromoles of quanta per second per square meter ($mmol.s^{-1}m^2$). Light profiles were obtained during November 2011 and February 2012.

3.2.1.2 ASSEMBLAGE COMPOSITION

The structure and species composition of the assemblages were determined by estimating percentage cover from photoquadrats. Photoquadrats on the shallow reef were obtained by SCUBA divers. At each station, divers swam a 50 m transect in eight predefined directions. Eight to ten photos were haphazardly taken within each transect using a Canon G9 camera (12.1 megapixels) mounted on a tripod. This strategy was employed to avoid resampling the same area and to maximise dive time. The tripod maintained a set distance from the substrate and sampled an area of 0.33 m^2. Deep reef photoquadrats were obtained with a ROV (Falcon Seaeye: 12177) fitted with a SubCControl 1Cam (12.3 megapixel HD camera). The 1Cam was fitted with two laser pointers set at 64.2 mm apart, permitting size approximation of the sampled area. Strong currents restricted the manoeuvrability of the ROV, and between 100 and 150 photoquadrats were obtained from a single longer transect as opposed to the strategy employed by the SCUBA divers. Care was taken to follow a depth contour when conducting all transects with the ROV, thereby standardising depth during sampling. According to the recommendations of Deter et al. (2012), 30 photos were selected haphazardly from each sample station, amounting to 180 photos per reef.

3.2.1.3 SIZE OF UPRIGHT MACROBENTHOS

The heights of macrobenthic species that demonstrated upright growth were measured at the same stations surveyed for the community analysis. The heights of invertebrates on the shallow reef were obtained by SCUBA divers. Divers swam along the 50 m transects and captured photos of all macrobenthos that demonstrated upright growth. Prior to capturing the photo, a second diver would place a measuring stick directly next to the specimen. Deep reef photos were obtained from the ROV footage when the camera was horizontal, recording measurements only when both laser pointers were fixed simultaneously on a specimen. Measurements of thin specimens were recorded when the lasers pointed at the substrate directly below the object. Photos were subsequently imported into in the software package Coral Point Count with Excel extensions (CPCe 4.1) to allow post calibration, after which the heights of the specimens were measured.

3.2.2 ANALYSES

3.2.2.1 LIGHT PROFILES

The light attenuation coefficient [$K_{d(PAR)}$ (m^{-1})] was determined by linear regression between depth and the natural logarithm-transformed irradiance (Lund-Hansen 2004). The attenuation coefficient is used to compare different water bodies with respect to availability of photosynthetically useful radiant energy (Kirk 1983). The coefficient *a* in the regression equation *f(x) = ax + b* equals the exponent K_d in the Lambert-Beer law for the vertical distribution of irradiance:

$$I = I_0 e^{-K_d(PAR)z}$$

where I_0 is the irradience just below the surface (here taken at 5 m), I the irradience at *z* depth (m) (Kirk 1983).

A useful rule-of-thumb in aquatic biology is that net-gain from photosynthesis occurs only to a critical depth, Z_{eu} (euphotic zone). Below Z_{eu} the respiratory carbon loss exceeds photosynthetic carbon gain (Kirk 1983). The euphotic zone can be determined by:

$$Z_{eu} = \frac{4.6}{K_d}$$

A likelihood ratio test (LRT) was conducted to determine if the light profiles differed significanlty between reefs and season (November 2011 and February 2012).

3.2.2.2 ASSEMBLAGE COMPOSITION

Photoquadrats obtained from both reefs were calibrated in CPCe 4.1, and 56 x 31cm (0.2m^2) blocks were superimposed onto individual images. The software CPCe facilitates automation of random point counts, a method commonly used to describe benthic communities from photographs (Kohler & Gill 2006). This method employs random points as substitutes in statistical power analyses to estimate the actual population composition (Kohler & Gill 2006), thereby avoiding the need to count each individual and substantially reducing analysis time. A species accumulation curve was plotted to estimate the number of points required to identify 95% of the macrobenthic species per photoquadrat. The accumulation curve analysis (see appendix, Figure A3.4 & Table A3.1) indicated that 54 points were required to analyse each photoquadrat, resulting in 1,620 points per transect. Under each point, species were identified to the nearest taxon (noting substrate cover where applicable) according to the invertebrate collection hosted by the South African Institute for Aquatic Biodiversity (SAIAB), Samaai & Gibbons (2005), Jones (2008) and Branch et al. (2010). The number of points were summed for each taxon and percentage cover estimated. All identifiable species were included in the species list; however, similar looking species were grouped during the CPCe analysis to avoid incorrect identifications in poor quality photographs. Many macrobenthic species from the deep reef could not be identified and were either grouped to a higher taxonomic level or those species from easily recognisable genera were accordingly labelled “sp. 1” etc. Identification of these species was achieved with help from taxonomic experts, namely T. Samaai (sponges), K. Sink (gorgonians), S. Parker-Nance (ascidians) and W. Florence (bryozoans).

A. SPECIES RICHNESS & COMPOSITION

Data on percentage cover of the macrobenthos were analysed using the software programme PRIMER v6 according to Clarke & Warwick (2001), Clarke & Gorley (2006) and Clarke et al. (2008). The macrobenthic abundance data were averaged for each station and 4th root transformed before calculation of a similarity matrix by means of the Bray-Curtis distance measure. This transformation was chosen to highlight the importance of rarer species (Clarke & Warwick 2001), as these should play an important role in a stable, well established community such as that expected in the TNP MPA.

Statistical analyses were conducted either *a priori* – as defined by the shallow and deep reefs, or *a posteriori* to determine if the macrobenthic assemblages cluster according to depth. To establish *a priori* if the benthic assemblages on the shallow and deep reefs differed in composition, two-way analysis of similarity (ANOSIM; sample stations nested in study sites) tests were performed on the percentage cover similarity matrix. The R statistic produced by the ANOSIM measures the degree of separation of pre-defined groups (reefs), where an R value of zero indicates no difference between

the communities, and an R value of one indicates that the communities are completely different. A p-value associated with this R value gives an indication of the significance level of the separation of the pre-defined groups. The major contributing and discriminatory species at each reef were initially determined by employing the similarity percentage (SIMPER) routine in PRIMER v6.

B. INDICATOR SPECIES

Due to their niche preferences, indicator species can be used to predict the diversity of other species within an area (community type) or reflect their ecological preferences (biotic or abiotic state of the environment; De Cáceres et al., 2012). In this study, indicator species were determined by following the indicator value index (*IndVal*) procedure (Dufrêne & Legendre 1997). The *IndVal* procedure was chosen over the two-way indicator species analysis (TWINSPAN; Hill 1979) because it allows for predefined partitioning of sites (shallow and deep), and because it is species specific and not influenced by the abundances of other species (Legendre 2013). The *IndVal* index can be defined by computing two values: specificity (A_{kj}) and fidelity (B_{kj}). For each species *j* in each cluster of *k* sites, A_{kj} and $B_{kj.}$ can be computed by:

$$A_{kj} = Nindividuals_{kj}/Nindividuals_{+k}$$

$$B_{kj} = Nsites_{kj}/Nsites_{k+}$$

$$IndVal_{kj} = A_{kj}B_{kj}$$

The specificity (*A*) is based on abundance values and describes the degree to which a species is found only in a group of pre-defined sites. Fidelity (*B*) is computed from presence/absence data, and describes the degree to which a species is present at all sites of a group (Legendre 2013). Statistical significance of the species-site group associations was determined by a permutation test. Indices were computed using the *multipat()* function from the 'indicspecies' package (De Cáceres & Jansen 2013) in R-Studio 2.15.3 (R Core Team 2013).

Indicator species for the shallow and deep reefs were calculated from the average species assemblage data from each station. The high number of species constrained to either the shallow or deep reefs resulted in very high numbers of indicator species. Thus, indicator species were reduced by grouping mobile species and leaving the data untransformed to decrease the importance of rare species.

C. GUILD STRUCTURE

A guild is a group of species that exploit the same set of resources in a similar manner, resulting in potentially significant overlaps in niche requirements (Root 1967). This concept is focused on the similarity in resource sharing, and does not consider the function or ecological consequences that may arise due to utilising the same resources (functional groups; Blondel 2003). Thus, through strong inter-specific competition for the same resource, members of a guild share structural and morphological adaptations to obtain a limited resource.

To determine if the depth gradient, and the associated changes in important limiting resources (habitat, food and light), translated into a significant change-over in guild composition, the macrobenthic species were assigned to guilds according to structural traits associated with resource exploitation. These morphological adaptations can be classified into the following categories: 1) height above seafloor (growth form: solitary, colonial and shape), and 2) size selection of food particles (feeding apparatus/mechanism). This classification scheme was developed from the concepts explained by Woodin & Jackson (1979), Jackson (1977) and Wildish & Kristmanson (1997).

Species were grouped according to the different strategies used to occupy and gain space (first two columns in Table 3.1). Thereafter, species were grouped according to the strategy and mechanism employed to obtain food (last two columns Table 3.1). Algae were separated from the rest of the macrobenthos because food in the form of light only affects autotrophs. Thus, reading from left to right, species were first subdivided by basic body plan and the functional organisation of tissues, including either solitary or colonial groups (Woodin & Jackson 1979). The second column splits solitary species based on mobility (sessile species are permanently attached to the substrate and mobile species are capable of limited movement), and colonial species according to how they occupy space (i.e. colony morphology; Woodin & Jackson 1979). There seems to be two main strategies: low encrusting growth and erect tree-like growth (and variations there-of). Encrusting species can outcompete other species for reef habitat through overgrowth, whereas the erect tree-like growth species avoid competition for space through growing vertically (McLean & Lasker 2013). The last two columns of Table 3.1 classify species according to feeding strategy and feeding mechanism (Wildish & Kristmanson 1997). For the majority of macrobenthic species found on reefs, food is available in the form of suspended particulate matter, thus suspension-feeders tend to be dominant. Suspension-feeders can be active, passive, facultatively active or combined passive-active (Wildish & Kristmanson 1997). Active suspension-feeders obtain food by expending energy to transport water over their feeding structures in the form of ciliary or muscular pumps (Wildish & Kristmanson 1997). Passive suspension-feeders rely completely on ambient flow to bring food particles into contact with their feeding structures (Wildish & Kristmanson 1997). Facultatively active suspension-feeders switch

between passive and active feeding depending on ambient flow (Wildish & Kristmanson 1997). Combined passive-active suspension-feeders employ both passive and active mechanisms at the same time, but never switch between active and passive feeding (Wildish & Kristmanson 1997).

Table 3.1. Guild assignment of macrobenthos. Macrobenthic species were grouped into guilds according to modified concepts of Woodin & Jackson (1979) and Jackson (1977) and Wildish & Kristmanson (1997).

Resource			
Space		Food	
Adaptation			
Body plan	Growth form	Feeding strategy	Feeding mechanism
Solitary	Mobile	Grazer Scavenger	Unknown Macro-benthos
	Sessile	Suspension feeder	Active Passive Facultative active Combined passive-active
Colonial	Sheet Mound Sheet-like mound Vine Tree-like		
Solitary (algae)	Sheet Tree-like	Primary producer	Autotroph

Solitary animals employ the different filter feeding strategies mentioned above, or animals such as gastropods and echinoderms feed by grazing and scavenging. To classify the macrobenthic species according to the guilds (Table 3.1), species specific trait information was obtained from Jones (2008b) and Branch et al. (2010). Thus, each species would have a combination of the adaptive traits assigned to it (obtained from Table 3.1). This procedure resulted in 21 different trait combinations that were considered guilds. For a list of the taxonomic groups represented by each guild, see Table A3.3 in the appendix.

To determine *a priori* if the guild composition differed between the reefs, an ANOSIM procedure was performed on a 4^{th}-root transformed Bray-Curtis similarity matrix based on the guild traits of each species (PRIMER v6). A SIMPER procedure was conducted to determine what functional groups contributed to the differences between the reefs.

D. POPULATION SIZE STRUCTURE

Size data (height [cm]) for the following species were obtained from both reefs: nippled sea fan (*Eunicella papillosa*), palmate sea fan (*Leptogorgia palma*), noble coral (*Stylaster nobilis*), gorgonian twig coral (*Homophyton verrucosum*) and elephant ear ascidian (*Gynandrocarpa placenta*). Although

sponges with tree-like growth were dominant on the deep reef, only the unidentified orange finger sponge was represented on both reefs. As a consequence, the heights of the same sponge species could not be compared between reefs. To determine if sponges demonstrated more upright growth on the deep reef, all measured sponges were grouped by reef regardless of species and compared.

Mean heights were tested for significance between the shallow and deep reefs. Data sets that demonstrated normal distribution (palmate sea fan, elephant ear ascidian and grouped sponges) were tested for significant differences in height between the reefs, using a student's t-test, and nippled sea fan, gorgonian twig coral and noble coral were tested for significant differences in height between the reefs by employing a non-parametric Mann-Whitney U test in STATISTICA v12, as these data sets did not demonstrate normality.

To test if the population size structure of upright growing species differed between the shallow and deep reefs, kernel density estimates (KDEs) were produced from the size frequency data on those species with sufficient sample sizes from each reef (Langlois, Fitzpatrick, et al. 2012). Kernel density estimates is a non-parametric approach that compares pairs of size frequency data (shallow and deep) via permutation. The analysis allows for testing for differences in both shape and location of size frequency data sets. The shape of the size frequency distribution curve provides information regarding a potential bias to a particular size class in a population. The location of the curve represents the mean height of the measured population. To account for within-population variance and sampling effect, only the shape of the size frequency curves was considered. To test for shape only, the data were standardised by median and variance. Because KDE is a data-driven method, the bandwidth selection avoids bootstrapping of large independent samples and subjective selection of size-bins. A statistical test between the KDEs from the shallow and deep reef was based on the null model of 'no difference' and a permutation test. Analyses were conducted employing the computer code suggested by Langlois et al. (2012) with the 'KernSmooth' (Wand 2012) and "sm" (Bowman & Azzalini 2013) packages in R.

To determine if the shapes of the size frequency distribution curves demonstrated significant bias towards a particular size class, analyses of skewness (g_1) were performed. Significant skewness indicates that the data are asymmetrical. Positively skewed data signifies the prevalence of small size classes in a population, and vice versa for negatively skewed distributions (Rossi et al. 2012). Analyses were conducted in R-Studio 2.15.3 (R Core Team 2013) employing Agostino's test from the 'moments' package (Komsta & Novamestrky 2012).

3.2.2.3 ENVIRONMENTAL PARAMETERS

Following the recommendations of Clarke & Gorley (2006), environmental data with non-normal distributions (reef profile and percentage rock cover) were log-transformed, after which all environmental data were normalised to make the variables directly comparable. To determine if the environmental parameters differed significantly between reefs, an ANOSIM was performed on the Euclidian distance matrix in PRIMER v6. To further clarify how these parameters affected species assemblages and guild composition, the global BEST test (Clarke & Gorley 2006) was performed. This procedure amalgamates the BIO-ENV and BVSTEP procedures from PRIMER v5 and searches for high rank correlations between species and environmental data. It provides an overall investigation of the parameters that drive species assemblage structure. The test was performed on 4^{th} root transformed Bray-Curtis similarity matrix species assemblage data. Correlations between the normalised environmental data and the 4^{th} root transformed species resemblance matrices were determined by calculating Spearman's rank correlation coefficients (ρ). Environmental parameters included depth (m), light intensity (mmol s^{-1} m^2), temperature (^oC), reef profile (categorised as low or high) and substrate type (categorised as bare rock, rubble, sand, shells or settled particulate matter). Reef profile for each transect was estimated by SCUBA divers on the shallow reef and from the ROV footage on the deep reef. Substrate type was estimated as percentage cover obtained from the photoquadrats. Significance of the correlations between species and environmental data was estimated with the global BEST match permutation test. The ρ (Spearman's rank correlation coefficient) obtained from the data (observed) was compared to that produced by 999 random permutations (predicted). If ρ was larger than any of the permuted values, the null hypothesis of 'no agreement in multivariate pattern' was rejected and the correlation was significant.

The global BEST test gives an indication of the environmental parameters responsible for broad-scale impacts on the species assemblage. Linkage trees (LINKTREE procedure), on the other-hand, identify the environmental variables that result from finer scale divisions of the biota (Clarke et al. 2008). The LINKTREE procedure is a non-linear and non-additive technique that links sample patterns to environmental variables (Clarke & Gorley 2006, Clarke et al. 2008). It is a procedure in PRIMER v6 based on De'ath (2002)'s multivariate regression trees (MRT). Clustering of the biotic samples is explained in terms of a sequence of inequalities on the environmental variables. Thus, a cluster of sample stations (based on species assemblage data) would have specific environmental gradients in common that influence their internal structuring. Linkage trees are constrained to consider only divisions that can be expressed as thresholds on an environmental variable. To test if the clusters differed significantly, a Similarity Profiles (SIMPROF) analysis, which tests for multivariate structure among biotic samples, was performed (Clarke et al. 2008). Similarity Profiles are produced by

calculating the similarity among samples and are ranked from smallest to largest. If the observed profile (based on actual species data) falls outside the expected distribution generated under the null hypothesis of 'no difference' in the community, the null hypothesis is rejected. A test statistic (π) is calculated from the total area between the observed profile and the mean profile under random permutations (Somerfield & Clarke 2013). LINKTREE thus produces three values; ANOSIM R, B% and π (and corresponding p-value). The difference of average rank dissimilarities between and within groups and in the LINKTREE application were estimated from the ANOSIM R, which is a measure of the degree of separation of the two groups. The measure B% determines how well two groups of samples split relative to the maximum separation achievable at the first partition (y-axis). The test statistic of the SIMPROF test (π) describes the extent of the difference between the observed profile and permuted profiles.

The environmental parameters identified by the global BEST test were used in the LINKTREE analysis to simplify interpretation, because a large number of explanations can result from the inclusion of too many abiotic variables (Clarke & Gorley 2006). However, running the LINKTREE analysis on different combinations of the measured environmental variables indicated exactly the same outcome. As a consequence, all variables were employed in the analysis. Analysis was performed on the 4^{th} root transformed Bray-Curtis similarity matrix. Environmental data were not normalised, as it did not change the outcome of the linkage tree procedure, and interpretations of non-normalised data were more straight-forward (Clarke & Gorley 2006).

3.3 RESULTS

3.3.1 LIGHT PROFILES

The light profiles for both November 2011 and February 2012 were significantly different when compared between the shallow and deep reefs (LRT: $p < 0.001$; Figure 3.1 A & B). Furthermore, significantly different light conditions occurred over time (LRT; $p < 0.001$), and less light penetrated the water column during November 2011 (shallow $K_d = 0.216$; deep $K_d = 0.152$) compared to February 2012 (K_d: shallow = 0.173 and deep = 0.091). The lower K_d values obtained above the deep reef during both November 2011 and February 2012 translated into more light penetrating to depth, with a euphotic depth of 30.1 m on the deep reef and 21.3 m on the shallow reef during November 2012, and an euphotic depth of 54.2 m on the deep reef compared to 26.6 m above the shallow reef in February 2012.

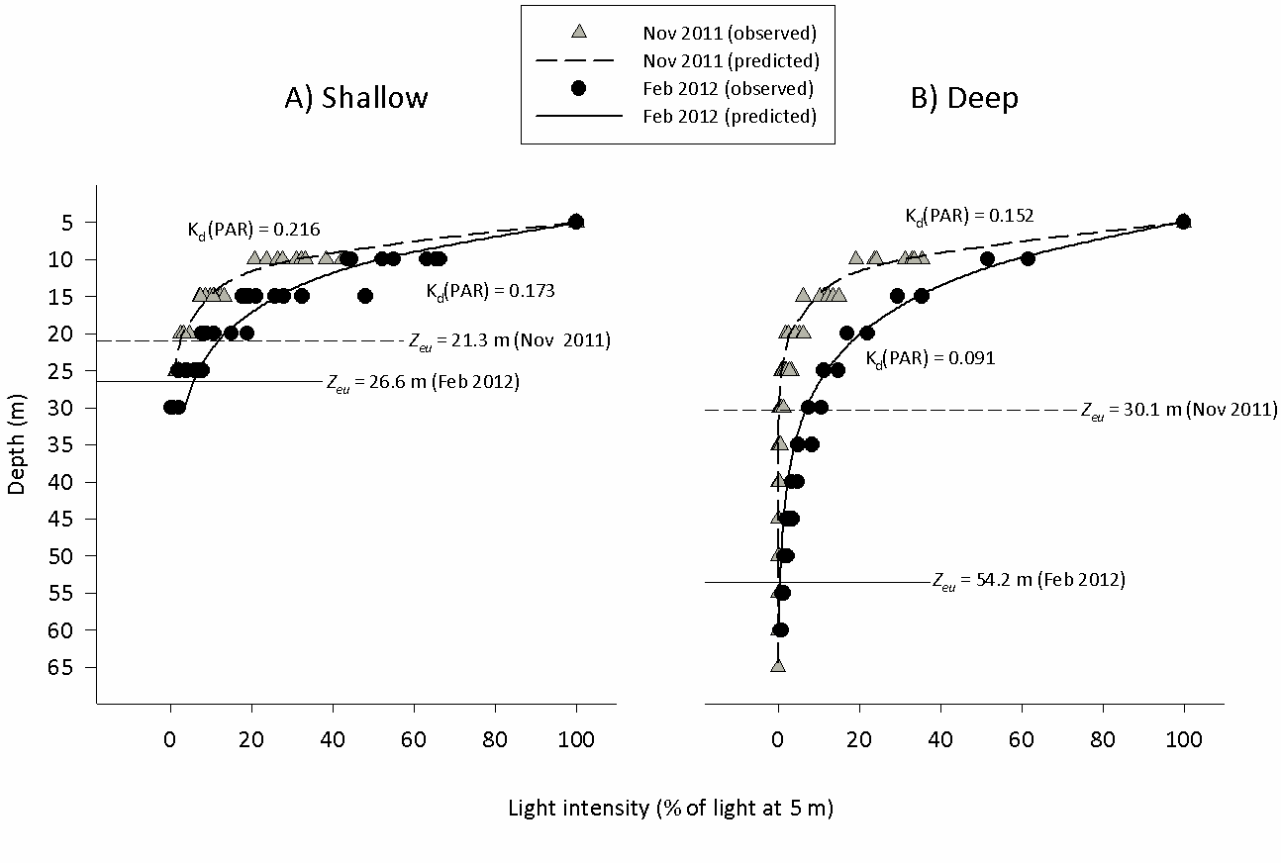


Figure 3.1. Light profiles. Irradiance versus depth as a percentage of subsurface irradiance measured throughout the water column above the shallow (A) and deep (B) reefs in Tsitsikamma. Samples represented by grey triangles and dashed lines were collected in November 2011; solid lines and circles represent February 2012 samples. The light attenuation coefficient (K_d) and euphotic depth (Z_{eu}) are indicated for each profile (February deep: n = 2, February shallow: n = 8, November deep: n = 8 and November shallow: n = 9).

3.3.2 ASSEMBLAGE COMPOSITION

A. SPECIES RICHNESS & COMPOSITION

Compared to the deep reef, on which less than half (48%) of the area was inhabited by macrobenthos, the shallow reef was covered predominantly by macrobenthic species (85%; Figure 3.2). The remaining (52%) of the deep reef comprised of different substrate types. Here, the dominant substrate type was settled particulate matter (settled PM; 38%; Figure 3.2 B), whereas the shallow reef was covered by near equal amounts of sand (5%), settled PM (4%) and rubble (3%; Figure 3.2 A).

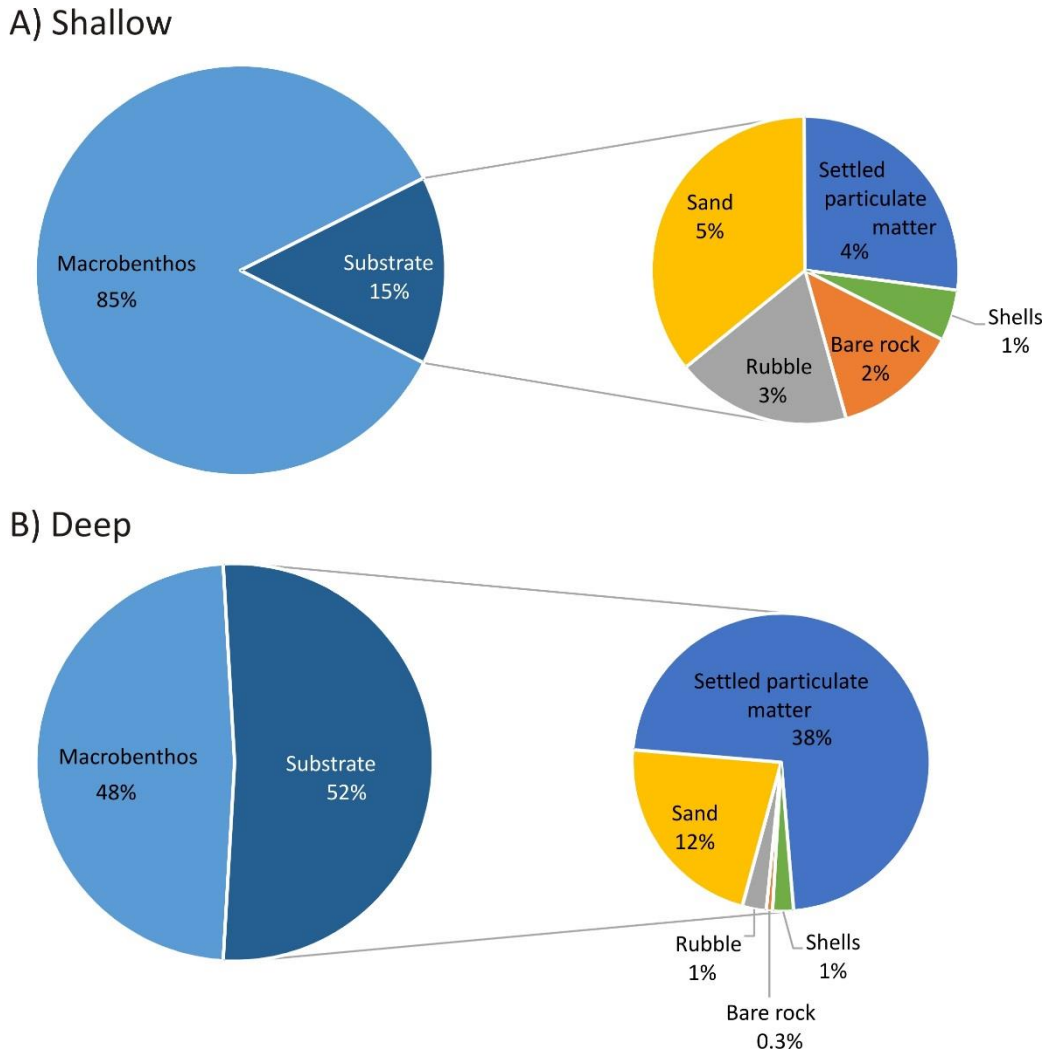


Figure 3.2. Substrate composition. The contribution of different substrate types to the shallow (A) and deep (B) reefs in Tsitsikamma (n = 360 photoquadrats).

Of the 161 identified specimens, 111 were identified to genus and 67 to species level (Table 3.2). The remaining specimens were either grouped to higher order taxa, identified to genus (but may include several species), or recognised as a species (but cannot be identified; see appendix Figure A3.2 for information on groupings). Similar proportions of unidentified specimens were present on the shallow and deep reefs (23.6 and 18.9%, respectively). The shallow reef demonstrated higher species richness than the deep reef, with 129 and 90 identified taxa, respectively.

Table 3.2. Species list for the shallow and deep reef macrobenthic assemblages. There are five levels of identification (see Figure A3.2 in appendix). Several species from the same taxonomic group that were unclassifiable from photoquadrats are written in normal font and designated as "spp." (e.g. Algae spp.). Taxa distinguishable as species that could not be formally identified to a genus are indicated in normal font as "sp." under the higher taxonomic level name (e.g. Didemnidae sp. 1). Taxa identified to genus are indicated in *italics* as "spp." and can include several species (e.g. *Sycozoa* spp.), and taxa identified to genus but distinguishable as a single species are designated "sp." if representing a single species (e.g. *Reteporella* sp. 1).

Phylum/Class	Order	Family	Species
			Algae spp.
Anthozoa	Zoantharia	Parazoanthidae	*Isozoanthus capensis*
	Actiniaria		Anemones spp.
		Actiniidae	*Anthostella stephensoni*
			Anthothoe spp.
			Alcyonacea spp.
			Alcyonacea sp. 1
	Alcyonacea	Alcyoniidae	*Alcyonium fauri*
			Eleutherobia variable
			Klyxum sp. 1
			Malacacanthus capensis
		Anthothelidae	*Homophyton verrucosum*
		Gorgoniidae	*Eunicella albicans*
			Eunicella papillosa
			Eunicella tricoronata
			Leptogorgia gilchristi
			Leptogorgia palma
		Melithaeidae	*Acabaria rubra*
		Nephtheidae	*Capnella thyrsoidea*
	Scleractinia	Caryophylliidae	*Caryophyllia* sp. 1
		Dendrophylliidae	*Balanophyllia bonaespei*
			Dendrophyllia sp. 1
Arthropoda	Cirripedia	Balanidae	*Austromegabalanus cylindricus*
Ascidiacea			Colonial ascidian spp.
			Colonial ascidian sp. 1
			Encrusting ascidian spp.
			Solitary ascidian sp. 1
			Solitary ascidian spp.
	Aplousobranchia	Didemnidae	Didemnidae spp.
			Didemnidae sp. 1
		Euherdmaniidae	*Euherdmania divida*
		Holozoidae	*Distaplia skoogi*
			Sycozoa arborescens
			Sycozoa spp.
		Polycitoridae	*Eucoelium pallidus*
			Eudistoma sp. 1
		Polyclinidae	*Aplidiopsis tubiferus*
			Aplidiopsis sp. 1
			Aplidium flavolineatum
			Aplidium mernooensis
			Polyclinum isipingense
		Pseudodistomidae	*Pseudodistoma africanum*
			Pseudodistoma sp. 1
			Pseudodistoma sp. 2
		Pycnoclavellidae	*Pycnoclavella filamentosa*
			Pycnoclavella sp. 1

	Phlebobranchia	Diazonidae	Rhopalaea sp. 1
	Stolidobranchia	Pyuridae	*Pyura stolonifera*
		Styelidae	*Botryllus* spp.
			Gynandrocarpa placenta
			Polyandrocarpa anguinea
			Polyandrocarpa sp. 1
Brachiopoda	Rhynchonellata	Kraussinidae	*Kraussina rubra*
Bryozoa			*Encrusting bryozoan* sp. 1
			False coral sp. 1
			False coral spp.
			Soft false coral spp.
	Cheilostomatida	Adeonidae	*Adeonella inaequalis*
			Adeonella purpurea
			Adeonella sp. 1
			Adeonella sp. 2
			Adeonella sp. 3
			Adeonella sp. 4
			Laminopora sp. 1
		Candidae	*Hoplitella armata*
			Menipea triseriata
		Chaperiidae	*Chaperia* spp.
		Cribrilinidae	*Membraniporella* spp.
		Gigantoporidae	*Gigantopora polymorpha*
		Lepraliellidae	*Celleporaria* sp. 1
		Microporellidae	*Flustramorpha* spp.
		Phidoloporidae	Phidoloporidae spp.
			Reteporella lata
			Reteporella sp. 1
			Schizoretopora sp. 1
		Tubuliporidae	*Tennysonia stellata*
			Tennysonia spp.
	Ctenostomatida	Buskiidae	*Cryptopolyzoon concretum*
Chlorophyta			Green algae spp.
	Bryopsidales	Codiaceae	*Codium* spp.
Echinodermata	Asteroidea		Asteroidea spp.
		Echinasteridae	*Henricia ornata*
		Goniasteridae	*Calliaster baccatus*
		Ophidiasteridae	*Austrofromia schultzei*
	Crinoidea	Tropiometridae	*Tropiometra carinata*
	Echinoidea		Echinoidea spp.
		Parechinidae	*Parechinus angulosus*
	Ophiuroidea		Brittlestar spp.
		Gorgonocephalidae	*Astrocladus euryale*
Hydrozoa			Hydrozoa spp.
			Hydroid sp. 1
			Hydroid sp. 2
			Sponge encrusted hydrozoan
	Anthoathecata	Solanderiidae	*Solanderia procumbens*
		Stylasteridae	*Stylaster nobilis*
	Leptothecata	Aglaopheniidae	*Aglaophenia pluma*
			Lytocarpia formosa
			Macrorhychia filamentosa
		Halopteridídae	*Antennella* sp. 1
			Gattya humilis
			Halopteris tuba
		Sertulariidae	*Amphisbetia operculata*
			Sertularella arbuscula

Mollusca		Calliostomatidae	*Calliostoma ornatum*
		Fissurellidae	*Fissurella mutabilis*
	Nudibranchia		Nudibranch spp.
		Proctonotidae	*Bonisa nakaza*
Ochrophyta			Brown algae spp.
	Dictyotales	Dictyotaceae	*Dictyota* spp.
			Exallosorus spp.
			Zonaria subarticulata
Porifera			Ball sponge spp.
			Encrusting sponge sp. 1
			Encrusting sponge sp. 2
			Encrusting sponge sp. 3
			Encrusting sponge spp.
			Fan sponge sp. 1
			Fan Sponge spp.
			Orange finger sponge sp. 1
			Finger sponge spp.
			Sponge sp. 1
			Sponge spp. 1
			Sponge spp. 2
			Sponge spp. 3
			Sponge spp. 4
	Axinellida	Axinellidae	*Axinella* sp. 1
	Dictyoceratida	Ircinidae	*Ircinia* sp. 1
			Psammocinia hawere
			Psammocinia sp. 1
	Haplosclerida	Chalinidae	*Haliclona* sp. 1
			Haliclona sp. 2
			Haliclona sp. 3
			Haliclona sp. 4
	Merliida	Hamacanthidae	*Hamacantha* sp. 1
	Poecilosclerida	Acarnidae	*Cornulum* sp. 1
		Chondropsidae	*Psammoclema* sp. 1
			Psammoclema sp. 2
			Psammoclema sp. 3
		Isodictyidae	*Isodictya ectofibrosa*
			Isodictya frondosa
			Isodictya grandis
		Latrunculiidae	*Latrunculiidae* sp. 1
			Tsitsikamma sp. 1
		Microcionina	*Clathria (Axosuberites) nervosa*
			Clathria (Clathria) axociona
			Clathria (Isociella) oudekraalensis
			Clathria (Thalysias) oxitoxa
		Tedaniidae	Tedania spp.
	Polymastiida	Polymastiidae	*Proteleia sollasi*
			Polymastiidae sp. 1
	Suberitida	Halichondriidae	*Ciocalypta* sp. 1
			Halichondria sp. 1
	Tethyida	Tethyidae	*Tethya magna*
	Verongiida	Aplysinellidae	*Aplysinellidae* sp. 1
Rhodophyta			Red algae spp.
	Corallinales	Corallinaceae	Red branching algae spp.
			Upright coralline algae spp.
		Hapalidiaceae	*Leptophytum* spp.
			Mesophyllum spp.
	Hildenbrandiales	Hildenbrandiaceae	*Hildenbrandia lecanellierii*

The multivariate species composition differed significantly between the shallow and deep reefs (Global R = 1, $p < 0.002$). From the 161 species identified, 78 were exclusive to the shallow and 38 to the deep reefs, and 45 species were common to both (Figure 3.3). Sponges were the most diverse taxonomic group with a total of 43 species, of which 19 species were exclusive to the deep reef. This pattern stands in sharp contrast to that observed for ascidians. Of the 29 identified ascidian species, 20 were exclusive to the shallow reef, and only one ascidian species was unique to the deep reef.

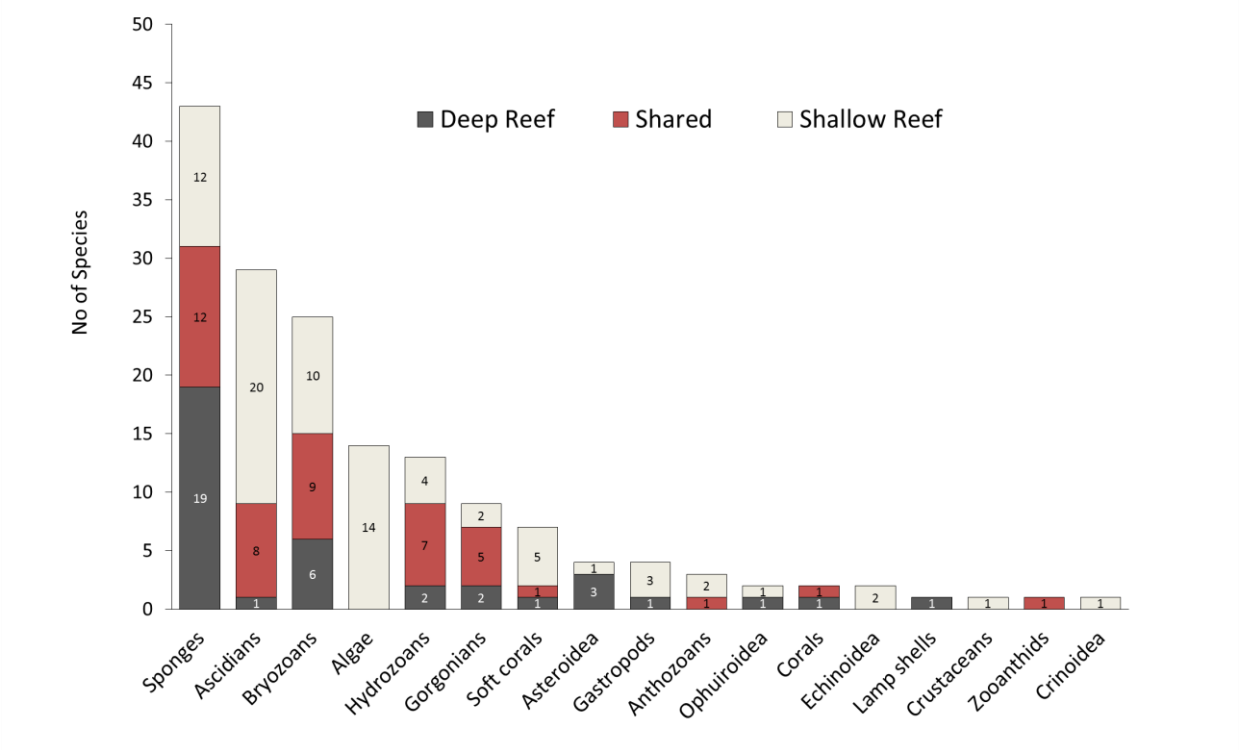


Figure 3.3. Macrobenthic species richness. The total number of species identified in each taxonomic group, indicating the contribution of species exclusive to the shallow (grey) and deep (black) reefs. Sections in red represent species found on both reefs.

When considering the contributions of the major taxonomic groups in terms of percentage cover, ascidians, algae and sponges represented similar contributions on the shallow reef (Figure 3.4). On the deep reef, sponges and bryozoans were the dominant taxa, and ascidians contributed very little to percentage cover. Although sponges were represented by more species on the deep reef, they contributed slightly less in terms of percentage cover (Figure 3.4) owing to the lower overall percentage cover of macrobenthos on the deep reef (Figure 3.1).

As indicated by the SIMPER procedure, the shallow macrobenthic assemblage was dominated by an encrusting pink coralline algae taxa (*Leptophytum* spp.; 3.66%), a colonial ascidian (*Polyclinum isipengense*; 3.21%) and a red encrusting sponge (*Clathria oudekraalensis*; 3.02%), with a within group similarity of 64.8%. The deep reef macrobenthic assemblage was dominated by encrusting sponges (5.39%), brown scrolled false coral (*Laminopora* sp. 1; 5.16%) and unidentified sponges

(grouped; 4.01%), with a within group similarity of 63%. The average dissimilarity between the shallow and the deep reefs was 79.26%.

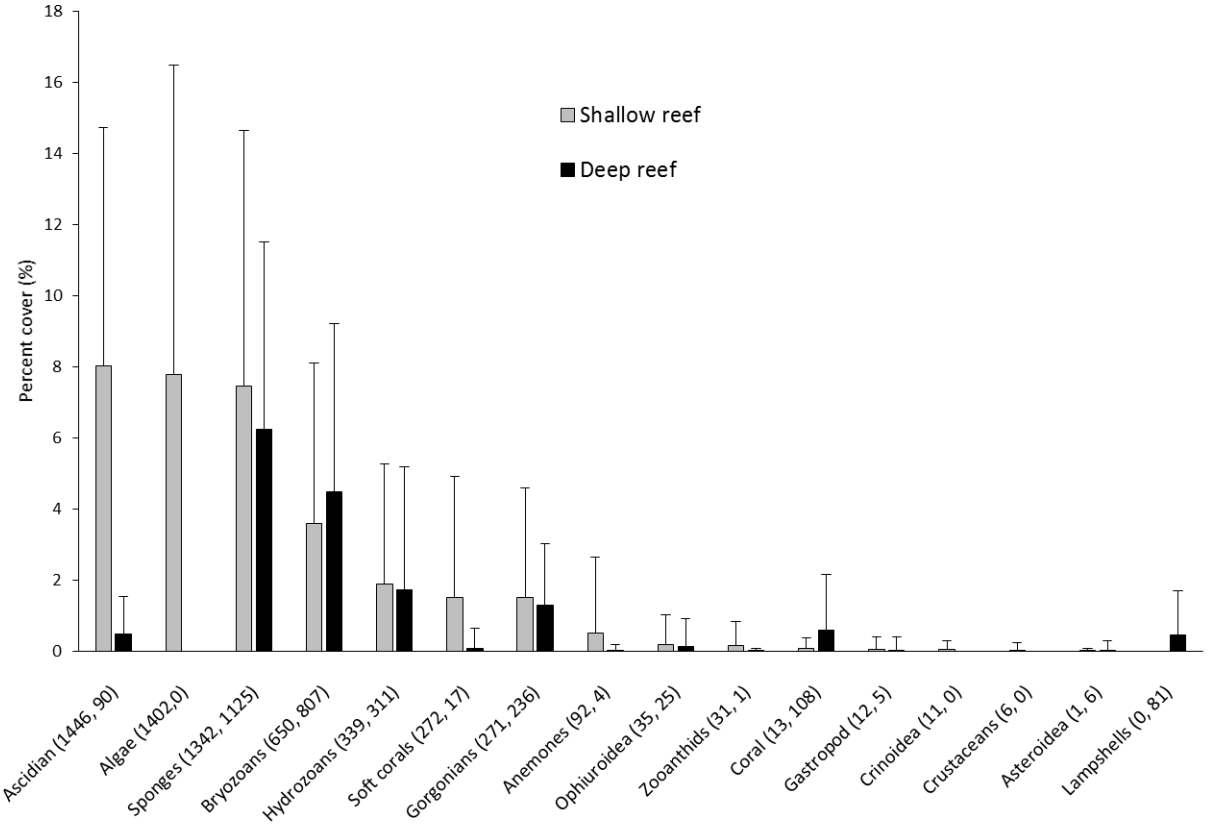


Figure 3.4. Average percent cover of the major taxonomic groups. Percentage cover of the shallow (grey) and deep (black) reefs in Tsitsikamma. Error bars represent standard deviation, values in brackets represent the sample size for the shallow and deep reefs, respectively.

B. INDICATOR SPECIES

Due to the small overlap in species composition between the shallow and the deep reefs, many species were identified as significant indicators. From the 148 species selected for the analyses, 57 were significant indicator species: 36 for the shallow reef and 21 for the deep reef. The high number of significant indicator species for the shallow reef was reduced to include only those species with an *IndVal* index of one, which amounted to 18 indicator species for the shallow reef (Table 3.3). A full list of significant indicator species for the shallow reef is provided in the appendix (Table A3.5).

On the shallow reef, most indicator species were either algae or ascidians. Other major taxonomic groups included the low growing and encrusting sponges, soft corals, hydroids and a single bryozoan species (Table 3.3). Indicator species for the deep reef were mostly sponges and bryozoans. Interestingly, the indicator species for the shallow reef were all low growing or encrusting forms, whereas many of the sponge indicator species for the deep reef demonstrated upright growth.

Table 3.3. Indicator species for the shallow (I) and deep (II) reefs. Species were identified using the *IndVal* method, where A is a measure of specificity (the degree to which a species is found only in a given group of sites), B is a measure of fidelity (the degree to which a species is present at all sites of a group), and the *IndVal* statistic is the degree to which a species is an indicator for the group of sites (i.e. reefs).

	Taxonomic categories or species	Common name	A	B	IndVal index	p value
I.	SHALLOW INDICATOR SPECIES					
	Algae					
	Corallinaceae spp.	Upright coralline algae	1.000	1.000	1.000	0.004
	Hildenbrandia lecanellierii	Black encrusting algae	1.000	1.000	1.000	0.004
	Leptophytum spp.	Pink thin coralline crust	1.000	1.000	1.000	0.004
	Mesophyllum spp.	Purple thin coralline crust	1.000	1.000	1.000	0.004
	Sponges					
	Haliclona sp. 1	Grey encrusting sponge	1.000	1.000	1.000	0.004
	Isodictya ectofibrosa	Wall sponge	1.000	1.000	1.000	0.004
	Tedania spp.	Oscular sponges	1.000	1.000	1.000	0.004
	Cnidaria					
	Anthozoa					
	Alcyonium fauri	Purple soft coral	1.000	1.000	1.000	0.004
	Hydrozoa					
	Lytocarpia formosa	Rusty feather hydroid	1.000	1.000	1.000	0.004
	Macrorhychia filamentosa	Smokey feather hydroid	1.000	1.000	1.000	0.004
	Bryozoans					
	Cryptopolyzoon concretum	Sand sausage	1.000	1.000	1.000	0.004
	Ascidians					
	Ascidian sp. 1	Orange glow ascidian	1.000	1.000	1.000	0.004
	Didemnidae sp. 1	Light didemnum	1.000	1.000	1.000	0.004
	Distaplia skoogi		1.000	1.000	1.000	0.004
	Polyandrocarpa anguinea	Large zooid ascidian	1.000	1.000	1.000	0.004
	Polyandrocarpa sp. 1	Small zooid ascidian	1.000	1.000	1.000	0.004
	Pseudodistoma sp. 1	Red lobed ascidian	1.000	1.000	1.000	0.004
	Pycnoclavella filamentosa	Feather sand ascidian	1.000	1.000	1.000	0.004
II.	DEEP REEF INDICATOR SPECIES					
	Sponges					
	Haliclona sp. 2		1.000	1.000	1.000	0.005
	Fan sponge		0.982	1.000	0.991	0.005
	Finger sponge		0.970	1.000	0.985	0.005
	Psammocinia sp. 1	Calcified cup sponge	1.000	0.833	0.913	0.027
	Proteleia sollasi	Papillae sponge	1.000	0.833	0.913	0.027
	Clathria (Thalysias) oxitoxa	Red fan sponge	1.000	0.833	0.913	0.027
	Isodictya frondosa	White hand sponge	1.000	0.833	0.913	0.027
	Clathria (Clathria) axociona	Thin finger sponge	1.000	0.833	0.913	0.027
	Cnidarians					
	Anthozoa					
	Scleractinia (hard corals)					
	Caryphyllia sp. 1	Cup coral	0.849	1.000	0.921	0.028
	Alcyonacea (sea fans)					
	Homophytum verrucosum	Gorgonian twig coral	0.992	0.833	0.909	0.023
	Bryozoans					
	Phidoloporidae	Deep lacy false coral	1.000	1.000	1.000	0.005
	Adeonella sp. 1	Forked false coral	1.000	1.000	1.000	0.005
	Soft false coral	Soft false coral	1.000	1.000	1.000	0.005
	Adeonella sp. 3	Soft forked false coral	1.000	1.000	1.000	0.005
	Tennysonia spp.	Thin branching false coral	1.000	1.000	1.000	0.005
	Laminopora sp 1.	Brown false coral	1.000	1.000	1.000	0.005
	Celleporaria sp. 1	Encrusting bryozoan	0.954	1.000	0.977	0.007
	Flustramorpha spp.	Branching moss animal	0.951	1.000	0.975	0.005

C. GUILD STRUCTURE

Twenty-one guilds were identified from the species assemblage data. Both reefs were dominated by animals with a colonial body plan, all of which were suspension-feeders (Figure 3.5). Solitary animals were considerably less common, contributing only 3.2% and 7.1% to the shallow and deep reefs, respectively (see appendix Figure A3.6 for the contributions of solitary animals with different feeding strategies to the reef communities). Nevertheless, the guild structure and composition differed significantly between the shallow and deep reefs (ANOSIM: Global $R = 1$; $p < 0.002$). The SIMPER procedure indicated that the dissimilarity (41.6%) in guild structure between the shallow and deep reefs was mostly attributable to the absence of primary producers on the deep reef. Upright and encrusting algae collectively contributed 21.1% to the dissimilarity between the reefs.

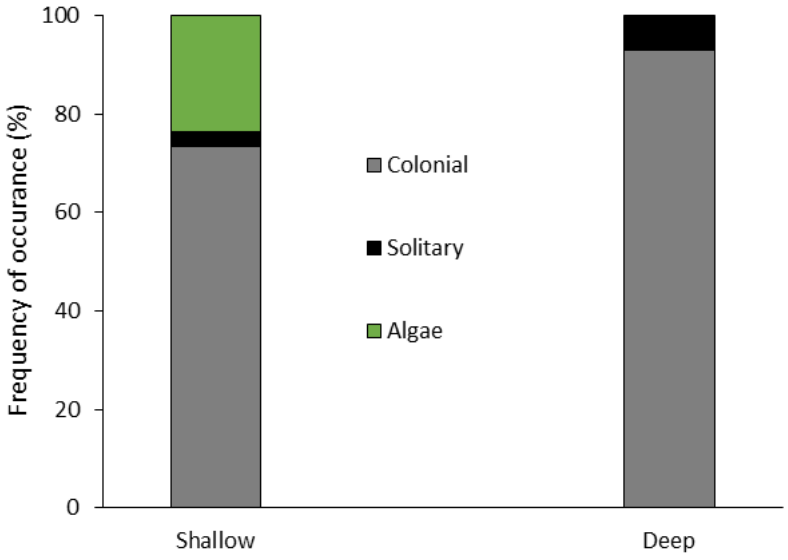


Figure 3.5. Occurrences of different body plans of the macrobenthos at the shallow and deep reefs. Percentage frequency of occurrence of different body plans exhibited by macrobenthos at the shallow and the deep reefs. While colonial animals, all of which were suspension-feeders, dominated both reefs, algae were absent from the deeper reef.

Examination of the different feeding strategies employed by the colonial suspension-feeders (Figure 3.6) revealed the presence of tree-like, combined active-passive suspension-feeders (sponges) on the deep reef. This guild was absent on the shallow reef and contributed 8.1% to the dissimilarity between the reefs. Feeding strategies of the tree-like growth forms on the deep reef were more diverse (included all forms of colonial suspension feeding strategies) and comprised numerous taxonomic groups (sponges, bryozoans, hydroids and sea fans), suggesting that conditions on the deep reef were favourable for upright growing forms. Almost all of the solitary suspension-feeders were passive feeders and included a diverse group of taxa (cup corals, anemones, basket, brittle and feather stars; see appendix for details, Figure A3.6 and Table A3.6).

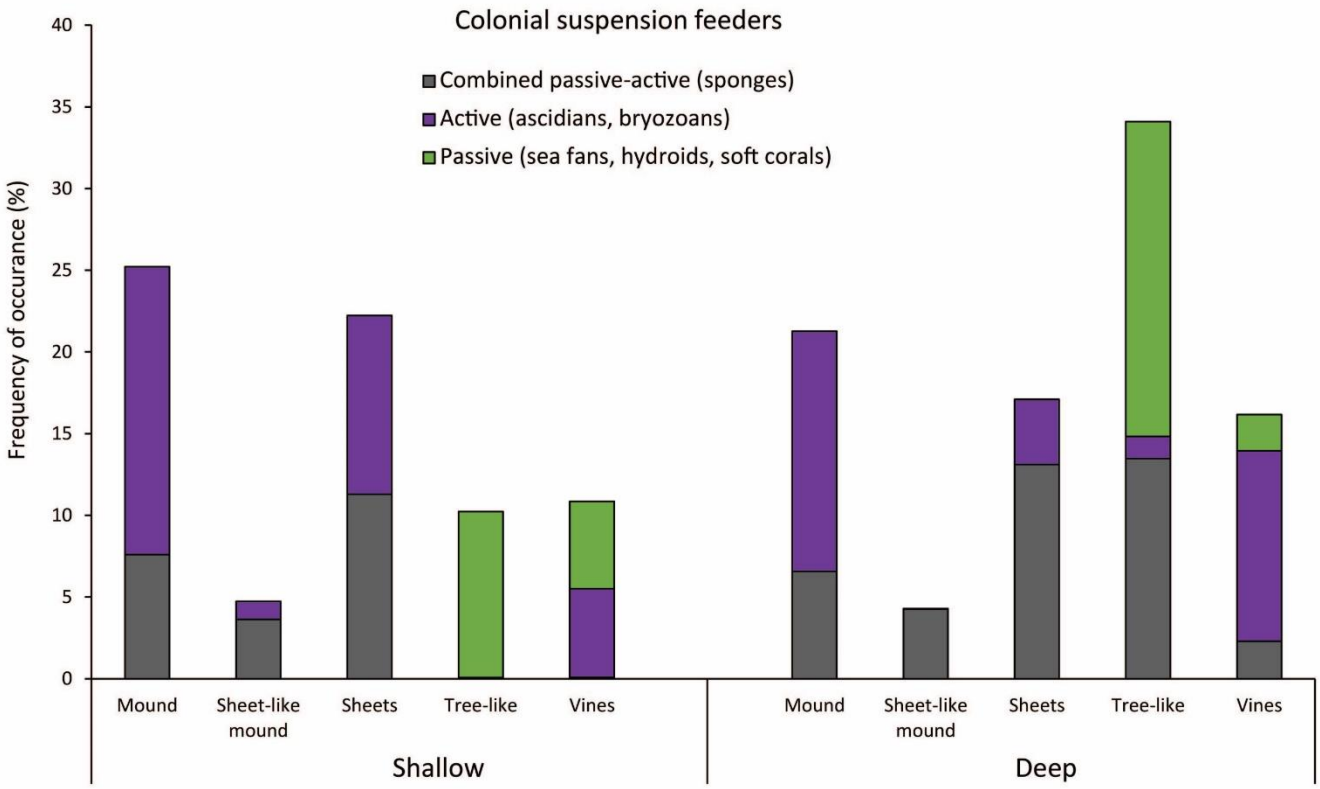


Figure 3.6. Frequency of occurrence of types of colonial suspension-feeders. The three feeding strategies employed by suspension-feeders are indicated for the shallow and deep reef separately.

D. POPULATION SIZE STRUCTURE

Six of the seven species or groups analysed attained greater mean heights on the deep reef compared to the shallow reef (Figure 3.7). Noble coral heights ranged from 1.8 to 41.8 cm and the mean height was significantly smaller (U = 41887; $p < 0.01$) on the shallow reef (7.8 ± 3.8 cm) compared to the deep reef (14.8 ± 9.4 cm). Similarly, the individuals of palmate sea fans were significantly smaller (U = 1629; $p < 0.01$; 21.8 ± 15.9 cm) on the shallow reef compared to the deep reef (29.7 ± 11.0 cm). In contrast, the nippled sea fans were significantly larger (U = 1140; $p < 0.001$) on the shallow reef (9.0 ± 3.6 cm) compared to the deep reef (5.4 ± 2.5 cm).

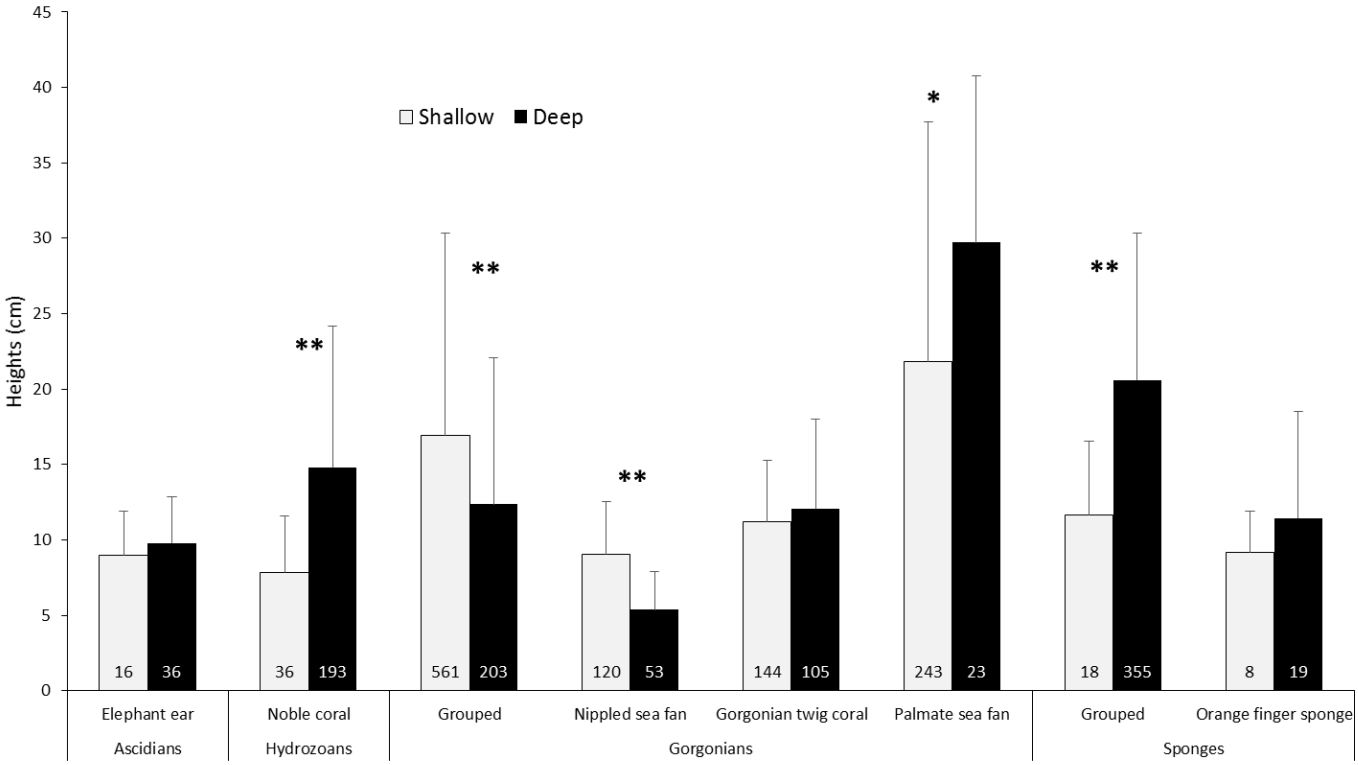


Figure 3.7. Heights of upright growing macrobenthos. Mean heights of upright growing macrobenthic species or groups on the shallow (grey) and deep (black) reefs. Values indicated at the base of each bar represent the number of individuals measured (* = $p < 0.01$; ** = $p < 0.001$; error bars indicate positive standard deviations).

Upright sponges were almost completely absent on the shallow reef, and as a result height comparisons of individual sponge species between the shallow and deep reefs were not possible. Of the 382 sponge measurements obtained overall, only 18 measurements were obtained on the shallow reef (Figure 3.8), and only orange finger sponge produced a representative sample from both reefs. Consequently, all remaining measured sponge heights were grouped to allow for statistical analysis (Figure 3.7). The heights of sponges ranged from 3.9 to 53.0 cm and were significantly smaller ($U = 1647$; $p < 0.001$) on the shallow reef (11.6 ± 4.9 cm) compared to the deep reef (20.6 ± 121 cm).

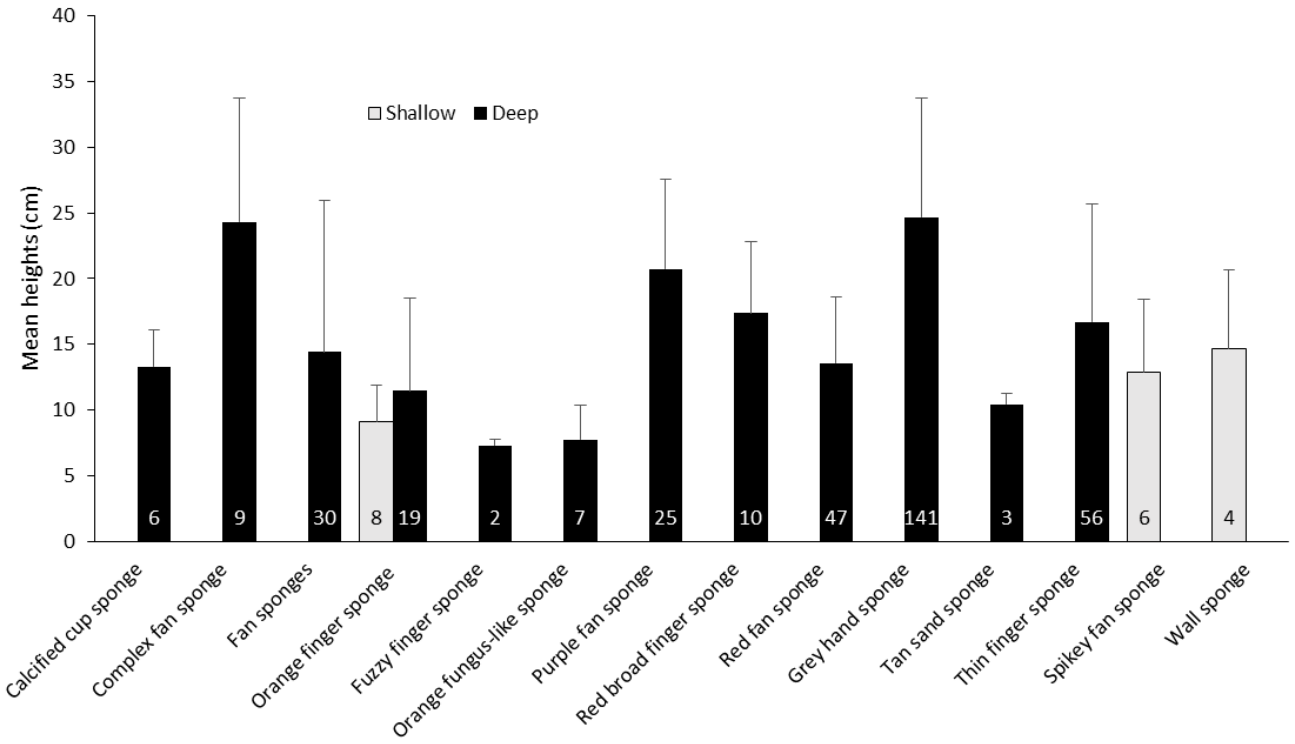


Figure 3.8. Heights of upright growing sponge species. Mean heights (cm) of sponge species at the shallow (grey) and deep reefs (black). Values indicated at the base of each bar represent the number of individuals measured. Error bars indicate positive standard deviations.

Similar to the trends in mean heights (Figure 3.7), the KDEs for the shallow and deep populations of noble corals and palmate sea fans were significantly larger on the deep reef (Figure 3.9 C, G). This finding contrasted the height structure of the nippled sea fan population, which were significantly smaller on the deep reef (Figure 3.9 E). The populations of gorgonian twig corals, palmate sea fans and noble corals demonstrated a bimodal distribution and the remaining species a single mode. With the exception of the deep population of palmate sea fans, the length frequency curves of all four species were positively skewed (g_1), suggesting a predominance of smaller size classes on both reefs. Skewness for the deep population of palmate sea fans and both shallow and deep populations of noble corals were near zero, suggesting a symmetric size distribution. Significantly positively skewed size distributions were observed in both populations of nippled sea fans (shallow $g_1 = 1.2$ and deep $g_1 = 1.08$; $p < 0.01$ for both) and gorgonian twig corals (shallow and deep $g_1 = 1.03$; $p < 0.01$). The shallow population of palmate sea fans was strongly positively skewed (1.7; $p < 0.01$). Tests for shape only (Figure 3.9 B,D,E) resulted in populations demonstrating the same size distribution shape with no significant differences between the reefs.

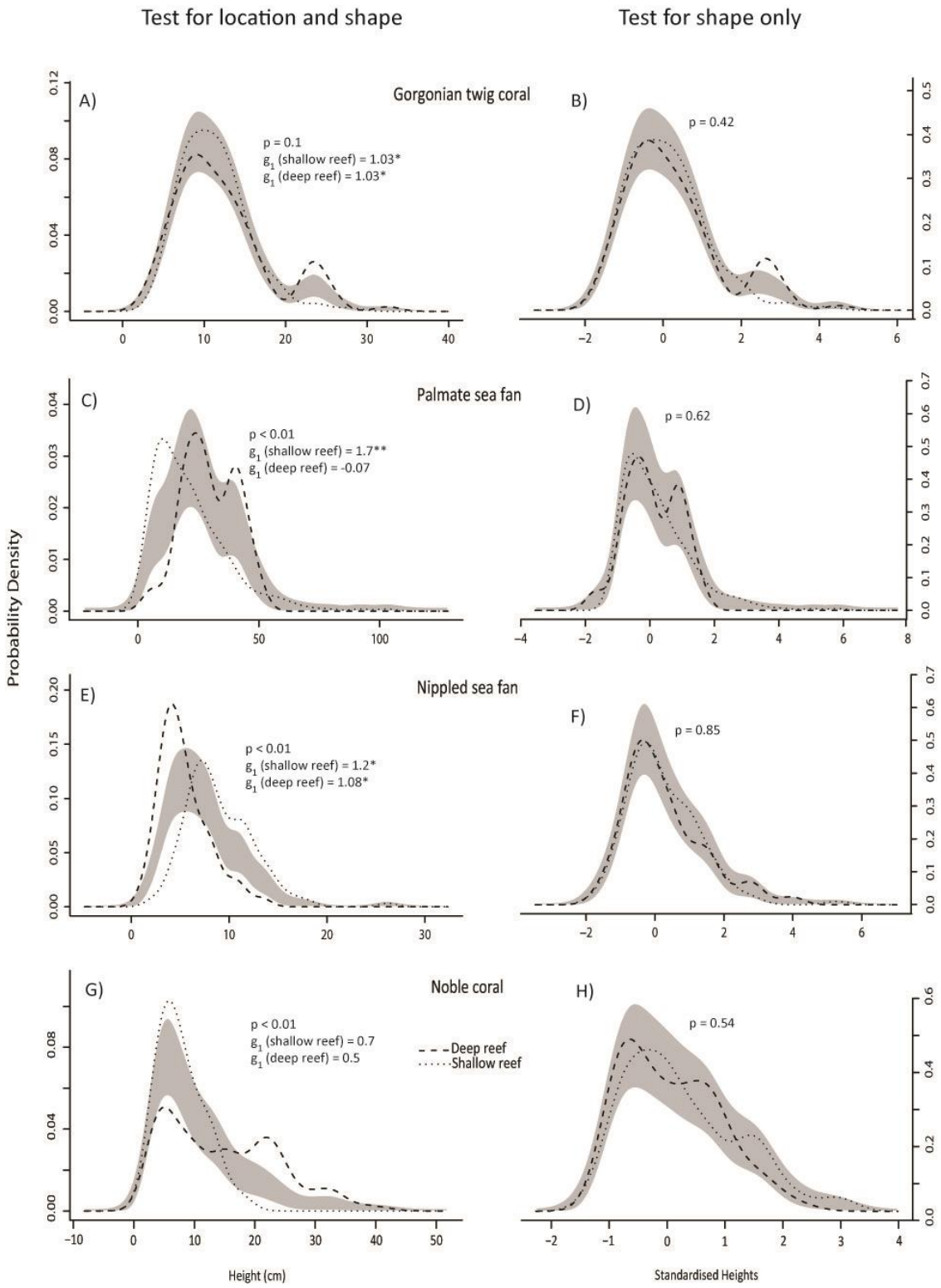


Figure 3.9. Kernel density estimates (KDEs) of upright macrobenthos. Comparison of KDEs of sea fan species (a – f) and a hydrozoan (g & h) sampled on the shallow (dotted lines) and deep (dashed lines) reefs. The grey areas denote one standard error above and below the null model of no difference between shallow and deep reef KDEs. If a size frequency distribution curve falls outside this band it is significantly different to that resulting from permutations of the data. Curves in the left column test for both shape and location, and the standardised data (right column) test only for differences in shape. Skewness (g_1) determines if the shape of the curve is significantly asymmetrical. Significance codes: * = p < 0.01; ** = p < 0.001.

3.3.3 ENVIRONMENTAL PARAMETERS

The ANOSIM procedure indicated that environmental conditions differed significantly between the shallow and deep reefs ($R = 0.7$; $p < 0.002$). Depth explained 92.2% of the variability observed between the shallow and deep reef macrobenthic assemblages (BEST test: Spearman correlation coefficient $\rho = 0.922$; $p < 0.002$). Because an increase of water depth typically results in corresponding changes in other physical parameters (e.g. decrease in light intensity), a second BEST procedure was run with depth excluded, thereby clarifying the importance of the remaining environmental variables on species composition. The results revealed that the percentage cover of settled PM on the reefs explained 79.3% ($p < 0.002$) of the differences observed in the macrobenthic assemblages between the shallow and deep reefs. Similarly, 89.1% ($p < 0.001$) of the differences in the guild composition between the reefs were due to the difference in depth. Excluding depth, settled PM explained 78.1% ($p < 0.002$) of the variability observed in guild structure. With an increase in depth there was a clear gradient from very little settled PM on the shallowest sample site (13m; 0.6% settled PM) to large amounts on the deepest site (73 m), on which 57% of the reef was covered by settled PM (Figure 3.10). Current speed was not measured during the present study. However, the increase in settled PM from the shallow to deep reef can be considered a proxy for current speed. This inference was made based on the fact that lower current velocities result in the increase in the settlement of particles from the water column (Sundborg 1956).

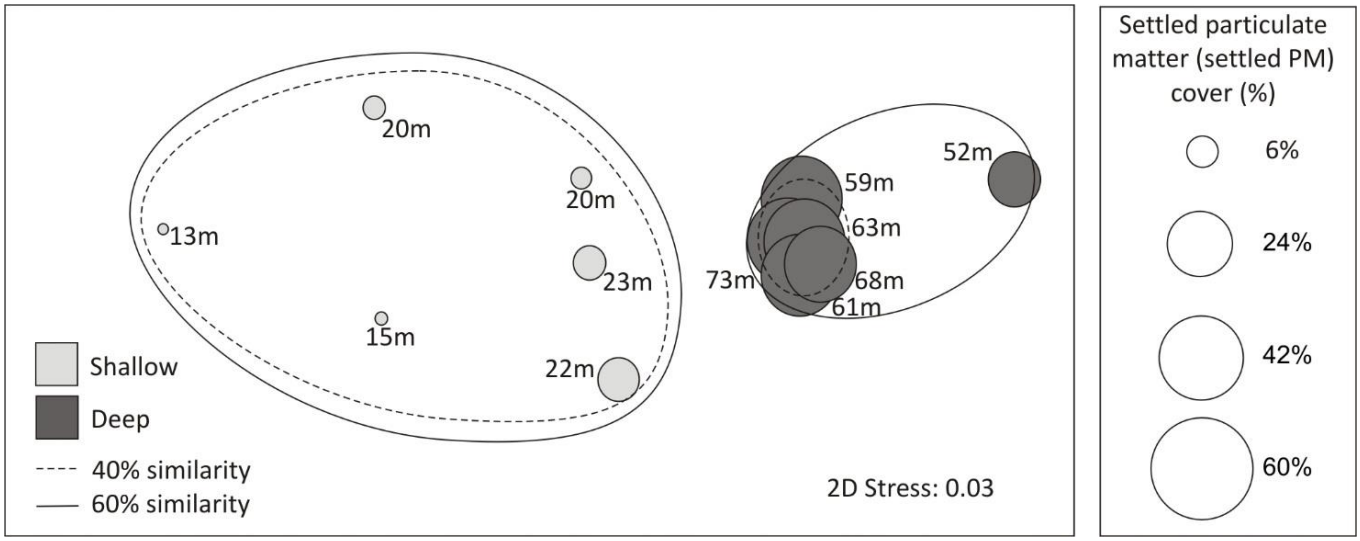


Figure 3.10. Multidimensional scaling plot (MDS). MDS of the species assemblage data employing Euclidian distance measures. The percentage cover of settled particulate matter at each site is superimposed as bubbles. Shallow reef sample sites are indicated in light grey and deep reef sample sites in dark grey. Sample site depths are provided in meters.

The BEST results indicated that depth plays a central role in structuring the macrobenthic assemblages. This result was further supported by the LINKTREE analysis, which produced four significantly different macrobenthic clusters, each associated with a specific depth range (Figure 3.11). To establish if the macrobenthic assemblages were influenced by the depth gradient, depth was excluded as a parameter in the LINKTREE analysis. Consequently, if species clustered according to a specific depth range, it provided confirmation of the importance of depth in structuring macrobenthic species. The four clusters separated into two shallower clusters confined to the shallow reef, and two deeper clusters confined to the deep reef. Each cluster had a set of associated environmental variables that was responsible for the internal structure of that cluster. The first split separated the shallow reef from the deep reef assemblage. The shallow reef was characterised by low settled PM cover and greater light intensity compared to the deep reef (Figure 3.11; ANOSIM $R = 1$; $\pi = 17.6$; $p < 0.001$). The linkage tree further split the shallow reef macrobenthic assemblage into clusters A and B. This split was based on higher light intensities and less settled PM cover observed in cluster A compared to B (ANOSIM $R = 0.75$; $\pi = 1.78$; $p < 0.003$). Further distinction of the deep reef into two smaller clusters was due to higher light intensities, and less settled PM and sand cover on the shallower cluster C (52m; in fact a single site) compared to the deeper cluster D (average depth: 64.8 m; ANOSIM $R = 1$; $\pi = 3.49$; $p < 0.001$).

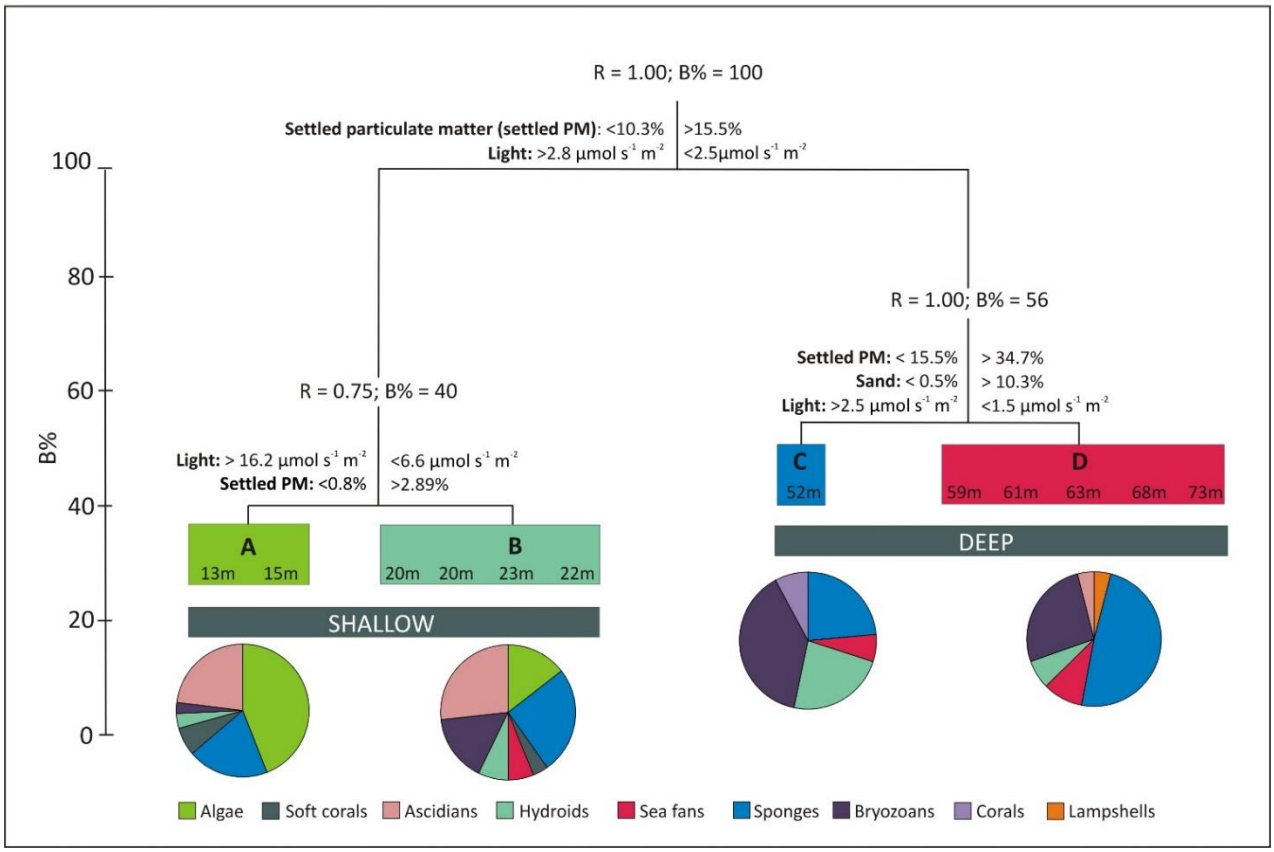


Figure 3.11. LINKTREE analysis. Linkage trees explain the division of each cluster (based on species assemblage data; percentage contribution indicated by pie charts) with a set of environmental variables specific only to that group. Global R provides information on the within group similarity and ranges from 0 to 1 (0 indicating 'no difference' and 1 signifying 'completely different' communities). On the y-axis, B (%) signifies an absolute measure of dissimilarity between the clusters.

As light intensity decreased and settled PM cover progressively increased with depth, a changeover of the dominant macrobenthic taxa became evident (see pie charts: Figure 3.11). Algal cover decreased from cluster A to B, and was absent from the deep reef. This loss can be explained by the rapid decrease in light intensity, and it seems that on the shallow reef algae were being replaced by ascidian and bryozoan cover with depth. On the deep reef, ascidians were again replaced by hydrozoans and bryozoans. Within the deep reef sites, hydrozoan cover declined from cluster C to D, and on cluster D sponges accounted for half of the macrobenthic cover. Sponges were consistently present at all depth ranges, but became the dominant cover of sites around 60m and deeper.

3.4 DISCUSSION

The main aim of this chapter was to determine if changes in abiotic variables associated with an increase in depth correlate with the patterns identified in macrobenthic assemblages of Tsitsikamma.

Specifically, a shallow (<25 m) and a deep (45 – 75 m) reef site were compared in terms of species richness and composition, indicator species, guilds and population size structure. These macrobenthic communities were further explored to establish if the depth gradient caused clear zonation patterns.

The results revealed a clear change in the macrobenthic assemblage and size structure related to depth. The shallow and deep reefs differed significantly in all measured aspects, and a finer scale depth zonation was apparent within each reef. Initial changes in environmental variables were drastic, and within the first few meters light intensity and current speed (as inferred from increase in settled PM with depth) declined rapidly. With an increase in depth, changes in light intensity became less pronounced. This pattern was mirrored in the macrobenthic assemblages, where initial changes occurred quickly, and with an increase in depth became less distinct. As such, the data supported the hypothesis that depth related changes in environmental parameters alter the macrobenthic assemblage structure and guild composition.

3.4.1 ASSEMBLAGE COMPOSITION

3.4.1.1 SPECIES RICHNESS & GUILD STRUCTURE

Overall, the rocky reef macrobenthic assemblage of the study area was dominated by colonial suspension-feeders, which contributed 83% to the reef cover (excluding substrate type). The rest of the macrobenthos consisted of algae (12%) and solitary suspension-feeders and grazers (5%). Space on which to live is one of the most important limiting resources for hard-substrate communities (Jackson 1977). Space is vital, because to survive, macrobenthic organisms need space to gain access to food or light (Sebens 1986). Colonial species monopolise hard-substrate habitats due to the competitive advantage associated with modular growth (Jackson 1977, 1979). Through asexual reproduction, indeterminate growth and morphological adaptations (to reduce fouling by other animals), colonial animals can outcompete solitary species for space (Jackson 1977). Consequently, most species that inhabit hard substrates demonstrate colonial growth (Woodin & Jackson 1979), which explains the dominance of colonial animals observed on the shallow and deep reefs in Tsitsikamma.

Because colonial macrobenthic species are sedentary and cannot forage for food, they rely on water movement to bring suspended particles into contact with their feeding structures (Okamura & Partridge 1999) and to disperse their propagules (Russ 1982, Palardy & Witman 2011). Consequently, water flow is key in determining the distribution of sedentary macrobenthic species (Gili & Coma 1998). A recent study conducted on the deep reef (Middlebank Reef; TNP MPA) indicated that

current velocity decreased with depth (Roberts & van den Berg 2005, Hancke 2010), a seemingly wide-ranging pattern observed on the Agulhas Bank (Boyd et al. 1992) and elsewhere around the South African coast (Roberts et al. 2006). This decrease in current speed explains the observed increase of settled PM with depth (Figure 3.10). As such, food (water movement) and light change predictably with depth, and can be incorporated into a niche axis (Figure 3.12) on which macrobenthic species are lost or gained, depending on strategies evolved to attain limiting resources. Because colonial suspension-feeders represent a guild of species that exploit the same set of resources, guild membership means strong competition for space and food. As a consequence, guild members evolved different structural adaptations that allowed for slight differences in resource acquisition. Modifications in resource acquisition can result in resource partitioning/niche differentiation that enables co-existence (Blondel 2003, Booth & Murray 2008), a mechanism thought to drive diversity (Silvertown 2004, Slatyer et al. 2013). Change-over in species on the niche axis resulted in depth zonation within the macrobenthos, and will be discussed below (Section 3.3.2).

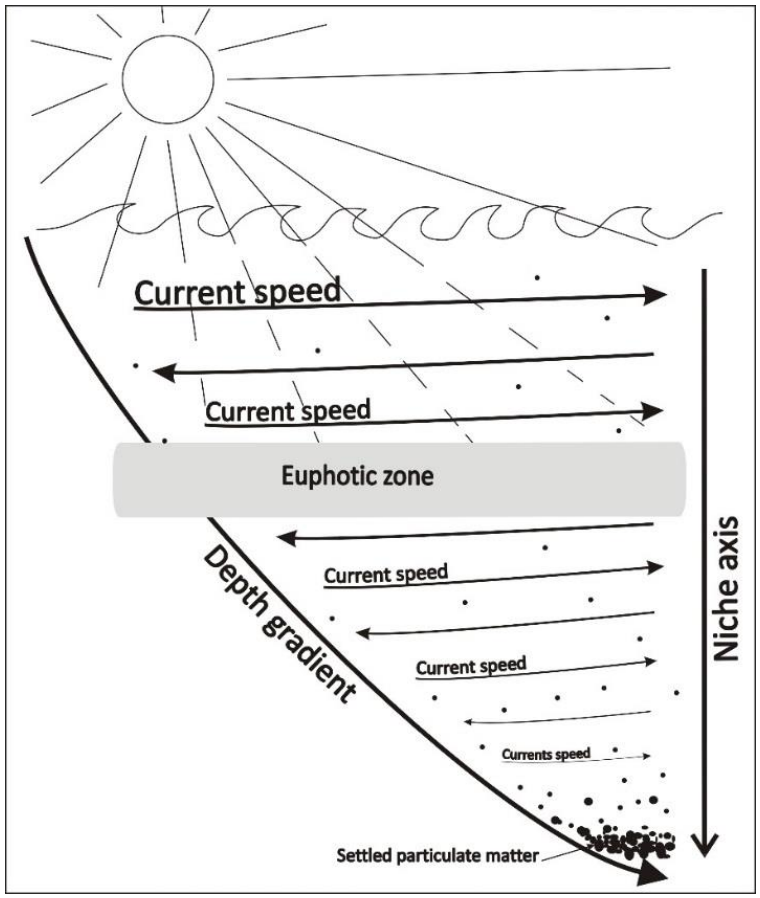


Figure 3.12. Niche axis. Conceptual diagram depicting the niche axis as an aid to explain the macrobenthic species distribution patterns in the study area. With an increase in depth, predictable environmental changes occur: light intensity and current speed decreases. These changes in environmental variables, in turn, impact the distribution of primary producers (which cannot photosynthesise below the euphotic zone). Current velocity influences settlement of PM on reefs, and the feeding of suspension feeding species.

Patterns in macrobenthic density and diversity observed here and elsewhere can be, apart from light intensity, explained by current speed. Several authours have demonstrated, both experimentally and emperically, that flow speed drives both denisty and diversity (Gili & Coma 1998, Palardy & Witman 2011). Their results proved that a decline in current intensity and speed resulted in the progressive decrease in species richness and density of suspension-feeders (Gili & Coma 1998, Palardy & Witman 2011), a trend also observed in this study. Loss of species was explained by the decline in delivery of reproductive propagules with decreased flow causing lower recruitment rates (Palardy & Witman 2011). Decline in the suspension feeder densities was explained by a reduction in the transport of suspended food particles, thus supporting fewer individuals. In Tsitsikamma (Middle Bank Reef), current speed decreased with depth, a change that explains the gradual loss of macrobenthic species and density from shallower towards deeper sites. An additional loss of species on the deep reef can be attributed to insufficient light for photosynthesis, which transpired in a loss of three guilds and their associated species (encrusting and upright algae and their grazers). In contrast, on the shallow reef, greater current speeds translated into higher recruitment rates and food availability. Higher concentrations of reproductive propagules and food particles increased macrobenthic diversity and density, which enhanced structural complexity (spatial variation in surface features), and in turn influencing patterns of near-bed water flow (Gili & Ballestros 1991, Cardinale et al. 2002). Small changes in water flow increased particle capture of suspension-feeders, thereby supporting greater diversity and biomass of suspension-feeders through interspecific, hydrodynamic facilitation (Cardinale et al. 2002).

3.4.1.2 POPULATION SIZE STRUCTURE

None of the size-frequency distribution curves of either the shallow or deep populations of noble corals, palmate sea fans, gorgonian twig corals or nippled sea fans found in Tsitsikamma were significantly negatively skewed (Figure 3.9). This finding suggests that healthy and viable populations represented by many small individuals exist. Healthy populations are positively skewed, indicating adequate recruitment into the population (Bak & Meesters 1998, Meesters et al. 2001, Ortiz 2011). Degraded populations tend to be more negatively skewed, an indication that the population is aging without replenishment (Bak & Meesters 1998). The KDEs testing for ‘shape only’ (Figure 3.7) indicated that the shallow and deep populations in Tsitsikamma did not differ significantly, thereby suggesting similar environmental effects and historical processes impacted both reefs in the past (Bak & Meesters 1998). The lack of differences in the shapes of the length frequency curves between reefs suggests that the TNP MPA is well established and stable, and that neither the shallow nor deep macrobenthic populations of the measured species have been recently exposed to strong disturbances.

3.4.2 DEPTH RELATED ZONATION

The environmental forcing associated with the depth gradient resulted in the formation of four significantly different macrobenthic clusters (Figure 3.11), all falling within a specified depth zone. The depth gradient can be interpreted as a niche axis, on which niche availability and consequently species or guilds are lost or gained (Figure 3.12). The depth at which the clusters split may represent a position on the niche axis where resources are gained, lost or altered (Silvertown 2004). This shift in resource availability may explain the observed depth zonation of the macrobenthos, as specific adaptations associated with resource acquisition limit their distribution (Wing & Jack 2012, Dubois & Colombo 2014).

Environmental filtering selects species with similar adaptive traits that allow them to survive in a particular habitat (Ingram & Shurin 2009). These species may be ecologically similar because they share common ancestry or because they independently evolved similar adaptive traits (Ingram & Shurin 2009). Here, for instance, colonial suspension-feeders formed a guild that exploits the same set of resources in a similar manner. However, exploitation of a similar resource results in potentially significant overlaps in niche requirements (Root 1967). Therefore, for members of the same guild to co-occur, they have to limit their similarity in resource acquisition (Ingram & Shurin 2009). The principal of limiting similarity suggests that although members of a guild share some structural and morphological adaptations due to environmental filtering (e.g. colonial suspension-feeders), competition for the same resource would further drive trait evolution to allow species co-existence (Macarthur & Levins 1967, Ingram & Shurin 2009). For colonial suspension-feeders, different feeding mechanisms that allow slight differences in resource acquisition can support species co-existence (Wing & Jack 2012). Because taxonomically closely related species (congeners) often share similar structural adaptations, it is not surprising that members of a guild often demonstrate a strong taxonomic link. This is also evident in the macrobenthic assemblages of Tsitsikamma, where several guilds are represented by a single higher taxonomic group (Table A3.2). Here, I attempted to relate the detected depth zonation with the structural adaptations (associated with resource acquisition) identified in major macrobenthic taxonomic groups. This comparison is achieved by identifying environmental parameters associated with each depth cluster, and comparing the physical adaptations of the major taxonomic groups most prolific in each cluster (Figure 3.11).

The first taxonomic groups lost along the niche axis were primary producers and their associated grazers. At the shallowest sites, encrusting and upright algae dominated. Light becomes limiting for photosynthesis below the euphotic zone which, depending on season and distance offshore, ranged between 21.3 and 54.2 m in Tsitsikamma (Figure 3.1). The remaining changes observed in the species

composition of the macrobenthos can be explained by the decrease in current speed with depth. The current speed at which different filter-feeders function optimally differs between taxonomic groups, and are caused by small differences in adaptations necessary to acquire suspended particles.

Active filter-feeders were present on both reefs; however, ascidians were present almost exclusively on the shallow reef and progressively replaced by bryozoan species with depth. Ascidians obtain food particles by actively filtering water through a mucous net (Petersen 2007), and grow best in high current speed conditions (Wing & Jack 2012). Bryozoans obtain food particles by actively beating ciliated tentacles (Hentschel & Shimeta 2008), and grow best at weaker current speeds (Eckman & Duggins 1993). These two mechanisms differ in terms of the current speed at which they can function optimally. Increased presence of settled PM on the deep reef (Figure 3.10) affected ascidian and bryozoan feeding. Where ascidian growth is slowed at high particle concentrations (due to the risk of clogging their filtering mechanisms, resulting in reduced retention and pumping rates; Kowalke 1999; Petersen 2007; Torre et al. 2012), bryozoans can discard unwanted particles through selective flicking, or expel their guts contents at unusually high particle concentrations (Riisgård & Manríquez 1997). In turbulent conditions, the feeding structures of bryozoans are deformed, thereby reducing growth and causing colony miniaturisation (Eckman & Duggins 1993, Okamura & Partridge 1999). These differences in feeding optimisation help to explain the absence of bryozoans at the very shallow sites (13 – 15 m; Figure 3.11) of Tsitsikamma, and their dominance at mid depths (52 m).

At the other extreme, sponges dominated depths where current speeds were low (cluster D: 59 – 73m; Figure 3.11). This pattern can be explained by the exceptional morphological plasticity of sponges, which are capable of modifying their body shape depending on prevailing flow conditions (Palumbi 1984, Okamura & Partridge 1999, Kaandorp 1999, Bell & Barnes 2000a). When current speeds are low, sponges can modify their growth to form upright tree-like shapes (Okamura & Partridge 1999, Kaandorp 1999). This morphology maximises particle capture through increased surface area, thereby feeding further in the water column (Wing & Jack 2012) and preventing sediment accumulation on sponge surfaces (Bell & Barnes 2000a). Furthermore, sponges are capable of feeding on a very large range of particle sizes (Jackson & Winston 1982). This flexibility provides sponges with a competitive advantage at all depths, but particularly on deeper reefs, and is reflected in the higher species richness and relative percentage cover at deeper sites within Tsitsikamma (Figure 3.1 & 3.2; respectively). Several studies conducted on sponge communities within different marine biogeographic regions, e.g. temperate reefs in Lough Hyne, Co Cork, Ireland (Bell & Barnes 2000a, b) or tropical reefs in south-east Sulawesi, Indonesia (Bell & Smith 2004), demonstrated similar findings regarding the morphological adaptations of sponges and their distribution related to current speed. These studies sampled sponge assemblages down to 30 m at sites that differed in

terms of current speed, intensity and direction (Bell & Barnes 2000a, b, Bell & Smith 2004). At very turbulent sites, Bell & Barnes (2000a) found that massive and encrusting forms dominated sponge communities. The shallowest sites in this study at Tsitsikamma were equivalent to the turbulent sites sampled by Bell & Barnes (2000a), and very little upright growth was observed here (Figure 3.8). Sites with low current speeds sampled by Bell & Barnes (2000a) were marked by high sedimentation, and upright sponges were most abundant. Similarly, Cluster D from the deep reef of Tsitsikamma can be regarded equivalent to the calm sites of Bell & Barnes (2000a), as low current speeds resulted in increased settlement of settled PM (Figure 3.10) and the prevalence of upright growth in sponges (Figure 3.8).

3.4.2.1 INDICATOR SPECIES

Indicator species reflect the prevailing environmental conditions (De Cáceres & Legendre 2009). It was therefore not surprising that there were strong correlations among the indicator species, the prevailing environmental conditions and the dominant taxa found within each cluster (zone), as it presents the realised niche space those species occupy on the niche axis. Although indicator species were not established for the individual clusters identified in Figure 3.11 (because cluster C comprised of only one site, and *Indval* statistics were not possible to calculate), the indicator species for the shallow and deep reefs demonstrated close associations with the zonation patterns described above. Indicator species for the shallow reef were represented by algae and encrusting and massive sponges and ascidians, which require either sufficient light to survive or are best suited for turbulent hydrodynamics. Indicator species for the deep reef were mostly bryozoans and upright growing sponges. Bryozoans are best adapted to intermediate current speeds, as described above, and the high number of bryozoan indicator species most likely represented the environmental conditions associated with the shallower zone of the deep reef (cluster C). The upright growing sponge species selected as indicators for the deep reef were best adapted to low current speed and intensity, and as such signified these environmental conditions in the deeper zone of the deep reef (cluster D).

3.4.3 CONCLUSIONS

The macrobenthic community of Tsitsikamma demonstrated a distinct changeover of species along the depth gradient. The changeover in species revealed a strong taxonomic link that could be explained by feeding adaptations best suited for particular environmental conditions associated with variable depths. Each zone represented a group of macrobenthic species that provides habitat for higher order consumers. The zonation patterns identified here represent specific habitat requirements for fish, and as such fish assemblages should demonstrate close association with the

depth zonation identified. Determination of fine-scale fish habitat association patterns provides further insight into the identification of priority habitats that require preferential consideration to ensure effective resource management, and this aspect was addressed in Chapter 4.

4 HABITAT SELECTIVITY OF THE SHALLOW & DEEP SUBTIDAL REEF FISH OF TSITSIKAMMA

4.1 INTRODUCTION

Extensive exploitation has led to the depletion of near-shore fish stocks, forcing fisheries to move further offshore and target deeper communities (Morato et al. 2006, Watson & Morato 2013). The increased fishing pressure on deep communities is of major concern as these are generally dominated by large predatory species (Macpherson & Duarte 1991). Furthermore, older and larger individuals are typically targeted by commercial and recreational fisheries, and these larger fish have exponentially greater fecundity, thereby contributing most to reproduction within a population (Birkeland & Dayton 2005, Garcia et al. 2012). The selective removal of larger individuals has further implications for critical life-history characteristics such as size at maturity, size at sex-change, sex ratio and growth rate (Buxton 1993, Law 2000, Allendorf & Hard 2009), and possibly causing cascading effects to lower level consumers and producers (Steneck 2012). It is therefore important to develop baselines on the distribution of fish assemblages inhabiting shallow and deep nearshore reefs to effectively manage our marine resources (Kahng et al. 2010, Fitzpatrick et al. 2012).

The spatial distribution of fish species is strongly influenced by the degree to which they are associated with a particular habitat type (habitat specialisation; Wilson et al. 2008). Reef fish are often highly resident, and characteristically occupy small home ranges (Buxton & Smale 1989, Edgar et al. 2004, Kerwath et al. 2007, Gunderson et al. 2008, Bryars et al. 2012). Ontogenetic shifts in habitat usage are common in most fish species, and are usually related to changes in diet and habitat preference (Booth & Buxton 1997, Griffiths & Wilke 2002, Wilson et al. 2008, 2010, Götz et al. 2008, Fitzpatrick et al. 2012). As a result, depth and habitat are two parameters that can explain a large amount of variability in fish assemblage patterns (Brokovich et al. 2008, Fitzpatrick et al. 2012). Depth and habitat are strongly related because large changes in abiotic variables (i.e. current speed, light intensity and temperature) occur within the first few meters of the water column (Garrabou et al. 2002, Brito 2013). These rapid changes in environmental variables are accompanied by

transformations in reef community structure and function, and so alter available habitat to fish (Friedlander & Parrish 1998, Brokovich et al. 2008).

In the global context, the Southern Africa's east and south coasts have been identified as a region that require preferential consideration for fisheries management (Worm & Branch 2012). The entire region harbours high levels of species richness and endemism, endures increasing catch trends but are characterised by ineffective management efforts (Tittensor et al. 2010, Worm & Branch 2012). Many South African endemic reef fish species are targets of commercial and recreational fisheries (Buxton 1992, Attwood et al. 2002). The majority of these endemics are sparids (family Sparidae), which are particularly susceptible to over-exploitation due to high levels of residency, longevity, vulnerability to barotrauma and likeliness of undergoing sex-change (Attwood et al. 1997; Turpie et al. 2000; Brouwer 2002; Cowley et al. 2002; Götz et al. 2008; Kerwath et al. 2013). A number of studies in South Africa showed that reef fish undergo depth related ontogenetic shifts in habitat use (Buxton & Smale 1989, Burger 1990, Mann & Buxton 1992, Götz 2005). Although the depths over which these studies were conducted are considered shallow (SCUBA diving depth; <30 m), the general and consistent finding of larger fish in the deeper regions of shallow reefs implies that more of the older and sexually mature individuals occur at depth.

With a clear understanding of fish-habitat associations, researchers can develop robust species distribution models. Such models are crucial in the design process of marine protected areas (MPAs) and MPA networks, which will ensure the protection of species throughout their entire life span, thereby improving conservation and fisheries management efforts (Moore et al. 2010, Young et al. 2010, Fitzpatrick et al. 2012).

4.1.1 STUDY AIM

With increasing demands and the mounting threats on South Africa's marine resources, compounded by a lack of information regarding patterns of fish habitat use on deep nearshore reefs, the main aim of this chapter was to identify patterns in habitat use of reef fish and characterise predictors that may explain these patterns.

More specifically, the depth gradient, characterised by predictable changes in abiotic variables, influences the macrobenthic community composition in Tsitsikamma (Chapter 3). Due to the specific niche/resource requirements of different fish species and life stages, I hypothesised that the fish assemblages in Tsitsikamma are influenced by the depth gradient and macrobenthic patterns.

The following specific hypotheses were tested:

iv) fish species composition differs between the shallow and deep reefs because resources change with depth and attract different suits of species

v) fish assemblages differ among the habitat types defined by the macrobenthos in Chapter 3 because each habitat type provides different niches for fish species

vi) fish species demonstrate depth related ontogenetic shifts in habitat use because diet preferences change with increasing size

If certain habitat types identified in Chapter 3 match certain resource requirements for a set of fish species, then habitat type may be investigated as a useful proxy to predict fish assemblage composition.

4.2 MATERIALS & METHODS

4.2.1 STUDY AREA

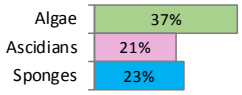
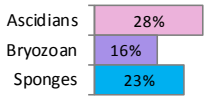
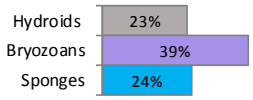
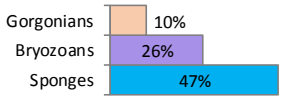
Research was conducted at the Middlebank and Rheeders Reef complexes situated close to the Storms River mouth in the Tsitsikamma National Park (TNP) MPA. A full study area description is provided in Chapter 2, Section 2.1.2.

4.2.2 SAMPLING STRATEGY

4.2.2.1 HABITAT TYPES

Habitat types were classified according to selected physical and biological parameters. Physical parameters included depth, light intensity and substrate type, whereas macrobenthic species assemblage data served to classify the biogenic environment. The macrobenthic assemblage structure and species composition were determined by estimating percentage cover from photoquadrats, and a LINKTREE analysis of these data identified four significantly different habitat types (Table 4.1). Details on the collection and processing of photoquadrats are provided in Chapter 3.2.2., along with a full description of the linkage tree (LINKTREE) procedure and results.

Table 4.1. Classification of habitat types. Habitat types were identified by means of LINKTREE analysis (Chapter 3). The major taxonomic groups, depth zones and the numbers of baited remote underwater stereo-video system (stereo-BRUVs) samples collected in each habitat are indicated.

	Reef	Depth zones	Physical characteristics	Dominant macrobenthic community	No of stereo-BRUVs samples
Habitat a	Shallow	11 - 17m	High light intensities (16.2 µmols $^{-1}m^{-2}$) < 0.2% rubble < 0.9% settled particulate matter		19
Habitat b	Shallow	18 - 25m	Med light intensities (2.8 - 6.6 µmols $^{-1}m^{-2}$) 2 - 6% rubble 3 - 10.3% settled particulate matter		9
Habitat c	Deep	45 - 55m	Low light intensities (2.5 µmols $^{-1}m^{-2}$) < 0.5% sand < 15.5% settled particulate matter		10
Habitat d	Deep	56 - 75m	Very low light intensities (< 1.4 µmols $^{-1}m^{-2}$) 10 - 16.8% sand 34 - 54% settled particulate matter		13

To determine whether the defined habitat types could predict the distribution of fish assemblages, stereo-BRUVs samples were allocated to the different habitat types (Table 4.1) according to the depth at which they were collected. Because the focus of this research was on reef communities, those stereo-BRUVs samples collected from sandy sites were excluded from the analyses, resulting in a somewhat uneven, but acceptable, stereo-BRUVs sample stratification.

4.2.2.2 FISH COMMUNITY SAMPLING

Reef fish assemblages were surveyed by means of non-destructive and non-extractive stereo-BRUVs. Stereo-BRUVs represent a standardised method that provides fisheries-independent data that allow for the estimation of relative abundance of fish species (Watson et al. 2005, 2010, Harvey et al. 2007, Langlois et al. 2010). The simultaneous application of two cameras (stereo camera configuration) allows for precise measurements of the lengths of fish (Harvey & Shortis 1995, Harvey et al. 2001, 2002, SeaGIS 2008). At each station, a stereo-BRUVs (Figure 4.1) was deployed from a boat and left on the seabed to record for a standard 60 minute period (Watson et al. 2005, Langlois, Fitzpatrick, et al. 2012, Bernard & Götz 2012). For stations located deeper than 50 m, a blue LED light was mounted on the frame between the cameras (Fitzpatrick et al. 2013). Bait was placed in a perforated polyvinyl chloride (PVC) container holding one kilogram of crushed sardine (*Sardinops sagax*) that was suspended approximately 1.2 m from the two cameras.

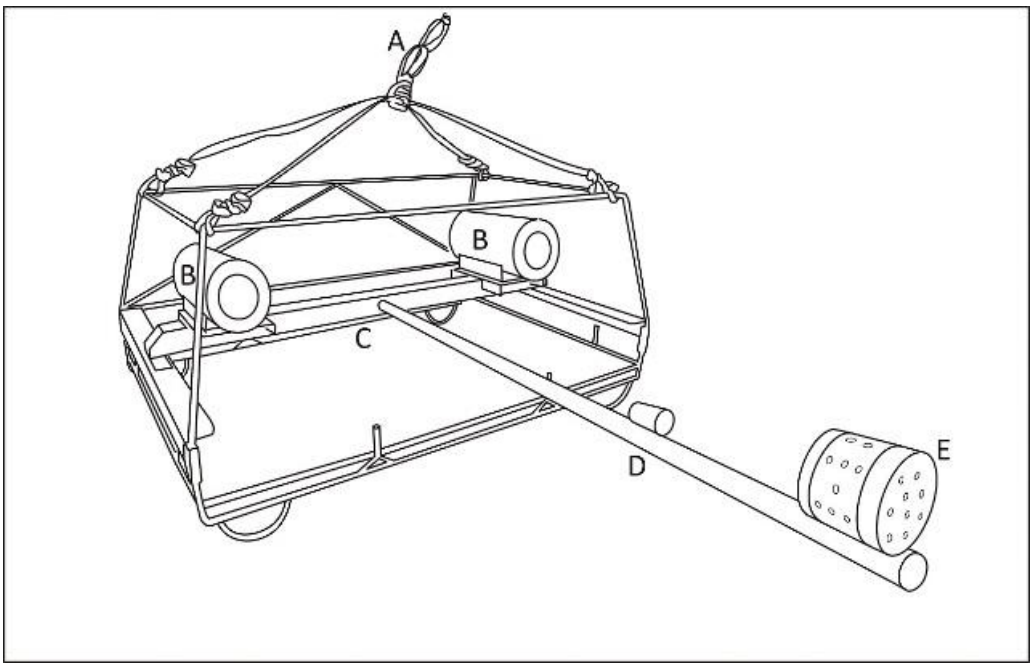


Figure 4.1. Diagram illustrating a stereo-BRUVs. Rope attached to surface buoy (A), underwater camera housings (B), solid bar with cameras mounted at eight degrees for overlapping field of view (C), bait arm suspended 1.2 from cameras (D) and a bait container filled with 1kg of crushed sardine (E). Modified from Harvey et al. (2001).

Sampling occurred at the midpoint of 300 x 300 m grid-cells, as described in Chapter 2 (Figure 2.2.1), during February and September 2013 and February 2014. Each grid was classified according to depth (shallow: 11-25 m; deep: 45-75 m) and profile (high or low). Sampling followed a stratified random approach, with even allocation of sampling efforts between the shallow and deep study sites and reef profiles.

4.2.2.3 VIDEO ANALYSIS

To estimate abundances of fish species and measure lengths of individuals recorded during each stereo-BRUVs deployment, the footage of both cameras was calibrated with the software program EventMeasure and calibration files derived from CAL v1.32 (SeaGIS 2008) software (Harvey and Shortis 1995). Subsequent to calibration, a 60-minute section of video footage was analysed in EventMeasure to obtain fish abundance and length data from each deployment. Because fish could be recounted upon leaving and re-entering the camera's field of view, the measure 'MaxN' was applied to estimate abundances (Willis & Babcock 2000). The measure MaxN is the number of individuals of a species found at one time (i.e. in one frame) throughout the one hour of video footage analysed (Cappo et al. 2006, Shortis et al. 2007). As a conservative measure of abundance (Willis & Babcock 2000, Cappo et al. 2004), MaxN is widely used and the current standard for analysing stereo-BRUVs footage. Length measurements were obtained from the MaxN video frame.

Environmental variables were recorded at each stereo-BRUVs station and included temperate, depth, visibility, percentage water column visible, and bottom type (Table 4.2).

Table 4.2. Environmental variables recorded at stereo-BRUVs stations. A list of the environmental variables included as covariates in statistical analysis**.**

Covariates	Description
Temperature	Average temperature recorded at depth during 60-minute stereo-BRUVs deployment.
Depth	Recorded from echo-sounder when stereo-BRUVs landed on the seabed.
Visibility	Estimated in EventMeasure by making a 3D point at the furthest distance that an object can accurately be identified from both cameras.
Percentage visible water column	Estimated using Vidana software by filling the region of visible water column with colour from which area is calculated (www.marinespatialecologylab.org).
Bottom type - in the field of view of the camera (substrate characteristics)	
Sand	100% sand
Sand inundated reef	Reef covered by a thin layer of sand
Patch-reef low	Mosaic of sand and reef, with visible reef varying by <1 m in height
Patch-reef high	Mosaic of sand and reef, with visible reef varying by >1 m in height
Reef low	100% reef varying by <1 m in height
Reef high	100% reef varying by >1 m in height

4.2.3 STATISTICAL ANALYSES

Both univariate and multivariate statistics were conducted using permutational analysis of variance (PERMANOVA) in PRIMER v6 with PERMANOVA+ (Anderson et al. 2008) unless otherwise indicated. This statistical approach was used as it is based on permutations, which makes the analyses distribution free, and allows for any distance measure to be applied (Anderson et al. 2008), thereby maintaining robustness.

4.2.3.1 FISH ASSEMBLAGE STRUCTURE

A. UNIVARIATE ANALYSIS

The univariate parameters were explored by PERMANOVA (Anderson 2001, Anderson et al. 2008, Fitzpatrick et al. 2012). For significance tests, *P*-values were estimated from 9999 permutations employing Euclidian distance measures calculated from the untransformed univariate data sets. The following univariate datasets were considered:

a) TOTAL MAXN

The total MaxN for each stereo-BRUVs sample was obtained by summing all MaxN values.

b) AVERAGE LENGTH

The average length per stereo-BRUVs sample was obtained by averaging the lengths of all individuals of all species measured in their respective MaxN video frames.

c) TROPHIC LEVEL

Trophic levels obtained from FishBase (Froese & Pauly 2014) were assigned to each individual fish counted in a stereo-BRUVs sample and averaged per sample.

d) SPECIES RICHNESS

Species richness was calculated as the number of species detected in each stereo-BRUVs sample.

e) SPECIES DIVERSITY

Shannon-Wiener diversity (H') was estimated for each stereo-BRUVs sample. This measure is influenced by both the presence of species and their relative abundance within the community. It provides an ecologically useful assessment of the composition diversity by indicating if a community is dominated by one or a few species and is calculated as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where *i* is the number of samples, p_i is the proportion of the total count represented by the *i*th species and *S* is the total number of species (Clarke & Warwick 1994).

B. MULTIVARIATE ASSEMBLAGE STRUCTURE

To establish the influence of localised (station specific) environmental variables on the fish assemblages, variables collected during stereo-BRUVs deployments were analysed with a forward stepping distance based linear model (distLM) using 9999 permutations (Anderson et al. 2008). The

distLM also served to test if visibility and percentage visible water column impacted the count data. The most parsimonious model was selected by means of the AIC procedure (Akaike Information Criteria; Akaike 1973; Anderson et al. 2008). The environmental variables included in this analysis were temperature, depth, bottom-type, visibility and percentage of water-column visible (Table 4.2). The analysis was performed on all samples including those from sandy sites and intermediate depths (26 – 44m). For the remaining analyses, samples collected from sandy sites and intermediate depths were not included to focus on stereo-BRUVs data collected from reefs within the depth zones of the different habitat types (Table 4.1).

To establish if there were significant differences in the multivariate assemblage data between reefs and among the habitat types, MaxN values were compared employing PERMANOVA (using 9999 permutations). Similarity percentages (SIMPER) were estimated to determine which fish species contributed most to the dissimilarities between the reefs and among habitat types. Non-metric multi-dimensional scaling (MDS) was employed to visualise the MaxN and length assemblage data, and overall summed MaxN and average length data at each station were superimposed as bubble plots, respectively (Clarke & Gorley 2006). Biomass values were not calculated because length-weight conversion data for one of the dominating species, Fransmadam (*Boopsoidea inornata*), was not available and results would thus be less meaningful. The vectors obtained from Spearman's rank correlations for the fish identified by the SIMPER procedure above were superimposed on MDS ordination plots to facilitate interpretation and visualize the importance of the vectors in the fish assemblage structure (Anderson et al. 2008).

Prior to analysis, data were square-root transformed to reduce the impact of schooling species (Watson et al. 2005, Heagney et al. 2007, Moore et al. 2010) and a modified Gower logbase 10 resemblance matrix was produced from the MaxN and length data. This distance measure was used because it places greater emphasis on the compositional change of a community rather than actual changes in MaxN (Anderson et al. 2006). This procedure was deemed necessary because MaxN is considered a conservative estimate of abundance.

4.2.3.2 HABITAT ASSOCIATION OF FISH

A canonical analysis of principal coordinates (CAP) was performed to establish if the habitat types identified from macrobenthic species and substrate type analysis (Table 4.1) were effective proxies to predict the fish assemblages (Anderson & Willis 2003, Anderson et al. 2008). As a constrained ordination procedure, CAP allows for any distance or dissimilarity measure commonly employed in ecological studies. Similar to traditional canonical methods, CAP uncovers important patterns in

multivariate data by accounting for a hypotheses in question, i.e. grouping by habitat type (Anderson & Willis 2003). When CAP maximises the separation of *a priori* groups, it is called generalised discriminant analysis based on distances (Anderson & Robinson 2003) and can predict group allocation from principle coordinate (PCO) axes. The PCO axes are generated from the fish assemblage data, and the strength of the canonical correlation provides a measure of the relationship between the PCO axes (fish assemblage data) and the grouping variable (habitat type).

To establish if the CAP model identified the correct number of PCO axes, and how well the PCO axes discriminated among grouping variables, cross-validation tests were performed to determine the misclassification error. The 'leave-one-out' procedure is a method that provides a statistical estimate of the misclassification error, where the misclassification error is the proportion of points that were placed in the wrong group. High percentage allocation success suggests good potential of the CAP model for making valid predictions and allocations.

To determine which components of the habitat groups and fish species were responsible for the groupings, vectors that corresponded to Pearsons correlations > 0.4 were superimposed on the CAP ordination plot. The percentage cover of the major taxonomic groups (excluding solitary species) and the different substrate types were used as habitat data. Because the CAP axes are specifically drawn to separate groups as well as possible, any variables that show either an increasing or decreasing relationship with these CAP axes are likely responsible for observed differences among the groups (Anderson et al. 2008). Prior to analysis, the fish assemblage data were fourth-root transformed to down-weight the dominance of schooling and abundant species, and a resemblance matrix was produced employing a modified Gower logbase 10 distance measure (Anderson et al. 2006).

4.2.3.3 ONTOGENETIC SHIFTS IN HABITAT USE

A. POPULATION SIZE STRUCTURE

For fish species that were sufficiently abundant on both the shallow and deep reefs, kernel density estimates (KDEs) were calculated to compare their length frequency distributions between these reefs (Langlois, Fitzpatrick, et al. 2012). Because sample sizes for some species were small, shallow reef samples included those collected to 30 m, and not 25 m as indicated in Section 4.2.2. The KDE procedure is a non-parametric approach to compare pairs of length-frequency data via permutation. Details on how the method was applied are provided in Section 3.3.

To determine if the shape of the length frequency distribution curves demonstrated a significant bias towards a particular size class, analysis of skewness (g_1) was performed. Significant skewness indicates that data are asymmetrical, and positively skewed data signifies the prevalence of smaller

size classes in a population, and vice versa for negatively skewed distributions (Rossi et al. 2012). Analyses were conducted in R-Studio 2.15.3 (R Core Team 2013) employing Agostino's test from the 'moments' package (Komsta & Novamestrky 2012).

B. HABITAT GENERALISTS & SPECIALISTS

Unique and rare fish species were identified from each habitat type. Unique species were defined as those found in only one of the habitat types, and rare species as those recorded fewer than three times. A habitat generalist was defined as a species that was distributed across all depths, and a habitat specialist was any fish species restricted to either the shallow or deep reef.

One-way PERMANOVA using the univariate MaxN and length data for each of the commercially important fish species classified as generalists were performed to test for differences between the shallow and deep reefs, and among habitat types. Significance was determined by 9999 permutations performed on the untransformed data employing Euclidian distance measure (Anderson et al. 2008, Fitzpatrick et al. 2012). Because the number of measured individuals for most species were often low and also unevenly distributed between the reefs, unique permutations were often low. When permutations were less than 100, Monte Carlo *P*-values were used, which are more reliable when sample sizes are small (Anderson et al. 2008).

C. HABITAT PREFERENCE RELATED TO DIET

To establish if ontogenetic changes in habitat use were related to diet, abundant fish species were separated into juveniles and adults applying the length at 50% maturity for each species (Mann 2013). The feeding guild (Table 4.3) associated with the different life stages (juvenile/adult) of each specimen were then assigned as indicators. The sum of all specimens assigned to each feeding guild was calculated for each stereo-BRUVs sample. A Bray-Curtis similarity matrix was produced from these untransformed data. The data were left untransformed because interest lies in identifying the habitat type for which a specific feeding guild demonstrated association due to high abundances. A CAP analysis was performed on the feeding guild data, with habitat type as the grouping factor. Vectors obtained from Pearson correlations > 0.4 on the feeding guilds and the different life stages of fish species and habitat types were superimposed on the ordination diagram.

Table 4.3. Fish feeding guilds assigned to juvenile and adult fish. Data for the classification of each fish species were obtained from Mann (2013). Details on which species were assigned to the different feeding guilds are available in the appendix (Table A4.1).

Feeding guild	Examples
1 Herbivores	All benthic algae
2 Omnivores	
a)Planktivores	Phytoplankton, copepods, crustacean larvae
b)Omnivores that feed on small invertebrates	Algae, amphipods, isopods, mysids
c)Omnivores that feed on large invertebrates	Algae, crabs, cephalopods, crinoids, gastropods
d)Benthic omnivores	Algae, sponges, ascidians, hydroids, anemones
3 Carnivores	
a)Carnivores of small invertebrates	Amphipods, isopods, mysids
b)Carnivores of large invertebrates	Crinoids, cephalopods, crabs
c)Benthic carnivores	Sponges, ascidians, hydroids, gorgonians
d)Carnivores of large invertebrates & fish	Octopus, squid, crabs, small bony fish

4.3 RESULTS

4.3.1 FISH ASSEMBLAGE STRUCTURE

In total, 2,979 individual fish representing 48 species were recorded with stereo-BRUVs. The distLM established that the variation observed in the fish assemblages was mainly explained by depth (18%), bottom type (6.1%) and temperature (4.1%; Table 4.4).

Table 4.4. The results of the forward selecting distance based linear model (distLM). A distLM was employed to identify the importance of station specific environmental variables on the fish assemblage data. AIC: Akaike Information Criteria; SS: sum of squares; Prop %: increased proportion of explained variation with each variable that is added; Cumul %: Cumulative total.

SEQUENTIAL TESTS

Variable	AIC	SS(trace)	Pseudo-F	P	Prop. %	Cumul. %
Depth	-82.423	5.1962	16.01	0.0001	18.0	18
Bottom type	-86.176	1.7495	5.7403	0.0001	6.1	24
Temp	-88.595	1.2554	4.3086	0.0001	4.3	28

4.3.1.1 UNIVARIATE ANALYSIS

The results of the univariate PERMANOVA indicated significant decreases in the overall MaxN, Shannon diversity and species richness from the shallow to the deep reef, a pattern that was consistent when progressing from the most shallow to the deepest habitat type (Figure 4.2 A,D & E). The average trophic level increased with a decrease in depth ($P < 0.001$; Figure 4.2 C). There was no significant difference in the average length between reefs (pseudo-$F = 1.417$; $P = 0.25$) or among the different habitat types (pseudo-$F = 0.869$; $P = 0.49$). All remaining univariate data (summed MaxN, average trophic level, species richness and Shannon diversity) measured within habitat A were significantly different from the rest of the habitat types. The highest average MaxN (62.5 ± 41.5), Shannon diversity (1.9 ± 0.3), number of species (13.6 ± 4) and numbers of unique and rare species (Figure 4.3) were recorded in habitat A, which also showed the lowest average trophic level (3.4 ± 0.1).

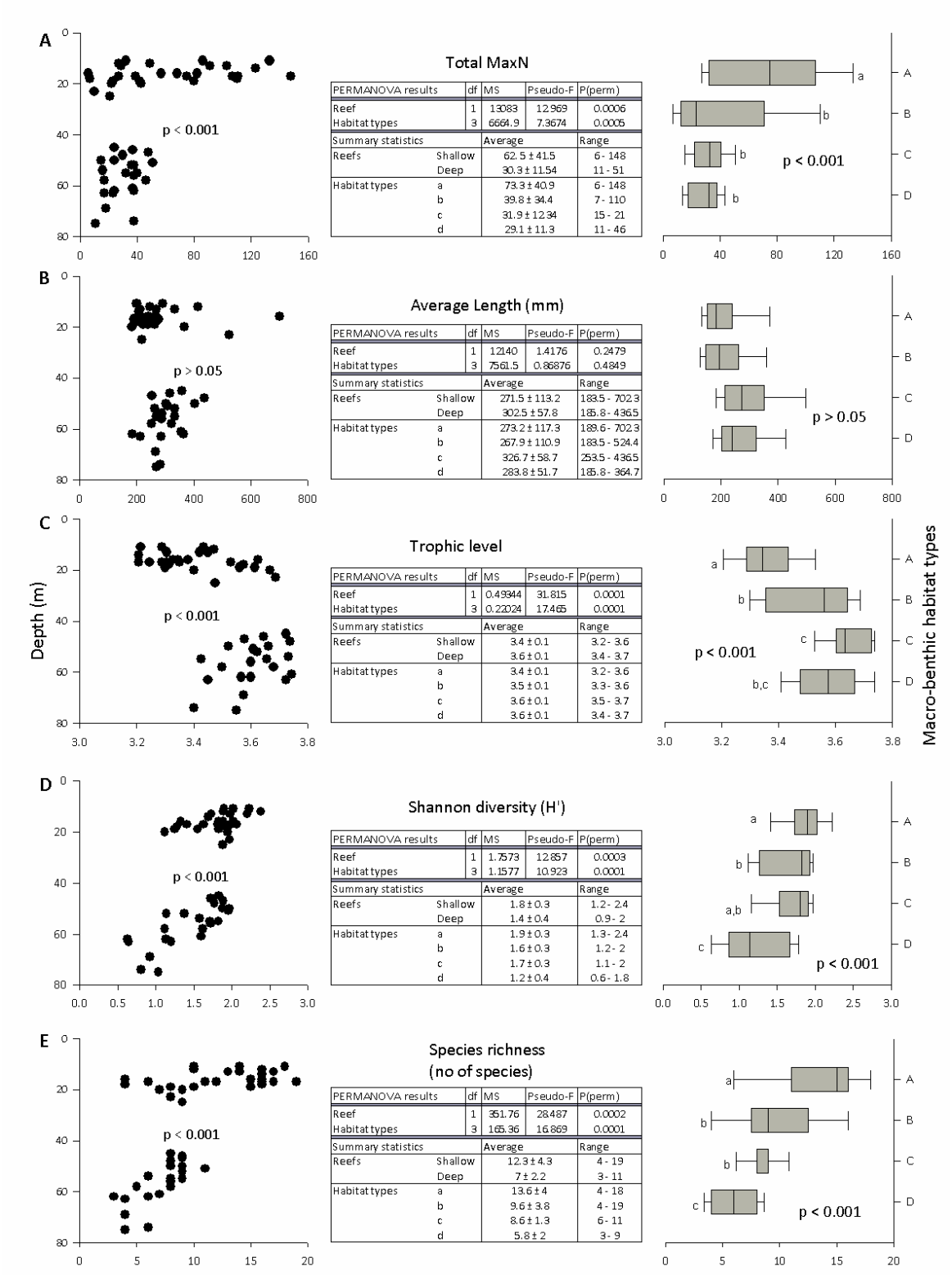


Figure 4.2. Univariate PERMANOVA results displaying the A) total MaxN, B) average length, C) trophic level, D) Shannon diversity and E) species richness of stereo-BRUVs samples. In the first column, data points represent a stereo-BRUVs sample, and were tested for significant differences between the shallow and deep reefs. The second column provides a numerical summary of the statistics associated with the univariate data sets. The third column provides tests of the univariate data between different habitat types, starting from the shallowest habitat type A to deepest D (error bars represent standard deviations; error bars followed by different letters indicate significant differences between habitat types).

4.3.1.2 MULTIVARIATE ASSEMBLAGE STRUCTURE

A. ABUNDANCE

a) REEFS

Multivariate PERMANOVA confirmed significant differences in the MaxN assemblage data between the shallow and deep reefs (pseudo-F = 19.72; P < 0.001) and among different habitat types (pseudo-F = 8.42; P < 0.001; Table 4.5). According to the SIMPER results, the fish assemblage on the shallow reef was 75% dissimilar compared with the deep reef assemblage (Table 4.5). The shallow reef assemblage was characterised by high numbers of fransmadam (*Boopsoidea inornata*) and steentjie (*Spondyliosoma emarginatum*), and the deep reef by high numbers of panga (*Pterogymnus laniarius*), carpenter (*Argyrozona argyrozona*) and hottentot (*Pachymetopon blochii*) (Figure 4.3 and Table 4.5), with hottentot found exclusively on the deep reef.

b) HABITAT TYPES

According to the results of the SIMPER procedure, the dissimilarity between habitats A and B (54.9%) was due to differences in abundance of fransmadam, steentjie, blacktail (*Diplodus capensis*), blue hottentot (*Pachymetopon aeneum*) and strepie (*Sarpa salpa*), all of which decreased in abundance from habitat A to B (Table 4.5). Dissimilarity between habitats B and C (64.6%) was attributed to the absence of carpenter and hottentot in habitat B, and the marked increase in panga abundance in habitat C. Both fransmadam and steentjie decreased markedly in abundance from habitat B to C. Dissimilarity between habitats C and D (48.8%) was due to the decrease in abundance of hottentot, roman (*Chrysoblephus laticeps*), blue hottentot, and striped catshark (*Poroderma africanum*) and a slight increase in steentjie abundance from habitat C to D.

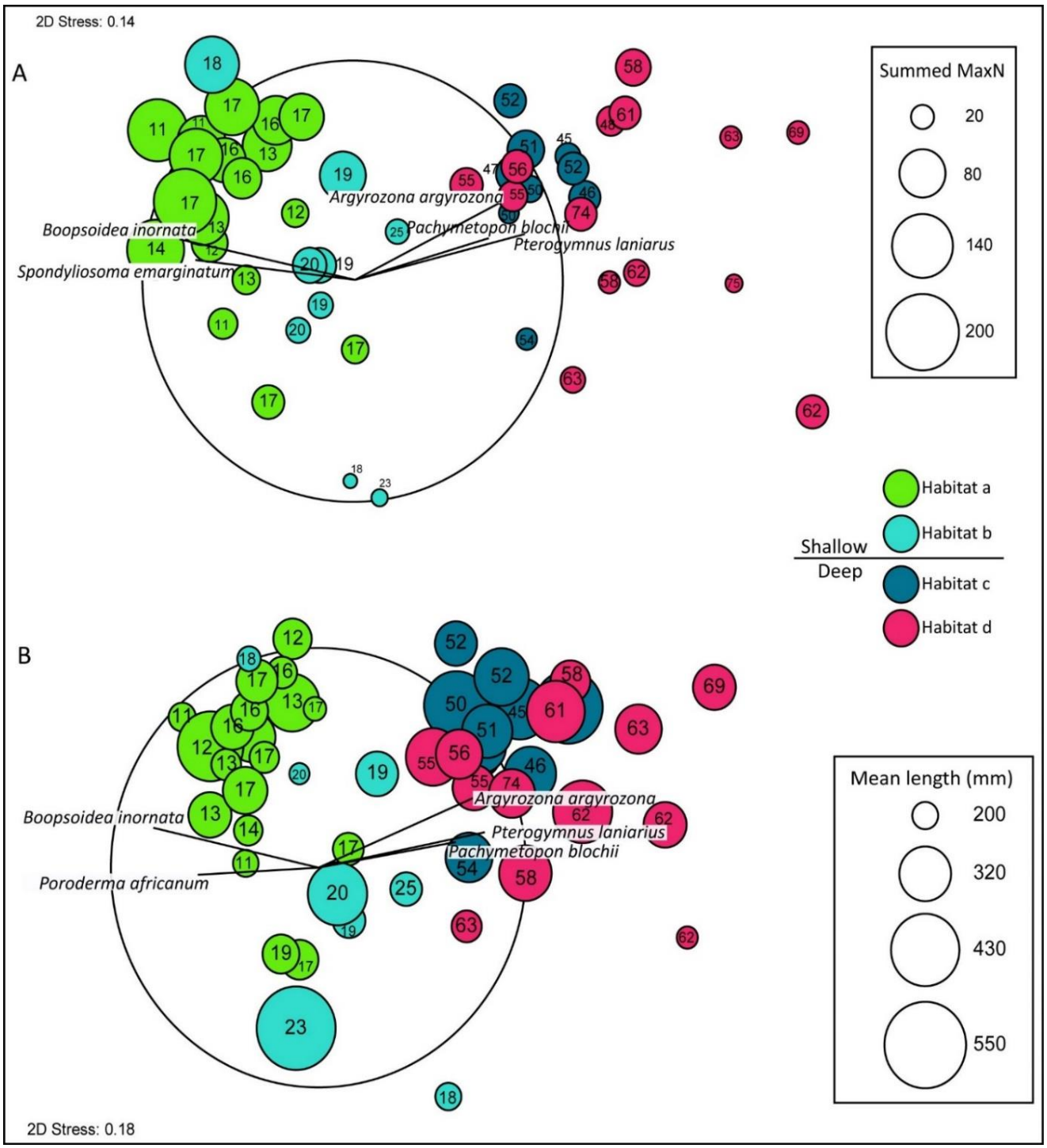


Figure 4.3. MDS plot of the multivariate MaxN (A) and length (B) fish data, with the overall MaxN and average length superimposed as bubbles. The size of the bubbles represents the value of the summed MaxN (A) and average length (B) per station (legend inserts) and the value in each bubble is the depth at which a sample was collected. The direction and magnitude of Spearman's rank correlations of the five fish species identified from the similarity percentage (SIMPER) procedure that contributed most to differences between the shallow and deep reefs are indicated as vector lines.

B. LENGTH

In contrast to the average length data (univariate PERMANOVA, Figure 4.2), the multivariate examination of length data indicated that the length of fish differed significantly between the reefs (pseudo-*F* = 15.339; *P* = 0.0001) and among habitat types (pseudo-*F* = 7.45; *P* = 0.0001; Figure 4.3; Table 4.5). When comparing the MaxN and length MDS plots (Figure 4.3 A, B, respectively), the lack of significant differences in the univariate average length PERMANOVA results can be explained by the elevated average lengths obtained at several stations in the shallow reef (Figure 4.3 B). The stations that demonstrated this increase in average length were marked by very low abundances (Figure 4.3 A) but high average lengths, trends resulting from the combining effects of low bony fish abundances and the presence of shy shark species. Indeed, the SIMPER results (Table 4.5) illustrated the importance of shark presence in the length data (underlined species in Table 4.5), as sharks attained consistently larger sizes than the bony fish species.

Table 4.5. Multivariate PERMANOVA and SIMPER results for MaxN and length data of fish assemblages. Pairwise comparisons were done only between adjacent habitat types. The shallowest and the deepest habitat types were not compared. Significance values *$p < 0.05$; ** $p < 0.001$.

		PERMANOVA					SIMPER							
		df	MS	Pseudo-*F*	P(perm)		Dis-similarity (%)	Species	Average Shallow	Average Deep	Average dissimilarity	Sim/SD	Contribution (%)	
REEFS	MaxN	1	4.912	19.72	0.0001		75.3	*Pterogymnus laniarius*	0.2	1.8	7.4	2.2	9.8	
								Spondyliosoma emarginatum	1.8	0.7	5.4	1.5	7.1	
								Argyrozona argyrozona	0.0	1.2	5.1	1.8	6.8	
								Boopsoidea inornata	1.4	0.3	5.0	1.7	6.6	
								Pachymetopon blochii	0.0	0.8	3.4	1.2	4.5	
	Length	1	6.456	15.339	0.0001		74.2	*Pterogymnus laniarius*	0.3	2.1	5.2	1.7	7.0	
								Argyrozona argyrozona	0.0	1.9	5.2	1.6	7.0	
								Pachymetopon blochii	0.0	1.5	4.3	1.1	5.7	
								Poroderma africanum	1.9	1.2	4.3	1.0	5.7	
								Boopsoidea inornata	1.6	0.5	3.6	1.2	4.9	
HABITAT TYPE						Pairwise comparisons			Habitat A	B	C	D			
	MaxN	3	1.995	8.4247	0.0001	Habitat A & B*	54.9	*Boopsoidea inornata*	1.6	0.9			3.5	1.3	6.4
								Spondyliosoma emarginatum	1.9	1.5			3.4	0.9	6.1
								Diplodus capensis	1.0	0.2			3.1	1.3	5.6
								Pachymetopon aeneum	1.1	0.9			2.9	1.2	5.3
								Sarpa salpa	0.7	0.3			2.5	0.8	4.6
						Habitat B & C**	64.6	*Pterogymnus laniarius*		0.2	1.7		7.1	2.2	10.9
								Spondyliosoma emarginatum		1.5	0.5		5.6	1.4	8.6
								Argyrozona argyrozona		0.0	1.2		5.5	2.3	8.6
								Pachymetopon blochii		0.0	1.0		4.8	1.6	7.5
								Boopsoidea inornata		0.9	0.3		3.6	1.3	5.6
						Habitat C & D**	45.5	*Pachymetopon aeneum*			1.3	0.7	4.6	1.3	10.0
								Chrysoblephus laticeps			1.4	0.7	4.3	1.1	9.5
								Pachymetopon blochii			1.0	0.6	4.0	1.2	8.8
								Spondyliosoma emarginatum			0.5	0.8	3.9	1.2	8.5
								Poroderma africanum			0.8	0.3	3.8	1.3	8.4
	Length	3	2.909	7.4488	0.0001	Habitat A & B**	62.6	*Poroderma africanum*	2.4	1.0			4.7	1.2	7.5
								Mustelus mustelus	1.5	0.4			3.9	0.8	6.2
								Galeichthys feliceps	0.9	1.2			3.3	0.9	5.2
								Haploblepharus edwardsii	1.3	0.3			3.1	1.0	5.0
								Diplodus capensis	1.4	0.2			3.1	1.3	4.9
						Habitat B & C**	67.9	*Pachymetopon blochii*		0.0	1.9		5.7	1.7	8.4
								Argyrozona argyrozona		0.0	1.9		5.4	1.8	8.0
								Petrus rupestris		0.8	2.2		5.4	1.3	8.0
								Poroderma africanum		1.0	1.9		5.3	1.1	7.8
								Pterogymnus laniarius		0.5	2.0		5.0	1.7	7.4
						Habitat C & D**	48.8	*Poroderma africanum*			1.9	0.7	5.5	1.2	11.2
								Petrus rupestris			2.2	0.8	5.4	1.3	11.0
								Notorynchus cepedianus			1.1	0.8	4.0	0.8	8.2
								Chrysoblephus laticeps			2.3	1.3	3.7	1.0	7.6
								Chrysoblephus gibbiceps			1.2	0.3	3.7	1.0	7.5

4.3.2 HABITAT ASSOCIATION OF FISH

Nine canonical axes (*m*) best described the variability in fish abundance data. The first two canonical correlation values, which indicated the strength of the associations between the fish abundance data and the grouping variable (habitat types), were large (0.98 and 0.74; Figure 4.4). The first canonical axes separated the shallow from the deep reef, and the second axis separated the different habitat types.

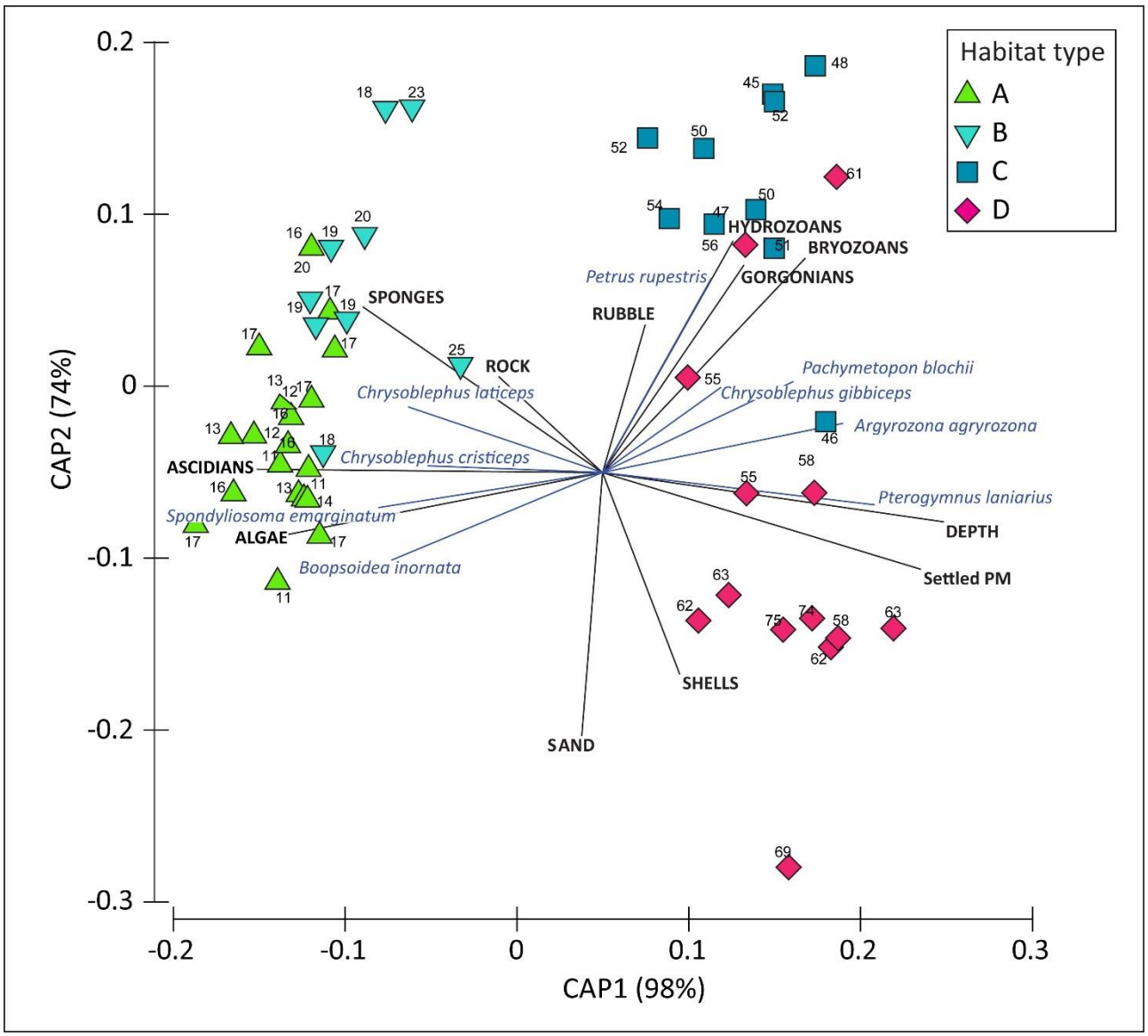


Figure 4.4. Ordination diagram of the first two axes from a canonical analysis of principle coordinates (CAP) using habitat type to group fish abundance data (MaxN). The number near each sample represents the collection depth. The different habitat types defined from macrobenthic species can be identified from the legend insert, and the Pearson's correlations (> 0.3) of the fish and macrobenthic percentage cover from each habitat type are superimposed as vectors. Settled PM = settled particulate matter.

The estimation of the misclassification error determined from the leave-one-out procedure indicated a high allocation success, with a total of 84.3% stereo-BRUVs samples correctly assigned to the defined habitat types. The highest allocation success occurred for habitat C (90%), followed by habitat B (88.9%), with the lowest for habitat D (76.9%; Table 4.6). This result indicated very distinct groups and goodness of fit of the model, and that habitat type, as defined by the macrobenthic assemblage and substratum data, was an appropriate proxy for predicting the fish assemblages associated with a habitat type.

Table 4.6. Results from the cross-validation test. The leave-one-out procedure indicates the allocation success of fish assemblages by habitat type.

Original group	A	B	C	D	Total no of samples per group	Correctly classified (%)
A	16	3	0	0	19	84.2
B	1	8	0	0	9	88.9
C	0	0	9	1	10	90.0
D	0	0	3	10	13	76.9

To determine which fish species and components of the habitat types (major macrobenthic taxa and substrate type) were responsible for the differences among the groups, vectors that corresponded to a Pearsons correlation >0.4 were superimposed on the CAP ordination plot. The shallowest habitat type (A) was characterised by high algal and ascidian cover, with steentjie, fransmadam and, to a lesser extent, dageraad (*Chrysoblephus cristiceps*) closely associated with this group (Figure 4.4). Moving slightly deeper, habitat B was typified by bare rock and sponge species, and dageraad were found in the shallower portions of this habitat. Roman abundances were highest at about 18m (Figure 4.6), and thus were an important component of the shallow end of habitat B. Habitat C was typified by hydrozoans (mostly noble coral), bryozoans, seafans (gorgonians) and rubble. Fish associated with this habitat type included red steenbras (*Petrus rupestris*), red stumpnose (*Chrysoblephus gibbiceps*) and, to a lesser extent, hottentot. The deepest habitat type (D) was characterised mostly by substrate type (percentage cover of sand, shells and settled particulate matter). Panga demonstrated the strongest relationship with this habitat type, whereas carpenters were associated with both habitats C and D.

4.3.3 ONTOGENETIC SHIFTS IN HABITAT USE

4.3.3.1 POPULATION SIZE STRUCTURE

The KDEs demonstrated significantly different length frequency distributions between the shallow and deep reef populations (Figure 4.5). This difference was especially evident in the steentjie, red steenbras, blue hottentot and fransmadam populations. All populations indicated larger modes at the deeper reef, which means that greater percentages of sexually mature individuals inhabited the deeper reef. This pattern was most pronounced in the blue hottentot population, where 85.7% was sexually mature on the deep reef, compared to only 21.8% on the shallow reef (Figure 4.5C). Similarly, all recorded red steenbras on the shallow reef were smaller than the length-at-50% maturity, whereas 37.5% of the deep reef population was sexually mature individuals (Figure 4.5F). Sixty percent of the deep reef panga population was sexually mature, compared to 25% on the shallow reef. Both the shallow and deep populations of fransmadam and roman were marked by

large numbers of sexually mature individuals, with the modes for roman differing only slightly between the shallow and deep reef populations (Figure 4.5E). Fransmadam demonstrated a bimodal size distribution, with the first mode on both reefs near the 150 – 180 mm size class and the second mode at 230 mm evident only on the deep reef (Figure 4.5B).

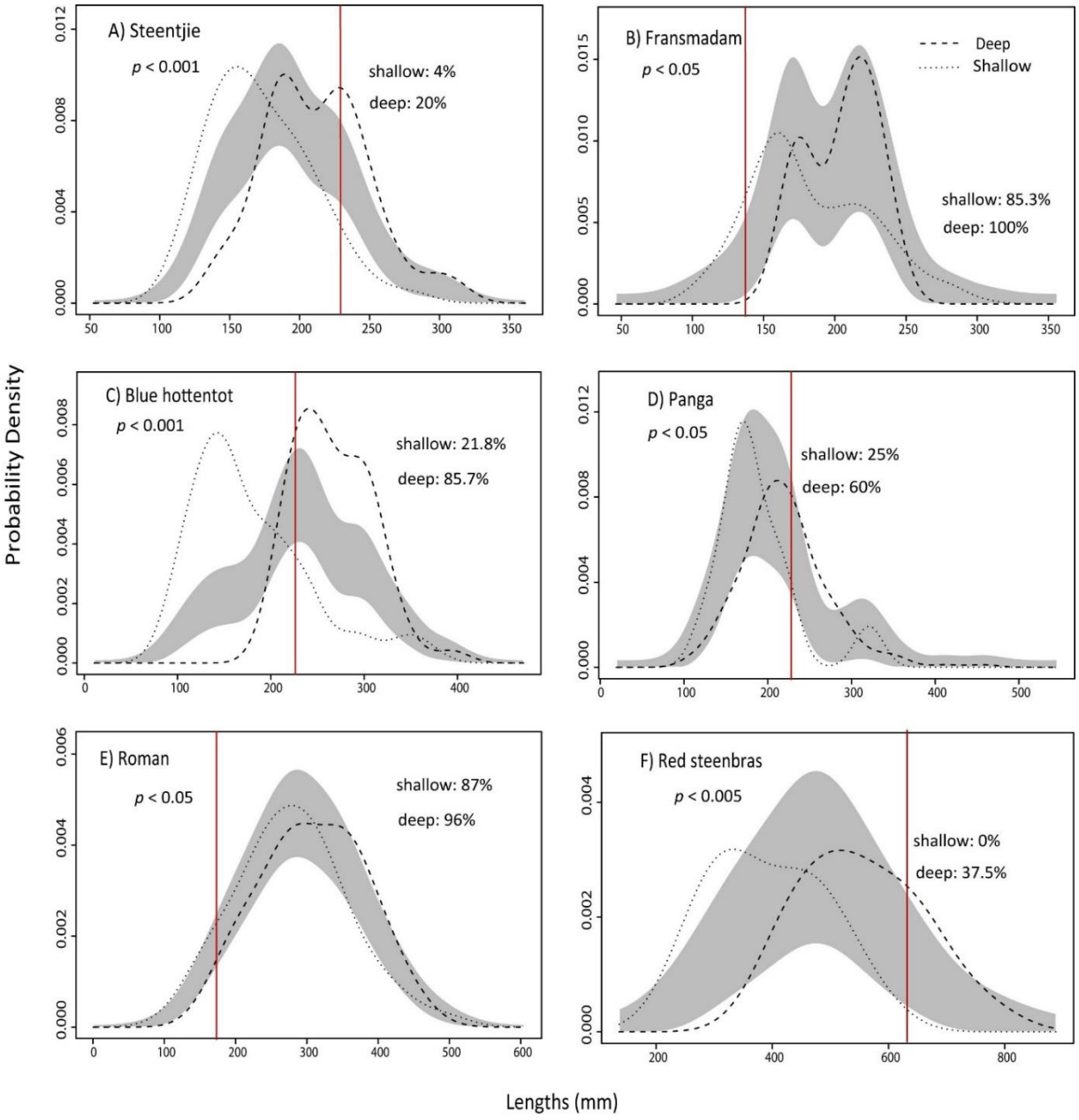


Figure 4.5. Kernel density estimates (KDEs) of commercially important fish species for the shallow (dotted lines) and deep (dashed lines) reef populations in Tsitsikamma. The grey areas represent one standard error above and below the null model of 'no difference' between KDEs. If a length frequency distribution curve falls outside this band, it is significantly different from the permuted model of 'no difference'. Red vertical lines indicate length at 50% maturity, with the percentage of individuals greater than this length to the right of the line.

The measure of skewness (g_1) showed that, apart from the deep populations of fransmadam and roman, which demonstrated slightly negative g_1 values, all other fish populations were positively skewed, indicating the presence of more smaller individuals (see Table 4.7). Only the shallow populations of steentjie and blue hottentot, and the deep population of panga, were significantly positively skewed (Table 4.7).

Table 4.7. Results of analysis of skewness (g_1) on the length frequency distribution curves. Analyses of skewness were performed only on the populations of commercially important fish species that demonstrated distributions across both reefs.

	Shallow			Deep		
	g_1	Z	*p* - value	g_1	Z	*p* - value
Steentjie	0.6	2.52	**0.012**	0.4	0.78	0.45
Fransmadam	0.45	1.63	0.102	-0.27	-0.31	0.756
Blue hottentot	1.09	2.5	**0.012**	0.59	1.21	0.226
Panga	1.51	1.7	0.089	0.97	3.18	**0.001**
Roman	0.32	1.09	0.275	-0.02	-0.05	0.961
Red steenbras	0.23	0.26	0.795	0.37	0.49	0.624

4.3.3.2 SPECIES SPECIFIC DISTRIBUTIONS

Assignments of fish as generalist or specialist habitat users (Table 4.8) indicated that more species were specialists than generalists. Of the 48 species identified, only 15 inhabited both the shallow and deep reefs, and 15 of the 33 habitat specialists were confined to a single habitat type. Most of the habitat specialists were from the shallow reef. The majority (62.5%) of the unique species were associated with habitat type A (Table 4.8).

Table 4.8. Assignment of habitat specialists and generalists within the reef fish community in Tsitsikamma. Commercially important species (indicated in bold) were examined in more detail.

Habitat Specialists		Total abundance	Habitat type
Unique species (confined to one habitat type)			
Geelbek	*Atractoscion aequidens*	2	A
Bluefin gurnard	*Chelidonichthys kumu*	2	D
Bank steenbras	*Chirodactylus grandis*	1	A
	Clinidae sp	4	A
Short-tail stingray	*Dasyatis brevicaudata*	1	B
Yellowbelly rockcod	*Epinephelus marginatus*	1	A
Redeye round herring	*Etrumeus whiteheadi*	1	A
Tiger catshark	*Halaelurus natalensis*	2	A
Dark shyshark	*Haploblepharus pictus*	1	A
Common eagle ray	*Myliobatis aqulia*	1	A
Piggy/pinky	*Pomadasys olivaceum*	41	A
Dane seabream	*Porcostoma dentata*	3	A
Cape stumpnose	*Rhabdosargus holubi*	10	A
Cape yellowtail	*Seriola lalandi*	1	B
Streaked gurnard	*Trigloporus lastoviza*	1	D
Species confined to a reef			Reef
Koester	*Acanthistius sebastoides*	9	Shallow
Carpenter	***Argyrozona argyrozona***	**90**	**Deep**
Barred fingerfin	*Cheilodactylus pixi*	7	Shallow
Santer	*Cheimerius nufar*	15	Shallow
Dageraad	***Chrysoblephus cristiceps***	**22**	**Shallow**
Red stumpnose	***Chrysoblephus gibbiceps***	**10**	**Deep**
Blacktail	*Diplodus capensis*	74	Shallow
Zebra	*Diplodus hottentotus*	10	Shallow
White sea catfish	*Galeichthys feliceps*	18	Shallow
Janbruin	*Gymnocrotaphus curvidens*	9	Shallow
Puffadder shysark	*Haploblepharus edwardsii*	16	Shallow
Sand steenbras	*Lithognathus mormyrus*	5	Shallow
Cape knifejaw	*Oplegnathus conwayi*	9	Shallow
Hottentot	***Pachymetopon blochii***	**43**	**Shallow**
Red tjor-tjor	*Pagellus bellottii natalensis*	34	Shallow
Jutjaw	*Parascorpis typus*	7	Deep
White stumpnose	*Rhabdosargus globiceps*	18	Shallow
Streepie	*Sarpa salpa*	156	Shallow
African seabass	*Serranus knysnaensis*	2	Shallow

Habitat Generalists		Total abundance
Evileye blaasop	*Amblyrhynchotes honckenii*	16
Fransmadam	***Boopsoidea inornata***	**278**
Redfingers	*Cheilodactylus fasciatus*	14
Two-tone fingerfin	*Chirodactylus brachydactylus*	27
Roman	***Chrysoblephus laticeps***	**221**
Smoothhound	*Mustelus mustelus*	17
Cowshark	*Notorynchus cepedianus*	13
Blue hottentot	***Pachymetopon aeneum***	**203**
Red steebras	***Petrus rupestris***	**21**
Striped catshark	*Poroderma africanum*	67
Leopard catshark	*Poroderma pantherinum*	5
Panga	***Pterogymnus laniarius***	**301**
Steentjie	***Spondyliosoma emarginatum***	**533**
Horse mackerel	*Trachurus trachurus*	107

Commercially important fish species that were classified as deep reef specialists included red stumpnose, carpenter, panga and hottentot (Table 4.8 & Figure 4.6). Dageraad was classified as a shallow reef specialist as no individuals of this species were counted on the deep reef. The remaining commercially important fish species that were considered in more detail (Figure 3.6) were habitat generalists and demonstrated depth related ontogenetic shifts (Figure 4.6).

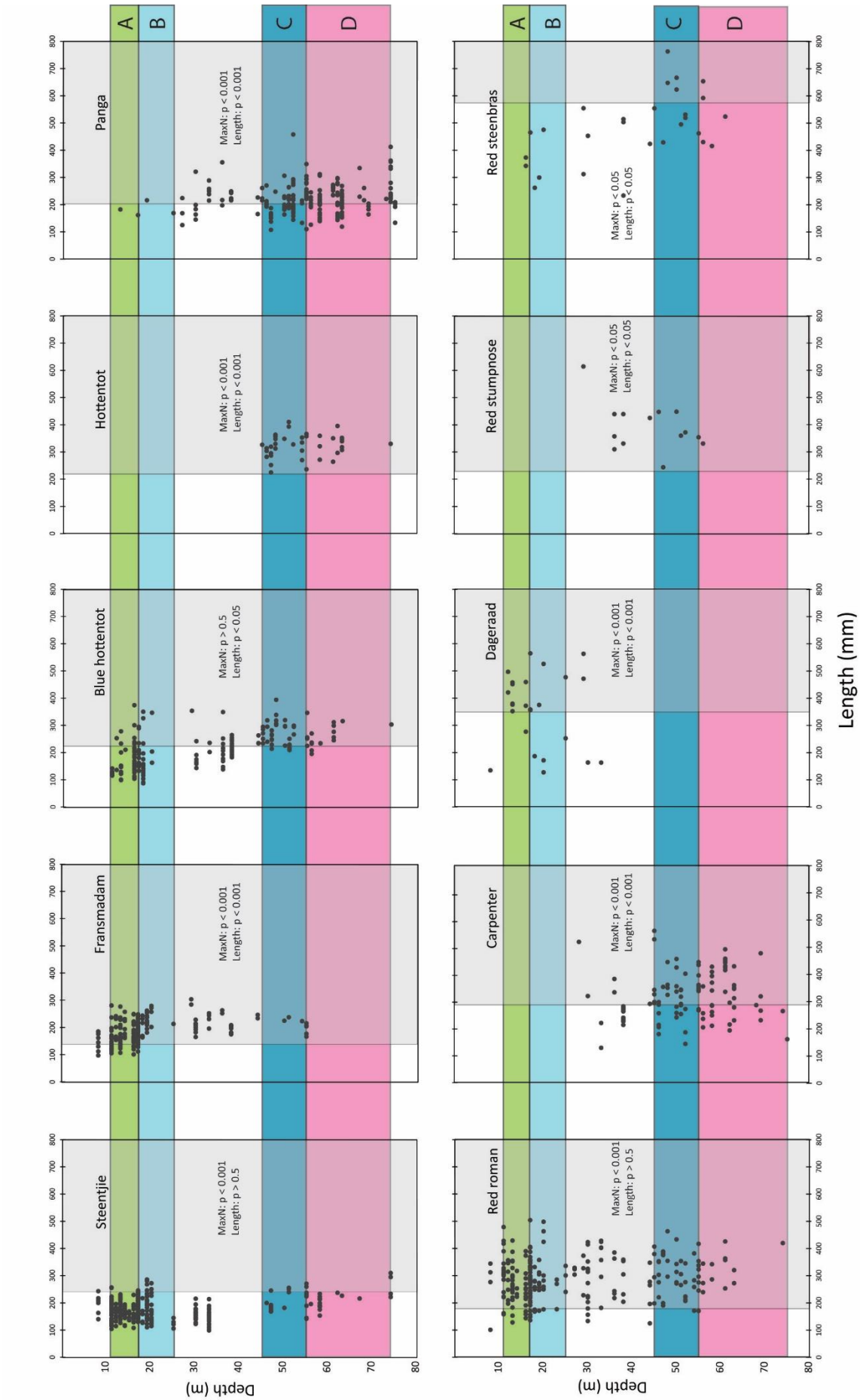


Figure 4.6. Depth distribution patterns of target fish species. Individual fish and corresponding lengths are indicated and plotted against the depth from which stereo-BRUVs samples were collected. The grey area represents length at 50% maturity for a species, horizontal coloured boxes designate the different habitat types. *P*-values are provided for differences in length and MaxN values for each species between the shallow and deep reef employing a univariate PERMANOVA.

C. HABITAT GENERALISTS

Abundances of blue hottentot did not differ significantly between reefs (Table 4.9), but populations demonstrated a significant increase in lengths from 17.8 ± 6.5 cm on the shallow reef to 26.3 ± 4.1 cm on the deep reef. This increase in average size on the deep reef suggests that, although blue hottentot can be considered a habitat generalist (distributed evenly across all depths), it demonstrated an ontogenetic shift in habitat use. In contrast, roman, also present at all depths, demonstrated a significant decrease in abundance and averaged 5.9 ± 2.7 fish on the shallow reef and 3.7 ± 1.4 on the deep reef, and did not differ significantly in terms of measured lengths (Table 4.9). Similarly, PERMANOVA results for steentjie did not demonstrate a significant increase in length from the shallow to the deep reef, although KDEs indicated a significantly larger mode on the deep reef (Figure 4.5 A). Steentjie were present across the entire sampled depth range, but demonstrated a preference for the shallow reef. Steentjie abundances differed significantly between reefs, with an average of 19.7 ± 14.1 fish recorded on the shallow reef compared to 3.3 ± 3.3 on the deep reef (Table 4.9). Red steenbras demonstrated an even distribution across the sampled depth range (Figure 4.6). However, there was a clear shift in habitat use indicated by differences in fish length, with larger individuals found deeper (also supported by the KDE results; Figure 4.8). The average length of red steenbras increased significantly from 36.9 ± 8.7 cm on the shallow reef to 55.4 ± 10.1 cm on the deep reef. Although fransmadam occurred to depths of 55 m, this species demonstrated a clear preference for the shallower reef, particularly habitat type A, which differed significantly in terms of abundance from habitat types B, C and D (Figure 4.5 & Table 4.10). There was also a significant increase in fransmadam size from the shallow to the deep reef (Table 4.10). These depth records are extensions of the fransmadam depth distribution from 30 m recorded in the literature (Mann 2013) to 75m in this study. Panga were found in very low abundances on the shallow reef (1.5 ± 1.0 fish) compared to 12.8 ± 7.5 on the deep reef. Panga inhabiting the deep reef were significantly larger (21.9 ± 5.2 cm) compared to fish on the shallow reef (18.2 ± 2.4 cm; Table 4.9).

Table 4.9. Univariate PERMANOVA and descriptive statistics for commercially important fish species. Comparative tests between the reefs were performed using MaxN and length data. Values indicated in bold were significantly different between reefs.

		PERMANOVA				Average MaxN	
		df	MS	Pseudo-*F*	*P* (perm)	Shallow	Deep
Abundance (MaxN)	Steentjie	1	3179.5	26.065	**0.0001**	19.7 ± 14.1	3.3 ± 3.3
	Fransmadam	1	1054.1	20.094	**0.0001**	11.7 ± 9.5	2 ± 1.2
	Blue hottentot	1	60.103	1.3886	0.2723	6.6 ± 9.1	4 ± 3.2
	Hottentot	-	-	-	**-**	-	2.8 ± 2.2
	Panga	1	2008.5	78.732	**0.0001**	1.5 ± 1	12.8 ± 7.5
	Roman	1	106.47	16.569	**0.0002**	5.9 ± 2.7	3.7 ± 1.4
	Carpenter	-	-	-	**-**	-	4.5 ± 3.3
	Red stumpnose	-	-	-	**-**	-	1.2 ± 0.8
	Dageraad	-	-	-	**-**	1.5 ± 0.6	-
	Red steenbras	1	2.4213	5.9524	**0.0194**	1.2 ± 0.4	1.3 ± 0.6
		PERMANOVA				Average length (cm)	
		df	MS	Pseudo-*F*	*P* (perm)	Shallow	Deep
Length (cm)	Steentjie	1	118.73	1.5975	0.2128	17.3 ± 3.8	21.6 ± 3.5
	Fransmadam	1	1184.9	17.825	**0.0002**	18.2 ± 4.1	20.4 ± 3.5
	Blue hottentot	1	639.3	5.5515	**0.0253**	17.8 ± 6.5	26.3 ± 4.1
	Hottentot	-	-	-	**-**	-	32.4 ± 4.3
	Panga	1	3410.5	131.92	**0.0001**	18.2 ± 2.4	21.9 ± 5.2
	Roman	1	41.951	0.36452	0.5402	27.9 ± 7.4	30.4 ± 7.2
	Carpenter	-	-	-	**-**	-	33.4 ± 9.2
	Red stumpnose	-	-	-	**-**	-	36.5 ± 7.0
	Dageraad	-	-	-	**-**	37.2 ± 11.9	-
	Red steenbras	1	5859.8	12.133	**0.0015**	36.9 ± 8.7	55.4 ± 10.1

D. HABITAT SPECIALISTS

Commercially important fish species classified as habitat specialists included hottentot, carpenter, red stumpnose and dageraad, the last being the only species restricted to the shallow reef. None of the commercially important habitat specialists varied significantly in abundance among the habitat types within the reefs they inhabited. Apart from red stumpnose, which had significantly larger individuals in habitat C compared to D, all remaining habitat specialists did not demonstrate any ontogenetic shifts in habitat use (Figure 4.5 & Table 4.10). No juvenile hottentot or red stumpnose were observed in the study area.

Table 4.10. Univariate PERMANOVA and descriptive statistics for the commercially important fish species in Tsitsikamma. PERMANOVA among habitat types for both MaxN and length data. Values indicated in bold font denote significant differences.

		PERMANOVA				Habitat - Average MaxN				Pairwise comparisons					
		df	MS	Pseudo-*F*	*P* (perm)	A	B	C	D	A,B	A,C	A,D	B,C	B,D	C,D
Abundance (MaxN)	Steentjie	3	1107.6	8.9228	**0.0002**	20.2 ± 15	19.8 ± 14	3.8 ± 4.3	3.1 ± 3.1	0.4333	**0.0009**	**0.0003**	**0.008**	**0.0037**	0.7616
	Fransmadam	3	523.42	11.975	**0.0001**	14 ± 9.8	5.25 ± 4.2	1.3 ± 0.6	3 ± 1.4	**0.0146**	**0.0011**	**0.0001**	**0.0212**	**0.0174**	1
	Blue hottentot	3	42.906	0.98261	0.4069	7.1 ± 10.2	3 ± 6.5	4.9 ± 3.9	2.9 ± 2	0.613	0.7555	0.1613	0.8003	0.2686	**0.0368**
	Hottentot	3	20.974	10.077	**0.0002**	0	0	3.6 ± 2.9	2 ± 1		**0.0002**	**0.0008**	**0.003**	**0.0238**	0.0608
	Panga	3	681.49	26.383	**0.0001**	2 ± 1.4	1	11.4 ± 8.8	13.9 ± 6.4	1	**0.0001**	**0.0001**	**0.0006**	**0.0001**	0.4466
	Roman	3	53.993	9.7847	**0.0001**	6.6 ± 2.8	4.1 ± 1.7	4.1 ± 1.3	3.1 ± 1.7	0.1059	**0.0443**	**0.0001**	0.7603	**0.0043**	**0.0058**
	Carpenter	3	66.002	11.878	**0.0002**	0	0	3.8 ± 2	5.1 ± 4.1		**0.0001**	**0.0001**	**0.0002**	**0.0051**	0.571
	Red stumpnose	3	0.62703	2.4239	0.0549	0	0	1	2 ± 1.4	0.3168	**0.0018**	0.1604	0.1375	0.7433	0.6109
	Dageraad	3	2.5988	5.3776	**0.0039**	1.5 ± 0.5	2 ± 0.9	0	0	1	**0.0087**	**0.0021**	**0.0303**	**0.0165**	
	Red steenbras	3	1.232	3.1035	**0.0322**	1.5 ± 0.7	1	1.1 ± 0.4	1.5 ± 1	0.4357	**0.0041**	0.3227	0.0534	0.8338	0.3026
		PERMANOVA				Habitat - Average length (cm)				Pairwise comparisons					
		df	MS	Pseudo-*F*	*P* (perm)	A	B	C	D	A,B	A,C	A,D	B,C	B,D	C,D
Length (cm)	Steentjie	3	113.4	1.5582	0.2181	17.2 ± 3.3	17.6 ± 5.1	20.4 ± 3.3	22.1 ± 3.6	0.3117	**0.0167**	0.6256	0.2962	0.8018	0.2254
	Fransmadam	3	448.77	6.8125	**0.001**	17.6 ± 3.9	22.4 ± 3.4	22.9 ± 0.7	19.1 ± 2.0	0.1906	**0.0031**	**0.0001**	0.2948	0.0589	0.4826
	Blue hottentot	3	453.49	4.3307	**0.0104**	17.6 ± 5.9	18.2 ± 8.5	26.9 ± 4.2	26.1 ± 4.1	0.1728	**0.003**	0.7704	**0.0022**	0.2346	0.0547
	Hottentot	3	1758.1	16.409	**0.0001**	0	0	32.3 ± 4.3	32.6 ± 4.5		**0.0001**	**0.0006**	**0.0004**	**0.0161**	0.3892
	Panga	3	1163.2	46.033	**0.0001**	17.1 ± 1.4	19.2 ± 3.3	20.4 ± 5.1	22.8 ± 5.2	0.37	**0.0001**	**0.0001**	**0.0012**	**0.0001**	0.1314
	Roman	3	279.06	2.7076	0.0523	27.8 ± 7.3	28.2 ± 7.9	30.5 ± 7.5	31.6 ± 6.6	0.4803	0.141	0.0941	**0.023**	0.3521	**0.0438**
	Carpenter	3	2453.8	27.695	**0.0001**	0	0	32.0 ± 9.5	34.4 ± 9.1		**0.0001**	**0.0001**	**0.0008**	**0.0005**	0.945
	Red stumpnose	3	863.05	7.9501	**0.0007**	0	0	37.4 ± 8.4	34.3 ± 1.6		**0.0033**	0.1558	**0.0323**	0.4974	**0.0355**
	Dageraad	3	1047.2	5.0479	**0.0053**	40.9 ± 7.5	30.2 ± 15.9	0	0	0.6723	**0.0263**	**0.0105**	**0.031**	**0.017**	
	Red steenbras	3	3771.2	9.732	**0.0002**	39.4 ± 6.4	34.4 ± 11.4	58.1 ± 10.3	51.3 ± 9.5	0.265	**0.0001**	0.0901	**0.0083**	0.737	**0.0109**

E. DEPTH RANGE EXTENSIONS

Some species (steentjie, fransmadam and hottentot) were sampled at depths beyond their previously recorded maxima (Table 4.11, Heemstra & Heemstra 2004, Mann 2013).

Table 4.11. Depth range extensions of three sparid fish species recorded in Tsitsikamma.

Species	Common name	Depth distribution (according to literature)	Reference	Depth distribution (present study)
Boopsoidea inornata	Fransmadam	30m 34m	Mann (2013) Heemstra & Heemstra (2004)	55m
Pachymetopon blochii	Hottentot	55m	Mann (2013) Heemstra & Heemstra (2004)	75m
Spondyliosoma emarginatum	Steentjie	50m 60m	Mann (2013) Heemstra & Heemstra (2004)	75m

4.3.3.3 HABITAT PREFERENCES RELATED TO DIET

Five canonical axes (*m*) best described the variability in the ontogenetic feeding guild data. The first two canonical correlations were large (0.95 and 0.65) and designated the strength of the associations between the feeding guild groups and habitat types (Figure 4.7). The first canonical axes separated the shallow and deep reefs from each other, and the second axis separated the shallow habitats from the deeper habitats within each reef.

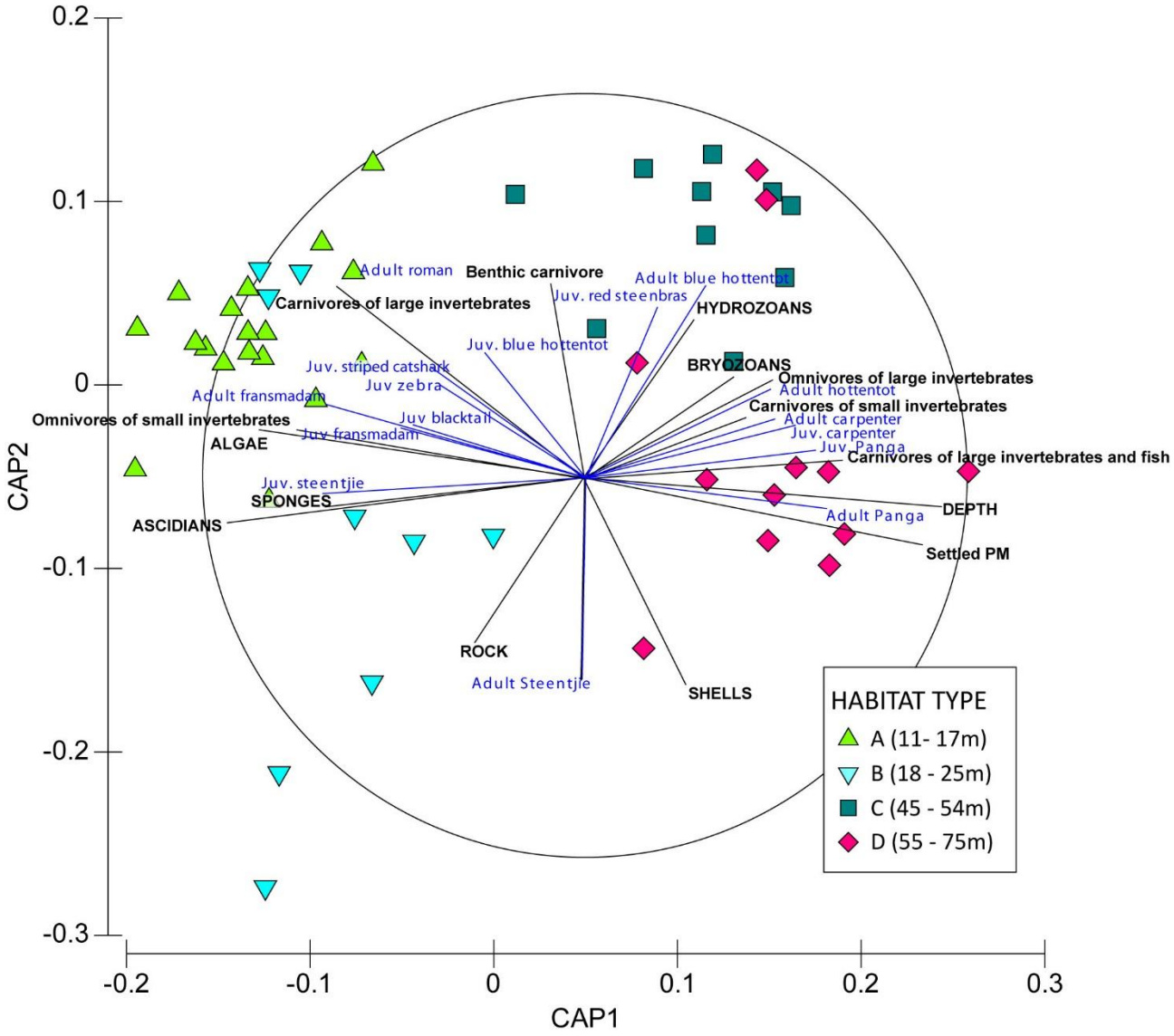


Figure 4.7. Canonical analysis of principle coordinates (CAP) performed on the feeding guilds of commercially important fish species and separated into adult and juvenile diets. The different habitat types defined from macrobenthic species can be identified from the legend insert, and the Pearson's correlation coefficients (> 0.4) of the percentage cover (habitat types; capital letters), fish life stages (blue italics) and feeding guilds (normal font) are superimposed as vectors. Settled PM = settled particulate matter. See Table 4.2 for details on the feeding guilds.

The estimation of the misclassification error determined from the leave-one-out procedure indicated a high allocation success, with80% of the feeding guild data correctly assigned to the defined habitat types (Table 4.12). The high allocation success is an indication of how well habitat types predicted the feeding guild composition, suggesting that patterns in fish distribution may be related to diet preferences or ontogenetic shifts in diet.

Table 4.12. Cross-validation results for the CAP using feeding guilds of the commercially important fish. Commercially important fish were divided into adults and juveniles and corresponding feeding guild assigned to each species.

Original group	A	B	C	D	Total no of samples per group	Correctly classified (%)
A	15	3	0	0	18	83.3
B	4	5	0	0	9	55.6
C	0	0	9	1	10	90.0
D	0	0	2	11	13	84.6

To better explain the relationships between fish feeding guilds and habitat types, the different feeding guilds and strongly associated life stages of the fish species were superimposed as vectors on the CAP ordination plot. On the shallow reef, habitat A (defined by algae) supported omnivorous fish life stages that fed on algae and small and large mobile invertebrates. Life stages associated with habitat A included mostly juveniles of blue hottentot, zebra, blacktail, fransmadam and striped catshark, and adult fransmadam and roman. Juvenile steentjie were associated with habitat B, which was defined by ascidians and sponges. On the deep reef, habitat C supported omnivores (adult hottentot) that fed on small mobile invertebrates and carnivores such as juvenile red steenbras. Habitat D was defined by high cover of bryozoan species, settled PM and gorgonians that supported carnivores fish (adult and juvenile panga and carpenter) that fed on large mobile invertebrates and fish (squid, octopus, crabs, etc.).

4.4 DISCUSSION

There was a clear change in fish assemblage structure with an increase in depth, and the fish communities of Tsitsikamma differed between reefs and among habitat types. The percentage cover of encrusting macrobenthos (algae, ascidians, sponges, bryozoans, hydrozoans and gorgonians) and substrate cover (rock, rubble, settled PM, shells and sand) of these habitat types successfully predicted the fish assemblage structure and species composition. The changes in habitat types were

strongly related to depth (Chapter 3) and suggested the importance of depth and habitat as predictors of fish distribution and assemblage patterns.

4.4.1 DISTRIBUTION OF FISH RELATED TO DEPTH, HABITAT & DIET

Fish assemblages differed among the habitat types due to rapid changes in abiotic variables, which in turn influenced niches available to the different fish species. Furthermore, fish species demonstrated depth related ontogenetic shifts in habitat use because diet preferences changed with increasing size.

The shallow reef fish assemblages were characterised by high abundance of mostly small fish species feeding at low trophic levels. This pattern stood in contrast to the deep reef fish assemblages, which were comprised of fewer low abundance species feeding at higher trophic levels. Indeed, this phenomenon of "smaller shallow and larger deep" is a commonly observed pattern in many demersal fish communities (Macpherson & Duarte 1991, Brokovich et al. 2008, Ryer et al. 2010, Fitzpatrick et al. 2012). With an increase in depth, predictable changes in abiotic conditions occur, such as decreased light, water movement, temperature and increased sedimentation (Garrabou et al. 2002). The impacts that these changes have on fish assemblage structure, composition and distribution are two-fold. Firstly, changes in abiotic conditions change the habitat (benthos) available to fish (Chapter 3). Different habitat types attract different types of fish due to specific resource requirements (food and shelter; Fischer et al. 2007) and physiological preferences (temperature, light, pressure and salinity; Macpherson & Duarte 1991). Secondly, fish behaviour influences habitat selection due to the variation in abilities of different species to avoid predators or obtain prey (Ryer & Olla 1999, Rypel et al. 2007). Thus, habitat use of fish is strongly influenced by habitat preference at different life stages and the trade-offs among resource availability, resource use and predator avoidance (Wolter & Freyhof 2004).

The loss of light with an increase in depth impacts fish assemblages and their distribution in two ways. Firstly, a decrease in light intensity results in the loss of primary producers. Loss of primary producers can reduce habitat complexity and niche availability to all levels of consumers. Primary producers increase niche availability through enhancing habitat complexity and by providing direct food sources to lower level consumers (Fischer et al. 2007). Primary production as a source of energy is essential for sustaining large abundances of small and juvenile fish, which tend to have high energy requirements. Lower level consumers, in turn, become prey and thereby improve the food quantity and quality to higher level consumers such as fish (Fischer et al. 2007, Félix-Hackradt et al. 2014). Secondly, loss of light changes fish behaviour (Rickel & Genin 2005), and therefore assemblage

structure and composition. Feeding efficiency of small fish species that forage in the water column near the benthos is highly dependent on the availability of sufficient light to feed (Ryer & Olla 1999). Low light conditions decrease the foraging efficiency of these fish and increase their vulnerability to predation (Ryer & Olla 1999, Rickel & Genin 2005), which may explain the preference of juvenile omnivorous fish species, especially steentjie, fransmadam, and blue hottentot, for habitat A. These fish species feed on a combination of small mobile invertebrates and algae, thus explaining their close association with a habitat type characterised by high light intensity and algal cover.

Since relative piscivory generally increases with fish size, and because there was a general trend of increased fish size with depth (Figure 4.6), it follows that abundances of larger piscivorous fish increase with depth (Ryer et al. 2010), a trend also found in Tsitsikamma. For instance, juvenile steentjie that fed on plankton and algae congregated on the shallow reef (mostly habitat A; Figure 4.6). As steentjie increase in size, this species migrates to depth, mostly feeding on large mobile invertebrates and small fish (Mann 2013). With an increase in size, a general ontogenetic change in the nervous system occurs, and larger fish have increased light and sound thresholds (Macpherson & Duarte 1991). The added sensitivity to light for larger fish gives them an advantage in darker (deep) environments, and consequently adult fish can exploit deep reefs that are not suitable for juveniles (Rickel & Genin 2005). Furthermore, higher numbers of predators at depth can either reduce juvenile numbers through predation (Ryer et al. 2010), or alter juvenile behaviour so that they preferentially choose shallower regions where predator numbers are lower and environmental conditions are optimal (more food and warmer temperatures).

Temperature plays an essential role in fish metabolism (Hanna et al. 2008) and behavioural responses (Valdimarsson et al. 1997). Shallow reefs generally demonstrate higher water temperatures compared to deeper reefs, a trend also found in the Tsitsikamma study area (Roberts & van den Berg 2005). Higher temperatures combined with abundant food on shallow reefs support the metabolic requirements and accelerated growth of juvenile fish (Macpherson & Duarte 1991). Accelerated growth decreases the time frame juveniles require to achieve size-refuge from predation and the ability to migrate to adult habitats. Larger, mostly piscivorous predators such adult panga, carpenter and red steenbras were found inhabiting the deeper reef in Tsitsikamma. In fact, larger fish were consistently found on the deeper reef, and this pattern can be explained by several benefits associated with migration to deeper reefs. With an increase in depth, temperature and light decreases. These changes in abiotic conditions benefit larger fish at depth through shifts in behavioural adaptations, life history parameters and longevity (Macpherson & Duarte 1991). Lower temperatures result in lower metabolic rates, so larger fish require less food compared to smaller fish. Lower temperatures extend fish lives by lowering metabolic costs, which increases their total

reproductive output (Macpherson & Duarte 1991). An increase in the total reproductive output is due to the combined effects of lowered metabolic cost and increased longevity. Lower metabolic costs means more energy directed to reproduction, and increased longevity affords a significant increase in the number of offspring produced over a lifespan (Macpherson 1998). As such, it seems that the different resource and niche requirements of fish at different stages of their life cycle implies migration from warmer shallow reefs to deeper cooler waters as part of their ontogeny.

4.4.2 HABITAT SPECIFICITY

4.4.2.1 HABITAT SPECIALISTS

Fish species that demonstrated habitat specificity restricted their movements and remained within a reef (shallow or deep) or a particular habitat type during their entire lifespan. Commercially important fish species classified as specialists included carpenter, red stumpnose, hottentot and dageraad. The majority of specialists not considered as commercially important were rare and unique species found only in habitat A. These rare and unique species included elasmobranchs such as the short-tail stingray (*Dasyatis brevicaudata*), common eagle ray (*Myliobatis aqulia),* dark shyshark (*Haploblepharus pictus*), and bony fish like bank steenbras (*Chirodactylus grandis*), yellowbelly rockcod (*Epinephelus marginatus*), and dane seabream (*Porcostoma dentata*; Table 4.8). The restricted movement of specialist species to a particular habitat type suggested that no depth related ontogenetic shifts occurred in habitat use, and that both juveniles and adults co-existed.

4.4.2.2 HABITAT GENERALISTS

Species categorised as habitat generalists (distributed across all depth ranges) demonstrated ontogenetic shifts in habitat use, with larger, sexually mature individuals found on deeper reefs, whereas juveniles congregated on shallower reefs (Figure 4.6). Depth related ontogenetic shifts were evident from the KDE results for steentjie, roman, fransmadam, blue hottentot and red steenbras (Figure 4.5). The degree to which fish species demonstrated habitat selectivity may be due to the combined effects of shifts in feeding strategy and behaviour. Although there was a strong connection between feeding strategy and habitat specificity, this pattern did not hold true for all fish species. For instance, roman was considered a habitat generalist in Tsitsikamma. Roman feed on a large variety of prey items, allowing them to forage within all habitat types, although they were most abundant in habitat type B. Similarly, Harvey et al. (2013) reported that predatory fish species such as the ocean jacket (*Nelusetta ayraudi*) were both prevalent and abundant within the Recherche Archipelago (Western Australia). Their abundance throughout the region suggested that ocean

jackets were habitat generalists. Ocean jackets feed on a selection of molluscs, crustaceans and cephalopods (similar diet to roman), and this varied selection of prey items means that they are able to cope with local disturbances (Harvey et al. 2013). However, dageraad, which have a diet similar to that of roman and the ocean jacket (Mann 2013), were found only on the shallow reef in Tsitsikamma. Dageraad are aggressive shoaling predators which demonstrate particular affinity for high profile reefs, and as such are easy targets for fishers (Griffiths 2000). In contrast, roman is an aggressive predator and a highly resident territorial species that lives either solitary or in small groups, and as such is distributed more evenly across reef habitats (Griffiths 2000). Therefore, the restriction of dageraad to the shallow reef of Tsitsikamma may be due to a combination of a history of previous exploitation and the competitive exclusion by more territorial species such as roman.

Variable patterns in species-specific ontogenetic shifts in habitat use such as those reported here are also common in coral reef communities (Lecchini & Galzin 2005, Ortiz & Tissot 2012). For instance, Lecchini & Galzin (2005) found that of the 20 most abundant coral reef fish species recorded in Moorea lagoon (French Polynesia), 12 species demonstrated ontogenetic habitat shifts and the rest remained in the same habitat throughout their lives. Ortiz and Tissot (2012) found similar patterns around the islands of Hawaii; many species shifted habitat, and others remained in the same habitat as they matured. Species that did demonstrate ontogenetic shifts in habitat use would either shift to more structured, or less structured substrates, and selection of habitat was species-specific (Ortiz & Tissot 2012).

4.4.3 DEPTH RANGE EXTENSIONS

An interesting depth range extension was observed in the hottentot. Although the literature indicates that hottentot are found only to depths of 55m and are omnivorous (Heemstra & Heemstra 2004, Mann 2013), they were encountered only on the deep reef in Tsitsikamma. The information on the biology of this fish species in the literature was collected in the colder Western Cape waters (Lechanteur & Griffiths 2003, and references therein), and might suggest that the restriction to deeper reefs might be due to the preference of hottentot for lower temperatures found only deeper in Tsitsikamma.

4.5 CONCLUSIONS

This study is the first to give detailed information on the depth distribution and habitat association of reef fish in the Agulhas warm-temperate ecoregion. Those species considered habitat generalists

demonstrated ontogenetic shifts in habitat use, whereas species that demonstrated habitat specificity did not follow this pattern. These findings validate the importance of considering both shallow and deep reef habitats in MPA design for the conservation of biodiversity and fisheries management efforts. Shallow reefs were characterised by higher diversity and abundances of unique and rare species in addition to juvenile life stages. The loss of protection of shallow reef habitat thus has important impacts on the conservation of biodiversity (important for ecosystem resilience) and on the population sizes and assemblage structure of fish species. Equally, deeper reefs were characterised by greater abundances of larger individuals and species, many of which are top predators important in top-down control of fish communities, thereby maintaining ecosystem stability. Those fish species that demonstrated no shallow reef affinity (carpenter, hottentot, red stumpnose) are afforded very little protection, as current MPAs extend only a few kilometres offshore and exclude deeper reef habitats (Fitzpatrick et al. 2012).

5 TROPHODYNAMICS OF THE SHALLOW AND DEEP REEFS IN THE TSITSIKAMMA MARINE PROTECTED AREA

5.1 INTRODUCTION

Marine ecosystems demonstrate complex interactions among species and their environment. Such complex interactions make it difficult to fully describe the trophodynamics and energy flow within a community (Piché et al. 2010). However, a clear comprehension of the ecological processes and the roles that different functional groups play in supporting a resilient ecosystem are needed for the implementation of effective management strategies (Pitcher 2008). Determining the diets of animals is essential for understanding their basic ecology (Thompson et al. 2012). Ecological processes such as predator-prey interactions, bottom-up and top-down control on prey populations, population dynamics, changes in species distribution and community level shifts in responses to biotic or abiotic variables can be better understood when clear trophic links can be established (Hairston et al. 1960, Leibold 1996, Thompson et al. 2012). In our quest to better understand processes that drive community structure and change in the marine realm, most research has been conducted on trawlable sites (soft bottoms) or reefs that lie within SCUBA diving depths. However, deep nearshore reefs host many sexually mature commercially important fish species (Chapter 4; Buxton & Smale 1989; Mann & Buxton 1992; Götz 2005) and unique macrobenthic assemblages (Chapter 3; Brokovich et al. 2008; Kahng et al. 2010; Gori et al. 2012). Thus, besides the inherent difficulties in gathering information on the ecological processes in marine ecosystems, additional logistical difficulties and higher expenses of sampling and observing communities at depth (Dodds et al. 2009) make deeper reefs even more challenging to study.

Traditional methods to study trophic interactions (stomach contents, faeces) provide snapshots of diets and can underestimate soft and highly digestible food items, but overestimate the most recently consumed items (Bowen 2000, Budge et al. 2006, Beck et al. 2007). Trophic studies on deeper reefs are even more challenging because the rapid change in pressure results in animals

expelling ingested food as they are brought up from depth (Dodds et al. 2009). Consequently, ecologists have developed indirect methods for examining trophic interactions. Fatty acid (FA) analysis has been used to study trophic interactions in many different marine communities; e.g. Arctic benthos (Graeve et al. 1997), tropical reefs (Piché et al. 2010), subtropical pelagic zooplankton (Richoux 2011), temperate pelagic fish (van der Bank et al. 2011) and warm-temperate rocky reefs (Gori et al. 2012). Fatty acids can be used as trophic tracers in marine food webs because marine FAs are extremely diverse and have a variety of structures (Budge et al. 2008). Furthermore, biochemical restrictions on the synthesis of FAs make it possible to identify FAs derived from their prey (Budge et al. 2008). Fatty acids are the main components of acyl lipids and during digestion FAs are released from the ingested lipid molecules but are not degraded (Iverson et al. 2004). The FAs consumed by a predator are deposited into lipid stores with little or predictable modifications, thus providing an integrated record of dietary intake over time (Budge et al. 2006).

Essential fatty acids (EFA) are so called due to the limited ability of animals to synthesise these components in appreciable amounts required for basic biological functions (Kainz et al. 2004, Arts & Kohler 2009). The inability of most animals to synthesise EFAs stems from the lack of enzymes that can produce n-3 and n-6 polyunsaturated fatty acids (PUFAs), and as such animals have to obtain them through their diet (Parrish 2009). These important FAs are needed for maintenance of membrane structure and function and are important precursors for prostaglandins, hormone-like molecules involved in many cellular activities (Parrish 2009). In marine fish, three FAs have been identified as essential: eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6-n3), and to a lesser extent, arachidonic acid (ARA, 20:4n-6). Both EPA and DHA are important in growth, immunity and stress resistance of finfish (Parrish 2009, 2013). Reduced levels of DHA in fish decrease fecundity, impair eye sight and ability to feed at low light, decrease survival in early life stages, lessen membrane function, and affect schooling behaviour (Arts & Kohler 2009, Parrish 2009). Arachidonic acid is important in both sea urchin and finfish eggs and required for finfish growth, survival and stress resistance (Parrish 2009). Virtually all PUFAs originate from primary producers (Iverson 2009), and as a result, identification of the sources of EFA in an ecosystem can provide insights into the processes that support and sustain the community.

On rocky subtidal reefs, the main sources of ARA usually originate from benthic primary production (Kelly & Scheibling 2012), whereas microalgae (planktonic primary production) are the main sources of EPA and DHA (Dalsgaard et al. 2003). The primary production on the shallow reef in Tsitsikamma differed from the deep reef, as there was an absence of benthic algae in the deep regions (Chapter 3). In addition, the FA composition of the deep reef plankton community might differ from the shallow reef due to reduced light intensities, lower temperatures (Mortensen et al. 1988), microbial

degradation during transit to depth (Galloway et al. 2013) and grazing impact by zooplankton at depth (Desvilettes et al. 1997). Lastly, terrestrial sources should be more important for the shallow reef community due to the increased proximity to the shore. These differences suggest that the FA composition of shallow reef consumer tissues should differ from those on the deep reef.

5.1.1 STUDY AIM

The main aims for this chapter are to determine if different processes support the shallow and deep reef communities of Tsitsikamma, and what these differences mean in terms of the nutritional condition of the two reef communities. The processes that are considered include the following:

i) the importance of benthic algae and terrestrial input as carbon sources on the shallow reef,
ii) modifications in FA composition of the plankton community due to transit to depth, and include bacterial degradation and grazing impact by zooplankton,
iii) differences in sources of EFA on the shallow and deep reefs.

Accordingly, the following objectives were addressed:

i) to determine the FA profiles of plankton, macrobenthos and fish and compare these between the shallow and deep reefs,
ii) to establish if the feeding guilds identified in Chapters 3 and 4 correspond to FA profiles,
iii) to compare the FA profiles of macrobenthos and fish feeding guilds and establish the most influential FAs that identify trophic interactions.

From the patterns identified through the above objectives the following hypothesis was tested:

Because suspension-feeders directly consume plankton, their FA profiles should indicate the importance of different processes that support the two reef communities. Different processes considered here include terrestrial input, pelagic vs benthic productivity, grazing by zooplankton and microbial degradation of plankton during transit to depth. I therefore hypothesised that the FA profiles of the deep reef suspension feeding community differ from the shallow reef, and specifically the deep reef consumers are marked by higher proportions of bacterial FA (BAFAs) and zooplankton markers, and lower proportions of terrestrial markers

5.2 MATERIALS & METHODS

5.2.1 STUDY AREA

Research was conducted on the Middlebank and Rheeders Reef complexes situated close to the Storms River mouth in the TNP MPA. A full study area description can be found in Chapter 2, Section 2.1.2.

5.2.2 SAMPLING STRATEGY

The sampling strategy for this section was divided into two components. The collection of (i) physico-chemical data and plankton samples, and (ii) tissue samples from animals representing different feeding guilds in the study area. Excluding long-term temperature data, all physico-chemical samples were collected three times over a one year period (July and November 2011, and Feb 2012), and plankton samples were collected in November 2011 and February 2012. During each of these fieldtrips, three randomly selected stations (Figure 2.5) from each reef complex were targeted. Unless otherwise indicated, three replicates of each sample type were obtained from each station. Physico-chemical and plankton sample stations are termed plankton sample stations throughout the thesis, and all samples were collected according to this strategy.

Due to the difficulty of obtaining animal samples from regions deeper than SCUBA diving depth (>25 m), tissue samples were not collected at the sample stations mentioned above (Figure 2.5), but rather collected opportunistically from each reef complex. For a full list of samples processed for FA analyses, see Table A5.1.

5.2.3 SAMPLE COLLECTION

5.2.3.1 PHYSICO-CHEMICAL PARAMETERS

A. TEMPERATURE PROFILES

Long-term temperature data were obtained from underwater temperature recorders (UTRs; Onset HOBO Pro v2) positioned in the centres of the shallow and deep study sites. A thermister array attached to an AR-60-E acoustic release (Sub Sea Sonics) was deployed to 80 m on Middlebank Reef and serviced every four months. Hourly temperature was recorded at 75 m, 65 m and 60 m to an accuracy of 0.01°C. To obtain temperature data for the shallow reef, two UTRs were permanently fixed on Rheeders Reef at 18 m depth and retrieved by SCUBA divers at the end of the study.

B. CHLOROPHYLL-*A* CONCENTRATION

Water samples were collected by lowering a Vertical Point Sampler to just above the reef. Three discreet water samples were collected at each station, employing the sampling strategy introduced in Section 5.2.2. Total chlorophyll-*a* (chl-*a*) concentrations were determined from three 200 ml water samples.

C. SALINITY, dO_2 & CONDUCTIVITY

A YSI 600XLM multi parameter water quality probe that allowed for simultaneous measurements of depth, salinity, conductivity and dissolved oxygen (dO_2) was lowered at each plankton sample station. As changes in these parameters were assumed to be minimal over small spatial scales due to horizontal mixing in the water column, the probe was lowered only once at each sample station. Data from the YSI water sampler were logged onto a 650MDS (Multiparameter Display System) data display and logging system, after which they were downloaded using ECOWatch software. Physico-chemical data were collected just above the reef to obtain a representation of the environmental conditions associated with the plankton collected.

5.2.3.2 PLANKTON

Plankton samples were collected as indicated in Section 5.2.2. Plankton samples were collected by lowering a KC Denmark Model 23.580 plankton pump to just above the reef and pumping water through a 65 µm mesh. After 15 minutes, the pump was recovered and samples were retrieved from the cod-end. Samples were size fractioned into >500 µm and between 65 and 500 µm.

5.2.3.3 MACROBENTHOS

Prior to macrobenthic and fish tissue collections, an extensive literature survey was conducted to determine the key and abundant species that represent each feeding guild within the bioregion (Buxton 1984, Buxton & Smale 1984, Burger 1990, Mann & Buxton 1992, Wood et al. 2000, Brouwer 2004). Target species, including algae, were collected opportunistically, and when possible three replicates of each species were obtained from both the shallow and deep reef sites. All samples were collected during February 2012. Macrobenthic samples were collected employing a number of strategies. Shallow reef (<25 m) samples were retrieved by SCUBA divers and deep reef samples with the Falcon SAAB Seaeye ROV fitted with a Hydro-lek five-function manipulator arm (HLK-43000). Smaller mobile invertebrates were sampled by employing a selection of un-baited traps.

5.2.3.4 REEF FISH

Fish tissue samples were obtained by fishing, spearing and deploying a selection of un-baited traps. Shallow reef samples were collected by spearing and fishing, and deep reef samples by fishing and trapping. To guarantee that fish were killed as humanely as possible, they were all pithed prior to being placed on ice. This procedure ensured a quick, stress-free death, and is considered by the South African National Parks (SANParks) as an acceptable method of euthanasia during field studies. The use of chemicals during euthanasia may contaminate body tissues, making the samples unsuitable for biochemical studies. Fish not sacrificed were immediately returned to sea. When fish obtained from the deeper reef suffered severe barotrauma, they were either vented by inserting a hypodermic needle into their swim bladder or assisted to depth by hooking a weighted barbless hook through their lower jaw and released with a gentle tug of the rod on return to depth.

For FA analysis, freshness of tissue is of utmost importance because lipolytic enzymes begin to degrade FAs straight after death (Budge et al. 2006). To prevent degradation, tissues should be freshly frozen to minimize losses of FAs (Budge et al. 2006). Due to logistical constraints in the field, fish and plankton samples were placed on ice and invertebrate samples were kept alive in cold sea-water, separated into sealed plastic bags to prevent feeding. On return to the field laboratory, samples were processed and initially frozen at -20°C between 1 and 5 hours after collection, and within three weeks transferred to -80°C for long term storage.

5.2.4 SAMPLE TREATMENT

5.2.4.1 PHYSICO-CHEMICAL PARAMETERS

CHLOROPHYLL-*A* CONCENTRATION

Upon return to land, aliquots of 200 ml water samples collected just above the reef were gently filtered (<5 mm Hg vacuum) through 47 mm Whatman glass fibre filters (GF/F) and extracted in 10 ml of 90% acetone for 24 h at -20°C. Extracted samples were centrifuged (5000 rpm) for five minutes and the total chl-*a* determined employing a Turner Designs 10AU fluorometer following the method of Holm-Hansen & Riemann (1978). Chlorophyll-*a* concentrations were expressed as $\mu g\ l^{-1}$.

5.2.4.2 PLANKTON

Similar to the chl-*a* procedure, samples collected with the plankton pump (>65 µm) were gently filtered (<5 mm Hg vacuum) filtered onto pre-ignited, pre-weighed G/FF (65 µm – 500 µm) and G/FC (>500 µm) filters. The filters were placed in individual foil pockets and stored at -20°C for the

remainder of the field trip (about 3 weeks), and on return to the laboratory stored at -80°C until further sample processing.

5.2.4.3 MACROBENTHOS & REEF FISH

When possible, invertebrates were dissected and muscle tissues removed, placed in individual foil pockets and frozen. Smaller invertebrates such as amphipods and isopods were pooled to obtain adequate signals. When pooling was done, care was taken to select individuals of the same species and similar size range. Animals that were sampled whole were initially allowed to clear their guts. However, high mortality rates allowed only short evacuation episodes of between two and three hours. Where possible, animals were identified to species level using Zsilavecz (2007), Jones (2008) and Branch et al. (2010). A small section of white dorsal tissue was dissected from sacrificed fish specimens. Care was taken to include only white flesh and not to contaminate the sample with scales, blood or skin. Tissue samples were labelled and placed in individual foil pockets and frozen for later processing.

5.2.5 SAMPLE PROCESSING

In the laboratory, samples were lyophilized (Virtis Benchtop 2K) at -60°C for at least 24 h. All invertebrate, algae and fish tissue samples were individually homogenised with a mortar and pestle. Aliquots of homogenised tissue samples were weighed (between 20 – 200 g of dry mass, depending on relative lipid content). Fatty acid samples were stored at -20°C under nitrogen in sealed test tubes with butylated hydroxytoluene (BHT) and chloroform ($CHCl_3$). Surplus homogenised samples intended for stable isotope analyses were stored for later processing (Chapter 6).

5.2.6 FATTY ACID ANALYSIS

Before FA analysis can be carried out, lipids must be separated (extracted) from the matrix in which they are embedded. Then, to obtain both quantitative and qualitative FA profiles, fatty acid methyl ester (FAME) derivatives must be formed through trans-esterification (Budge & Parrish 2003). Numerous methods exist for both steps, but here macrobenthic and plankton samples were processed using a modified version of a one-step method described by Indarti et al. (2005), and fish samples were processed by combining and modifying methods from Folch et al. (1957), Budge & Parrish (2003) and Budge et al. (2006). Fish samples were processed differently from the macrobenthos and plankton samples to remove phospholipids (PL). These PL are part of the structural components of cell walls, and due to their specialised functions, organisms tend to

conserve the FAs in PL. As a consequence, PL are highly robust to dietary changes and not informative as diet indicators, especially in higher order predators (Budge et al. 2006).

5.2.6.1 PLANKTON & MACROBENTHOS

Fatty acid methyl esters were analysed by modifying the one-step method of Indarti et al. (2005). Briefly, an internal standard (19:0) and 2 ml anhydrous methanol-sulphuric acid mixture were added to each sample and placed in an oven at 100°C for 30 min. After cooling samples to room temperature, 1 ml of distilled water (dH_20) was added to each sample and centrifuged. Centrifuging separated the samples into two phases: the upper aqueous phase, which was discarded, and the lower FAME phase, which was passed through a pipette packed with pre-rinsed cotton wool and Na_2SO_4 (a drying agent) into a 2 ml vial. The solvent was evaporated to dryness under a gentle stream of nitrogen (N_2), and topped with hexane prior to gas chromatography-mass spectrometry (GC/MS) injection.

5.2.6.2 FISH SAMPLES

A. LIPID EXTRACTION: MODIFIED (Folch et al. 1957)

One millilitre of methanol (MeOH) and 2:1 ($CHCl_3$: MeOH) were added to each of the stored samples (2 ml $CHCl_3$and BHT). Samples were sonicated on ice for 4 min and stored at -20°C for at least 24 h. Samples were filtered through pre-rinsed cotton wool-plugged pipettes into a freshly prepared lipid cleaned test tube. Following the addition of 1.5 ml of a 0.9% potassium chloride (KCl) solution, samples were centrifuged at 3000 RPM for 3 min and the top layer of the stratified contents discarded. Next, 0.5 ml of 0.9% KCl in dH_20 and 0.5 ml of methanol were added to the samples, which were then centrifuged and the top aqueous layer discarded. The remaining solvent was dried with Na_2SO_4 and filtered through a cotton wool-plugged pipette into another lipid cleaned test tube. Solvents were evaporated to dryness under a gentle stream of N_2, and 1.5 ml of Na_2SO_4-dried $CHCl_3$was added and stored under N_2.

B. FRACTIONATION OF LIPID EXTRACT (Budge & Parrish 2003)

Neutral lipids (NL) were fractionated from acetone-mobile polar lipids and PLs using column chromatography on silica gel. A small amount of glass wool was placed in the tapered end of a Pasteur pipet and combusted at 500°C for 5 h. The pipet was packed with approximately 0.8 g of silica gel that had been activated by heating at 100°C for 1 h. The column was then rinsed sequentially with 6 ml MeOH, $CHCl_3$ and 98:1:0.5 ($CHCl_3$/MeOH/formic acid). Approximately 5 mg of lipid extract in $CHCl_3$ was placed at the top of the column, and NL were recovered by eluting approximately 6 mL of

98:1:0.5 through the column. Samples were evaporated to small volumes and stored in 1.5 ml dichloromethane under N_2 until FAME synthesis.

C. TRANS-ESTERIFICATION (Budge et al. 2006)

Known quantities of the internal standard (19:0) were added to each sample, followed by 3 ml of Hilditch reagent (1.5 ml H_2SO_4 to 100 ml of anhydrous Na_2SO_4 MeOH), and placed in an oven at 100°C for 1 h. After cooling to room temperature, 3 ml hexane and 1 ml dH_2O were added to each sample and centrifuged at 3000 RPM for 5 min. This process was repeated using only 1 ml of dH_2O. The top layer (FAMEs in solvent) was pipetted into a new test tube, evaporated to dryness under a gentle stream of N_2 and finally transferred into a 2 ml vial with 0.5 ml of hexane prior to GC/MS injection.

5.2.6.3 GAS CHROMATOGRAPHY

Fatty acid compositions were determined using an Agilent 7890A/7000 Triple Quadrupole GC/MS equipped with Zebron ZB-WAXplus capillary GC columns (30 m length x 0.32 mm inner diameter I.D., 0.25 µm film thickness) and both flame ionization (FID) and MS detectors. Helium was the carrier gas at 1.664 ml min^{-1} for both FID and MS analyses, and 1 µl of each FAME sample was auto-injected at 250°C with the oven set at 70°C. After 1 min, the oven temperature was raised to 170°C at 40°C min^{-1} and held for 3 min, then increased to 250°C at 2.5 min sec^{-1} and held for 4 min (total run time 40 min). The FID was kept at a constant 300°C. Fatty acid methyl ester peaks were identified in representative samples of each species using MassHunter B05.00 and the NIST 08 MS library. Retention times and external standards (marine PUFA no. 1, 37 component FAMEs, SUPELCO) were used to interpret FID chromatograms integrated by Chemstation 04.02. Quantification of FAME peaks was accomplished by comparing FAME peak areas with that of the internal standard, and the data were reported as the fatty acid weight per mg dry mass (µg FA mg^{-1} DM). Each FA was also measured as a proportion of the total fatty acids (% TFA). Fatty acids were named according to A:Bn-X, where *A* is the number of carbon atoms, *B* is the number of double bonds and *X* is the position of the first double bond from the methyl end of the molecule. With this naming system, it is assumed that all FAs are methylene-interrupted (i.e. each double bond is separated by a CH_2 group). However, unusual FAs do occur with double bonds that are non-methylene interrupted (NMI). Their presence in the food chain is due to synthesis by marine invertebrates such as bivalves and sponges (Barnathan 2009). Fatty acids without double bonds are saturated (SFA) and FAs with one double bond are monounsaturated FAs (MUFA). Fatty acids with two or more double bonds are polyunsaturated FAs (PUFA). Some FAs contain a methyl branch on the second or third carbon closest to the terminal methyl group. A methyl branch at the second carbon is indicated by prefacing the FA name with an "*i*" (iso) and "*ai*" (anti-iso) indicating a methyl branch at the third carbon (Budge et al. 2006).

5.2.7 STATISTICAL ANALYSES

5.2.7.1 PHYSICO-CHEMICAL PARAMETERS

A. TEMPERATURE

To test if the deep and shallow reef temperatures obtained from the long term UTRs were significantly different, temperature data were averaged for each reef and a t-test was performed on the normally distributed data using STATISTICA (v12).

B. CHLOROPHYLL-*A* CONCENTRATION

To test if chl-*a* concentrations were significantly different between reefs and among sampling periods (season), Mann-Whitney U and Kruskal-Wallis tests were performed, respectively, as assumptions of normality for parametric tests were not met. Analyses of all datasets were performed using STATISTICA (v12).

C. SALINITY, TEMP, dO_2 & CONDUCTIVITY

To test if salinity, specific conductivity (ms cm^{-1}), and dO_2 (mg l^{-1}) measurements yielded significantly different values between reefs and among the sampling periods (seasons), non-parametric Mann-Whitney U and Kruskal-Wallis tests were employed as the data were not normally distributed. Analyses of all datasets were performed using STATISTICA (v12).

5.2.7.2 FATTY ACID ANALYSIS

All multivariate and univariate analyses were conducted using PRIMER v6 (Clarke & Gorley 2006) with the PERMANOVA + add-on package (Anderson et al. 2008). All data sets were left untransformed to avoid giving weight to FA found in low quantities, as these FA found are not important for community analyses (Kelly & Scheibling 2012). Univariate permutational multivariate analyses of variance (PERMANOVA) were performed using Euclidean distance measures, and multivariate PERMANOVAs were performed using Bray-Curtis distance measures, as these are most suited for ecological data (Clarke & Warwick 2001).

For comparison of the pooled univariate data using only one factor (reefs), *P*-values were obtained from 9999 unrestricted permutations of the raw data (Anderson et al. 2008). For multivariate PERMANOVA where more than one factor was included, 9999 permutations of the residuals under a reduced model were computed for each term to obtain *P*-values (Anderson et al. 2008). Significant interactions were investigated with pairwise analyses based on 9999 permutations. Because the replicates for a species were few and unevenly distributed between the reefs, unique permutations were often small. To ensure that the permutation results were reliable, Monte Carlo (MC) *P*-values

were calculated. If permutations were less than 100, then MC *P*-values were used. Due to the opportunistic sampling conducted to obtain tissue samples intended for FA analysis, and because some FA samples were lost during processing, the data set was unbalanced. To account for the unbalanced structure of the PERMANOVA design, the procedures were run by selecting Type III sums of squares, ensuring complete independence of all factors tested (Anderson et al. 2008).

A. PLANKTON

To establish the influence of environmental parameters on the FA composition of the plankton community, a forward stepping distance based linear model (distLM) using 9999 permutations was performed on an untransformed Bray-Curtis similarity matrix (Anderson et al. 2008). The best model was selected using the AIC procedure (Akaike Information Criteria; Anderson et al. 2008). Environmental parameters included were temperature, chl-*a* concentration, light intensities at depth, depth and salinity.

Plankton samples (n = 57) included FA signatures collected at different dates (season) and consequently the multivariate PERMANOVA experimental design consisted of three factors each with two levels: 'reef' (shallow, deep), 'season' (November 2011, February 2012) and 'size class' (65 – 500 µm and >500 µm). To visualise the results, non-metric multi-dimensional scaling (MDS) ordinations were constructed with superimposed bubble plots for the most discerning FAs.

B. MACROBENTHOS & FISH

Apart from certain fish samples (n = 13), all remaining tissue samples for fish (n = 44) and macrobenthos (n = 89) intended for FA analysis were collected during February/March 2012. Fish samples collected during July 2011 were included to increase the sample size for fish species found on both the shallow and deep reefs.

Typically, the FA compositions of animals demonstrate a strong taxonomic link (Budge et al. 2002), and the differences in species-specific FA can overshadow any other patterns of interest. Due to opportunistic sampling on the deep reef, very few samples of the same species for both the shallow and deep reefs were available. Thus, to allow for comparisons between the shallow and deep reefs, samples were grouped according to different variables and tested using a canonical analysis of principal coordinates (CAP) analysis (Anderson & Willis 2003, Anderson et al. 2008). The grouping variable that performed best and was most relevant was used to test the effect of 'reef' employing multivariate PERMANOVA. For the macrobenthos, the grouping variables tested by the CAP analysis included higher order taxa (class), feeding guild (as defined in Chapter 3), growth form (excludes feeding mechanism), feeding mechanism (excludes growth form) and broad feeding guild (filter-

feeders, suspension-feeders, deposit-feeders, etc.). For the fish FAs, the grouping variables tested were higher order taxa, feeding guild (as defined in Chapter 4), broad feeding guild (which excluded separating species into juvenile and adults), and species. Following this procedure, a multivariate PERMANOVA was conducted on the macrobenthos and fish data to evaluate differences between the reefs and the identified grouping variable. Additionally, the results of the CAP analyses also served to establish whether the feeding guilds assigned in Chapter 3 and 4 corresponded to FA profiles and if the ontogenetic shifts in habitat use of fish were related to diet. To establish which CAP model best explained the FA composition in the macrobenthos and fish assemblages, misclassification errors were calculated. The 'leave-one-out' procedure is a method that provides a statistical estimate of the misclassification error, where the misclassification error is the proportion of points that were placed in the wrong group. High percentage allocation success suggests not only a high potential of the CAP model in making valid predictions, but also gives an indication of how distinct groups are. A detailed explanation of CAP analysis is in Section 4.2.3.2 of Chapter 4.

To explain the variation in FA patterns among the different feeding guilds, important and discriminating FAs were identified from the similarity percentage (SIMPER) procedure by comparing each feeding guild with the rest of either the macrobenthic or fish assemblages. The SIMPER procedure also provided information on the within-group similarities and dissimilarities between each feeding guild and the rest of the reef community. These FAs were superimposed as vectors (Pearson's correlations) on the CAP ordination plot.

To evaluate differences in the nutritional condition of food sources, both the proportions (% TFA) and concentrations (µg FA mg^{-1} DM) of total and essential fatty acids were compared between the reefs. Nutritional condition was inferred from fatty acids because lipids and their constituent fatty acids are important sources of nutritional energy (Iverson et al. 2002, Tocher 2003, Kainz et al. 2004, Daly et al. 2010). Essential fatty acids are particularly important as these FAs cannot be synthesised *de novo* in appreciable levels by consumers and must be obtained from primary producers. Due to their paramount importance in many biological functions, levels of EFAs can represent the health, or nutritional condition of a community or species.

Bacterial fatty acids (BAFAs) include odd-numbered carbon chains and iso- (*i*) and anteiso- (*ai*) branches (Budge et al. 2006), and EFAs include 20:4n-6 (ARA), 20:5n-3 (EPA) and 22:6n-3 (DHA; Parrish 2009). The ratio 18:1n-9/18:1n-7 is indicative of the relative level of carnivory, and 22:6n-3/20:5n-3 indicates the prevalence of diatoms over dinoflagellates in environments dominated by these algae groups (Dalsgaard et al. 2003). Terrestrial markers include the sum of 18:2n-6 and 18:3n-

3 (Budge & Parrish 1998) and several studies have indicated the predominance of 20:1 and 22:1 isomers as indicative of feeding on copepods (Graeve et al. 1997, Cripps & Atkinson 2000).

5.3 RESULTS

5.3.1 PHYSICO-CHEMICAL PARAMETERS

5.3.1.1 TEMPERATURE PROFILES

Temperature profiles recorded at the deep and shallow study sites were typical for Tsitsikamma (Figure 5.1; Roberts & van den Berg 2005). Water temperatures for the duration of the study ranged between 9.2 and 22.6°C (16.8 ± 2.5°C) on the shallow reef (18 m) and between 8.9 and 18.2°C (11.6 ± 2.3°C) on the deep reef (75 m), and were significantly different between reefs ($F_{1,113} = 892.35$, $p < 0.001$). A vertically stratified water column was present during the summer months (December to February 2012; Figure 5.1) and at times surface and bottom temperatures differed by as much as 10°C. During the rest of the year, the water column was mostly isothermal, particularly during the early summer months of November and December 2011. A prolonged upwelling event occurred during the February 2012 sampling period which lasted for seven days (4th – 10th of February 2012), coinciding with the collection of plankton samples (red arrows in Figure 5.1).

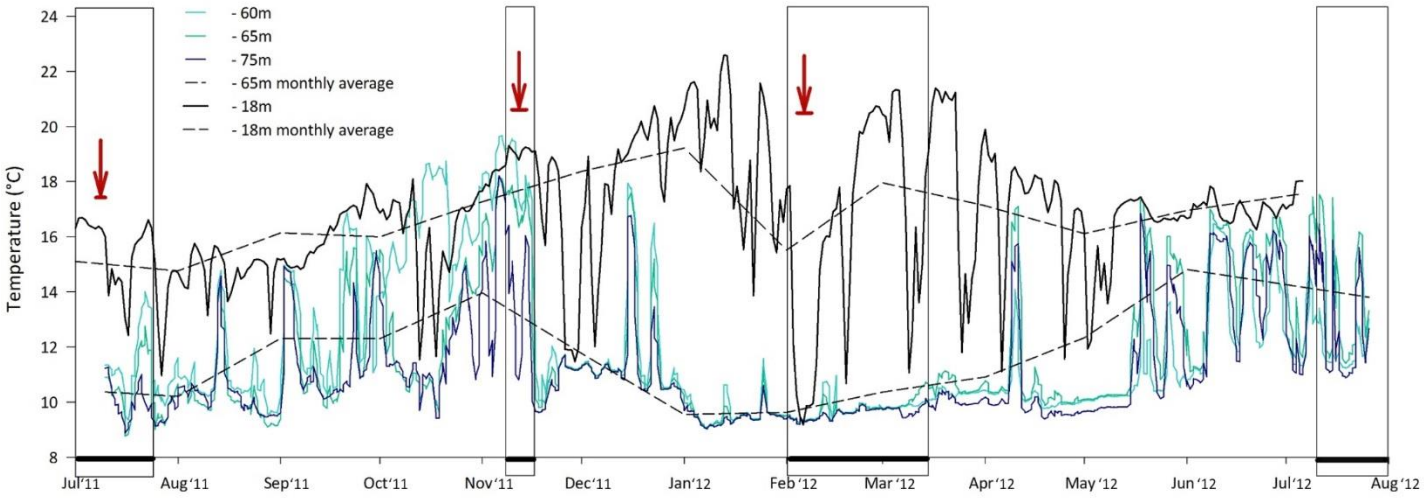


Figure 5.1. Temperature data recorded from July 2011 - August 2012 on the shallow (solid black line) and the deep (blue lines) reefs in Tsitsikamma. Framed sections indicate sampling seasons and red arrows depict the days when plankton and physico-chemical parameters were collected.

5.3.1.2 CHLOROPHYLL-*A* CONCENTRATION

Overall, the pooled chl-*a* concentrations did not differ significantly between the shallow and deep reefs (U = 187, Z = 0.285, p > 0.5; Figure 5.2 B). However, considering the chl-*a* samples of each sampling period in isolation, samples collected during July 2011 from the shallow reef did demonstrate significantly higher concentrations compared to those from the deep reef (U = 0, p < 0.05). Furthermore, a significant effect of season was observed (H = 23.099, p < 0.001) and chl-*a* concentrations obtained in November 2011 (0.61 ± 0.57 µg L^{-1}) were significantly higher compared to those collected during July 2011 (0.14 ± 0.19 µg L^{-1}) and February 2012 (0.06 ± 0.05 µg L^{-1}; U = 19, Z = -2.6, p < 0.001).

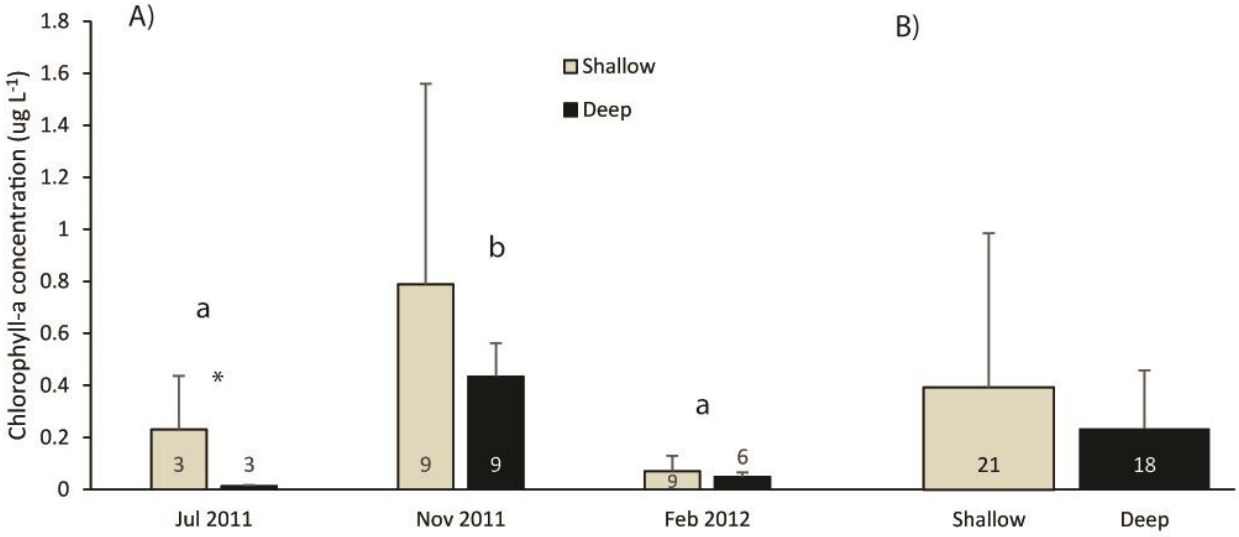


Figure 5.2. Chlorophyll-*a* concentrations. The average chlorophyll-*a* (chl-*a*) concentrations (µg L^{-1}) at the shallow (grey) and deep (black) reefs in Tsitsikamma. A: average chl-*a* concentrations obtained from each sampling event, and B: pooled chl-*a* data collected at all three sampling events to compare concentrations between the reefs. Error bars represent positive standard deviations. Different lower case letters indicate significant differences between seasons (A); * p < 0.05.

5.3.1.3 SALINITY, DO_2 & CONDUCTIVITY

Salinity and specific conductivity were not significantly different when compared between the shallow and deep reefs (salinity: U = 25, Z = 1.01; p > 0.05; specific conductivity: U = 33, Z = -0.24, p > 0.05) or among the sampling seasons (salinity H = 5.539, p > 0.05; specific conductivity: H = 2.774, p > 0.05). Dissolved oxygen was significantly higher on the deeper reef (U = 7, Z = -2.74, p < 0.05; Table 5.1), but did not demonstrate any seasonality (H = 1.471, p > 0.05).

Table 5.1. Physico-chemical variables of Tsitsikamma reefs. Data obtained from the YSI 600XLM multi parameter water quality probe from the shallow and deep reefs during July and November 2011 and February 2012. Values indicated in bold were significantly different ($p < 0.05$) between the deep and shallow reefs.

	Shallow reef	Deep reef	Shallow reef			Deep reef		
	(pooled dates)		Jul 2011	Nov 2011	Feb 2012	Jul 2011	Nov 2011	Feb 2012
Salinity (‰)	36.0 ± 0.3	35.7 ± 0.5	35.92 ± 0.14	36.35 ± 0.05	35.64 ± 0.01	35.78 ± 0.04	35.61 ± 0.95	35.78 ± 0.16
Specific conductivity (ms cm^{-1})	54.5 ± 0.2	54.2 ± 0.8	54.36 ± 0.13	54.81 ± 0.06	54.37 ± 0.001	54.4 ± 0.01	53.87 ± 1.28	54.62 ± 0.21
Dissolved oxygen (mg l^{-1})	**2.8 ± 1.4**	**5.8 ± 2.1**	3.46 ± 0.69	1.66 ± 1.56	3.29 ± 1.81	8.21 ± 0.34	4.41 ± 0.11	4.11 ± 0.46

5.3.2 FATTY ACIDS

5.3.2.1 UNIVARIATE

A. PLANKTON

The plankton samples were dominated by SFAs (up to 68% TFA, most of which were 16:0), followed by PUFAs (up to 54 % TFA, mostly from the EFAs 22:6n-3 and 20:5n-3), and MUFAs contributed the least to the % TFA of the plankton samples (Table 5.2). Quantitative concentrations of FAs in the plankton samples were on average 7.2 ± 4.7 µg mg^{-1} DM on the shallow reef and 10.9 ± 5.9 µg mg^{-1} DM on the deep reef. Saturated fatty acids were slightly higher on the shallow reef (43.1 ± 7.9 % TFA), but did not differ significantly from the deep reef SFAs (39.7 ± 8.2 % TFA). Monounsaturated fatty acids and the marker for copepods were significantly higher on the shallow reef compared to the deep reef (Tables 5.2 A & 5.3; Figure 5.3 A). Essential fatty acids were significantly higher on the deep reef (36.4 ± 12.6% TFA) compared to the shallow reef (29.4 ± 11.7% TFA; Tables 5.2 A & 5.3; Figure 5.3 A). Bacterial fatty acids were moderate and similar in the plankton samples from both the shallow and deep reefs (5.1 ± 1.4 % TFA and 5.2 ± 3.4 % TFA, respectively). Conversely, PUFAs were lower in the plankton samples from the shallow reef (35.6 ± 11.2 % TFA) compared to the deep reef (41.2 ± 12.2 % TFA; Table 5.2 A; Figure 5.3 A). Terrestrial signatures (∑ [18:2n-6, 18:3n-3]) were low but slightly higher in the shallow reef plankton samples (Figure 5.3 A).

B. MACROBENTHOS

In contrast to the plankton samples, the macrobenthic samples were dominated by PUFAs (up to 62% TFA, most of which were the EFAs 20:5n-3 and 22:6n-3), followed by roughly equivalent proportions of SFAs and MUFAs (Table 5.2 B; Figure 5.3 B). Quantitative concentrations of FAs in the macrobenthos were on average 15.6 ± 30.8 µg mg^{-1} DM on the shallow reef and 13.0 ± 11.0 µg mg^{-1} DM on the deep reef (Table 5.2 B). Similar proportions of SFAs, PUFAs, EFAs, MUFAs, BAFAs, and the copepod marker were evident in the pooled macrobenthic samples on both reefs and did not differ significantly (Figure 5.3 B, Table 5.3). The terrestrial signals were significantly higher in macrobenthic

animals on the shallow reef (2 ± 1.6% TFA) compared to the deep reef, (1.5 ± 0.8% TFA; Table 5.3; Figure 5.3 B).

Table 5.2. The fatty acid profiles (percent total fatty acids [% TFA]; mean ± standard deviation) of different components A) plankton (total lipids), B) macrobenthos (total lipids) and C) fish (neutral lipids) of the Tsitsikamma reef communities. Fatty acids included were those >1% TFA. Sum of saturated fatty acids (∑ SFAs), polyunsaturated fatty acids (∑ PUFAs), essential fatty acids (∑ EFAs), monounsaturated fatty acids (∑ MUFAs) and bacterial fatty acids (∑ BAFAs), terrestrial markers (∑ [18:2n-6, 18:3n-3]), copepods marker (∑ [22:1, 20:1]) and quantitative concentrations of TFA (µg mg^{-1} DM).

	A) Plankton (n = 57)		B) Macrobenthos (n = 86)		C) Fish (n = 57)	
Fatty acids (% TFA)	**Shallow**	**Deep**	**Shallow**	**Deep**	**Shallow**	**Deep**
14:0	5.7 ± 1.6	5.7 ± 1.9	3.1 ± 2.1	3.5 ± 3.6	2.8 ± 1.3	3.1 ± 1.7
i-15-0	0.6 ± 0.2	0.5 ± 0.2	0.7 ± 1.1	0.7 ± 0.6	0.3 ± 0.2	0.4 ± 0.5
ai-15:0	0.5 ± 0.3	0.3 ± 0.2	0.4 ± 0.6	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.2
15:0	0.7 ± 0.4	0.7 ± 0.3	0.7 ± 0.4	0.8 ± 0.5	1.0 ± 0.3	1.1 ± 0.3
i-16:0	0.6 ± 0.5	1.4 ± 3.2	0.4 ± 0.5	0.8 ± 0.8	0.3 ± 0.2	0.2 ± 0.2
ai-16:0	0.3 ± 0.3	0.2 ± 0.3	0.5 ± 0.5	0.5 ± 1.3	0.4 ± 0.7	0.4 ± 0.6
16:0	24.3 ± 4.2	23.0 ± 3.9	14.6 ± 11.3	12.9 ± 5.4	23.5 ± 5	22.8 ± 4.4
i-17:0	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.5	1.2 ± 0.7	0.5 ± 0.3	0.4 ± 0.3
ai-17:0	0.4 ± 0.3	0.4 ± 0.2	0.7 ± 0.6	0.9 ± 0.9	0.4 ± 0.4	0.3 ± 0.2
17:0	1.0 ± 0.4	0.8 ± 0.2	1.2 ± 0.8	1.3 ± 0.7	1.8 ± 0.5	1.7 ± 0.5
i-18:0	0.4 ± 0.3	0.5 ± 0.2	0.8 ± 0.7	0.8 ± 0.4	0.5 ± 0.5	0.4 ± 0.3
18:0	8.5 ± 3.3	7.4 ± 3.9	6.9 ± 3.3	7.7 ± 3.8	10.3 ± 4.3	10.9 ± 3.4
20:0	1.0 ± 1.1	0.8 ± 0.7	1.0 ± 0.8	1.0 ± 0.9	0.9 ± 0.4	0.9 ± 0.3
21:0	0.2 ± 0.3	0.3 ± 0.4	1.7 ± 3.6	0.2 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
22:0	1.3 ± 2.3	0.7 ± 1.3	1.1 ± 1.6	1.1 ± 0.9	1.9 ± 1.2	2.3 ± 1.6
23:0	0.4 ± 0.5	0.3 ± 0.5	0.3 ± 0.5	0.5 ± 0.5	0.7 ± 0.4	0.8 ± 0.7
25:0			0.1 ± 0.5		0.4 ± 0.5	0.6 ± 0.6
26:0					0.6 ± 0.5	0.7 ± 0.5
28:0					0.7 ± 0.7	0.9 ± 0.8
∑SFA	**43.1 ± 7.9**	**39.7 ± 8.2**	**30.7 ± 10.2**	**28.9 ± 9.3**	**45.1 ± 10.6**	**46.3 ± 9.7**
14:1n-5	0.4 ± 0.3	0.5 ± 1.1	0.3 ± 1	0.2 ± 0.5	0.1 ± 0.1	0.2 ± 0.2
16:1n-7	4.3 ± 2.2	4.9 ± 1.5	2.5 ± 2.3	4 ± 2.7	3.5 ± 1.9	4.1 ± 2.4
16:1n-5	0.3 ± 0.7	0.5 ± 1.1	0.4 ± 1	0.5 ± 0.6	0.2 ± 0.2	0.2 ± 0.1
17:1n-7	0.3 ± 0.2	0.2 ± 0.1	2.2 ± 2.9	1.7 ± 2.1	0.5 ± 0.2	0.5 ± 0.3
18:1n-9	4.8 ± 1.6	4.3 ± 2.1	5.1 ± 2.7	5.5 ± 4	10.5 ± 4.6	10.6 ± 3.2
18:1n-7	2.4 ± 0.8	2 ± 0.6	2.7 ± 2.6	3.2 ± 2.5	3.0 ± 1.5	2.7 ± 1.0
18:1n-5	0.1 ± 0.2	0.1 ± 0.1	0.3 ± 0.5	0.3 ± 0.5	0.1 ± 0.1	0.1 ± 0.1
20:1n-9	1.1 ± 0.6	0.7 ± 0.5	5.9 ± 7.4	4.0 ± 5.5	1.2 ± 1.1	1.3 ± 0.9
20:1n-7	0.8 ± 0.8	0.4 ± 0.3	1.7 ± 3.8	1.6 ± 2.1	1.1 ± 0.5	1.6 ± 0.5
22:1n-9	2.5 ± 3.2	1.2 ± 2	0.7 ± 0.9	0.6 ± 1.0	0.7 ± 0.8	0.7 ± 0.5
24:1n-9	0.4 ± 0.8	0.2 ± 0.6	1.0 ± 1.6	1.1 ± 1.3	0.6 ± 0.8	0.9 ± 0.9
26:1n-9			1.3 ± 7.5	0.8 ± 3.0		
∑MUFA	**17.9 ± 4.3**	**15.5 ± 3.5**	**24.6 ± 11.4**	**24.2 ± 8.5**	**21.9 ± 7.4**	**23.1 ± 6.3**
16:2n-4	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 1.0	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.2
16:3n-4	0.4 ± 0.4	0.4 ± 0.4	0.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
18:2n-6	1.6 ± 0.4	1.6 ± 0.6	1.4 ± 1.1	1.0 ± 0.7	1.2 ± 1.3	0.7 ± 0.2
18:3n-6	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
18:3n-3	0.2 ± 0.9		0.6 ± 0.9	0.4 ± 0.2	0.4 ± 0.3	0.2 ± 0.1
18:4n-3	1.5 ± 1.2	1.2 ± 0.5	0.5 ± 0.5	0.7 ± 0.5	0.8 ± 0.5	0.4 ± 0.4
20:2n-6	0.3 ± 0.2	0.2 ± 0.2	1.3 ± 1.8	0.9 ± 0.8	0.3 ± 0.2	0.2 ± 0.1
20:3n-6	0.3 ± 0.5	0.2 ± 0.3				
20:3n-7	0.1 ± 0.2	0.1 ± 0.2	0.6 ± 0.9	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
20:4n-6	0.9 ± 0.4	0.6 ± 0.3	10.9 ± 10	7.7 ± 6.9	3.3 ± 1.8	2.7 ± 1.7
20:4n-3	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.5	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
20:5n-3	11.6 ± 4.2	13.6 ± 3	12.5 ± 7	13.1 ± 7	6.1 ± 3.1	5.0 ± 2.1
22:4n-6	0.1 ± 0.1		1.9 ± 3.9	1.3 ± 1.5	0.8 ± 0.4	0.9 ± 0.9
22:5n-6	0.5 ± 0.4	0.2 ± 0.2	0.8 ± 2.3	1.1 ± 1.9	0.7 ± 0.5	0.6 ± 0.4
22:5n-3	0.7 ± 0.8	0.3 ± 0.5	2.4 ± 5.4	2.8 ± 3.5	2.6 ± 0.9	3.0 ± 1.5
22:6n-3	16.9 ± 8.9	22.2 ± 10.2	4.8 ± 5.2	9.1 ± 5.1	13.3 ± 7.3	13.7 ± 4.6
26:2(17,21)			1.2 ± 7.3	0.9 ± 4.2		
∑PUFA	**35.6 ± 11.2**	**41.2 ± 12.2**	**40.5 ± 9.4**	**41.1 ± 11.5**	**30.5 ± 9.5**	**28.3 ± 6.2**
∑EFA	**29.4 ± 11.7**	**36.4 ± 12.6**	**28.1 ± 11.8**	**29.9 ± 9.1**	**22.7 ± 9.4**	**21.4 ± 5**
∑BAFA	**5.1 ± 1.4**	**5.2 ± 3.4**	**6.0 ± 2.5**	**7.4 ± 3.9**	**5.3 ± 2.3**	**5.0 ± 1.8**
∑ Terrestrial	**1.7 ± 1.0**	**1.5 ± 0.6**	**2.0 ± 1.6**	**1.5 ± 0.8**	**1.6 ± 1.4**	**0.9 ± 0.2**
∑ Copepods	**4.3 ± 3.6**	**2.3 ± 2.4**	**8.3 ± 8.5**	**6.2 ± 6.3**	**3.0 ± 1.7**	**3.5 ± 1.2**
TFA (µg mg^{-1} DM)	**7.2 ± 4.7**	**10.9 ± 5.9**	**15.6 ± 30.8**	**13.0 ± 11.0**	**10.0 ± 10.3**	**7.2 ± 7.8**

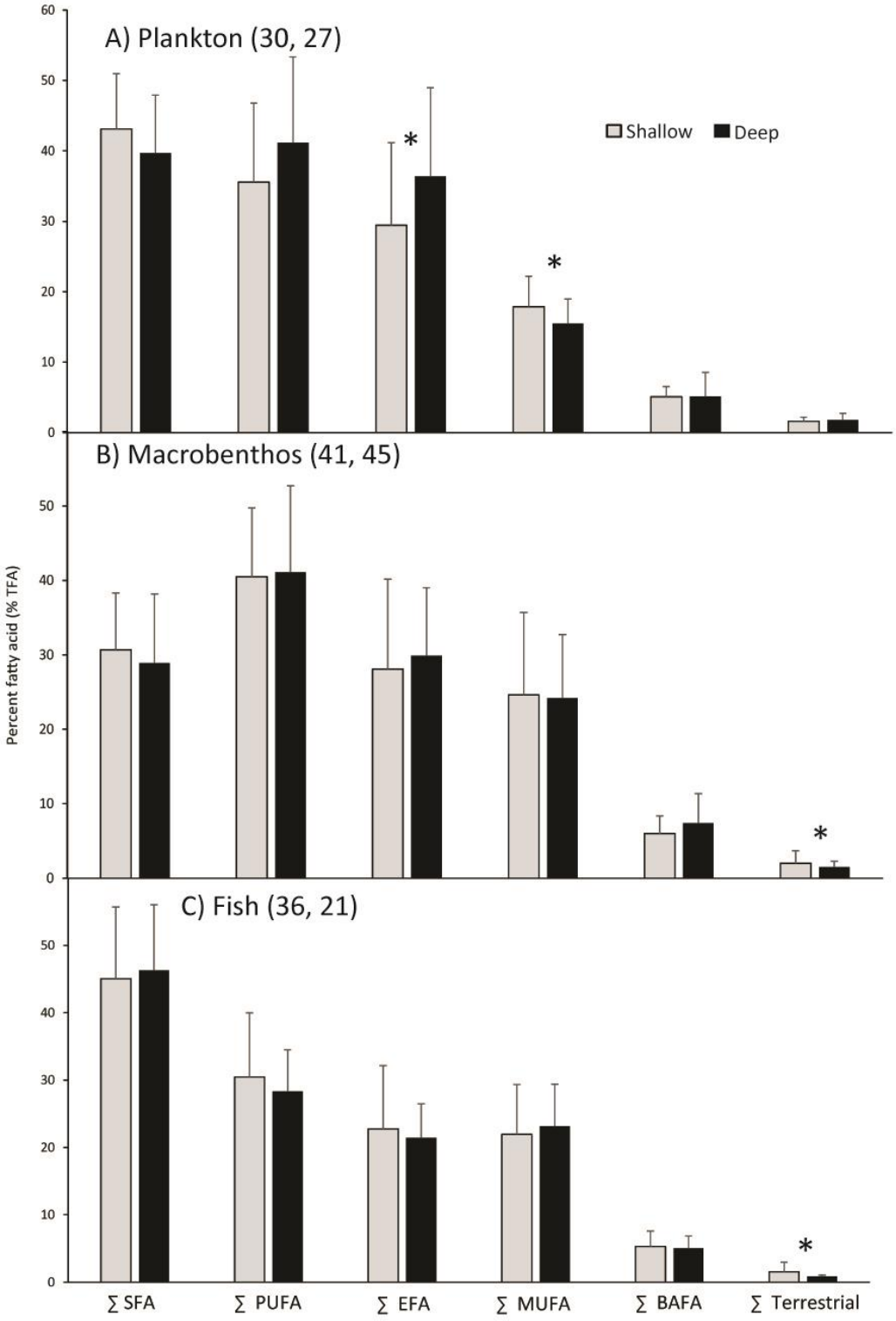


Figure 5.3. Fatty acid groupings (percent of total fatty acids; % TFA) in organisms from the shallow and deep reefs in Tsitsikamma. Sum of saturated fatty acids (∑ SFAs), polyunsaturated fatty acids (∑ PUFAs), essential fatty acids (∑ EFAs), monounsaturated fatty acids (∑ MUFAs), bacterial fatty acids (∑ BAFAs), terrestrial markers [∑ (18:2n-6, 18:3n-3)] and copepod marker (∑ [22:1, 20:1]) in A) plankton, B) macrobenthos and C) fish. Values in brackets represent sample sizes for the shallow and deep reef, respectively. Error bars represent positive standard deviations. * $P < 0.05$.

C. FISH

Fatty acid profiles in fish were dominated by SFAs (up to 69% TFA), followed by PUFAs (up to 58.5% TFA) and MUFAs (up to 37% TFA; Table 5.2 C; Figure 5.3 C). Concentrations of FAs in fish were on average 10.0 ± 10.3 µg mg^{-1} DM on the shallow reef and 7.2 ± 7.8 µg mg^{-1} DM on the deep reef. Similar proportions of SFAs, PUFAs, MUFAs, EFAs, BAFAs and the copepod marker were evident in fish on both reefs and did not differ significantly (Figure 5.3 C, Table 5.3). The terrestrial signals were significantly higher in fish collected from the shallow reef (1.6 ± 1.4% TFA) compared to the deep reef (0.9 ± 0.2% TFA; Table 5.3; Figure 5.3 C).

Table 5.3. Univariate PERMANOVA of the qualitative fatty acid data, based on Euclidian distances. PERMANOVAs for the sum of saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), essential fatty acids (EFAs), bacterial fatty acids (BAFAs) terrestrial (∑ 18:2n-6, 18:3n-3) and copepods (∑ [22:1, 20:1]) for the shallow and deep reefs guilds in Tsitsikamma. MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability level based on permutations. Values indicated in bold are significantly different between reefs.

	∑ SFAs			**∑ PUFAs**			**∑ MUFAs**			**∑ EFAs**			**∑ BAFAs**			**∑ Terrestrial**			**∑ Copepods**		
PERMANOVA (reefs)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)
Plankton	177.7	2.722	0.102	463.5	3.377	0.073	81.46	5.214	**0.027**	706.4	4.760	**0.0361**	0.079	0.012	0.917	0.44	0.661	0.4843	58.15	6.010	**0.0101**
Macrobenthos	2.15	0.057	0.822	5.36	0.055	0.817	0.56	0.006	0.941	63.07	0.722	0.4003	18.80	2.118	0.152	6.81	4.142	**0.0430**	2.91	0.055	0.8156
Fish	120.6	1.685	0.196	146.4	2.513	0.123	0.92	0.022	0.873	76.23	1.417	0.2426	0.165	0.056	0.819	5.956	4.541	**0.0085**	2.196	0.904	0.3469

5.3.2.2 MULTIVARIATE ANALYSES

A. PLANKTON

Results from the distLM indicated that 43.3% of the variation in the plankton FA data were explainable by changes in temperature (27.5%) and light intensity at depth (15.8%; Table 5.4). Both temperature (Figure 5.1) and light intensity at depth (Figure 3.1) demonstrated seasonality, with significantly higher temperatures recorded on both reefs during November 2011 compared to February 2012, and significantly less light penetrating to depth during November 2011, suggesting plankton FA profiles were variable over time and related to season.

Table 5.4. Results of the forward selecting distance based linear model (distLM). A distLM was employed to identify the importance of station specific environmental variables such as temperature and light intensity on the FA composition in the plankton samples. AIC: Akaike Information Criteria; SS: sum of squares; Prop %: increased proportion of explained variation with each variable that is added; Cumul %: Cumulative total.

Variable	AIC	SS(trace)	Pseudo-F	P	Prop. %	Cumul. %
SEQUENTIAL TESTS						
Temperature	317.81	5324.2	20.882	0.0001	0.275	0.28
Light intensity at depth	305.76	3063.5	15.094	0.0001	0.158	0.43
MARGINAL TESTS						
Chl-*a* concentration		1382.2	4.2315	0.0121	0.0714	
Depth		1264.1	3.8447	0.0163	0.0653	
Salinity		308.56	0.89138	0.4163	0.0159	
Tempertature		5324.2	20.882	0.0001	0.275	
Light intensity at depth		1493.4	4.6004	0.0078	0.0771	
Secchi disk		341.85	0.98926	0.3669	0.0176	

The FA composition of plankton samples collected from the shallow reef differed significantly from those of the deep reef (Pseudo-*F* = 2.847, *P* = 0.0394; Table 5.5). Plankton collected during November 2011 and February 2012 differed significantly in terms of their FA composition (Pseudo-*F* = 33.52, *P* = 0.0001; Table 5.5). The SIMPER results indicated that the proportions of EFA 22:6n-3, an indicator for dinoflagellate dominance, were higher in November 2011 (25.9% TFA) compared to February 2012 (9.9% TFA) and contributed most to the dissimilarity between the plankton collected from different seasons (Table 5.5; Figure 5.4 A). Size fractions (<500 µm and >500 µm) of the plankton samples differed significantly in their FA profiles, mostly due to higher proportions of 22:6n-3 in the smaller fraction (Table 5.5), especially during November 2011. The larger size class (>500 µm) had higher proportions of copepod indicators [∑ (22:1, 20:1)].

Significant interactions were evident between reef and season (Pseudo-*F* = 3.548, *P* = 0.0091) and reef and plankton size fraction (Pseudo-*F* = 4.141, *P* = 0.0031; Table 5.5). Pairwise comparisons indicated that although the shallow and deep reef plankton FAs differed significantly when seasons were pooled (November 2011 and February 2012), it was the FAs from November 2011 (mostly 22:6n-3) that contributed to the overall significant difference between reefs (t = 2.982, *P* = 0.0001; Table 5.5), while February 2012 samples did not differ between the reefs (t = 0.771, *P* > 0.05; Table 5.5).

Table 5.5. Multivariate PERMANOVA and SIMPER analyses on the fatty acid (FA) plankton data. PERMANOVAs comparing the FA profiles of plankton samples collected from the shallow and deep reefs from different seasons (Nov 2011 and Feb 2012). Values indicated in bold represent a significant effect. df = degrees of freedom; MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability level based on permutations.

PERMANOVA					SIMPER						
	df	MS	Pseudo-*F*	*P* (perm)	Dis-similarity (%)	FA compounds	Average		Average dissimilarity	Sim/SD	Contribution (%)
							Shallow	Deep			
REEFS	1	955.32	2.8471	**0.0394**	24.5	22:6n-3	16.9	22.2	5.8	1.4	23.8
						16:0	24.3	23.0	2.2	1.2	9.1
						20:5n-3	11.6	13.6	2.1	1.2	8.6
						18:0	8.5	7.4	2.0	1.3	8.3
						22:1n-9	2.5	1.2	1.2	0.8	5.1
							Nov 11	Feb 12			
SEASON	1	6309.70	33.5290	**0.0001**	29.5	22:6n-3	25.9	9.9	8.29	2.61	28.07
						18:0	5.9	10.9	2.72	1.73	9.23
						16:0	22.9	24.9	2.39	1.37	8.08
						20:5n-3	13.3	11.3	2.26	1.37	7.65
						22:1n-9	1.2	2.9	1.35	0.8	4.59
							< 500 µm	> 500 µm			
SIZE CLASS	1	1018.20	5.4104	**0.0002**	24.4	22:6n-3	20.0	18.9	5.58	1.39	22.87
						16:0	25.0	22.4	2.38	1.31	9.76
						20:5n-3	12.0	13.0	2.12	1.34	8.7
						18:0	8.2	7.7	1.99	1.22	8.14
						22:1n-9	1.1	2.6	1.23	0.8	5.05
PAIRWISE COMPARISON							Shallow	Deep			
REEF X SEASON	Nov 2011		2.982	**0.0001**	18.2	22:6n-3	22.14	29.58	3.9	1.0	21.3
						16:0	24.36	21.33	2.1	0.9	11.3
						20:5n-3	11.6	15.03	1.9	1.1	10.6
	Feb 2012		0.770	0.6962	23.4	16:0	24.21	25.76	2.4	1.5	10.3
						20:5n-3	11.58	11.04	2.3	1.4	9.7
						22:6n-3	10.1	9.63	2.2	1.5	9.6
REEF X SIZE CLASS	< 500 µm		1.617	**0.0257**	21.8	22:6n-3	19.49	20.48	5.5	1.3	25.1
						18:0	7.97	8.45	2.3	1.2	10.4
						16:0	24.62	25.48	2.0	1.4	9.1
	> 500 µm		1.998	**0.0036**	26.4	22:6n-3	14.65	23.77	6.3	1.5	23.9
						20:5n-3	11.24	15.04	2.7	1.3	10.1
						16:0	24.01	20.65	2.2	1.0	8.2

When superimposing the dietary indictor for copepods (∑ [22:1, 20:1]) as bubbles on the MDS plot of the November 2011 plankton data, it becomes clear that differences between the shallow and deep reefs resulted from the greater contributions of the copepod marker on the shallow reef compared to the deep reef (Figure 5.4 B). The pairwise analysis of the univariate PERMANOVA using ∑ (22:1, 20:1) also showed significant differences between reefs (t = 4.441, *P* = 0.0004). Although plankton from February 2012 had overall higher proportions of the copepod marker, no differences were apparent between the reefs. In February 2012 the copepod marker in plankton averaged 4.6 ± 4.3 % TFA compared to 2.5 ± 1.9 % TFA in November 2011.

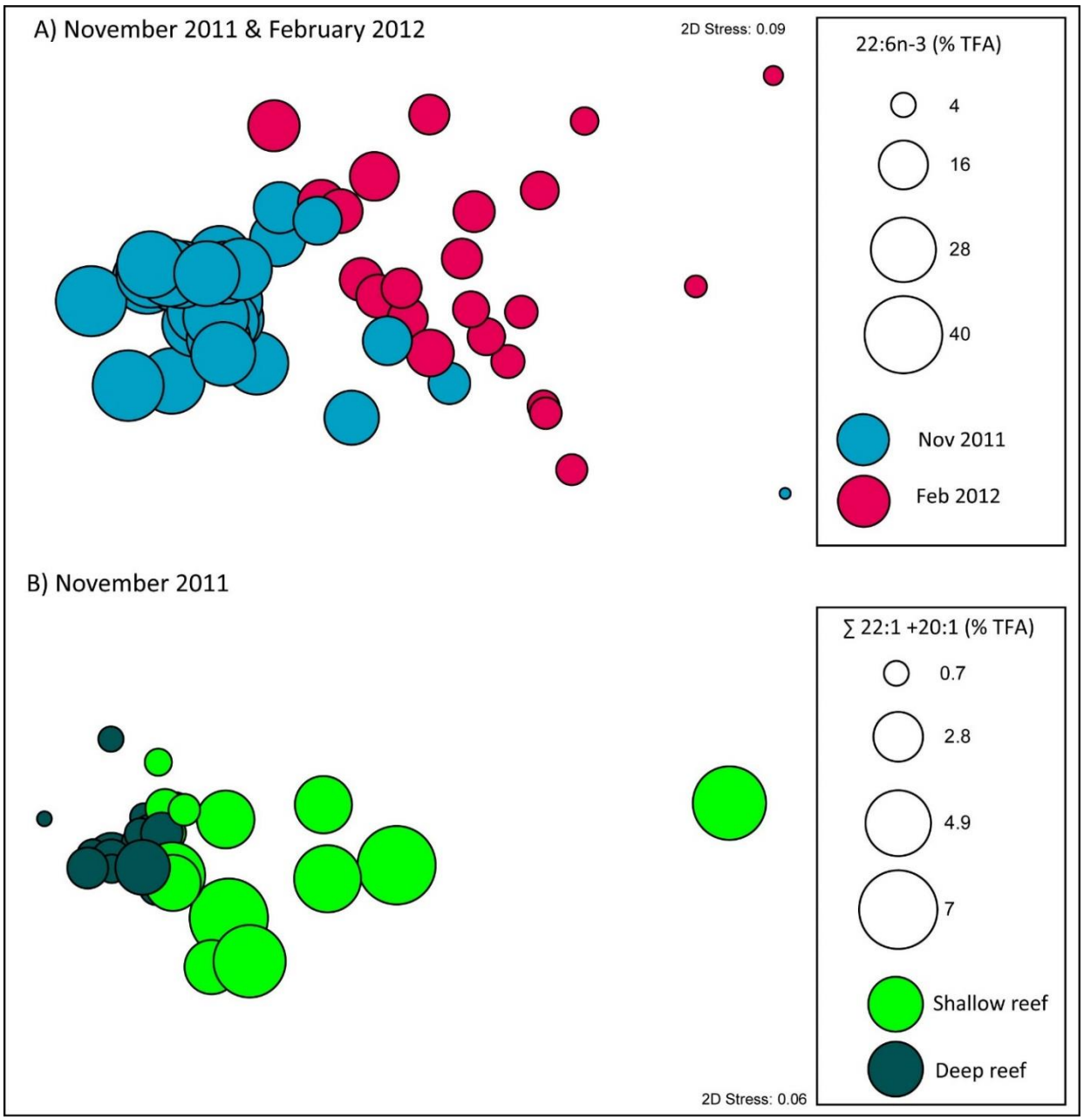


Figure 5.4. Non-metric multi-dimensional scaling (MDS) ordinations of the untransformed plankton data, based on a Bray-Curtis similarity matrix. The MDS biplot depicts the variation in fatty acid (FA) composition between sampling periods (A) and between the shallow and deep reefs (B) during November 2011. The bubble plot indicates the percentage contributions of A) the essential FA 22:6n-3, also associated with dinoflagellates, and B) ∑ 22:1 and 20:1, a dietary indicator for copepod contributions.

B. MACROBENTHOS

Amphipods and isopods were collected from two different habitats. Half were collected from traps, and thus suggested a detritus based diet, and the remaining half were picked from upright suspension-feeders (sea fans and sponges). These specimens feed directly on plankton in the water column (Amsler et al. 2009) and were considered suspension-feeders. No significant difference was

detected when the two feeding groups of both amphipods and isopods were compared based on their FA profiles, and as such they were grouped and described as detrivore/suspension-feeders.

Comparison of the different CAP models run to explain the variations in the FA composition of the macrobenthos (Table 5.6) indicated that all the grouping variables were good at discriminating amongst the groups. The two models with the highest classification success were those where FA compositions were grouped according to higher order taxa (class) and feeding guild, with classification successes of 90.1 % and 80.5 %, respectively. Due to the high allocation success of the grouping variable 'class', statistical tests for comparison between reefs were based on this variable.

Table 5.6. Comparison of different canonical analysis of principal coordinates (CAP) models. Each CAP model involved a different grouping variable to explain the variation in fatty acid compositions of the macrobenthic community. m = number of principle coordinate axes selected to maximise the classification success, CAP 1 and 2 are the first two axes and the variation explained by each axes (%), and classification success is number of samples correctly allocated to its original group.

Grouping variable	m	Correlation (%)		Classification success (%)
		CAP 1	CAP 2	
Class (higher order taxa)	15	98.8	98.4	90.1
Feeding guild	9	97.3	94.8	80.5
Growth form	15	96.1	94.9	79.3
Feeding mechanism	15	98.3	95.7	79.6
Broad feeding guild	16	95.6	93.5	74.4

The FA profiles were significantly different between the shallow and deep reef macrobenthos and when compared among the different macrobenthic taxonomic classes (Table 5.7). There was also a significant interaction between reef and class for the macrobenthos, although only eight out of the 14 classes were represented on both reefs (Table 5.7). Pairwise analysis of the reef/class interaction indicated that only ascidians differed in their FA composition when compared between the reefs (t = 2.88; P (MC) = 0.02).

For the pooled shallow and deep macrobenthic data, the SIMPER procedure indicated that the average dissimilarity in the FA profiles between the shallow and deep reefs was 46.6%, mostly due to higher proportions of 20:4n-6 on the shallow reef (11.1 % TFA) compared to 7.7 % TFA on the deep reef.

Table 5.7. Multivariate PERMANOVA for macrobenthic fatty acid (FA) profiles. Comparison of the FA profiles of macrobenthic species collected on the shallow and deep reefs, and among higher order taxa (class). Values indicated in bold represent a significant effect. ** term has empty cells; df = degrees of freedom; MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability level based on permutations.

	df	MS	Pseudo-*F*	*P* (perm)
Reefs	1	933.55	2.50	**0.02**
Class	13	4394.1	11.79	**0.0001**
Reefs x class **	7	828.66	2.22	**0.0011**

To get a better understanding of feeding interactions and identify the most influential FAs to help establish trophic interactions of the macrobenthos, the CAP model employing feeding guilds as a grouping variable was considered in greater detail (Figure 5.5). To clarify the relationship between the FA compositions of the macrobenthos and the feeding guilds, strongly associated dietary indices and dominant FAs based on Pearson's correlation coefficients >0.4 were superimposed as vectors on the CAP ordination plot (Figure 5.5). These were compared with SIMPER results for each feeding guild, and then compared against the rest of the macrobenthos.

Fifteen canonical axes (*m*) best described the variability in the FA compositions of the macrobenthic feeding guild data in the CAP analysis, and the first two canonical correlations were large (0.99 and 0.98), suggesting a strong association between the FAs and feeding guild groups (Figure 5.5). The first CAP axis separated the sponges from the rest of the macrobenthic community, and the second axis separated benthic algae from the remaining groups. The high allocation success (80.5%) suggested that the grouping factor (feeding guild) assigned to the different species was useful at discriminating between the different feeding guilds based on their FA compositions.

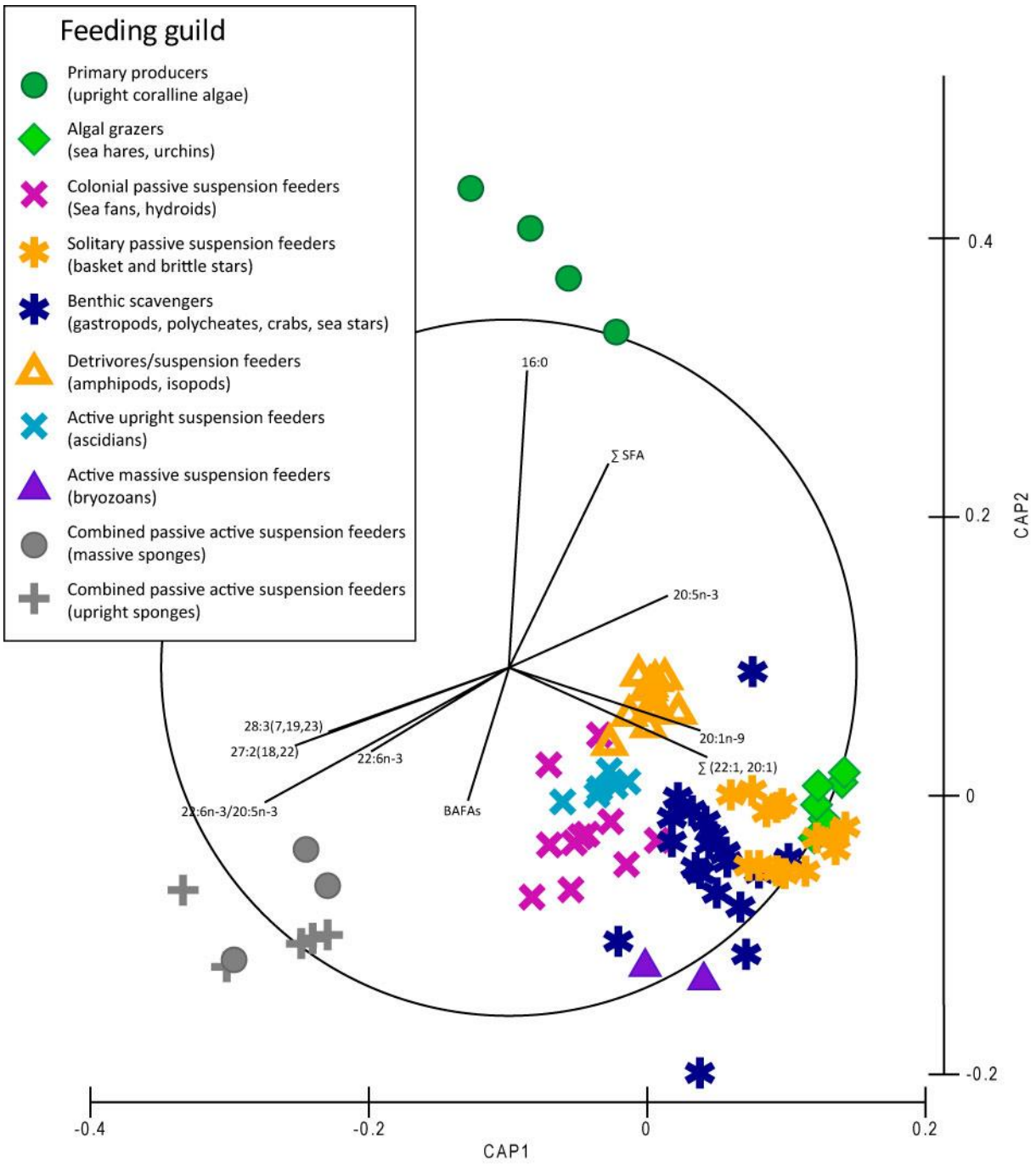


Figure 5.5. Canonical analysis of principle coordinates (CAP) performed on the fatty acid (FA) profiles of the Tsitsikamma macrobenthic community, using feeding guilds as the grouping variable. The different feeding guilds assigned to the macrobenthic species can be identified from the legend insert and Table 5.1 A, and the Pearson's correlation coefficients of important FA and dietary indices are superimposed as vectors.

According to the CAP bi-plot (Figure 5.5) benthic algae were most dissimilar from the rest of the macrobenthos and had high proportions of the EFA 20:4n-6, which occurred at an average of 19.5% TFA in algae compared to an average of 8.9% TFA in the rest of the macrobenthic community (Table 5.8).

Algal grazers, comprised of urchins and sea hares, had an average dissimilarity of 50.4% from the rest of the macrobenthic community, mostly due to the high proportions of 20:4n-6 (Table A5.2). Arachidonic acid (ARA; 20:4n-6) is a marker of benthic productivity (Piché et al. 2010) and it was important in algal FA profiles in Tsitsikamma. Arachidonic acid averaged 13.9% TFA in grazers compared to 9.03% TFA in the other macrobenthos (Table 5.8). Interestingly, the copepod marker was high in the grazers at 18.1% TFA (primarily in the sea urchins) compared to 6.3% TFA other macrobenthos (Table A5.3).

Solitary detritivores/suspension-feeders, comprising of amphipods and isopods, were all correctly classified to their feeding guild. The EFA 20:5n-3 (average 20.8% TFA) was best at discerning this group (other macrobenthos: 11.3% TFA; Table 5.8). The sum of EFAs were also high in this feeding guild, with an average of 36.3% TFA compared to 28% TFA in the remaining macrobenthos (Table A5.2).

Solitary passive suspension-feeders, which comprised basket and brittle stars, were 92.9% correctly allocated to their feeding guild, and demonstrated a high within-group similarity of 74.8% according to the SIMPER procedure (Table A5.2). Copepods were important components of solitary passive suspension-feeders' diets, as ∑ (22:1, 20:1) averaged 15.8% TFA compared to 5.5% TFA in the other macrobenthic species (Table A5.3). Furthermore, the dinoflagellate/diatom marker 22:6n-3/20:5n-3 was less than one (0.1%) compared to 1.1% in other macrobenthos, indicating the importance of diatoms in the diets of these suspension-feeders (Table A5.3). A ratio of larger than one indicates increasing importance of dinoflagellates compared to diatoms (Budge & Parrish 1998).

Sponges, which use a combination of passive and active feeding to obtain food, were characterised by distinct FA profiles, as seen in the CAP biplot (Figure 5.5). This distinction might be explained by the presence of long chained NMI FA such as 28:3(7,9,23) and 27:2(18,22), which were not present in other macrobenthic species.

Passive colonial suspension-feeders, which included sea fans and hydroids, were marked by high proportions of the EFA 20:4n-6 (21.8% TFA), compared to 7.49% TFA in the remaining macrobenthos (Table 5.8). Bacterial contributions to passive suspension feeding diets were slightly higher at 8.8% TFA compared to 6.4% TFA in others (Table A5.3).

Active colonial suspension-feeders, which consisted of mound shaped and upright active suspension-feeders, were both marked with higher than average BAFAs. In active mound shaped suspension-feeders (bryozoans), BAFAs contributed 11.7% TFAs compared to 6.6% TFA in the rest of the macrobenthic community (Table A5.3). Bacterial FA in active upright suspension-feeders,

represented by one species of ascidian (*Gynandrocarpa placenta*), averaged 10.4% compared to 6.4% in the rest of the macrobenthic community (Table A5.3).

Table 5.8. Similarity percentage (SIMPER) results comparing the feeding guilds within the macrobenthic community. The SIMPER results indicate the average dissimilarity, and important fatty acids that typify each feeding guild. Ave = average, Diss = dissimilarity, SD = standard deviation.

Feeding guild	Examples	Ave. dissimilarity (%)	Fatty acid compounds	Ave. abundance All	Ave. abundance Feeding guild	Ave. dissimilarity	Diss/SD	Contribution (%)
Colonial								
Combined passive-active suspension feeders	Upright sponges	59.5	16:0	14.2	4.4	5.04	1.17	8.47
			20:5n-3	13.18	4.86	4.54	1.43	7.63
			26:2(17,21)	0.62	8.33	4.35	0.84	7.31
Combined passive-active suspension feeders	Massive sponges	72.1	26:1n-9	0.48	17.78	8.88	0.83	12.31
			26:2(17,21)	0.52	16	8.06	0.75	11.18
			20:5n-3	13.1	1.33	5.91	1.81	8.19
Passive suspension feeders	Sea fans, hydroids	45.5	20:4n-6	7.49	21.77	7.73	1.3	16.96
			20:5n-3	13.52	7.17	3.97	1.46	8.72
			22:6n-3	6.77	7.4	3.32	1.37	7.3
Active massive suspension feeders	Bryozoans	44.7	22:6n-3	6.65	15.24	4.44	1.72	9.93
			20:5n-3	12.87	4.81	4.37	1.47	9.76
			20:4n-6	9.59	1.36	4.2	0.98	9.4
Active upright suspension feeders	Ascidians	43.5	20:5n-3	12.38	15.91	4.27	1.57	9.82
			16:0	13.43	15.55	4.06	0.89	9.35
			20:4n-6	9.74	5.6	3.6	0.95	8.28
Solitary								
Benthic scavengers	Gastropods, polycheates, crabs, sea stars	45.5	20:4n-6	10.09	7.18	3.93	1.02	8.64
			20:5n-3	12.81	12.25	3.73	1.41	8.21
			16:0	13.92	12.6	3.58	0.87	7.86
Detrivore/suspension feeders	Amphipods & isopods	44	20:5n-3	11.43	20.83	4.99	1.69	11.34
			20:4n-6	10.23	3.87	3.91	0.96	8.87
			16:0	13.15	16.59	3.7	1.08	8.4
Passive suspension feeders	Basket & brittles stars	47.9	20:1n-9	3.05	14.44	6.12	2.11	12.78
			20:5n-3	11.56	18.19	4.34	1.53	9.06
			16:0	14.63	8.56	3.92	0.96	8.18
Algal grazers	Sea hares & urchins	50.4	20:4n-6	9.03	13.91	6	1.53	11.91
			20:1n-9	4.55	10.35	4.45	1.35	8.83
			22:6n-3	7.38	0.19	3.6	1.33	7.13
Primary producers	Upright coralline algae	55.5	16:0	12.08	43.68	15.8	2.93	28.47
			20:4n-6	8.88	19.45	6.38	1.75	11.5
			22:6n-3	7.18	0.43	3.41	1.26	6.13

C. FISH

To determine which variable best grouped the FAs of the fish community, four different CAP models were run and compared (Table 5.9). The CAP model with the highest classification success was observed when fish were grouped according to higher order taxa, with 87.3% of the samples correctly allocated (Table 5.9). The CAP model which grouped fish FAs according to species performed second best, with 67.3% of the samples correctly allocated. To run the PERMANOVA, the grouping variable 'species' was employed, and not 'class' (higher order taxa), because 'class' consisted of six groups, with one very large group (Sparidae). The Sparidae group comprised of 43 specimens, and the remaining classes had fewer than three specimens each. Such variable sample numbers made the

PERMANOVA design highly unbalanced and only the Sparidae were comparable between reefs. Similar to the macrobenthos, to investigate the feeding interactions of the fish community, FA variability were further examined using the broad feeding guild CAP analysis (Figure 5.6).

Table 5.9. Comparison of different canonical analyses of principal coordinates (CAP) models. Each CAP model included a different grouping variable to explain the variation in fatty acid compositions of the fish community. m = number of principle coordinate axes selected to maximise the classification success, CAP 1 and 2 are the first two axes and the variation explained by each axes (%), and classification success is the proportion of samples correctly allocated to its original group.

Grouping variable	m	Correlation (%)		Classifcation success (%)
		CAP 1	CAP 2	
Class (higher order taxa)	10	89.9	82.7	87.3
Feeding guild	10	94.3	89	43.7
Broad feeding guild (excl. ontogeny)	9	94.7	87.9	61.9
Species	8	96.9	94.7	67.3

The multivariate PERMANOVA employing 'reef' and 'species' as fixed factors did not demonstrate significant effects when the FA profiles of fish from the two reefs were compared (Table 5.10). The FA profiles of the different fish species did differ significantly (Pseudo-*F* = 5.29; *P* = 0.0001), and a significant interaction between 'reef' and 'species' was observed (Pseudo-*F* = 1.99; *P* = 0.0158; Table 5.10). Pairwise comparisons indicated that the FA composition of blue hottentot differed significantly when compared between the shallow and deep reefs (*t* = 4.623; *P* (MC) = 0.0225).

Table 5.10. Multivariate PERMANOVA for fish fatty acid (FA) profiles collected in Tsitsikamma. PERMANOVA comparing the FA profiles of fish collected on the shallow and deep reefs, and among species. Values indicated in bold represent a significant effect. ** term has empty cells; df = degrees of freedom; MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability level based on permutations.

	df	MS	Pseudo-*F*	*P* (perm)
Reefs	1	263.88	2.16	0.0749
Species	15	644.92	5.29	**0.0001**
Reefs x species**	4	242.7	1.99	**0.0158**

Nine canonical axes (*m*) best described the variability in the FA compositions of the fish feeding guild data in the CAP analysis. The first two canonical correlations explained a large amount of the variability (94.7% and 87.9%, respectively; Figure 5.6). Axis one separated herbivores and omnivores from the carnivores. Axis 2 separated fish based on their different degrees of omnivory (herbivores, omnivores that feed on small invertebrates, omnivores that feed on large invertebrates and benthic omnivores) and the differing degrees of carnivory (benthic carnivores, carnivores of small invertebrates, carnivores of large invertebrates, and carnivores of large invertebrates and fish). The vectors for 20:4n-6 and 22:6n-3 pointed in opposite directions, with the former associated with herbivores and omnivores and the latter with carnivores (Figure 5.6). The remaining carnivores were situated between these two opposite feeding habits (blue squares in Figure 5.6).

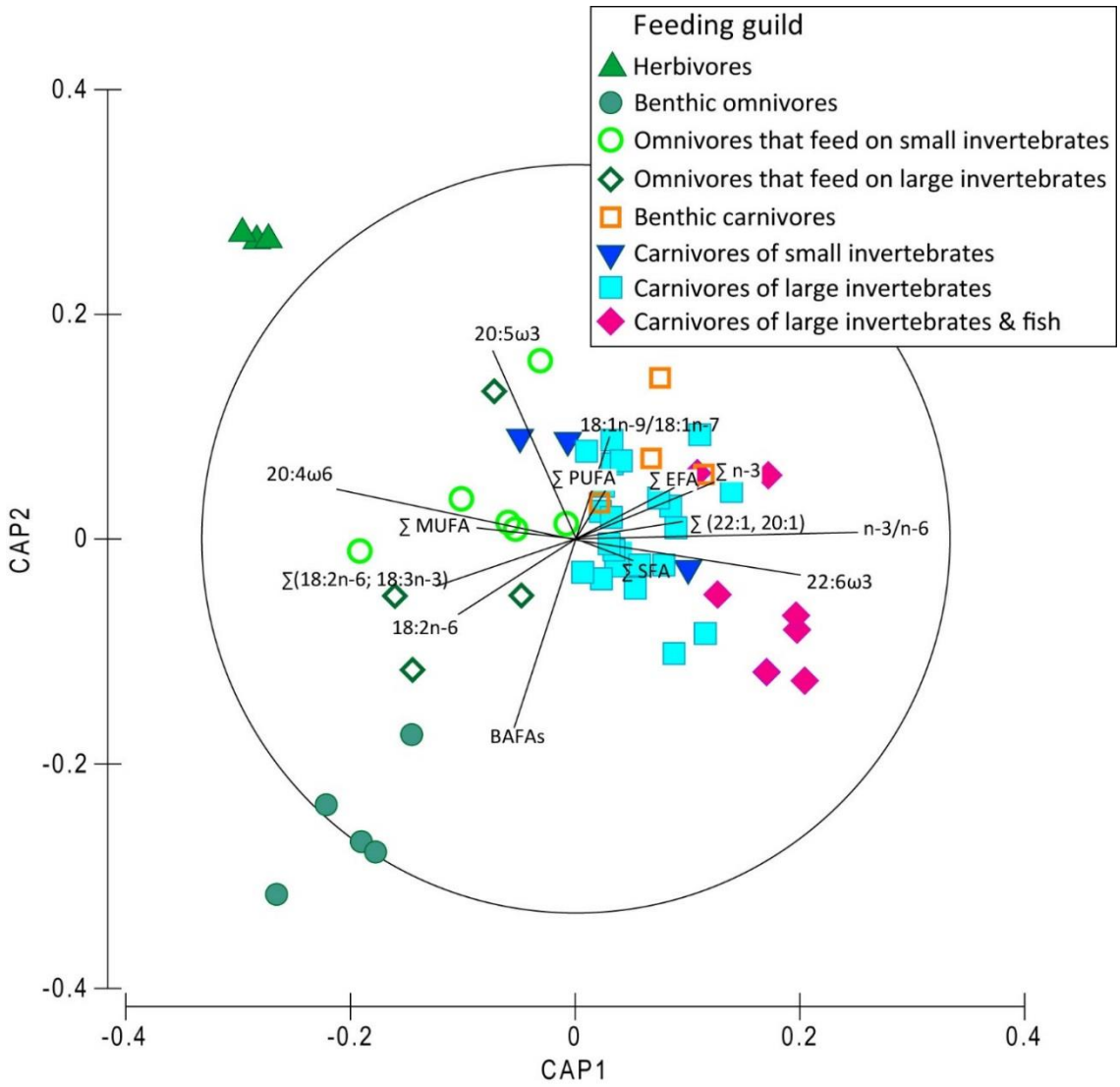


Figure 5.6. Canonical analysis of principle coordinates (CAP) performed on the fatty acid (FA) profiles of the Tsitsikamma fish community using feeding guilds as the grouping variable. The different feeding guilds assigned to the macrobenthic species can be identified from the legend insert and Table 5.1 B, and the Pearson's correlation coefficients of important dietary indices are superimposed as vectors.

Herbivores, represented only by strepie (*Sarpa salpa*), were low in the EFA 22:6n-3, which averaged at 5.2% TFA compared to 14.4% in the rest of the fish. In contrast, the EFAs 20:4n-6 (ARA) and 20:5n-3 (EPA) were proportionally greater in the herbivores (ARA 6.9%; EPA 9.22% TFA) compared to averages of 3.0% and 5.7% TFA, respectively, in the remaining fish community (Table 5.11).

Benthic omnivores, which consisted of janbruin (*Gymnocrotaphus curvidens*) and cape knifejaw (*Oplegnathus conwayi*) collected from the shallow reef, were separated from other fish in the lower left corner of the CAP biplot (Figure 5.6). The vector for BAFAs was strongly correlated with this group, a result supported by the SIMPER output, and benthic omnivores had higher proportions of BAFAs (average of 7.21% TFA) compared to the remaining fish community (4.8% TFA; Table A5.4). Benthic omnivores were characterized by proportionally less PUFAs, n-3 and EFAs at 25.7%, 16.1% and 17% TFA, respectively, compared to 30.8%, 24.6%, 23.5% TFA, respectively, in the other fish feeding guilds (Table A5.4). The terrestrial indicator [Σ(18:2n-6;18:3n-3)] was on average 2.8% TFA in benthic omnivores, compared to 1.2% in the rest of the fish (Table A5.4).

Omnivores preying on large mobile invertebrates were represented only by hottentot (*Pachymetopon blochii*). According to the CAP biplot (Figure 5.6), hottentot fed on food that originated from the terrestrial habitat (however, the simper results indicated the opposite).

Benthic carnivores, comprised of blue hottentot (*Pachymetopon aeneum*), received the highest allocation success (100%), as all samples were correctly placed in this group. Blue hottentot profiles were characterised by high proportions of 18:1n-9 (13.39% TFA), consistent with a carnivorous diet (Table 5.11). Blue hottentot samples were also marked with lower than average proportions of SFA (38.4% TFA) compared to the rest of the fish community (44.9% TFA), and on average had higher proportions of PUFAs, MUFAs, n-3 and EFAs (Table A5.4).

Carnivores of small mobile invertebrates, represented by klipfish (*Clinidae* spp.), had only one out of three samples correctly allocated to this group. Values of a marker for carnivory, (18:1n-9/18:1n-7), were very high, averaging at 14.5 compared to 3.9 in the rest of the community (Table A5.4). This group demonstrated high proportions of SFA (average of 49.6% TFA) compared to other fish (44.1% TFA; Table A5.4).

Carnivores of large mobile invertebrates were represented by many fish species including roman (*Chrysoblephus laticeps*), dageraad (*Chrysoblephus cristiceps*), panga (*Pterogymnus laniarius*), juvenile red steenbras (*Petrus rupestris*), striped catshark (*Poroderma africanum*) and two-tone fingerfins (*Chirodactylus brachydactylus*). Due to the many species associated with this group, no particular patterns were evident.

Carnivores of large invertebrates and small fish, represented by carpenter (*Argyrozona argyrozona*) and kingklip (*Genypterus capensis*), were characterised by high proportions of SFA (49.2% TFA), n-3 (27.2% TFA) and EFA (26.1% TFA) compared to the rest of the fish community (43.7, 23.3 and 22.5% TFA, respectively; Table A5.4). Proportions of the EFA 22:6n-3 were higher in this feeding guild and averaged at 20.8% TFA compared to 12.8% TFA in all other fish (Table A5.4).

Table 5.11. Similarity percentage (SIMPER) results which compared each feeding guild with the rest of the fish community. SIMPER results indicating the average dissimilarity, and important fatty acids that typified each feeding guild. Ave = average, Diss = dissimilarity, SD = standard deviation.

Feeding guild	Ave. dissimilarity (%)	Fatty acid compounds	Ave. abundance All	Ave. abundance Feeding guild	Ave. dissimilarity	Diss/SD	Contribution (%)
Herbivore	15.8	∑ n-3	24.07	19.05	2.11	1.28	13.34
Strepie		∑ EFA	23.02	21.39	2.05	1.12	12.97
		18:2n-6	0.97	1.16	0.17	0.59	1.1
Benthic omnivore	18.7	∑ n-3	24.56	16.12	2.92	1.41	15.59
Jan bruin & cape knifejaw		∑ EFA	23.53	16.95	2.65	1.26	14.17
		BAFAs	4.75	7.21	0.85	1.47	4.53
Omnivores that feed on small invertebrates	13.2	∑ SFA	44.75	41.62	2.51	1.35	18.98
Fransmadam & steentjie		∑ MUFA	22.63	25.84	1.97	1.59	14.9
		18:2n-6	0.96	1.15	0.18	0.59	1.33
Omnivores that feed on large invertebrates	15.4	∑ SFA	45.22	34.03	3.64	1.57	23.72
Hottentot		∑ MUFA	22.5	29.1	2.55	1.49	16.6
		∑ PUFA	30.06	34.35	2.13	1.19	13.88
Benthic carnivore	16	∑ SFA	44.88	38.41	2.89	1.41	18.08
Blue hottentot		∑ PUFA	30.24	32.1	2.6	1.43	16.27
		∑ n-3	23.6	26.22	2.33	1.48	14.58
Carnivores of small invertebrates	16.9	18:1n-9/18:1n-7	3.93	14.54	3.05	1.06	18.09
Klipfish		∑ SFA	44.11	49.59	2.81	1.35	16.63
		∑ EFA	23.03	21.25	1.75	1.15	10.35
Carnivores of large invertebrates	16.1	∑ SFA	43.7	45.39	2.94	1.35	18.29
Roman, dageraad, panga, juvenile red steebras		∑ PUFA	30.13	30.7	2.44	1.17	15.19
catsharks, fingerfins		∑ n-3	23.09	24.77	2.28	1.19	14.22
		∑ EFA	22.33	23.78	2.24	1.09	13.94
Carnivores of large invertebrates & fish	15.3	∑ SFA	43.71	49.21	2.37	1.35	15.54
Carpenter and kingklip		∑ n-3	23.29	27.24	2.12	1.33	13.88
		∑ EFA	22.47	26.12	2.06	1.27	13.5

5.3.2.3 TRACING ESSENTIAL FATTY ACIDS THROUGH THE FOOD WEB

SOURCES OF ESSENTIAL FATTY ACIDS

Only plankton and upright coralline algae were sources of the EFA in Tsitsikamma, and upright coralline algae were identified as the only source of 20:4n-6 since only trace levels of this EFA were recorded in the plankton samples (algae: 19.4 ± 6.6 % TFA; plankton: 0.8 ± 0.3 % TFA). In contrast, 22:6n-3 (DHA) proportions were highest in plankton samples (19.7 ± 9.5 % TFA) and very low in

upright coralline algae (0.5 ± 0.3 % TFA). Thus ARA and DHA could be traced through the shallow and deep food webs of Tsitsikamma. The EFA 20:5n-3 (EPA) did not demonstrate such clear patterns, and proportions of EPA in plankton and upright coralline algae were similar (plankton: 12.7 ± 3.4 % TFA; algae: 10.1 ± 3.7 % TFA).

A. BENTHIC ALGAE (ARACHIDONIC ACID; 20:4N-6)

a) SHALLOW REEF

The importance of ARA in the FA profiles of the Tsitsikamma shallow reef community is indicated in Figure 5.7 A. A clear gradient was apparent in the MDS output (Figure 5.7 A), with high proportions of ARA occurring in specimens positioned in the upper left hand corner and decreasing proportions in specimens located towards the lower right hand corner of the figure. Overall, the highest proportions of ARA were detected in algae and direct grazers of algae (urchins and sea hares; top middle towards lower left corner of the figure; Figure 5.7 A), although there were high proportions of ARA in colonial suspension-feeders (especially in one nippled sea fan, *Eunicella papillosa*). Slightly lower proportions of ARA were evident in scavengers, with the highest proportions in reticulated starfish (*Henricia ornata*; bottom left-hand corner of the figure; Figure 5.7 A). The lowest proportions of ARA occurred in plankton, with slightly higher proportions occurring in fish. In the fish samples, the greatest proportions of ARA were evident in strepie (a herbivore).

b) DEEP REEF

Examination of the deep reef community (Figure 5.7 B) revealed that ARA was important in the diets of colonial suspension-feeders, as this group was marked by the highest proportions of this FA. The highest values of ARA were recorded in the nippled sea fan sample. However, the absence of algae and associated grazers resulted in overall lower proportions of ARA in the deep reef organisms compared to those on the shallow reef (Figure 5.7 B). A two-way univariate PERMANOVA, excluding sources (algae and plankton), with reef and species as fixed factors indicated that ARA proportions were significantly higher in the tissues of the shallow reef community compared to the deep reef community (Pseudo-F = 28.472; P = 0.0001). There was also a significant interaction between ‘reef’ and ‘species’, and pairwise analysis indicated that basket stars (*Astrocladus euryale*) and amphipods both contributed to this effect (basket stars: t = 8.037; P (MC) = 0.0004; amphipods: t = 3.732; P (MC) = 0.0319).

B. DOCOSAHEXAENOIC ACID (22:6N-3)

a) SHALLOW REEF

A completely contrasting trend to ARA was observed in DHA proportions in the community (Figure 5.7 C & D), with highest proportions of DHA occurring in the plankton, which gradually decreased to

the top left of the biplot. Very low proportions of DHA were observed in grazers (none in sea hares), passive colonial and solitary suspension-feeders and scavengers (Figure 5.7 C). Intermediate proportions of DHA occurred in the tissues of active suspension-feeders, amphipods and isopods.

b) DEEP REEF

The deep reef community demonstrated a very similar pattern in DHA compared to the shallow reef, with plankton samples marked by the highest proportions of DHA, followed by similar proportions in fish, amphipods and isopods, scavengers and active colonial suspension-feeders (Figure 5.7 D). Scavengers were marked with significantly higher proportions of DHA on the deep reef compared to the shallow reef (Pseudo-*F* = 221.5; *P* = 0.0022).

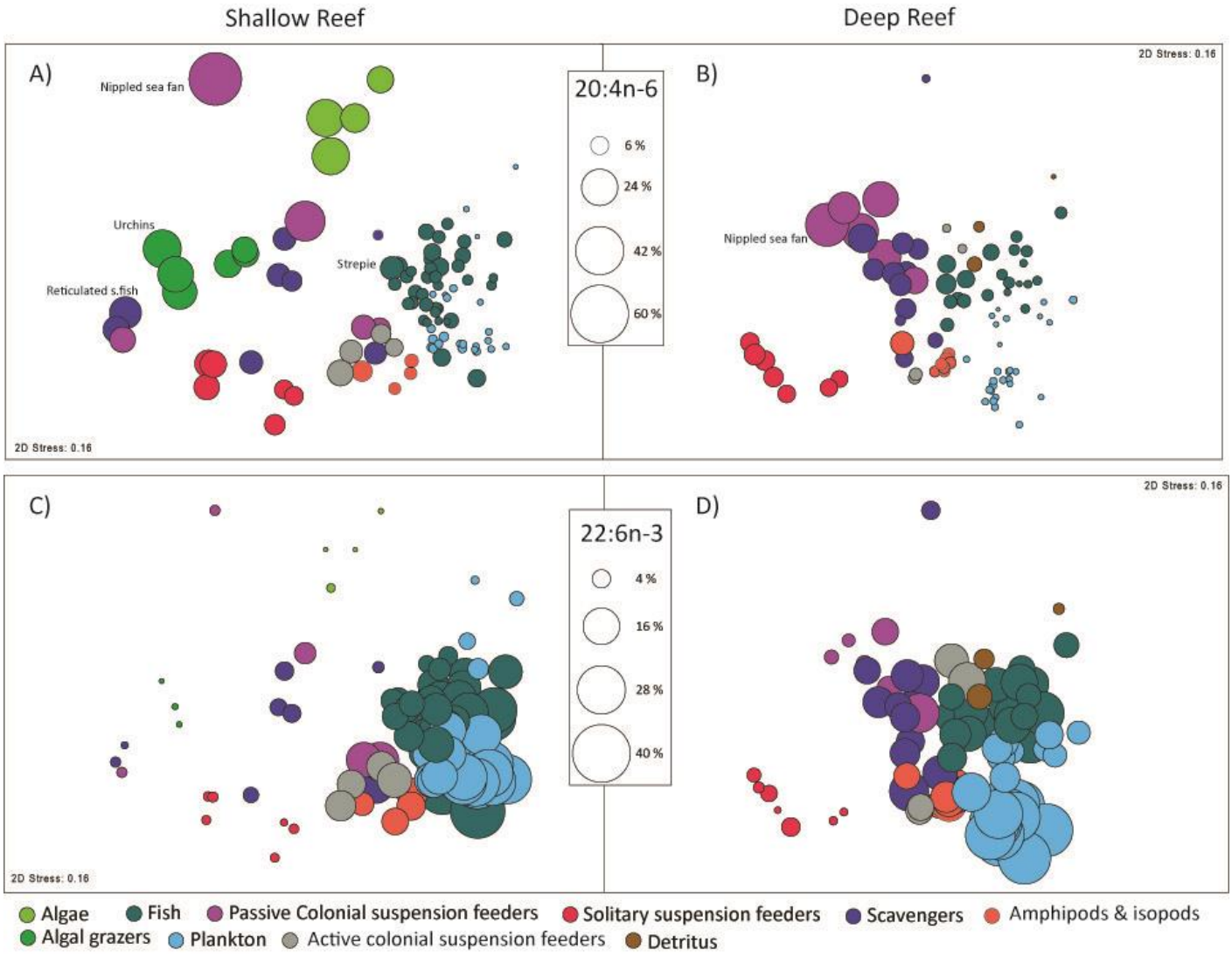


Figure 5.7. Non-metric multi-dimensional scaling (MDS) ordinations of the Tsitsikamma community, excluding sponges. MDS ordinations were based on a Bray-Curtis similarity matrix using untransformed data and depict the variation in fatty acid composition between shallow (left column) and deep (right column) reefs. The bubbles indicate the percentage contributions of A and B: 20:4n-6 (ARA), associated with benthic algae, and C and D: 22:6n-3 (DHA) a dietary indicator for dinoflagellate contribution.

C. DIETS OF SUSPENSION-FEEDERS

Suspension-feeders represent a direct link between the plankton (main source of EFA) and the rest of the reef community. Thus, direct inferences can be made about the importance of the different components of the plankton community in suspension feeding animals. Although the marker for dinoflagellates (DHA; 22:6n-3) occurred in greater proportions in the plankton samples than the marker for diatoms (EPA; 20:5n-3; Figure 5.8 A), this pattern was not reflected in the suspension-feeders' tissues (Figure 5.8 B). In the tissues of suspension-feeders, the marker for diatoms demonstrated a more constant pattern over time (Figure 5.8 A) and was a consistent indicator of diatoms as a basal food source for most suspension-feeders. Amphipods, isopods, brittle stars and ascidians all had EPA proportions >19% TFA. Two suspension-feeders (particularly the nippled sea fan) were marked by higher than average proportions of 20:4n-6, reflecting the influence of benthic algae (Figure 5.8 B). The marker for copepods [∑ (22:1, 20:1)] was important only in the diets of basket stars and brittle stars. Bacteria contributed equally to the nutrition of all suspension-feeders.

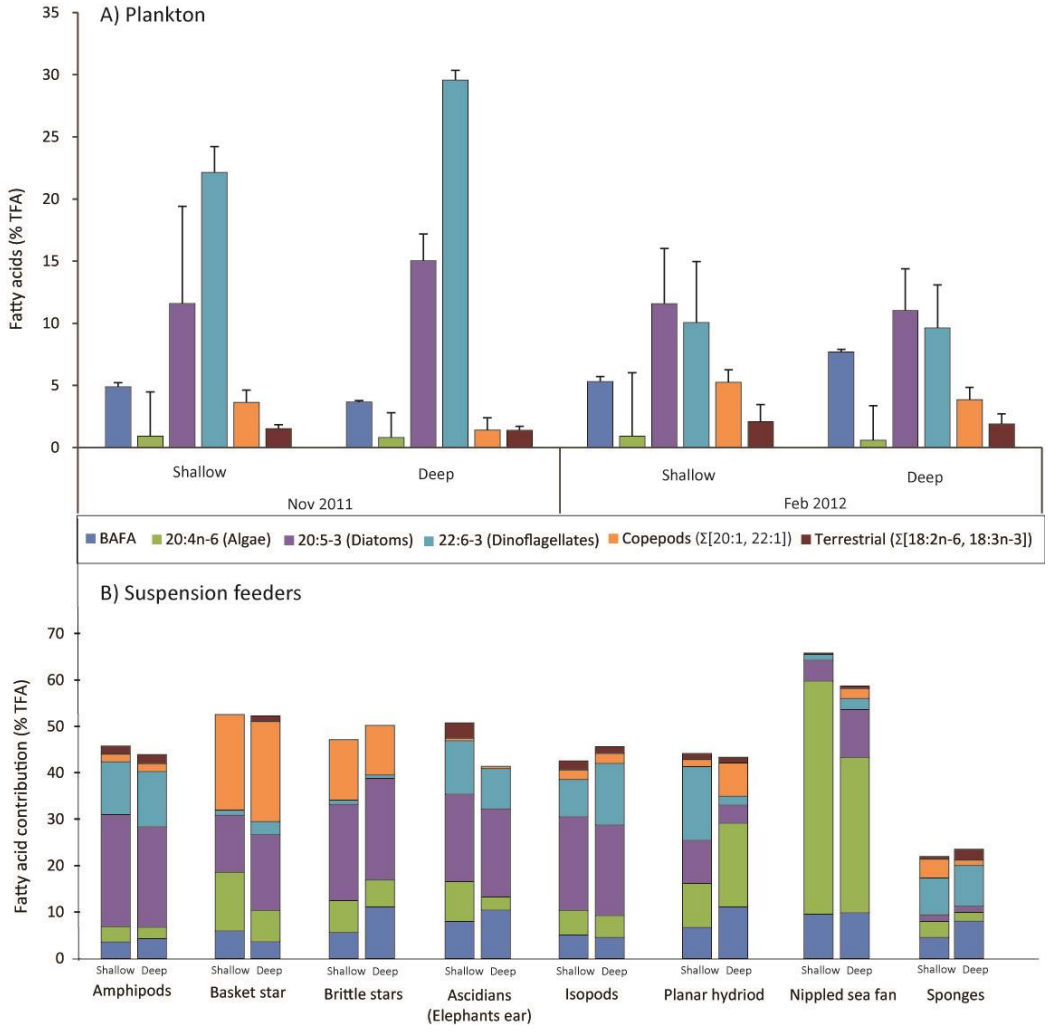


Figure 5.8. The fatty acid compositions of the plankton community (A) and the corresponding markers in suspension-feeders (B). Error bars in (A) represent standard deviations.

5.3.2.4 NUTRITIONAL CONDITION

A. PROPORTIONS OF FATTY ACIDS (% TFA)

The EFA 22:6n-3, a marker for dinoflagellates, occurred in significantly higher proportions in the deep reef community, except in the pooled fish data (Table 5.12; Figure 5.9). Plankton from the deep reef had significantly greater proportions of EPA and DHA and significantly lower proportions of ARA compared to the shallow reef (Figure 5.9 B; Table 5.12). This trend was reflected in the ratio ∑n-3/∑n-6, as significantly higher ratios were observed in samples (except fish) from the deep reef due to the higher proportions of both EPA and DHA (Table 5.12; Figure 5.9 B). No patterns in the EFAs were evident in the pooled fish FA data (Figure 5.9 D).

B. CONCENTRATIONS OF FATTY ACIDS (µG FA MG^{-1} DM)

For the entire Tsitsikamma community, significantly higher concentrations of ∑EFAs and 20:6n-3 were evident on the deep reef (Figure 5.9 B; Table 5.12 B). The effect of reef on the entire Tsitsikamma community was probably due to the highly significant effect of reefs on the plankton community (Figure 5.9 D; Table 5.12 B). Apart from 20:4n6, all remaining FA values recorded in plankton samples were significantly higher on the deep when compared to the shallow reef. When considering the macrobenthic community, only the EFA 20:6n-3 occurred in significantly higher concentrations in the tissues of deep reef consumers (Figure 5.9 F; Table 5.12 B). Compared to the results described above, the concentrations of FAs for the fish community demonstrated a contrasting trend. Total concentrations of FA, ∑EFA and 20:5n-3 were all significantly higher in fish collected from the shallow reef when compared to the deep reef (Figure 5.9 H; Table 5.12 B).

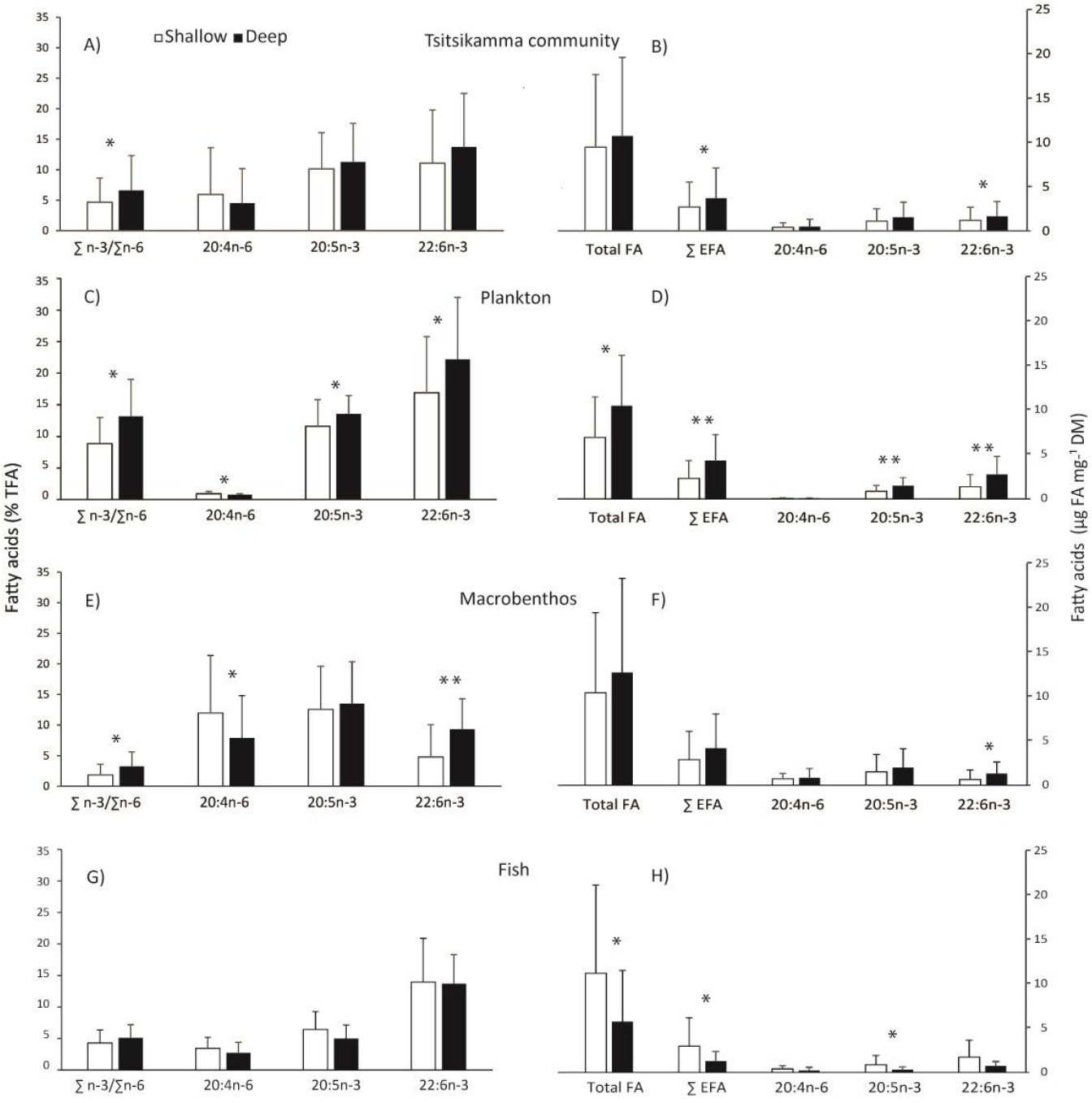


Figure 5.9. Comparison of the nutritional conditions of communities on the shallow (white) and deep (black) reefs in Tsitsikamma. The left hand column represents qualitative fatty acid data (percent total fatty acids; % TFA) and the right hand column the concentrations of fatty acids (µg FA mg^{-1} DM). The ratio of the sum of all n-3 and the sum of n-6 fatty acids (∑n-3/∑n-6), and the three essential fatty acids (20:4n-6; 20:5n-3 and 22:6n-3), concentration of all fatty acids (Total FA) and sum of essential fatty acids (∑EFA). Error bars represent standard deviations. * $P < 0.05$; ** $P < 0.001$.

Table 5.12. Univariate PERMANOVA comparing A) qualitative fatty acid data (percent total fatty acids; % TFA) and concentrations of fatty acids (µg FA mg^{-1} DM) between the shallow and deep reefs communities in the study site of Tsitsikamma. PERMANOVA was based on Euclidian distances for the ratio of the sum of all n-3 and the sum of n-6 fatty acids (∑n-3/∑n-6), and the sum of essential fatty acids (∑EFA) and the three EFA; 20:4n-6, 20:5n-3 22:6n-3. Values underlined and indicated in bold are significantly different.

A) Proportions of Total Fatty Acid (% TFA)

	∑ n-3/∑ n-6			**20:4n-6 (ARA)**			**20:5n-3 (EPA)**			**22:6n-3 (DHA)**		
PERMANOVA (reefs)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)
Community	180.07	7.62	**0.007**	128.88	2.76	0.094	66.31	1.88	0.172	350.04	4.52	**0.037**
Plankton	266.84	10.17	**0.003**	0.51	6.04	**0.017**	54.69	4.03	**0.047**	395.87	4.38	**0.042**
Macrobenthos	41.00	9.31	**0.003**	378.71	5.58	**0.018**	37.17	0.80	0.373	453.92	17.73	**0.000**
Fish	8.26	1.88	0.174	6.81	2.32	0.140	26.53	3.92	0.055	0.94	0.02	0.880

B) Concentrations of fatty acids (µg FA mg $^{-1}$ DM)

	Total Fatty Acids			**∑ EFA**			**20:4n-6 (ARA)**			**20:5n-3 (EPA)**			**22:6n-3 (DHA)**		
PERMANOVA (reefs)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)	MS	Pseudo *-F*	*P* (perm)
Community	76.86	0.98	0.325	45.96	4.35	**0.036**	0.25	0.49	0.505	9.19	3.48	0.060	10.58	3.97	**0.044**
Plankton	188.83	6.66	**0.014**	60.40	8.95	**0.005**	0.00	0.12	0.729	5.99	8.96	**0.004**	28.57	8.93	**0.004**
Macrobenthos	111.63	1.04	0.312	35.03	2.52	0.121	0.32	0.39	0.574	5.60	1.23	0.270	8.94	5.72	**0.020**
Fish	302.96	4.01	**0.047**	29.52	4.21	**0.043**	0.30	2.31	0.135	3.23	4.35	**0.040**	9.57	3.82	0.054

5.4 DISCUSSION

The main aim of this chapter was to determine if there were differences in the processes that support the shallow and deep reef food webs of Tsitsikamma. The processes considered were additional food sources on the shallow reef, changes in FA profiles of plankton during transit to depth, and different sources of EFAs for the shallow and deep reef communities. Indeed, the shallow reef was supported by more diverse food sources, as benthic algae (indicated by 20:4n-6) and terrestrial signals were significantly higher in shallow reef macrobenthic tissues, but only terrestrial signals were significantly higher in shallow fish samples. Modifications of FAs due to transit to depth (bacterial degradation and grazing impact) were not important for the macrobenthos or fish communities of Tsitsikamma. The shallow and deep reef communities demonstrated similar nutritional condition (same proportions of EFAs), although sources of EFAs differed between the shallow and deep reefs. Both shallow and deep reef communities had similar proportions of EPA (20:5n-3), which occurred in samples consistently over space and time. However, the overall pattern indicated that to balance the sum of EFAs between the two reefs, the shallow reef acquired EFAs in the form ARA (20:4n-6; benthic primary production), and the deep reef obtained EFAs from pelagic origin in the form of DHA (22:6n-3). However, DHA and ARA perform different physiological and biochemical functions in organisms,

which suggests possible differences in how nutrition is interpreted for the reef communities. For instance, both DHA and ARA are important in stress response and growth of fin fish and invertebrates, but DHA is especially important in membrane fluidity, whereas ARA is used to produce eicosanoids (e.g. prostaglandins), important compounds in chemical defence and inflammation control (Cimino & Ghiselin 1999). High proportions of ARA in marine organisms may imply an eicosanoid system with different enzyme activity levels (Copeman & Parrish 2003).

5.4.1 PRIMARY PRODUCERS

5.4.1.1 CHLOROPHYLL-*A*

The coastal waters of south-eastern South Africa are oligotrophic, characteristic of the Agulhas Current that prevails within the region (Machu et al. 2005). Generally, chlorophyll concentrations for this region range between 0.19 and 0.99 µg l^{-1} (Machu et al. 2005, Jury 2011), similar to the average range obtained in Tsitsikamma during November 2011 (0.1 – 0.5 µg l^{-1}), although lower values were obtained on the deep reef during July 2011 (0.01 ± 0.003 µg l^{1}), and on both reefs during February 2012 (0.04 – 0.07 µg l^{-1}).

5.4.1.2 PLANKTON

There was a strong temporal effect on the FA composition in the plankton community, as temperature and light intensity, both of which differed significantly between seasons, were selected by the DistLM as the most important factors to impact the FA compositions of the plankton community in Tsitsikamma. The EFA 22:6n-3 (DHA), a FA often associated with dinoflagellates (Broglio et al. 2003), dominated during November 2011, especially on the deep reef, and roughly correlated with the high chl-*a* concentrations observed during this time (Figure 5.2 A). The high proportions of 22:6n-3 during November 2011 may represent a post bloom event. Bloom events in upwelling regions are usually characterised by sharp increases in diatom concentrations, and as silica concentrations are depleted, the diatoms are replaced by dinoflagellates (Pitcher et al. 1993, Bruland et al. 2001), a trend observed by Mitchell-Innes (1988) during an upwelling event in Tsitsikamma. Jury (2011) indicated that primary production in the Agulhas region peaks from March to May, with a secondary peak during September to October, a period prior to when samples were collected and thus supporting the post bloom explanation. The plankton samples collected during February 2012 occurred from the 4th to 7th of February 2012, and on 4 February 2012 a drop in temperature by 5.75 ˚C in less than 24 hours was recorded, indicating an upwelling event (Figure 5.1). During this time, chl-*a* and the FAs associated with diatoms and dinoflagellates were less prevalent compared to those during November 2011, suggesting a lag-phase between the upwelled nutrients and bloom

development. Such lag events can range from 0.4 to 3.3 days, depending on the concentrations of upwelled nutrients and the size of the seed population (Pitcher et al. 1993). The different plankton communities observed during my study verified that the composition of the plankton community was highly variable over time and affected by seasonal differences in water column properties.

5.4.2 CONSUMERS

The results from the CAP analysis were congruent with results from other studies that suggested a strong phylogenetic link regarding FA signatures (Budge et al. 2002, Piché et al. 2010, Galloway et al. 2013). Higher order taxonomy (i.e. class) was selected by the CAP analysis as the best grouping variable for both macrobenthos and fish samples. Considering that very few samples of the same species were collected from both reefs, this strong taxonomic link associated with FAs might overshadow differences between reefs.

5.4.2.1 MACROBENTHOS

Overall, the macrobenthos was dominated by PUFAs, mostly the EFAs 20:5n-3 and 22:6n-3. PUFAs were on average 40.5 ± 9.4% TFA, SFAs were 29.8 ± 9.5% TFA and MUFAs averaged at 24.6 ± 9.5 (Table 5.3). Copeman & Parrish (2003) studied a benthic community in Gilbert Bay, Labrador, and the pooled average of a diverse selection of macrobenthic species demonstrated similar PUFA, SFA and MUFA proportions at 48.0 ± 7.3%, 25.0 ± 5.1% and 26.0 ± 6.7%, respectively.

A. SHALLOW VS DEEP

The significant effect of 'reef' detected in the multivariate macrobenthic community data may be explained by different sources of particular FAs reaching the shallow and deep reefs. The pooled macrobenthic data indicated that the tissues of species collected on the shallow reef were marked with significantly higher proportions of a terrestrial marker and the EFA 20:4n-6, a marker for benthic productivity (Kelly & Scheibling 2012; Figure 5.3 B & Figure 5.9 C). In contrast, the tissues of deep reef macrobenthos had significantly higher proportions of 22:6n-3, a FA most often associated with dinoflagellate productivity (Dalsgaard et al. 2003).

Galloway et al. (2013) found consistent differences in the FA composition of five solitary macrobenthic species when compared between shallow (10 – 15 m) and deep (90 – 100 m) sites at three locations in the San Juan Archipelago, Washington. Three plausible hypotheses were suggested to explain these differences in FA composition (Galloway et al. 2013). The first hypothesis was that food sources differ between the shallow and deep reefs and thus change FA compositions of consumers; the second hypothesis was that abiotic parameters such as temperature, light and

pressure alter the metabolism and behaviour of consumers, thereby changing their FA compositions; and the third hypothesis was that the same food sources are available to both shallow and deep benthic communities, but during transport from shallow to deep habitats, the FAs are altered through biochemical processes related to microbial degradation. From their results, Galloway et al. (2013) concluded that differences in the FA profiles of the investigated species was best explained by diagenesis (i.e. the third hypothesis).

The first hypothesis of Galloway et al. (2013), that food sources available to the macrobenthos differ between the shallow versus deep habitats, seems plausible for the community studied in Tsitsikamma (at least for some of the species). Benthic algae were not observed on the deep reef (Chapter 3), and as such FAs derived from algae should feasibly be more important on the shallow reef. Indeed, the marker for benthic productivity, 20:4n-6, was significantly more prevalent in shallow reef macrobenthic tissues. The significantly higher proportions of terrestrial FAs in organisms from the shallow reef also suggested that different FA sources were available to consumers. Higher proportions of FAs from terrestrial input can be explained by the closer proximity of the shallow reef to the shore and the mouth of the Storms River. Similar proportions of terrestrial FAs were found in nearshore sites in Notre Dame Bay and Trinity Bay, Newfoundland (1 – 4% TFA; Budge and Parrish 1998; Budge et al. 2001). In contrast to what was expected, proportions of the marker for copepods (∑ [22:1, 20:1]) were significantly higher in the shallow plankton samples collected in November 2011, although no difference between reefs were evident during February 2012. Furthermore, as proportions of ∑ [22:1, 20:1] in macrobenthos from both shallow and deep reefs were similar, the grazing impact on phytoplankton by zooplankton during transit to depth did not influence the consumers of Tsitsikamma.

The second hypothesis of Galloway et al. (2013), that abiotic parameters alter the metabolism and thereby the FA compositions of organisms, may apply to Tsitsikamma, as temperature and light intensity differed significantly between the reefs. Homeoviscous adaptation, which is increased membrane elasticity in ectotherms exposed to cold temperatures, has been linked to high levels of DHA (Arts & Kohler 2009). However, although significantly higher proportions of DHA were observed in macrobenthic tissues from the deep reef, it is highly unlikely that this trend is related to the requirement for ectotherms to increase membrane fluidity in response to lower temperatures, as the temperature differences observed here were not pronounced enough for such physiological adaptations (Cossins et al. 1977, Parrish 2009). Thus, higher proportions of DHA in deep reef macrobenthic tissues observed in Tsitsikamma were likely related to a greater supply of this EFA to consumers on the deep reef, as was evident in the plankton data (Figure 5.9 B).

The third hypothesis of Galloway et al. (2013), that FA profiles of animals that feed at depth differ from shallow reef consumers because FAs are altered by microbial degradation during transit to depth, is not likely a factor at Tsitsikamma. If microbial degradation was a factor, then deep reef plankton and primary consumers would demonstrate higher proportions of BAFAs. Although the data indicated a general increase in BAFAs with depth in the macrobenthos (Figure 5.3 B), the pooled data for the macrobenthos in Tsitsikamma did not support this hypothesis, as no significant difference in BAFAs were evident when comparing the shallow and deep reefs.

B. DIET

Benthic algae were most abundant at the very shallow sites in Tsitsikamma, as identified in Chapter 3, and they more important in the tissues of shallow compared with deep reef consumers. The main sources of ARA (20:4n-6) were benthic algae (here coralline algae; Rhodophyta), which was comparable to results by Allan et al. (2010), who found similar proportions of ARA in other Rhodophytes (*Gelidium pristoides and G. enterobium*) collected from the south coast of South Africa.

The transfer of ARA to algal grazers was evident, and high proportions were apparent in the tissues of sea hares (*Aplysia parvula*), urchins (*Parechinus angulosus*) and the shallow starfish species (reticulated starfish, *Henrica ornata*; Figure 5.7 A). Apart from the direct grazers, high proportions of the marker for benthic algae were recorded in sea fans, especially nippled sea fans from the shallow reef. Both urchins and sea fans revealed ARA proportions higher than those present in the coralline algae, possibly suggesting either selective retention or biosynthesis of this FA. Selective retention or biosynthesis of certain FAs may occur in consumers when their diets are deficient in that particular FA (Spychalla et al. 1997, Castell et al. 2004, Hall et al. 2006, Iverson 2009, Kelly & Scheibling 2012). Selective retention or biosynthesis was evident in several echinoderms from Gilbert Bay, Labrador, which had high proportions of ARA even when sources of this FA were negligible (Copeman & Parrish 2003). However, in Tsitsikamma, ARA was available as a direct source to the urchins and thus it is plausible that the proportions of ARA in their tissues were due to diet. Similar results were obtained by Sargent et al. (1983) from urchins collected in northern Norway, and from herbivorous amphipods collected in the South Orkneys (Nyssen et al. 2005). On the other hand, the high proportions of ARA in the tissues of sea fans in Tsitsikamma seemed unusual, as sea fans obtain food from the water column, suggesting high proportions of ARA should be present in the plankton. Suspended particulates collected in TNP and in other regions along the South African coastline (e.g. Allan et al. 2010, Antonio & Richoux 2014) did not show proportions of ARA high enough to support the very elevated proportions observed in sea fans. Similar high values of ARA were recorded in the closely related *Eunicella singularis* collected from Cap de Creus in the north-western Mediterranean Sea (Gori et al. 2012), and *Veretillum cynomorium*, an azooxanthellate octocoral studied near the Sado

estuary, Portugal (Baptista et al. 2012). In fact, ARA was the most important FA in azooxanthellate octocorals in several studies (Imbs et al. 2007, 2009, Baptista et al. 2012). Octocorals use prostaglandins, derived from ARA, as a chemical defence against grazers (Cimino & Ghiselin 1999), and may explain either selective retention or biosynthesis.

Algal grazers, especially urchins, displayed high proportions of the copepod marker, ∑ (20:1; 22:1). In contrast to the proportions of ARA in urchins, which can be explained by the direct consumption of benthic algae, the source of 20:1n-9 was most likely pelagic in origin, from wax esters in copepods (Sargent & Falk-Petersen 1988). Because it is unlikely that urchins feed directly on copepods, Sargent et al. (1983) suggested *de novo* biosynthesis of 20:1n-9 occurs in echinoderms. This possibility was verified by Castell et al. (2004), who demonstrated that the urchin *Strongylocentrotus droebachiensis* elongated 18:1n-9 to 20:1n-9 when it was fed an artificial diet low in the latter FA. Finally, the FA compositions of urchins collected in Tsitsikamma were very low in 22:1n-9. If copepods were the source of 20:1n-9, then 22:1n-9 should also contribute to the high proportions observed in urchins, as 22:1n-9 is a component of the copepod marker. The basket and brittle stars (Ophuirodea) also demonstrated high proportions of the copepod marker, but probably indicated direct feeding on copepods (Fig. 5.8A). Basket stars (here *Astrocladus euryale*) extend their arms into the water column and consume copepods and other large zooplankton species (Rosenberg et al. 2005). Drazen et al. (2008) also found high levels of 20:1n-9 in abyssal ophuiroids collected from the North-East Pacific Ocean, but did not exclude *de novo* synthesis of this FA.

Overall, the marker for diatoms (20:5-n3; EPA) were important in the diets of most macrobenthic species in Tsitsikamma, and no differences in FA compositions were detected between the shallow and deep reefs. High levels of EPA in diverse groups of marine invertebrates were reported by several authors studying different locations including the Arctic and Antarctic regions (Graeve et al. 1997, 2001, Nyssen et al. 2005), a shallow-water hydrothermal ecosystem (Kharlamenko et al. 1995), and temperate waters (Guest et al. 2009, 2010). Here, the marker for diatoms occurred in high proportions in amphipods, isopods, ascidians, brittle stars, basket stars (Figure 5.8 B) and in non-suspension feeding animals such as crabs and polychaetes. These findings suggest that diatoms can enter the food web either through direct feeding from the water column by suspension-feeders, from sediments by detrivores, and/or from the grazing of epiphytes (see Graeve et al. 2001, Howell et al. 2003, Richoux & Froneman 2008, Parrish et al. 2009).

Compared to the proportions of EPA, the EFA 22:6n-3 (DHA), likely derived from dinoflagellates, was less important in the diets of the macrobenthos in Tsitsikamma. Nonetheless, DHA occurred in the tissues of most suspension feeding animals including hydroids, sponges, ascidians, isopods, and

amphipods (Figure 5.8 B). Similar proportions of DHA were reported in ascidians (Guest et al. 2009), hydroids (Imbs 2013), amphipods (Nyssen et al. 2005) and sponges (Thurber 2007) in other marine regions. In contrast to most suspension-feeders, low proportions of DHA were evident in the tissues of passive suspension feeding sea fans in Tsitsikamma. This finding was consistent with Baptista et al. (2012), who reported very low proportions of DHA but high levels of ARA in the octocoral *V. cynomorium*.

5.4.2.2 FISH

Fatty acid profiles of fish in Tsitsikamma were dominated by SFAs (up to 78% TFA), followed by PUFAs (up to 58.5% TFA; Table 5.3 C). Similar proportions of SFAs were detected in the tropical reef fish species *Bodianus rufus* and *Caranx hippos* in the Gulf of Mexico (Carreón-Palau et al. 2013), and fish collected in the Hawaiian Archipelago, especially in the genus *Acanthurus* (Piché et al. 2010). Much lower levels of SFAs were detected by Budge et al. (2002) in cold water species collected from the Scotian Shelf, and Iverson et al. (2002) in the Prince William Sound, Alaska. Although the water temperatures measured in Tsitsikamma (8.9 – 22.6°C) were lower than those of the tropical studies (20 - 30°C; Carreón-Palau et al. 2013), the relatively high proportions of SFAs in the fish from Tsitsikamma were probably related to the ability of fish to adapt to cold temperatures through behavioural adaptations (Arts & Kohler 2009). Behavioural responses such as moving to warmer waters decreases the requirements of fish to maintain the very high proportions of PUFAs in warm to mid-temperature regions relative to those in more polar regions, where maintaining membrane fluidity becomes critical (Cossins et al. 1977, Wallaert & Babin 1994, Saito et al. 1999, Copeman & Parrish 2003).

A. SHALLOW VS DEEP

Contrary to expectations, fish FA profiles demonstrated no clear distinctions between the shallow and deep reefs. Apart from significantly higher terrestrial markers on the shallow reef, the other univariate and multivariate analyses indicated no differences in the FAs of fish between reefs. This result stands in contrast to Piché et al. (2010), who found clear differences in FA compositions of fish and invertebrates collected from shallow habitats around the north-western Hawaiian Islands compared to deep sub-photic zones. High proportions of 18:1n-9, and low proportions of 20:4n-3 were evident in the deep-water samples collected by Piché et al. (2010). However, one key difference in the study by Piché et al. (2010) was their larger depth range analysed (10 – 500 m), compared to 12 – 75 m in the present study. This comparison suggests that the lack of differences in the FA profiles of fish between the reefs in Tsitsikamma was caused by the smaller depth range and mobility of fish. Although clear differences in the size and species composition of fish were detected (Chapter 4), fish

are mobile and most are able to forage on both shallow and deep reefs in the same regions (Papastamatiou et al. 2015). Furthermore, fish that live at depth during the day often migrate to shallower water at night to feed (Papastamatiou et al. 2015), which may result in similar FA profiles in fish collected from different depths. The majority of the fish species that were investigated in Tsitsikamma (>13 species) were generalist omnivores and carnivores and they demonstrated considerable dietary overlap. Guest et al. (2009) also had difficulty in drawing conclusions about the diet of the southern rock lobster (*Jasus edwardsii*) due to its generalist predatory feeding habits. It becomes increasingly difficult to trace biomarkers to higher trophic level predators, as FAs that originate at the base of the food web become relatively ubiquitous throughout higher trophic levels (Iverson 2009).

For the remaining fish species in Tsitsikamma that demonstrated some degree of specialist feeding (blue hottentot and strepie), differences in their FA with depth were not large enough to generate a statistical distinction between reefs. Strepie were restricted to the shallow reef, and thus species-specific analysis between the reefs could not be conducted, and only one sample of blue hottentot was collected from the deep reef, making statistical analysis unfeasible. Preliminary qualitative analysis did suggest potential differences between the reefs (Figure 5.10 C), but this potential trend needs to be substantiated with further sampling.

5.4.3 NUTRITION

The sources of EPA and DHA in Tsitsikamma were from pelagic production, as plankton samples had EPA and DHA ranges of 11 ± 2.8 to 15 ± 2 and 9.6 ± 3.3 to 29.6 ± 2.2 % TFA, respectively. These values were high compared to those in suspended particulate samples collected close to the study area at Plettenberg Bay [EPA: 2.4 ± 0.6 % TFA; DHA: 2.2 ± 0.7 % TFA; Allan et al. (2010)]. However, the plankton samples analysed here were for size classes >65 µm and therefore more representative of the zooplankton community. Indeed, the markers for copepods were more dominant in the larger size class (>500 µm) of the plankton samples. Similar proportions of EPA (20:5n-3) and DHA (22:6n-3) were recorded in zooplankton from surface and demersal samples in Praia de Tofo, Mozambique (Couturier et al. 2013), and from towed plankton samples from a polar frontal zone near the subtropical convergence (Richoux 2011). The main source of ARA (20:4n-6) in Tsitsikamma was benthic algal production (coralline algae; Rhodophyta) and not pelagic sources, as confirmed by the low proportions of this FA recorded in plankton samples (Figure 5.9 D). Furthermore, Allan et al. (2010), Ackman & Mchlachlan (1977), Kharlamenko et al. (1995) and Kelly & Scheibling (2012) all found similar proportions of ARA in the tissues of a variety of benthic algal species.

Significantly lower n-3/n-6 ratios on the shallow reef macrobenthos supported the overall higher input of 20:4n-6, derived from benthic algae, to the shallow reef macrobenthos compared to the deep reef. This finding suggested that although the shallow and deep macrobenthic assemblages did not differ in terms of the overall nutritional condition (both for proportions and concentrations of ∑ EFAs), it seems that sources of EFAs differed between the reefs, as evident in the significantly higher proportions of 20:4n-6 in the tissues of shallow reef macrobenthos. The differences in shallow reef EFA compositions were made up by input from benthic productivity, contrasting with the deep macrobenthic community, which demonstrated a stronger bentho-pelagic connection since the source of DHA originated from pelagic primary production. This pattern was not mirrored by the proportional (%) fish data, and no differences with depth in any of the EFAs or the n-3/n-6 ratio were evident. In fact, results from the concentrations of FA indicated that fish from the shallow reef were actually feeding on food sources of greater nutritional quality.

5.5 CONCLUSIONS

When the sums of EFAs were considered for the consumers of Tsitsikamma, no difference was observed in the pooled macrobenthic data; however, the fish community demonstrated significantly higher concentrations of EFAs and total FA on the shallow reef. The pattern observed in the tissues of the macrobenthos can be explained by the higher proportions of the EFA 20:4n-6 available to the shallow macrobenthic community. The deep reef macrobenthic community was marked with significantly higher proportions and concentrations of the EFA 22:6n-3. The third EFA, 20:5n-3, occurred at similar proportions and concentrations in macrobenthos from both reefs. The differences in the FA profiles in macrobenthic tissues when compared between the reefs can be explained by additional supplies of food sources to the shallow reef. These additional sources include terrestrial materials and benthic algae. Although these sources were not as important for the deep reef community, a greater supply of DHA from pelagic production seemed to support the deep reef macrobenthic community. The pattern observed for the macrobenthic community demonstrated a strong bentho-pelagic link, especially for the primary consumers of plankton. The contrasting trend observed in the fish community might be explained by active selection of nutritional food items by fish, which were more abundant on the shallow reef due to the greater species richness observed here (Chapter 3). .

6 TROPHIC ORGANISATION OF THE SHALLOW & DEEP REEFS IN THE TSITSIKAMMA MARINE PROTECTED AREA

6.1 INTRODUCTION

The impacts of human activities on ocean ecosystems has resulted in habitat loss and local extinctions of species (Halpern et al. 2008). Loss of species has consequences for community structure and the ecological function that a particular assemblage performs in a community (Walker 1992, Micheli & Halpern 2005). Species and functional diversity have a strong positive correlation, suggesting that a biodiverse community is functionally diverse (Micheli & Halpern 2005). Functional diversity equates to ecological redundancy, because a redundant community is characterised by many distinct taxa which perform similar ecological functions in a community (Micheli & Halpern 2005). Thus, a community with low ecological redundancy is vulnerable to disturbance because functional traits that maintain the integrity of ecosystem function are rapidly lost when diversity declines (Walker 1992, Micheli & Halpern 2005). To effectively manage our marine resources, a better understanding of the vulnerability of communities is needed. This enhanced knowledge would allow managers to identify habitats and communities that require preferential protection or consideration.

In addition to fatty acid (FA) analysis, stable isotope ratios (SI) can provide general insights into the structural differences among communities. Stable isotope techniques are popular in ecology because the data produced provide a temporally-integrated depiction of the trophic dynamics of an organism (Post 2002). Where FA analysis can give an indication of the species composition of consumer diets (Iverson et al. 2004), SI estimate the trophic position of an organism, and also provide an indication of the source of primary production at the base of a consumer's diet (Post 2002). Trophic position represents the assimilation of energy through different trophic pathways to an organism (Post 2002) and can be measured because the ratio of the light to heavy nitrogen isotopes ($\delta^{15}N$) typically differs by about 3.4‰ in consumer tissues relative to its diet (Post 2002,

Jennings et al. 2008, Guest et al. 2010). In contrast, ^{13}C and ^{12}C are fixed at different ratios by primary producers, but the ratio between $^{13}C/^{12}C$ differs very little when assimilated by consumers (typically between 0 - 1‰), and can therefore be used to identify main sources of carbon at the base of the food web (Post 2002, Guest et al. 2010). In aquatic ecosystems, benthic and pelagic carbon values are usually distinct, and consequently researchers can derive information about paths of different carbon sources in a community (Post 2002).

Statistical procedures have advanced extensively since SI were first introduced to study trophic ecology (Phillips et al. 2014). Recent developments include the estimation of diet through the use of sophisticated mixing models (IsoSource; Phillips & Gregg 2003), which have been extended to incorporate Bayesian approaches including MixSIR (Moore & Semmens 2008), SIAR (Parnell et al. 2010) and MixSIAR (Stock & Semmens 2013). In addition, Layman et al. (2007) proposed the quantification of niche space by using the convex hull area occupied by species in $\delta^{13}C$-$\delta^{15}N$ iso-space, which was extended by Jackson et al. (2011) to account for differences in sample size. The community-wide metrics described by Layman et al. (2007) provide information on the trophic structure of communities and indicate the range of carbon sources, the trophic length and the level of trophic redundancy in a community.

6.1.1 STUDY AIM

Depth impacts not only the assemblage structure of macrobenthos and fish (Chapters 3 & 4; Garrabou et al. 2002; Vermeij & Bak 2003; Brokovich et al. 2008; Fitzpatrick et al. 2012; Zintzen et al. 2012; Wing & Jack 2012), but also their trophic ecology, especially in lower level consumers (Chapter 5; Piché et al. 2010; Galloway et al. 2013). The general patterns identified thus far in Tsitsikamma are that species richness decreases with depth, larger and sexually mature fish species occur in deeper locations, and shallow reef organisms have additional sources of primary production.

I hypothesised that the shallow reef consumers utilise a more diverse range of carbon sources due to the additional benthic primary production locally available (Chapter 5). Secondly, due to the larger sizes of fish on the deep reef (Chapter 4), and the general trend of increasing trophic level with size (Jennings et al. 2008), I hypothesised that the deep reef community has a longer food chain length, and because benthic primary productivity is absent on the deep reef, consumers on the deep reef feed at higher trophic levels. Finally, I hypothesised that due to the decrease in species and functional richness with depth (Chapters 3 & 4), the shallow reef has higher levels of trophic redundancy.

6.2 MATERIALS & METHODS

6.2.1 STUDY AREA

Research was conducted on the Middlebank and Rheeders Reef complexes situated close to the Storms River mouth in the Tsitsikamma National Park (TNP) marine protected area (MPA). A full study site description is provided in Chapter 2, Section 2.1.2.

6.2.2 SAMPLING STRATEGY

Tissue samples intended for SI analysis were obtained from the same samples collected for FA analysis. However, in contrast to the FA plankton samples, which consisted of plankton pump samples (micro and meso zooplankton), the SI samples consisted of filtered water samples of suspended particulate matter (SPM). For a full list of samples processed for SI analyses, see Table A5.1. Water samples were collected by lowering the Vertical Point Sampler to just above the reef. Three discreet water samples were collected at each station, separated into 2 l sampling jars and placed in a cooler-box for later processing. In the laboratory, the 2 l aliquots were gently filtered (<5 mm Hg vacuum) onto pre-weighed, pre-ignited (450°C overnight) GF/F Whatman glass fibre filters. An in-depth description of the sampling strategy is documented in Chapter 5, Sections 5.2.2 and 5.2.3.

6.2.3 ANALYSES

6.2.3.1 SAMPLE TREATMENT

Animals, plants and filters (SPM) were lyophilised at -60°C for 24 h (VirTis BenchTop 2K). Animal and plant samples were individually homogenised using a mortar and pestle and weighed separately into tin capsules. Species with high inorganic carbon (starfish, sea fans, urchins and coralline algae) were treated with 1M HCl to remove carbonates, rinsed with distilled water and re-dried for 24 h at 60°C (Fry & Wainright 1991). The $\delta^{13}C$ and $\delta^{15}N$ values of prepared samples were determined using a mass spectrometer with a Europa Scientific 20-20 IRMS linked to an ANCA SL prep unit. Isotope abundances were calibrated in relation to in-house standards; casein was used as a protein standard, nitrogen was expressed relative to atmospheric nitrogen, and carbon was expressed relative to beet sugar and ammonium sulphate. Isotope ratios are expressed in the δ unit notation following the equation:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where δ(‰) is either the $\delta^{13}C$ or $\delta^{15}N$ and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the standard and sample, respectively.

Lipids are isotopically lighter than other biochemical components and potentially cause variation in carbon isotope signatures due to variable lipid content among species. To minimise the effect of lipids on SI, a lipid correction model proposed by Post et al. (2007) was applied:

$$\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times (C:N)$$

where C:N is the ratio of carbon-to-nitrogen. The C:N ratios for the SPM samples were not available to perform lipid corrections. Thus C:N values of the marine SPM samples collected by Antonio & Richoux (2014) from the Agulhas Ecoregion were averaged and used to correct SPM samples. For long lived consumers, which includes most animals sampled here, trophic position should be estimated from long-lived primary consumers that capture the spatial and temporal variability in $\delta^{15}N$ (Post 2002). Here, algal grazing sea hares (*Aplysia parvula*) represented the basal trophic level for the shallow reef, and the suspension-feeding planar hydroids (*Sertularella arbuscula*) represented the base level for the deep reef consumers. The trophic positions (λ) of these grazers and suspension-feeders were set at 2.0 and 2.6, respectively. Trophic position of consumers was calculated according to Post (2002):

$$Trophic\ position = \lambda + (\delta^{15}N_{sc} - \delta^{15}N_{base})/\Delta_n$$

where λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (see above), $\delta^{15}N_{secondary\ consumer}$ ($\delta^{15}N_{sc}$; or any higher consumer) are the measured values for the consumers, and Δ_n is the enrichment factor set at 3.4‰ (Vander Zanden & Rasmussen 2001, Post 2002).

6.2.3.2 STATISTICAL ANALYSES

A. COMMUNITY BASED METRICS & NICHES

Food chain length (FCL) was estimated following Vander Zanden & Fetzer (2007):

$$FCL = (\delta^{15}N_{top\ predator} - \delta^{15}N_{base})/3.4 + \lambda\ .$$

To determine if the shallow and deep reef communities differed in their trophic structure, community wide metrics were applied to consumer tissues (Layman et al. 2007, Jackson et al. 2011). Jackson et al. (2011) accounted for differences in sample size and issues associated with

variance in trophic discrimination factors through the application of Bayesian inference; briefly, the six metrics include:

- $\delta^{15}N$ range (NR), which provides information on the trophic diversity and trophic length of the community,
- $\delta^{13}C$ range (CR), which is an indication of the diversity of basal sources,
- mean distance to centroid (CD) provides information about the average degree of trophic diversity and species spacing,
- mean Euclidean distance to nearest neighbour (NND) is a measure of the density and clustering of species, where lower values indicate many species of similar trophic ecologies and thus increased trophic redundancy,
- standard deviation of the nearest neighbour distance (SDNND) is a measure of evenness of spatial density packing, and also provides information on the ecological redundancy of a community.

Metrics were calculated in the package stable isotope Bayesian ellipses in R (SIBER; Jackson et al. 2011) in R-studio (R Core Team 2013). Bayesian estimates of Layman's metrics were computed, excluding sources, on the entire consumer community, macrobenthos and fish. Only fish collected during February 2012 were included in the analyses due to significant difference among samples by season for fish (Table 6.1), and because the remaining macrobenthic samples were all collected during February 2012, making the metrics more comparable.

The total area (TA) as originally proposed by Layman et al. (2007) is a direct measure of niche area and is calculated from a convex hull drawn on extreme values in a SI biplot. However, because the convex hull is drawn to include extreme values, additional samples would result in an increase of the hull area (Jackson et al. 2011). Thus, because sample sizes differed in this study, the isotopic niches of both the shallow and deep reef fish and macrobenthic assemblages were quantified using standard ellipse areas (SEA; Jackson et al. 2011). Standard ellipse area is the multivariate equivalent of the standard deviation, it uses the variance and covariance of bivariate isotope data to include about 40% of the data, and it is not affected by sample size. To minimise bias caused by small sample sizes, SEA_C was calculated by correcting SEA using the equation:

$$SEA_C = SEA \times [\frac{n-1}{n-2}].$$

Furthermore, the Bayesian standard ellipse area (SEA_B) was calculated to obtain confidence intervals for the isotopic niche areas. These confidence intervals allow for statistical comparisons of the isotope niche areas among populations. Differences in the isotopic niche position were

examined following Turner et al. (2010). Tests were based on nested linear models and residual permutation procedures from which null distributions were generated to test for differences between centroids of samples. Tests were conducted in R – Studio (R Core Team 2013). Prior to analyses all data sets were tested for multivariate normality using the Shapiro-Wilk test [mshapiro.test() function in R – Studio].

The combined C and N isotope value ($^{\delta}$CN) summarises the changes in δ^{13}C and δ^{15}N and is an indication of the benthic vs pelagic resource use, with lower values indicating reef derived resource use, and higher values indicating pelagic or oceanic resource use (Fry et al. 2008, Wyatt et al. 2012):

$$^{\delta}CN = \delta^{15}N - \delta^{13}C.$$

To determine if shallow and deep isotopic data differed, and if there was an effect of season, univariate and multivariate analyses of variance (PERMANOVA) were conducted using PRIMER v6 (Clarke & Gorley 2006) with the PERMANOVA + add-on package (Anderson et al. 2008). All PERMANOVA were performed from matrices based on Euclidean distance measures. For comparisons of the pooled data using only one factor (reef), *P*-values were obtained from 9999 unrestricted permutations of the raw data (Anderson et al. 2008). For PERMANOVA where more than one factor was included, 9999 permutations of the residuals under a reduced model were computed for each term to obtain *P*-values (Anderson et al. 2008). To account for the unbalanced structure of the PERMANOVA design, the procedures were run by selecting Type III sums of squares, ensuring complete independence of all factors tested (Anderson et al. 2008).

6.3 RESULTS

6.3.1 TROPHIC STRUCTURE

6.3.1.1 FOOD SOURCES

The multivariate plankton signatures of δ^{13}C and δ^{15}N differed significantly between reefs and sampling seasons (Table 6.1; Figure 6.1). However, univariate analyses of δ^{13}C and δ^{15}N revealed that differences between the reefs were due to variation in δ^{15}N rather than δ^{13}C (Table 6.1), and δ^{15}N was lower in plankton on the deep reef compared to the shallow reef (Table 6.2). Seasonal differences in plankton isotope signatures were due to variation of both δ^{13}C and δ^{15}N (Tables 6.1 & 6.2). For instance, δ^{13}C values in SPM samples collected from the shallow reef during February

2012 were on average -18 ± 1.4 compared to -12.8 ± 1.1 in July 2011, with slightly lower values on the deep reef (Table 6.2). Furthermore, pairwise comparisons including reef and season indicated that the SPM collected during November 2011 from the deep reef was significantly lower in $\delta^{13}C$ compared to the shallow reef (t = 2.094; P < 0.05). The $\delta^{15}N$ values were lowest in SPM collected during February 2012, especially from the deep reef, and highest in SPM samples collected during November 2011 (Table 6.2). A single sample of red algae represented the lowest $\delta^{13}C$ signature (-27 ‰), and coralline algae represented the highest (-9.7 ± 3.2 ‰).

Table 6.1. Multivariate and univariate PERMANOVAs based on Euclidian distances. PERMANOVA were conducted on a) plankton samples collected over three seasons, b) macrobenthos, only sampled during February 2012 and c) fish, sampled during July 2011 and February 2012. Values indicated in bold represent a significant effect; MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability based on permutations.

		$\delta^{13}C$ & $\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
PERMANOVA		MS	Pseudo - *F*	*P* (perm)	MS	Pseudo - *F*	*P* (perm)	MS	Pseudo - *F*	*P* (perm)
a) Plankton/SPM	Reef	159.190	8.779	**0.0027**	12.099	3.134	0.0912	147.090	10.306	**0.0038**
	Season	304.770	16.807	**0.0001**	156.780	40.609	**0.0001**	147.980	10.368	**0.0002**
	Reef & season	29.508	1.627	0.1881	2.542	0.658	0.5347	26.966	1.889	0.1672
b) Macrobenthos	Reef	53.035	7.866	**0.0017**	0.289	0.090	0.7631	52.746	14.870	**0.0004**
c) Fish	Reef	18.200	14.787	**0.0002**	0.194	0.321	0.5892	18.006	28.776	**0.0001**
	Season	24.572	19.964	**0.0001**	1.920	3.172	0.0765	22.652	36.202	**0.0001**
	Reef & season	5.405	4.391	**0.023**	3.110	5.140	**0.0238**	2.295	3.667	0.0658

6.3.1.2 CONSUMERS

Because macrobenthic tissue samples were collected only during February 2012, no seasonal comparisons could be made. Comparisons between reefs indicated significantly different SI values for macrobenthos collected from the shallow reef when compared to the deep reef (Table 6.1; Figure 6.1). In contrast to the SPM samples, the difference between the macrobenthos from the shallow and deep reefs was due to an overall increase, rather than decrease, in $\delta^{15}N$ from the shallow to the deep reef. For instance, the colonial ascidian, elephant's ear (*Gynandrocarpa placenta*), demonstrated an increase in $\delta^{15}N$ of 3.6‰, equivalent to more than one trophic level, from the shallow to the deep reef (Table 6.2). Similarly, sponges demonstrated an average increase in $\delta^{15}N$ of 3.7‰ from the shallow to deep reef.

Fish collected from both the shallow and deep reefs during July 2011 differed significantly to those collected in February 2012 (Table 6.1). Again, differences in fish SI for both season and reef were due to variations in $\delta^{15}N$ and not $\delta^{13}C$ (Table 6.1). The general pattern for fish was lower $\delta^{15}N$ in

samples collected during February 2012 compared to July 2011, and higher $\delta^{15}N$ from the samples collected from the deep reef compared to the shallow reef (Figure 6.1). For instance, $\delta^{15}N$ signatures in roman collected during July 2011 measured on average 15.7 ± 0.3‰, compared to 14.5 ± 0.01‰ in February 2012. Values of $\delta^{15}N$ in roman collected during February 2012 from the shallow reef measured on average 14.5 ± 0.01‰, compared to 15.4 ± 0.1‰ on the deep reef (Table 6.2).

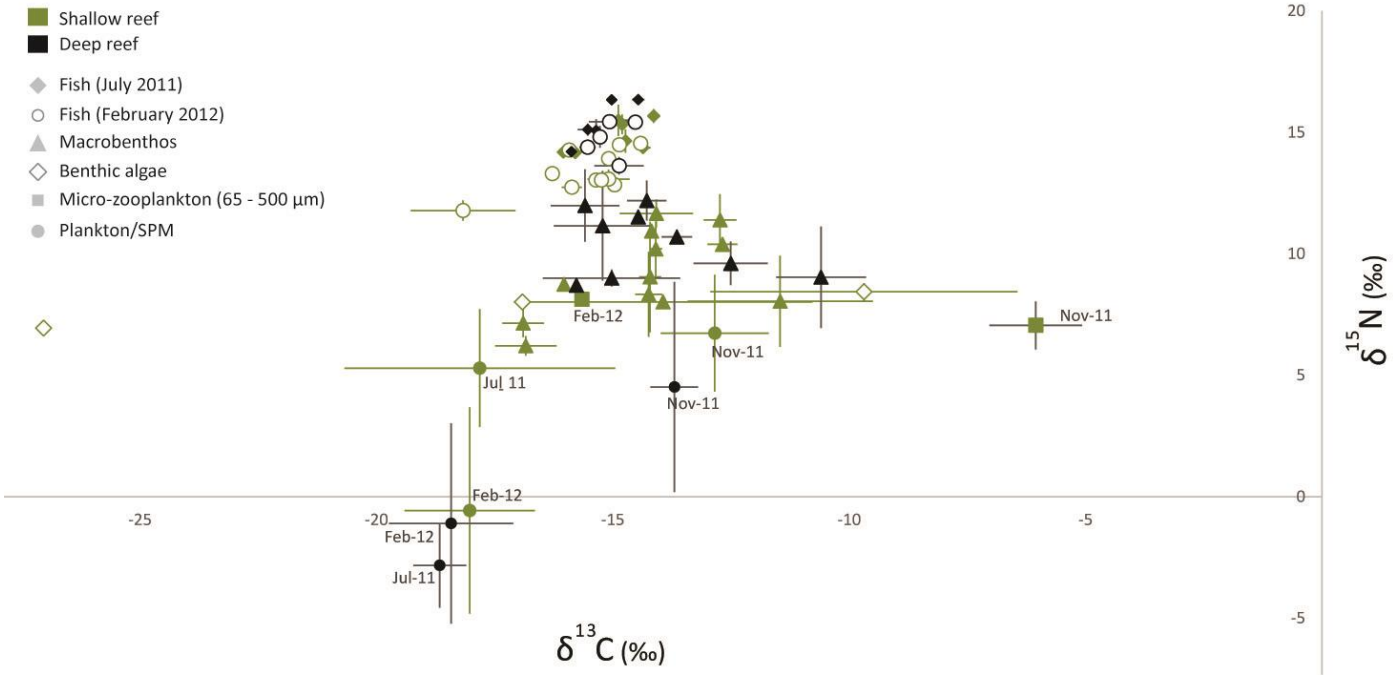


Figure 6.1. The trophic structure of the shallow (green) and deep (black) reef communities of Tsitsikamma. Trophic structure based on mean stable isotope values for organisms and food sources. Dates on the plot area represent the different sampling seasons for plankton collections. Error bars represent standard deviation.

Table 6.2. Stable isotope signatures, trophic levels and lengths (cm) of fish. A) The shallow reef community and B) the deep reef community sampled in Tsitsikamma.

	A) SHALLOW REEF					B) DEEP REEF				
	n	δ^{13}C (‰)	δ^{15}N (‰)	Trophic level	Length (cm)	*n*	δ^{13}C (‰)	δ^{15}N (‰)	Trophic level	Length (cm)
Phytoplankton/SPM										
Jul-11	4	-17.8 ± 2.9	5.3 ± 2.4	1.7 ± 0.7		3	-18.7 ± 0.6	-2.8 ± 1.7	-0.7 ± 0.5	
Nov-11	9	-12.8 ± 1.1	6.7 ± 2.4	2.2 ± 0.7		9	-13.7 ± 0.5	4.5 ± 4.3	1.3 ± 1.3	
Feb-12	9	-18 ± 1.4	-0.6 ± 4.2	0.01 ± 1.2		6	-18.4 ± 1.3	-1.1 ± 4.1	-0.1 ± 1.2	
Micro zooplankton (65 - 500 µm)										
Nov-11	2	-6.1 ± 1	7.1 ± 1	2.3 ± 0.3		-	-	-	-	
Feb-12	1	-15.7	8.1	2.6		-	-	-	-	
Algae										
Red algae	1	-27	6.9	2.2		-	-	-	-	
Coralline algae	3	-9.7 ± 3.2	8.4 ± 0.4	2.7 ± 0.1		-	-	-	-	
Macrobenthos										
Amphipods	3	-14.2 ± 0.2	9 ± 2.3	2.8 ± 0.7		3	-15 ± 1.5	9 ± 0.3	2.8 ± 0.1	
Whelks	3	-14.1 ± 0.8	11.7 ± 0.6	3.6 ± 0.2		6	-14.3 ± 0.4	12.2 ± 0.8	3.8 ± 0.2	
Crabs	1	-14.2	10.9	3.4		2	-10.6 ± 0.9	9 ± 2.1	2.8 ± 0.6	
Elephant's ear	4	-16.9 ± 0.4	7.1 ± 0.6	2.3 ± 0.2		3	-13.6 ± 0.3	10.7 ± 0.3	3.3 ± 0.1	
Isopods	3	-11.5 ± 2	8 ± 1.9	2.5 ± 0.6		5	-12.5 ± 0.8	9.6 ± 0.9	3 ± 0.3	
Sea fans	1	-16	8.7	2.7		1	-14.5	11.5	3.6	
Sponges	5	-14.2 ± 0.3	8.3 ± 1.7	2.6 ± 0.5		8	-15.6 ± 0.7	12 ± 1.5	3.7 ± 0.4	
Planar hydroid	3	-13.9 ± 3.2	8 ± 0.2	2.5 ± 0		1	-15.8	8.7	2.7	
Polychaetes	2	-14.1 ± 0.1	10.2 ± 1.1	3.2 ± 0.3		2	-15.2 ± 1	11.2 ± 2.3	3.5 ± 0.7	
Reticulated sfish	3	-12.7 ± 0.3	11.4 ± 1.1	3.5 ± 0.3		-	-	-	-	
Sea hare	3	-16.8 ± 0.6	6.2 ± 0.4	2 ± 0.1		-	-	-	-	
Cape urchin	3	-12.7 ± 0.3	10.4 ± 0.1	3.2 ± 0		-	-	-	-	
Ruby lampshell	-	-	-	-		1	-11.5	7.2	2.3	
Fish (July 2011)										
Blue hottentot	1	-15.8	14.2	4.3	25.9	1	-15.9	14.2	4.4	28.1
Carpenter	-	-	-	-	-	3	-15.5 ± 0.2	15.1 ± 0.2	4.6 ± 0.1	26.3 ± 2.9
Dageraad	3	-14.9 ± 0.3	15.5 ± 0.6	4.7 ± 0.2	33.9 ± 1.5	-	-	-	-	-
Fransmadam	3	-14.7 ± 0.1	14.6 ± 0.5	4.5 ± 0.1	23.3 ± 1.2	-	-	-	-	-
Hottentot	-	-	-	-	-	2	-15.4 ± 0.1	15.1 ± 0.5	4.6 ± 0.1	34.7 ± 1.6
Houndshark	2	-14.4 ± 0.2	14.4 ± 0.2	4.4 ± 0	68.8 ± 3.2	-	-	-	-	-
Panga	2	-14.8 ± 0.1	15.3 ± 0.4	4.7 ± 0.1	22.7 ± 7.6	-	-	-	-	-
Roman	3	-14.1 ± 0.1	15.7 ± 0.3	4.8 ± 0.1	35.4 ± 1.1	3	-14.5 ± 0.1	16.3 ± 0.2	5 ± 0.1	34.7 ± 8.1
Red Steenbras	1	-16	14.2	4.3	33.6	1	-15	16.3	5	42.8
Steentjie	3	-15.1 ± 0	13.9 ± 0	4.3 ± 0	17.1 ± 0.6	-	-	-	-	-
Fish (Feb 2012)										
Blue hottentot	3	-16.3 ± 0.1	13.3 ± 0.1	4.1 ± 0	23.1 ± 2.5	-	-	-	-	-
Cape knifejaw	3	-15 ± 0.1	12.8 ± 0.2	4 ± 0.1	38.6 ± 1.2	-	-	-	-	-
Carpenter	2	-15.9 ± 0.1	14.3 ± 0	4.4 ± 0	23.2 ± 0.3	3	-15.5 ± 0.2	14.4 ± 0.2	4.4 ± 0.1	39.8 ± 11.3
Fransmadam	3	-15.1 ± 0.1	13.9 ± 0.3	4.3 ± 0.1	19.1 ± 2.9	-	-	-	-	-
Hottentot	-	-	-	-	-	3	-15.3 ± 0.1	14.8 ± 0.4	4.5 ± 0.1	37.6 ± 2.4
Jan Bruin	3	-15.9 ± 0.2	12.7 ± 0.2	3.9 ± 0.1	27.1 ± 4	-	-	-	-	-
Klipfish	3	-15.1 ± 0.4	13.1 ± 0.4	4 ± 0.1	14 ± 11.7	-	-	-	-	-
Panga	1	-14.9	14.5	4.4	22.4	3	-15.1 ± 0.4	15.4 ± 0.2	4.7 ± 0.1	25.9 ± 1.9
Pyjama catshark	-	-	-	-	-	3	-14.9 ± 0.5	13.6 ± 0.4	4.2 ± 0.1	55.7 ± 12
Roman	3	-14.4 ± 0.1	14.5 ± 0	4.5 ± 0	34 ± 7.1	3	-14.5 ± 0	15.4 ± 0.1	4.7 ± 0	32.5 ± 4.4
Steentjie	2	-15.4 ± 0	13 ± 0.2	4 ± 0.1	18.1 ± 7.3	-	-	-	-	-
Strepie	3	-18.2 ± 1.1	11.8 ± 0.4	3.6 ± 0.1	16.1 ± 4.2	-	-	-	-	-
Twotone fingerfin	3	-15.2 ± 0	13 ± 0.2	4 ± 0.1	39.3 ± 19.5	-	-	-	-	-

On the shallow reef, similar numbers of consumers were feeding between the 2nd and 3rd and the 3rd and 4th trophic levels, with the highest number of consumers feeding between the 4th and 5th trophic levels (Figure 6.2). In contrast, the lowest number of consumers were feeding between the

2nd to 3rd trophic levels at the deep reef, with similar numbers of consumers feeding between the 3rd and 4th trophic levels.

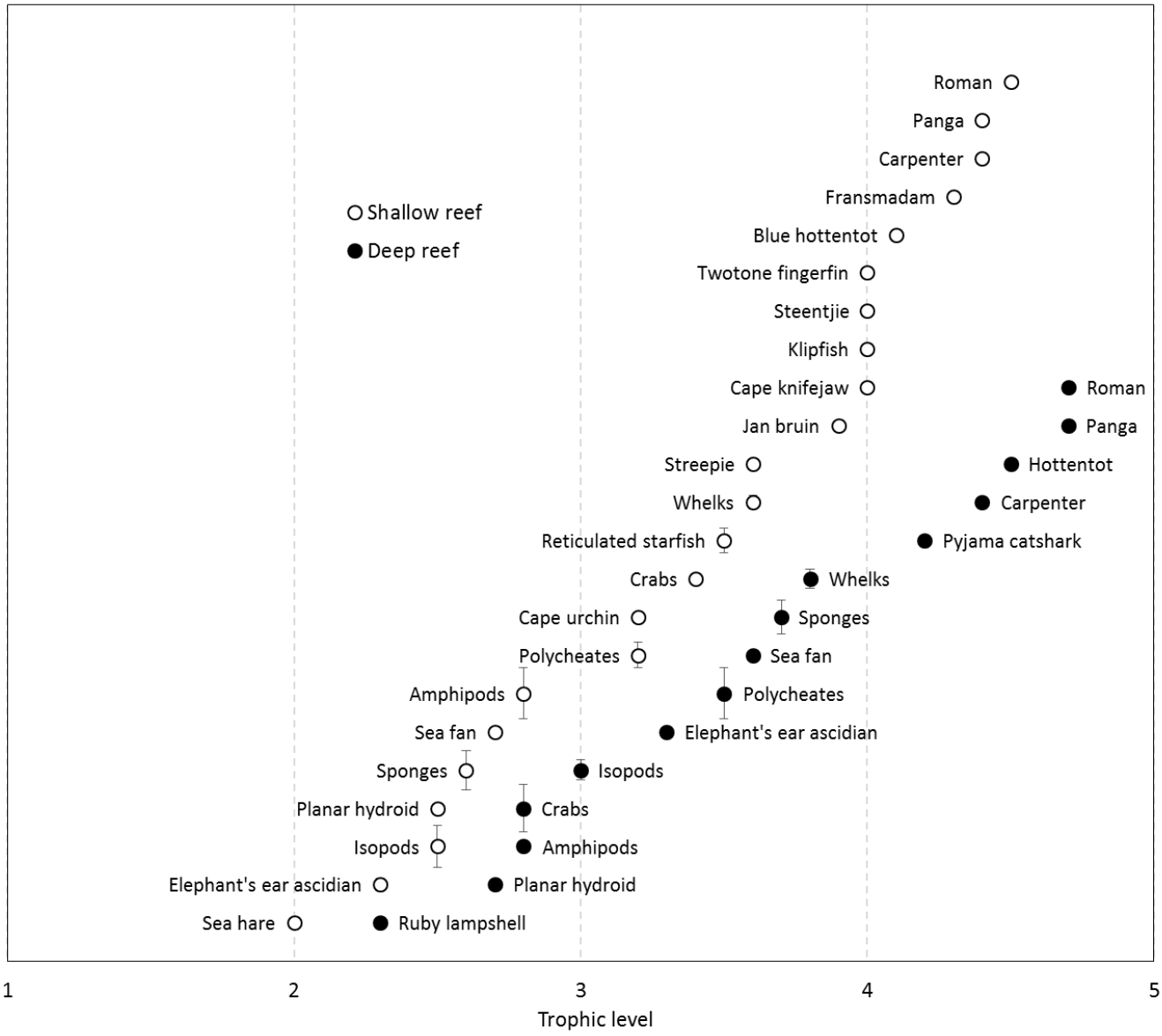


Figure 6.2. Average trophic level of consumers sampled in Tsitsikamma. The trophic level for shallow (white) and deep (black) reef consumers collected during February 2012. Species are arranged in ascending order of their trophic level values. Error bars represent standard deviation.

The average length of the pooled fish, which included samples from both July 2011 and Feb 2012, was significantly larger on the deep reef compared to the shallow reef (Pseudo-F = 6.49, P = 0.01). Further investigation into seasonal differences indicated that fish collected during February 2012 were significantly larger on the deep reef (t = 3.482, P = 0.0009), but this pattern was not evident in the fish samples from July (t = 0.072, P = 0.946).

6.3.2 COMMUNITY BASED METRICS

The centroids calculated for the macrobenthos collected from the shallow ($\delta^{13}C$ = -14.26, $\delta^{15}N$ = 8.96) and deep reefs ($\delta^{13}C$ = -14.13, $\delta^{15}N$ = 10.748) occupied different locations in bivariate isotopic space (distance = 1.79, P = 0.002; Hotelling's T^2 =; P = 0.001; Figure 6.3). Carbon values did not differ between the reefs (Table 6.1), and the significant change in centroid position for the macrobenthos was due to the overall higher $\delta^{15}N$ (8.959 vs 10.748) on the deep reef. The core niche size (SEA_C) of the macrobenthos was larger on the shallow reef (10.62) compared the deep reef (8.52), but they did not differ significantly (p > 0.05). There was a 41% overlap between the core niche areas of the shallow and deep reef macrobenthic communities (Figure 6.3).

Similar to the macrobenthos, the centroids calculated for the fish collected from the shallow ($\delta^{13}C$ = -15.32, $\delta^{15}N$ = 13.43) and deep reefs ($\delta^{13}C$ = -15.06, $\delta^{15}N$ = 14.7) occupied different locations in bivariate space (distance = 1.28, P = 0.002; Hotelling's T^2 = 32.39; P < 0.0001; Figure 6.3). The direction of change in the centroid position was mostly due to higher $\delta^{15}N$ in fish from the deep reef, and a slight increase in $\delta^{13}C$ with depth (Figure 6.3). The core niche sizes (SEA_C) were similar (p > 0.05) for fish from both the shallow (1.19) and deep (0.96) reefs, and demonstrated a 6% overlap.

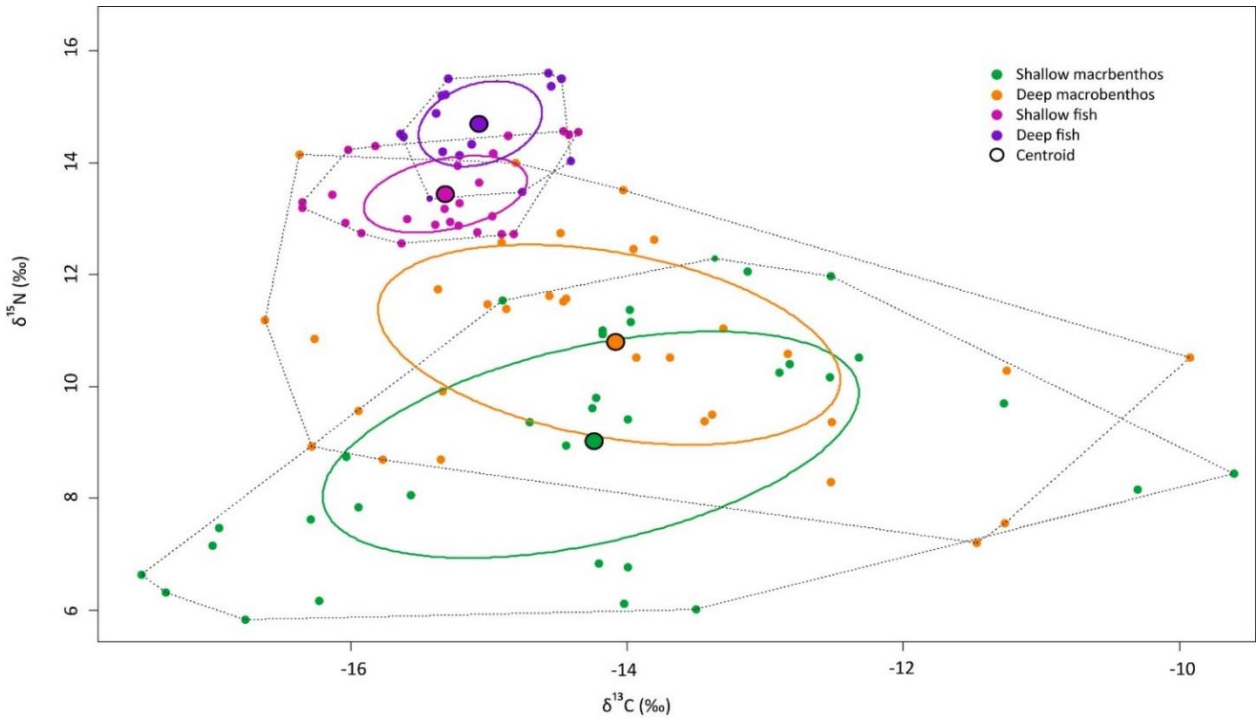


Figure 6.3. A $\delta^{13}C$ and $\delta^{15}N$ biplot of the fish and macrobenthic communities from the shallow and deep reefs in Tsitsikamma. The thin dotted lines indicate the convex hulls of total niche width (Layman et al. 2007). Solid lines indicate the sample size-corrected standard ellipse areas (SEA_C), which include a core niche of about 40% (Jackson et al. 2011). Dots (see legend) represent samples of consumers collected during February 2012, with the centroid of each group, denoted by a larger filled circle with the group's respective colour.

The shallow and deep reef communities did not differ in terms of diversity (CD: mean distance to centroid; Figure 6.3; Table 6.3 A). Differences in trophic structure between the reef communities were due to slightly longer food chain length (FCL) and $\delta^{15}N$ range on the deep reef, greater $\delta^{13}C$ range on the shallow reef, and lower levels of both NND (mean distance to nearest neighbour) and SDNND (standard deviation of nearest neighbour distance) on the shallow reef, suggesting closer packing of species in isotopic niches space thus greater redundancy on the shallow reef (Table 6.3 A). When considering the benthos and fish in isolation, it was evident that the fish contributed most to the differences in the metrics between the shallow and deep reefs, with lower average trophic diversity and $\delta^{15}N$ and $\delta^{13}C$ ranges occurring in the deep reef fish community (Table 6.3 C).

Table 6.3. Bayesian estimates of Layman's metrics and food chain length (FCL) for macrobenthos and fish collected during February 2012. Comparisons between the shallow and deep reef communities in Tsitsikamma of Layman's metrics for A) the entire community, B) the macrobenthos and C) the fish community. CD = mean centroid distance; NND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

	$\delta^{15}N$ range	FCL	$\delta^{13}C$ range	CD	NND	SDNND
A) Community						
Shallow reef	10.08	4.4	8.16	2.92	0.97	0.85
Deep reef	10.15	4.6	7.93	2.88	1.15	1.06
B) Macrobenthos						
Shallow reef	6.94		6.99	2.46	1.18	0.89
Deep reef	6.66		6.27	2.41	1.4	0.92
C) Fish						
Shallow reef	4.15		4.82	1.44	0.88	0.83
Deep reef	3.09		2.6	1.24	0.96	0.74

Higher $^{\delta}CN$ values were evident on the deep reef in both macrobenthos and fish (during both sampling seasons), indicating the greater contribution of pelagic resource use on the deep reef compared to the shallow reef community (Table 6.4). This pattern was even more distinct for fish collected during July 2011 (February 2012: Pseudo-*F* = 11.664, *P* = 0.002; July 2011: Pseudo-*F* = 19.637, *P* = 0.0002).

Table 6.4. Univariate PERMANOVA based on Euclidean distances of $^{\delta}$CN for consumers of the shallow and deep reefs in Tsitsikamma. Averages and standard deviations of $^{\delta}$CN indicated. Values in bold represent a significant effect; MS = mean square; Pseudo-*F* = F-ratios; *P* (perm) = probability level based on permutations.

		$^{\delta}$CN			Average	
PERMANOVA		MS	Pseudo - *F*	*P* (perm)	Shallow	Deep
a) Macrobenthos	Reef	45.230	7.638	**0.0074**	23.2 ± 2	24.9 ± 2.8
b) Fish	Reef	14.460	26.138	**0.0001**	29.2 ± 0.9	30.1 ± 0.8
	Season	11.384	20.577	**0.0001**	29.2 ± 0.9	30 ± 0.8
	Reef & season	0.062	0.112	0.7369		

6.4 DISCUSSION

Community wide metrics of consumers were calculated to determine if the trophic organisation of the shallow and deep reefs in Tsitsikamma differed. Although the reefs were both characterised by similar average trophic diversity (CD), the shallow reef community was characterised by a greater diversity of carbon sources and higher functional redundancy, whereas the deep reef community demonstrated a longer FCL and overall higher trophic levels for consumers. Furthermore, the greater $^{\delta}$CN values on the deep reef suggested that these consumers relied more on oceanic-derived resources compared to the shallow reef community, which relied more on reef-derived resources.

Values of SPM from both the shallow and deep reefs were similar to those from studies conducted in temperate south-eastern Australia (Davenport & Bax 2002), the warm-temperate south coast of South Africa (Hill et al. 2006, Richoux et al. 2014) and the Mediterranean (Rau et al. 1990), although δ^{13}C values of SPM collected during November 2011 were much higher (12.8 ± 1.1‰) compared to February 2012 (18 ± 1.4‰) and July 2011 (17.7 ± 2.9‰). Similarly high values of δ^{13}C in SPM were recorded by Hill et al. (2006) at Plettenberg Bay, a location about 60 km west of Tsitsikamma.

The very low values of δ^{15}N in SPM during July 2011 and February 2012 might be explained by the stable water column during these sampling trips (Figure 5.1). The absence of vertical mixing to replenish nitrates would cause plankton to rely on recycled nitrogen in the form of ammonia, which is a more ^{15}N-depleted source of nitrogen (Polunin et al. 2001). In contrast to the consumers (which demonstrated a general increase in δ^{15}N from the shallow to the deep reefs), SPM samples were more depleted in ^{15}N on the deep reef compared to the shallow reef. The pattern of relatively ^{15}N-

depleted SPM on the deep reef could have been a temporal mismatch between SPM and consumers. The SI of the primary consumers of SPM (suspension-feeders) represents an integrated view of their dietary components over several weeks. These ratios indicated that their diet (SPM) was more enriched in ^{15}N on the deep reef in their recent feeding history compared to the SPM signatures measured during July and November 2011. Unfortunately, no micro-zooplankton SI samples from the deep reef were available, and as a result, it could not be established whether the zooplankton community demonstrated a similar patter to the SPM or consumers.

Zooplankton samples from the shallow reef demonstrated an increase in both $\delta^{13}C$ and $\delta^{15}N$ values compared to the smaller SPM component collected at the same time. This pattern was consistent with that of Rau et al. (1990), who found an increase of both elements with increasing size class. One possible explanation of the ^{13}C-enrichment in the study by Rau et al. (1990) was that the smaller size particles were dominated by terrestrial signals (detritus), and as size classes increased the isotopic signatures became more similar to those of pelagic plankton. In Tsitsikamma, terrestrial input should be important, especially as the shallow reef is in close proximity to the Storms River Mouth. This idea was further supported by the significantly greater levels of FAs derived from terrestrial origin in the tissues of both macrobenthos and fish in collected from the shallow reef in Tsitsikamma (Chapter 5).

The general increase of $\delta^{15}N$ in consumer tissues with increased depth is a commonly reported phenomenon (Rau et al. 1989, Polunin et al. 2001, Mintenbeck et al. 2007, Williams & Grottoli 2010, Colaço et al. 2013). An increase in $\delta^{15}N$ with depth is often explained either by deeper consumers feeding at higher trophic levels, or by higher $\delta^{15}N$ at the base of the food web (SPM) related to microbial degradation during transit to depth (Polunin et al. 2001, Mintenbeck et al. 2007). The latter is often used to explain a constant increase in $\delta^{15}N$ with increasing depth in bathyal studies, where primary producers are absent and thus not necessarily related to trophic level. Here, the overall higher trophic levels and increased $\delta^{15}N$ on the deep reef could be explained by a combination of these processes. The first, the diagenesis (bacterial degradation) hypothesis, was only partially confirmed by the FA data as a general trend of increasing levels of BAFAs in the tissues of deep reef consumers was observed (Chapter 5), but not significantly so. However, the isotopic data from the SPM did not support an overall higher $\delta^{15}N$ of basal sources on the deep reef. The primary consumers, which are indicators of SPM dynamics integrated over time, demonstrated higher $\delta^{15}N$ values on the deep reef compared to shallow reef. This pattern was particularly evident in the elephant's ear ascidian and the sponges.

Similar to Post et al. (2000), Vander Zanden & Fetzer (2007) and Takimoto & Post (2013), the length of the food chain in Tsitsikamma was not associated with productivity. This finding was inferred from the higher light intensities and thus increased productivity on the shallow reef community in Tsitsikamma, which demonstrated a shorter FCL compared to the deep reef. An increase in FCL can be due to the addition of a predator at the top of the food chain (Post et al. 2000). Alternatively, if the same species are found at the top of the food chain in two communities, a longer FCL can be explained by a combination of increased trophic diversity between the bottom and the top of the food chain or by decreased omnivory at any level of the food chain (Post et al. 2000). Trophic diversity, as measured by centroid distance (CD; Table 6.3), was similar on both the shallow and deep reefs. However, if the diagenesis hypothesis is at least partly true, then the additional trophic level added by bacterial degradation of SPM would increase the FCL and increase the $\delta^{15}N$ at the base of the food web. This possibility was supported by the overall higher trophic positions of primary consumers of SPM, the suspension-feeders, on the deep reef. Decreased omnivory on the deep reef could be explained by the loss of benthic algae and algal grazers, as confirmed by the macrobenthic and fish surveys described in Chapters 3 and 4, and the importance of 20:4n-6, identified as a marker of benthic primary productivity, in the primary consumer tissues on the shallow reef (Chapter 5). Wyatt et al. (2012) conducted research on a fringing coral reef in Western Australia, and ascribed the increase in $\delta^{15}N$ in fish tissues with depth to changes in resource use. Fish collected from shallow waters were supported by benthic or reef productivity, whereas fish collected from deeper sites were supported by oceanic resources (Wyatt et al. 2012). Here, the significantly higher $^{\delta}CN$ values (lower $^{\delta}CN$ values associated with benthic productivity) on the deep reef in both macrobenthos and fish supported the explanation of differences in $\delta^{15}N$ arising from changes in resource use. Shallow reef consumers relied more on benthic production, and deep reef consumers on pelagic production. Furthermore, an overall trend of offshore depletion in ^{13}C in the SPM samples, similar to that observed by Hill et al. (2006) near the study site, was also evident here, especially during November 2011. Hill et al. (2006) explained the offshore depletion in carbon by the input of more enriched benthic algae (coralline algae averaged -9.7 ± 3.2‰ from the shallow reef; Table 6.2) from the intertidal to more depleted offshore organic carbon from a pelagic origin. On average, the change from nearshore to pelagic carbon occurred between 500 m to 1 km offshore (Hill et al. 2006), similar to the distance of the deep study site in Tsitsikamma to the shore. The larger $\delta^{13}C$ range on the shallow reef indicated greater diversity of carbon sources for shallow reef consumers, which provides further supportive evidence for these explanations.

Lastly, the results generally indicated that the increase in $\delta^{15}N$ with depth was due to overall higher $\delta^{15}N$ values in basal sources on the deep reef. Although this was certainly true for primary

consumers, the significantly larger size of fish measured on the deep reef, both here and in Chapter 4, and the generally accepted idea that trophic level increases with size in aquatic ecosystems (Jennings et al. 2008), suggest that fish might feed at higher trophic levels on the deep reef.

Higher trophic redundancy, as inferred from decreased NND and SDNND on the shallow reef, was expected due to the greater species and functional richness on the shallow reef and the strong correlation between species richness and functional diversity (Micheli & Halpern 2005). However, diversity measures do not always translate into the realised functional role or niche of a species (Layman et al. 2007). As such, the information gained from the SI analysis provided further evidence in support of closer packing of species with similar roles in isotopic niche space on the shallow reef compared to the deep reef.

6.5 CONCLUSION

The results from this chapter provided valuable information on the importance of deep nearshore reefs as priority sites for conservation and fisheries management strategies. The lower trophic redundancy identified in the deep reef community indicated that this community is less resilient to change and disturbance. Consequently the deep reef community can easily be altered by the removal of just a few species, which may result in undesirable changes in trophic organisation and ultimately in regime shifts. Furthermore, the greater diversity of carbon sources in addition to the increase trophic redundancy of shallow reef consumers may suggest that in the face of environmental uncertainty, the shallow reef will be more resilient to variations in primary production due to climate change.

7 SYNTHESIS AND RECOMMENDATIONS

7.1 SYNOPSIS

7.1.1 THESIS RATIONALE

The purpose of this thesis was to determine if deep nearshore reef ecosystems differed in structure and function when compared to the relatively well-studied shallow reefs that lie within SCUBA diving depths. I provided, for the first time in South Africa, baseline information on both shallow and deep nearshore reefs within a large and well-established marine protected area (MPA). With a clear understanding of how and why shallow and deep subtidal reefs differ, we can establish the role that deep nearshore reefs play in sustaining marine resource delivery. Additionally, in the face of global change, a strong grasp of the processes that support our subtidal reefs will allow for identification of threats that might compromise the ecosystem services that subtidal reefs provide. This information can then be used further to determine if deep nearshore reefs should be included in future MPA planning.

Subtidal research is logistically difficult and expensive. Consequently, what we understand about reef communities is usually based on knowledge gained from research conducted within SCUBA diving depths. Hence, the general lack of information on deep nearshore reefs exists because it becomes progressively more difficult and expensive to conduct research in deeper regions. However, with the growing demand on food resources and the rising popularity of consumption of marine derived resources (associated with favourable fatty acids and the consequent health benefits; Arts et al. 2001), shallow water fish stocks have been depleted. As a result, fisheries are increasingly targeting deeper reefs to keep up with consumer demands. However, very little is known about the deep nearshore reefs, and damage caused by harvesting these reefs may result in detrimental, and possibly irreversible, ecosystem degradation. This process, in turn, ultimately threatens the well-being of humans and increases the need for research on deep nearshore reefs.

7.1.2 APPROACH

With advances in technology the difficulties in obtaining data from deeper reefs are now surmountable. Here, the application of underwater video techniques allowed for the study of reef macrobenthos and fish communities below SCUBA diving depths. The results from these video techniques provided valuable information not only on the diversity of both fish and macrobenthos, but also on unknown macrobenthic species and depth extensions of fish species. Furthermore, video footage allows researchers to better understand how species interact with each other and their environment. This valuable information affords the researcher more intuitive insight when explaining patterns observed in the data. Moreover, the molecular information obtained from fatty acid and stable isotope biomarker techniques brings an additional angle towards understanding the processes involved in shaping and supporting communities.

To record baseline information, data should ideally be collected in the centre of a well-established MPA. Furthermore, deep and shallow reefs should be situated in close proximity to each other to exclude confounding factors. It is for these reasons that data were collected from the well-established Tsitsikamma National Park MPA. Due to the sharp topographical incline associated with this region of the South African coastline, deep nearshore reefs are still well within the borders of this MPA.

7.2 KEY FINDINGS

Differences between the shallow and deep reefs were anticipated, but the magnitude with which the reefs differed was not expected. Apart from the fatty acid data on fish, all other study components indicated some level of dissimilarity between the shallow and deep reefs. At the bottom of the food web, although seasonal differences in the plankton community resulted in variable supplies of different fatty acids, there was evidence that at times the plankton community differed significantly between the reefs. The macrobenthic community demonstrated some major changes in community structure with increasing depth. The changeover of species on the depth gradient resulted in classification of four habitat types, each of which fell within a set depth range (Figure 7.1). On the shallowest habitat (habitat A; Figure 7.1), high light intensities supported diverse benthic algal growth, which was gradually lost with increasing depths due to lower light conditions. The presence of benthic algae had several implications for the structure and function of the shallow reef community. Firstly, the upright growth of benthic algae increased structural diversity. The increased structural diversity provided habitat and food for lower level consumers, which in turn increased food quality and quantity to higher level consumers. Benthic algae were

also a direct source of food for grazers, which was confirmed by fatty acid profiles of urchins, sea hares and strepie, a commonly occurring herbivorous fish (Chapter 5). The importance of benthic algae on the shallow reef was further confirmed by greater diversity of food sources on the shallow reef, and more benthic resource use by shallow reef consumers (Chapter 6). Furthermore, benthic algae were the main source of one of the essential fatty acids, arachidonic acid (ARA: 20:4n-6), which was present in much lower levels in the tissues of deep reef consumers (Chapter 5). Additionally, terrestrial input was an important food subsidy on the shallow reef for both macrobenthos and fish species. In contrast, the deep reef had lower species and functional diversity, and resource use was more pelagic in origin (Figure 7.1).

The identified habitat types represented the prevailing environmental conditions and the macrobenthic cover on the reefs (Figure 7.1). On the shallowest high-energy sites, intense water movement prevented small particles from settling and upright growing species from thriving, and higher light intensities permitted the faster growing algae species to dominate. Moving deeper, light intensities decreased, algae were lost and upright growth prevailed due to high abundances of particulates which settled on the surfaces of encrusting species, thereby clogging their feeding mechanisms. The resources provided by the different habitat types attracted particular fish assemblages. Data from the baited remote underwater stereo-video systems (stereo-BRUVs; Chapter 4) indicated that smaller and juvenile fish were mostly associated with the shallow reef, especially habitat A. Evidently, lowest average trophic levels were recorded in habitat A, but this habitat was characterised by the highest fish abundances and the greatest diversity and numbers of rare and unique fish species. It was therefore not surprising that the shallow reef community demonstrated closer species packing, and was therefore more resilient to disturbance.

On the deep reef, the fishes associated with habitat C were the same two species (red stumpnose and red steenbras) that obtained the largest maximum sizes. This habitat was characterised by calcified benthos such as bryozoans (false corals) and hydrozoans (noble coral), which resulted in great topographical complexity. Finally, the largest habitat, habitat D, which spanned almost a 20 m depth range, had only a quarter of the reef covered by macrobenthic species. Both panga and carpenter demonstrated particular association with this habitat. Overall, the deep reef was characterised by lower fish diversity and abundances, but most fish were large, sexually mature individuals which fed at higher trophic levels compared with the shallow reef. The loss of benthic algae, and the increased dependence on pelagic sources, led the deep reef community to feed at higher trophic levels, although other processes such as bacterial degradation of plankton during transit to depth could not be excluded.

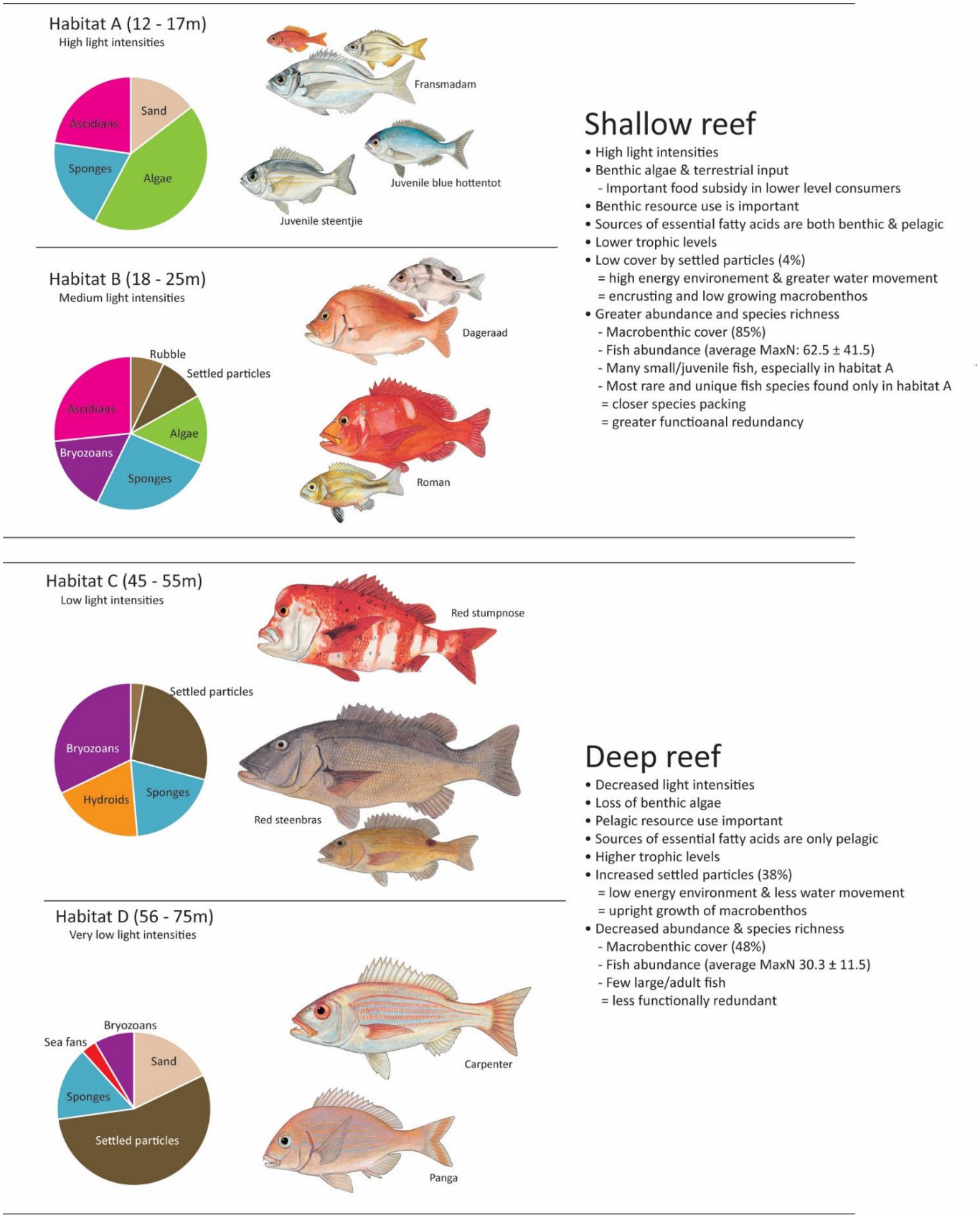


Figure 7.1. Simplified comparison of the shallow and deep reef communities of Tsitsikamma.

7.3 CRITICAL EVALUATION

Ideally, data should be collected from more than one study area within an ecoregion to ensure that the patterns identified in one region can be generalised across larger areas. This increase in spatial scale could have been achieved in this study if fewer components had been addressed. However, considering that this study in the Tsitsikamma was the first of its kind in South Africa, focus was directed towards obtaining a comprehensive understanding of how the deep nearshore reefs differed from the shallow reefs in this particular region, rather than a less detailed image of differences at a larger geographic scale. Lessons learned here can serve as a starting platform from which researchers can plan further research that focusses on those specific aspects that best described the patterns and processes of shallow versus deep nearshore reefs.

Comparative data should be collected using standardised methods to ensure that the results are representative of the specific questions asked, and not altered by some bias associated with the implementation of different techniques. For this study in Tsitsikamma, standardised sampling was implemented for the physico-chemical surveys, the plankton community surveys (niskin water samplers and plankton pumps), the fish abundance surveys (stereo-BRUVs), and to a lesser extent, the fish tissue sampling. The majority of fish samples intended for use in biomarker analyses was obtained through angling. However, some additional fish samples were collected on the shallow reef by spearing, so one could question whether the different samples collected from the deep reef are ecologically comparable. However, according to the data collected by stereo-BRUVs, the fishes collected for biomarker analyses from the deep reef were suitably representative of the deep reef fish assemblage.

Two sampling components that were not standardised across the reefs were the collection of photoquadrats and macrobenthic tissue samples. The shallow reef photoquadrats were obtained by SCUBA divers using a tripod with a downward facing camera. The deep reef photoquadrats were collected from remotely operated vehicle (ROV) footage when the ROV camera faced straight down. On the shallow reef, the ROV was very difficult to control due to strong wave action and consequent surges, resulting in poor quality photos. In contrast, deep reef photoquadrats could not be collected by SCUBA divers due to time and decompression limits. As such, although the methods employed were the best available at the time of study, the differences could have added variability from non-ecological factors to the data sets collected.

The collection of macrobenthic tissue samples from both reefs was very opportunistic. Again, as SCUBA diving is not feasible for deep reef sample retrievals, tissue sample collections from the

shallow and deep reefs differed. The only method that would allow for standardised collections of macrobenthos samples from both reefs would be dredging. However, dredging was unsuitable due to the risk of snagging in a hard-bottom environment, and the destructive nature of sampling. This method cannot be used to collected samples from within a no-take MPA, as it negatively affects the pristine condition of such environments and jeopardises conclusions that represent baseline findings for comparison with future studies.

To improve on the shortcomings identified here, I suggest the following improvements for future research. Since the onset of this study, an additional method (jump-camera) has been introduced and tested. A jump-camera allows for standardised collections of photoquadrats over the entire depth range studied. It consists of a tripod setup with LED lights and a GoPro camera in a deep-water housing fitted at a known distance from the bottom. With the boat slowly moving along an isobath, the tripod is repeatedly lifted and lowered onto the benthos, thereby conducting photoquadrat transects with minimised bias in spatial autocorrelation of neighbouring photoquadrats. To answer questions related to trophodynamics, I suggest restricting the collection of samples to answer specific questions, and to limit the species collected to those that can reliably be collected from two or more comparative sites. Species that are easily collected would allow for increased replication, which will make conclusions drawn from the analyses more robust. For instance, questions pertaining to the variability in sources of carbon and the essential fatty acids can be answered by collecting upright growing species such as the nippled sea fan, *Eunicella papillosa*. By selecting a filter feeding animal, questions regarding the variability in the supply of certain sources of plankton can be answered, and hence provide an indication of the bottom up processes that support different reef components. Furthermore, nippled sea fans are found in high numbers on both reefs in Tsitsikamma, and due to its upright growth, this species is an easy target for collection with the ROV manipulator arm. Considering the fish communities, I suggest targeting a common reef resident found throughout the depth range studied. Here, roman (*Chrysoblephus laticeps*), an aggressive general carnivore, was an important member of the warm-temperate reef community and due to its aggressive nature, it was easily caught at all depths by logistically simple angling. Ideally, data should be collected both over space and time, allowing for a more comprehensive understanding of the processes that act on these species.

7.4 MANAGEMENT RECOMMENDATIONS & FUTURE WORK

Keeping in mind that the data were collected in one geographic location within the Agulhas Ecoregion, the results obtained here have shed substantial light onto differences in the structure

and function of shallow and deep nearshore reefs. These findings provided the first step towards establishing whether deep nearshore reefs should be included in MPA network planning.

The shallow reef was identified as more resilient owing to the greater diversity of carbon sources, additional sources of essential fatty acids and the higher number of unique and rare species (increased biodiversity). In contrast, the deep reef was less resilient to disturbance due to the presence of fewer species that perform similar functions, making the community less redundant. Furthermore, the absence of terrestrial inputs and benthic algae as additional food sources meant that deep reef consumers had fewer carbon sources at the base of the food web. A loss of benthic algae results in less arachidonic acid (ARA) available to consumers, an essential fatty acid important in many hormonal pathways. Furthermore, the fragile calcareous macrobenthos characteristic of the deep reef are sensitive to physical damage from activities such as trawling, seabed mining and anchoring, adding to the greater vulnerability of the deep reef. However, the deep reef hosted many new and undescribed macrobenthic species, and the majority of the commercially important large predatory reef fish species, the bulk of which were sexually mature. Large predatory fish are important components of reef community structure and function, as they control prey populations through top down control (Myers & Worm 2003). Removal of large predatory fish can result in trophic cascades and regime shifts (Shears & Babcock 2002). Furthermore, large sexually mature female fish have exponentially greater reproductive output when compared to smaller adult females (Berkeley et al. 2004, Birkeland & Dayton 2005). Consequently, removal of such individuals has several negative implications for recruitment and maintenance of fish populations. These impacts are of major concern, as fisheries typically target large fish (Myers & Worm 2003, Birkeland & Dayton 2005). Furthermore, the lack of significant differences of fatty acids in fish between reefs could be explained by foraging of larger piscivorous fish on the shallow reef at night (Papastamatiou et al. 2015), which implies that the shallow reefs are important for sustaining the large predatory fish. Although the shallow reef community appeared to be more resilient than the deep reef community, shallow reefs hosted most of the unique and rare species and represent nursery grounds for juvenile fish. The deep reefs are important habitats for the larger predatory species, and they host many of the sexually mature individuals of those species that demonstrated depth-related ontogenetic shifts in habitat use. The findings outlined here provide compelling evidence to support an expansion of our MPAs to include deep nearshore reefs, and continued protection of the shallow reefs.

Additional research at other sites within the Agulhas Ecoregion needs to be conducted both inside and outside MPAs. Data need to be collected in a standardised manner, as explained above, in order to allow for further identification and interpretation of the general patterns. Also, to determine the

ideal depth to which MPAs should be expanded, deeper sites must be targeted. The depth at which species turnover becomes redundant would give an indication of the depths to which MPA expansion would be most effective. In other words, the rapid changes of environmental variables within the first few meters of the water column translate into similar rapid changes in species composition. Moving deeper, environmental variables become more stable and species changeover less pronounced. This means that few additional species would be afforded protection beyond a certain depth, so further offshore MPA expansion would be unwarranted. Additionally, because light intensity is an important driver behind changeover of species along a depth gradient, sites exposed to different levels of terrestrial run-off and distance offshore should be targeted. Terrestrial run-off alters water column properties and subsequent primary production. Such changes in the water column properties could further influence the depth to which species turnover becomes redundant. By conducting these additional studies, we can establish the impacts of fishing on macrobenthic and fish community structure and function, and we can identify the depth range that would be most effective for the conservation of biodiversity and management of our fisheries resources. Finally, these additional studies would permit the determination of general depth-related patterns in nearshore reefs in various marine ecoregions.

7.5 CONCLUSION

The research in this thesis addressed its main aim to describe dissimilarities in the structure and function of shallow and deep nearshore reefs of Tsitsikamma. This research was done to determine whether deep nearshore reefs need to be included in future MPA planning.

The results from this study have provided managers with a better understanding of the ecological differences between warm-temperate shallow and deep reefs, albeit only from Tsitsikamma. To provide further recommendations for an offshore expansion of our MPA networks, similar studies need to be conducted in additional regions. Such studies should aim at identifying the depth to which the MPAs should be extended, and the impact that fishing has on the structure and function of reef communities.

It is clear from the data collected here that there is an urgent need for additional research specifically on deep nearshore reefs. Typically, South Africa's current MPA network does not include deep nearshore reefs, yet they host the majority of our large predatory and commercially important fish species, many of which demonstrate depth-related ontogenetic shifts in habitat use. These shifts result in many of the sexually mature individuals occurring almost exclusively on deep reefs, and as such are afforded no protection at present. This problem is further compounded by

the fact that the deep reefs are likely to be less redundant, and consequently less resilient to disturbance. Consequently, even the removal of just a few species could dramatically alter the functioning of deep nearshore reefs.

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APPENDIX

SPECIES ACCUMULATION CURVES

ANALYSIS

Species accumulation curves were plotted to determine the number of points required to identify 95% of the macrobenthic species present in a photoquadrat. Between five and seven photoquadrats were selected from each station to ensure that a representative population was sampled. Five, 10, 20, 40 and 80 points were randomly superimposed on each photoquadrat. The number of species obtained at each point interval was analysed employing a non-linear mixed effects model (NLME). To accurately determine the number of points required to reach 95% saturation level, a 2-parameter logistic-ogive function was fitted to the NLME model (Bernard & Götz 2012). Calculations involving the NLME model package (Pinheiro et al. 2011) were performed in R-Studio 2.15.3 (R Core Team 2013).

RESULT

The species accumulation curves were not significantly different between the reefs ($F=2.493$, $p > 0.1$). On the shallow reef, 54.34 ± 2.37 points were required to account for 95% of the species found in a photoquadrat, compared to 48.17 ± 4.0 points for the deep reef. Although fewer points were required for the deep reef, for simplicity, both shallow and deep reef photoquadrats were analysed with 54 randomly distributed points.

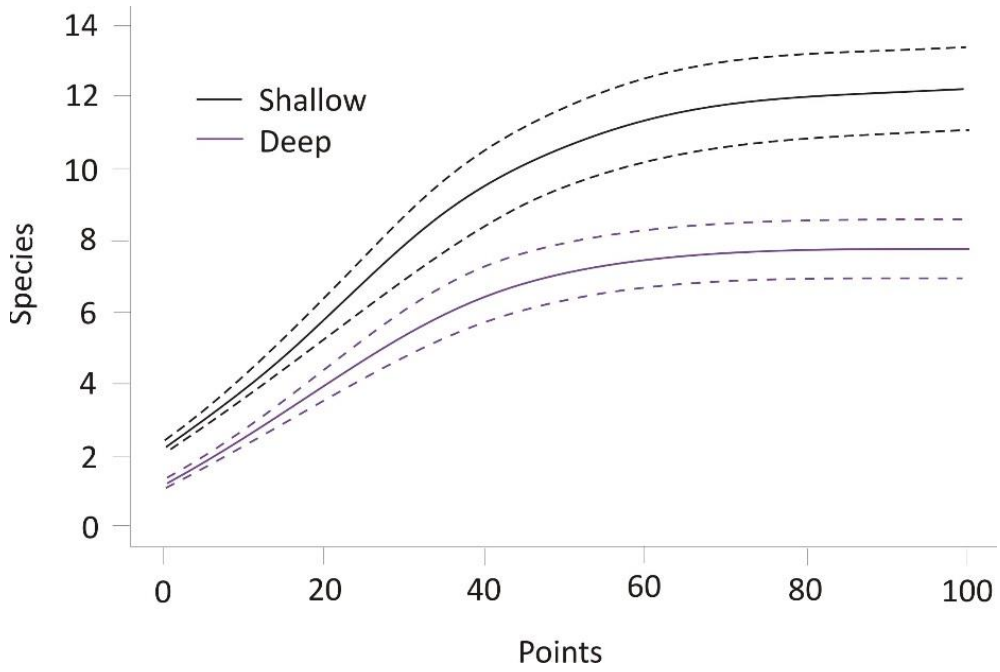

Figure A3.1. Species accumulation curves. Predicted accumulation of species from the non-linear mixed effect model for the shallow (black) and deep (purple) reefs. Dashed lines indicate 95% confidence intervals.

Table A3.1. Detailed results from the non-linear mixed effect analysis on the shallow and deep reef data comparing the mean number of points (± SE) at which species accumulation were at the 50% and 95% saturation levels. The observed number of species for the predicted number of points is provided along with the significance levels for the comparison of the number of points required to obtain saturation levels (50% & 95%) between the shallow and deep reefs.

	Shallow				Deep				Comparisons of points	
	Points		No. of Species		Points		No. of Species			
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*F-test*	*p-value*
50%	20.625	0.816	5.831	0.255	18.945	1.343	3.656	0.196	0.000	> 0.1
95%	54.393	2.367	11.126	0.505	48.163	3.965	7.109	0.396	2.493	> 0.1

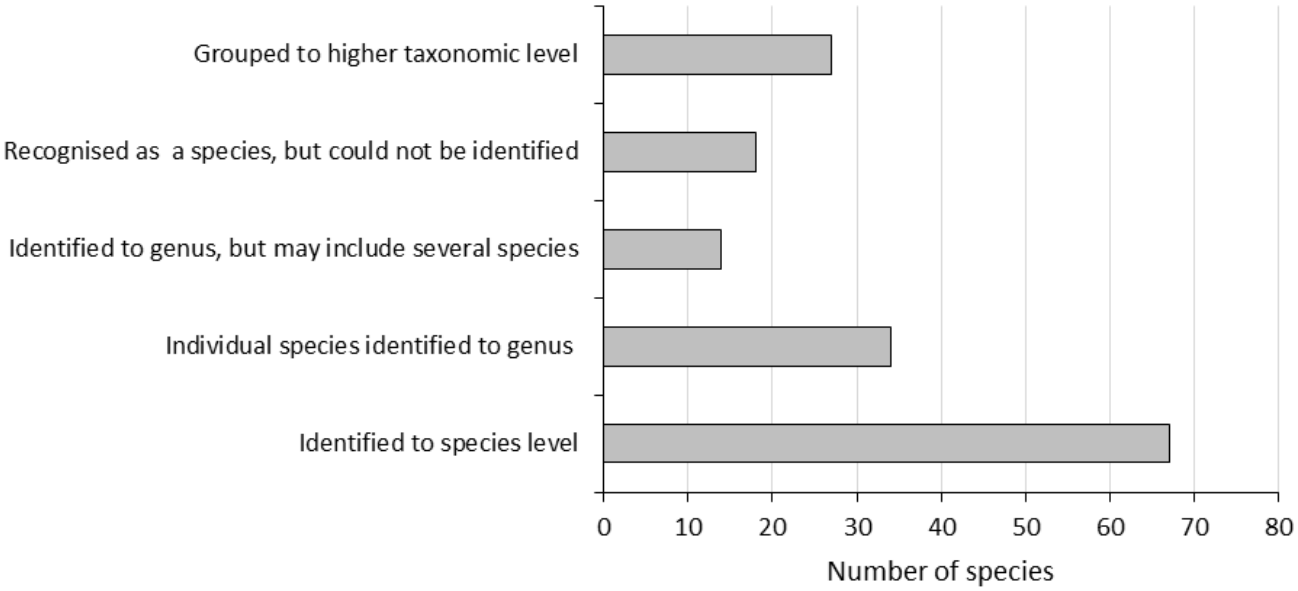

Figure A3.2. Macrobenthic species identified from photoquadrats. The contributions of the different levels of classification success during the analysis of macrobenthic assemblage data from photoquadrats.

Table A3.2. Details for the indicator species analysis that typify the shallow reef assemblage structure. Species were identified using the *IndVal* analyses, where A is the measure of specificity (the degree to which a species was found only in a given group of sites), B is a measure of fidelity (the degree to which a species was present at all sites of a group) and the *IndVal* statistic (the degree to which a species was an indicator for a group of sites [reefs]).

Taxonomic categories or species	Common name	A	B	IndVal index	p value
SHALLOW INDICATOR SPECIES					
Algae					
Hildenbrandia lecanellierii	Black encrusting algae	1.000	1.000	1.000	0.004 **
Leptophytum	Pink thin coralline crust	1.000	1.000	1.000	0.004 **
Mesophyllum	Purple thin coralline crust	1.000	1.000	1.000	0.004 **
Corallinaceae	Upright coralline algae	1.000	1.000	1.000	0.004 **
Rhodophyta		0.987	1.000	0.994	0.005 **
Porifera					
Haliclona sp. 1	Grey encrusting sponge	1.000	1.000	1.000	0.004 **
Isodictya ectofibrosa	Wall sponge	1.000	1.000	1.000	0.004 **
Tedania spp.	Oscular sponge	1.000	1.000	1.000	0.004 **
Psammoclema sp. 2	Thick yellow encrusting sponge	0.981	1.000	0.991	0.004 **
Clathria (Isociella) oudekraalensis	Red encrusting sponge	0.953	1.000	0.976	0.004 **
Purple encrusting sponge		1.000	0.833	0.913	0.021 *
Cnidaria					
Anthozoa					
Actiniaria (anemones)					
Anthothoe spp.	Square mouth anemone	0.931	1.000	0.965	0.009 **
Alcyonacea (soft corals)					
Alcyonium fauri	Purple soft coral	1.000	1.000	1.000	0.004 **
Eleutherobia variable	Variable soft coral	1.000	0.833	0.913	0.020 *
Zoanthidea (zoanthids)					
Isozoanthus capensis	Cape zoanthid	0.969	0.833	0.899	0.021 *
Hydrozoa					
Lytocarpia formosa	Rusty feather hydroid	1.000	1.000	1.000	0.004 **
Macrorhychia filamentosa	Smokey feather hydroid	1.000	1.000	1.000	0.004 **
Gattya humilis	White feather hydroid	0.955	1.000	0.977	0.005 **
Bryozoa					
Cryptopolyzoon concretum	Sand sausage	1.000	1.000	1.000	0.004 **
Membraniporela spp		1.000	0.833	0.913	0.020 *
Tennysonia stellata	Small antler false coral	1.000	0.833	0.913	0.021 *
Adeonella sp. 4	Yellow rimmed false coral	1.000	0.833	0.913	0.021 *
Chordata					
Tunicata					
Ascidiacea					
Distaplia skoogi		1.000	1.000	1.000	0.004 **
Pycnoclavella filamentosa	Feather sand ascidian	1.000	1.000	1.000	0.004 **
Polyandrocarpa anguinea	Large zooid ascidian	1.000	1.000	1.000	0.004 **
Didemnidae sp. 1	Light didemnum	1.000	1.000	1.000	0.004 **
Ascidian sp. 1	Orange glow ascidian	1.000	1.000	1.000	0.004 **
Pseudodistoma sp. 1	Red lobed ascidian	1.000	1.000	1.000	0.004 **
Polyandrocarpa sp. 1	Small zooid	1.000	1.000	1.000	0.004 **
Aplidiopsis tubiferus	Red stalked sandy ascidian	0.968	1.000	0.984	0.007 **
Didemnidae		0.968	1.000	0.984	0.004 **
Polyclinum isipingense	Sand ascidian	0.959	1.000	0.979	0.004 **
Pseudodistoma sp. 2	Gel ascidian	0.884	1.000	0.940	0.018 *
Encrusting ascidians		1.000	0.833	0.913	0.026 *
Euherdmania divida	Sandy bush ascidian	1.000	0.833	0.913	0.026 *
Aplidium flavolineatum	White ringed ascidian	1.000	0.833	0.913	0.026 *

Significance codes: *** = 0.001; ** = 0.01; * = 0.05

Table A3.3. Taxa comprising the different guilds. The major taxonomic groups that were assigned different trait combinations associated with resources acquisition.

Body plan	Feeding strategy	Growth form	Type of feeding strategy	Taxa
Colonial	Suspension Feeder	Mound	Combined passive active	Sponges
			Active	Bryozoans and ascidians
		Sheet-like mound	Combined passive active	Sponges
			Active	Ascidians
		Sheets	Combined passive active	Sponges
			Active	Bryozoans and ascidians
		Tree-like	Combined passive active	Sponges
			Active	Bryozoans
			Passive	Hydroids and sea fans
		Vines	Combined passive active	Sponges
			Active	Bryozoans and ascidians
			Passive	Soft corals, zooanthids and hydroids
Solitary	Suspension Feeder	Sedentary	Passive	Anemones, basket, brittle and feather -stars
		Sessile	Combined passive active	Lampshells
			Active	Ascidians
			Passive	Hard corals
			Facultative active	Barnacles
	Scavenger	Sedentary	Macro-benthos	Starfish
	Grazer	Sedentary	Unkown	Gastropods
	Primary producer	Sheets	Autotroph	Algae
		Tree-like	Autotroph	Algae

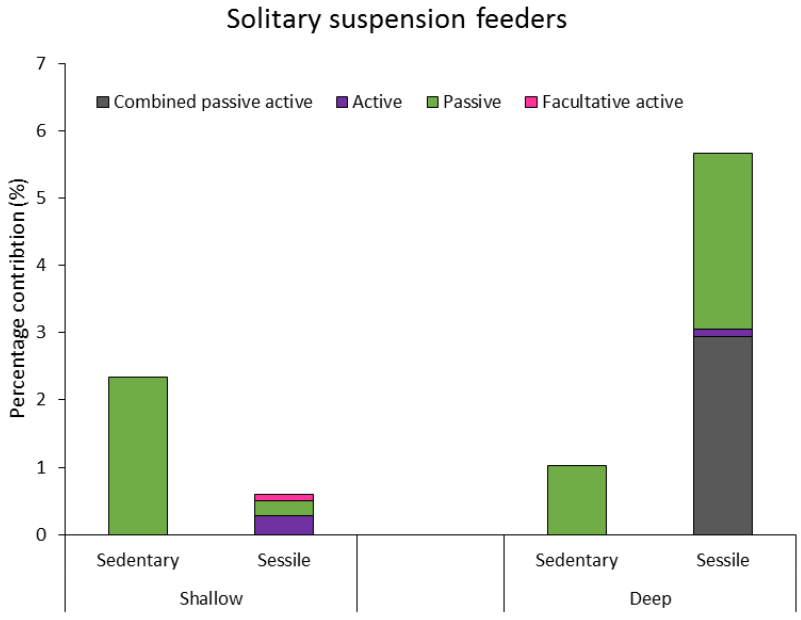

Figure A3.3. Percentage contributions of solitary suspension-feeders to the shallow and deep reef cover classified on the basis of mobility. Four feeding strategies (legend insert) employed by suspension-feeders (Wildish & Kristmanson 1997) are indicated as a percentage within each mobility category.

Table A4.1. List of the fish species recorded in Tsitsikamma with some species-specific characteristics. Species characteristics were obtained from Mann (2013) and trophic levels from FishBase (Froese & Pauly 2014).

Family	Scientific name	Common name	Fisheries importance	Length at 50% maturity (cm)	Trophic level	Max length (TL;cm)	Lifestage	Feeding guild
Ariidae	*Galeichthys feliceps*	White seacatfish	Tertiary	30.5	3.5	55	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
Carangidae	*Seriola lalandi*	Giant yellowtail	Primary	61.5	4.1	143	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
	Trachurus trachurus	Maasbanker	Tertiary	23.9	3.6	70	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
Cheilodactylidae	*Cheilodactylus fasciatus*	Redfingers	non-Target	ND	3.4	30	Adult	C.Mob.S.I
							Juvenile	ND
	Cheilodactylus pixi	Barred fingerfin	non-Target	ND	3.2	180	Adult	C.Mob.S.I
							Juvenile	ND
	Chirodactylus branchydactylus	Twotone fingerfin	Tertiary	ND	3.5	40	Adult	C.Mob.S.I
							Juvenile	ND
	Chirodactylus grandis	Bank steenbras	Secondary	ND	3.3	100	Adult	C.Mob.S.I
							Juvenile	C.Mob.S.I
Clinidae	*Clinid spp.*	Clinid spp.	non-Target	ND	ND	ND	Adult	ND
Dasyatidae	*Dasyatis brevicaudata*	Shorttail stingray	Tertiary	ND	3.9	430	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
Dussumieriidae	*Etrumeus whiteheadi*	Whitehead's herring	Primary	ND	3.4	22	Adult	O.Plank
							Juvenile	ND
Haemulidae	*Pomadasys olivaceum*	Piggy	Tertiary	15	2.6	55	Adult	C.Mob.S.I
							Juvenile	C.Mob.S.I
Hexanchidae	*Notorynchus cepedianus*	Spotted sevengill cowshark	Tertiary	175	4.6	300	Adult	C.Mam.Pisc
							Juvenile	C.Mob.S.I
Myliobatidae	*Myliobatis aqulia*	Eagleray	Tertiary	37	3.6	147	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.L.I
Oplegnathidae	*Oplegnathus conwayi*	Cape knifejaw	Secondary	ND	2.7	90	Adult	O.Ses.Mac
							Juvenile	ND
Parascorpididae	*Parascorpis typus*	Jutjaw	non-Target	ND	3.2	60	Adult	O.Plank
							Juvenile	ND
Rajidae	*Rostroraja alba*	White skate	Tertiary	ND	4.4	230	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
Sciaenidae	*Atractoscion aequidens*	Geelbek	Primary	90	3.9	130	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.S.I
Scyliorhinidae	*Halaelurus natalensis*	Tiger catshark	By-catch	ND	4.2	45	Adult	C.Mob.L.I
							Juvenile	ND
	Haploblepharus edwardsii	Puffadder shyshark	By-catch	ND	3.8	59	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
	Haploblepharus pictus	Dark shyshark	By-catch	ND	4.2	57	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
	Poroderma africanum	Striped catshark	By-catch	86.5	3.6	100	Adult	C.Mob.L.I
							Juvenile	C.Mob.L.I
	Poroderma pantherinum	Leopard catshark	By-catch	64	4.1	84	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
Serranidae	*Acanthistius sebastoides*	Koester	non-Target	ND	4	35	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
	Epinephelus marginatus	Yellowbelly rockcod	Primary	71.1	3.7	112.5	Adult	C.Mob.L.I
							Juvenile	C.Mob.L.I
	Serranus cabrilla	Comber	non-Target	17.5	3.4	40	Adult	C.Mob.L.I.Pisc
							Juvenile	ND

O.Plank: Feeds on zooplankton; O.Mob.S.I: Feeds on algae & small mobile invertebrates; O.Mob.L.I: Feeds on algae & large mobile invertebrates; O.Ses.Mac: Feeds on algae & sessile colonial animals; C.Mob.S.I: Feeds on small mobile invertebrates; C.Mob.L.I: Feeds on large mobile invertebrates; C.Ses.Mac: Feeds on colonial macrobenthic species; C.Mob.L.I.Pisc: Feeds on small fish & cephalopods; ND: no data

Table A4.1. Continued.

Family	*Scientific name*	Common name	Fisheries importance	Length at 50% maturity (cm)	Trophic level	Max size size (TL;cm)	Lifestage	Feeding guild
Sparidae	*Argyrozona argyrozona*	Carpenter	Primary	29.5	3.5	80	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.S.I
	Boopsoidea inornata	Fransmadam	Secondary	14	3.3	30	Adult	O.Mob.S.I
							Juvenile	ND
	Cheimerius nufar	Santer	Primary	34	3.5	75	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.L.I
	Chrysoblephus cristiceps	Dageraad	Primary	35.5	3.7	70	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Chrysoblephus gibbiceps	Red stumpnose	Primary	23.05	3.7	75	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Chrysoblephus laticeps	Roman	Primary	18	3.8	51	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Cymatoceps nasutus	Black musselcracker	Primary	53	3.6	109	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Diplodus capensis	Blacktail	Secondary	21.1	2.7	40.3	Adult	O.Mob.L.I
							Juvenile	C.Mob.S.I
	Diplodus hottentotus	Zebra	Secondary	28	3.6	60	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Gymnocrotaphus curvidens	Janbruin	Secondary	ND	3.4	50	Adult	O.Ses.Mac
							Juvenile	O.Ses.Mac
	Lithognathus mormyrus	Sand steenbras	Tertiary	20.4	3.4	37.2	Adult	C.Mob.L.I
							Juvenile	C.Mob.S.I
	Pachymetopon aeneum	Blue hottentot	Primary	22.5	3.2	60	Adult	C.Ses.Mac
							Juvenile	C.Ses.Mac
	Pachymetopon blochii	Hottentot	Primary	22	3.4	54	Adult	O.Mob.L.I.
							Juvenile	O.Mob.S.I
	Pagellus bellottii natalensis	Red tjor-tjor	Tertiary	ND	3.3	35	Adult	O.Mob.L.I.
							Juvenile	ND
	Petrus rupestris	Red steenbras	Primary	63	4.5	200	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.L.I
	Porcostoma dentata	Dane	Tertiary	15	3.3	42	Adult	C.Mob.L.I
							Juvenile	ND
	Pterogymnus lanarius	Panga	Secondary	20.4	3.7	45	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.S.I
	Rhabdosargus globiceps	White stumpnose	Primary	19.4	2.9	47.2	Adult	C.Mob.L.I
							Juvenile	O.Mob.S.I
	Rhabdosargus holubi	Cape stumpnose	Tertiary	19	2.6	45	Adult	C.Mob.L.I
							Juvenile	Herb
	Sarpa salpa	Strepie	Tertiary	14.5	2	30	Adult	Herb
							Juvenile	Herb
	Spondyliosoma emarginatum	Steentjie	Secondary	24.2	2.8	45	Adult	O.Mob.S.I
							Juvenile	O.Plank
Tetraodontidae	*Amblyrhynchotes honckenii*	Evileye blaasop	By-catch	ND	3.3	30	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
Triakidae	*Mustelus mustelus*	Smooth-hound	By-catch	107.8	3.8	173.2	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.S.I
	Triakis megalopteris	Spotted gullyshark	By-catch	138.5	4	207	Adult	C.Mob.L.I.Pisc
							Juvenile	C.Mob.L.I
	Chelidonichthys kumu	Bluefin Gurnard	Secondary	23	3.7	60	Adult	C.Mob.L.I.Pisc
							Juvenile	ND
	Trigloporus lastoviza	Streaked gurnard	Primary	15	3.4	40	Adult	C.Mob.L.I
							Juvenile	ND

O.Plank: Feeds on zooplankton; O.Mob.S.I: Feeds on algae & small mobile invertebrates; O.Mob.L.I: Feeds on algae & large mobile invertebrates; O.Ses.Mac: Feeds on algae & sessile colonial animals; C.Mob.S.I: Feeds on small mobile invertebrates; C.Mob.L.I: Feeds on large mobile invertebrates; C.Ses.Mac: Feeds on colonial macrobenthic species; C.Mob.L.I.Pisc: Feeds on small fish & cephalopods; ND: no data.

Table A5.1. List of samples processed for fatty acid and stable isotope analyses. SPM: suspended particulate matter. Values represent sample size.

	Phylum	Common name	Species name	Sample month	Fatty Acids		Stable Isotopes	
					Shallow	Deep	Shallow	Deep
Algae	Rhodophyta	Unknown rhodophyte		Feb 2012			1	
		Upright coraline algae		Feb 2012	4		3	
Detritus		Detritus		Feb 2012		3		
Plankton/SPM		Suspended particulate matter (SPM)		July 2011			4	3
				Nov 2011			9	9
				Feb 2012			9	6
		Micro-zooplankton (65-500 µm)		Feb 2012	6	5	1	
				Nov 2011	8	8	2	
		Meso-zooplankton (>500 µm)		Feb 2012	7	5		
				Nov 2011	9	9		
Macrobenthos	Annelida	Polycheates		Feb 2012	2	3	2	2
	Arthropoda	Amphipods		Feb 2012	1	4	3	3
		Cape Long-legged spider crab	*Macropodia falcifera*	Feb 2012		2		2
		Cape rock crab	*Plaguisa chabrus*	Feb 2012	1		1	
		Isopods		Feb 2012	2	4	3	5
	Brachiopoda	Ruby lamp shell	*Kraussina rubra*	Feb 2012		1		1
	Bryozoa	Bryozoan 1		Feb 2012		1		
		Lacy false coral		Feb 2012		1		
	Chordata	Elephants ear	*Gynandrocarpa placenta*	Feb 2012	4	3	4	3
	Cnidaria	Nippled sea fan	*Eunicella papillosa*	Feb 2012	1	1	1	
		Noble coral	*Stylaster nobilis*	Feb 2012		1		
		Palmate sea fan	*Leptogorgia palma*	Feb 2012	1			
		Planar hydriod	*Sertularella arbuscula*	Feb 2012	2	1	3	1
		Sinuous sea fan	*Eunicella tricoronata*	Feb 2012	1			
		Warty sea fan	*Homophyton verrucosum*	Feb 2012		3		1
	Echinodermata	Basket star	*Astrocladus euryale*	Feb 2012	3	4		
		Brittle stars		Feb 2012	3	3		
		Cape urchin	*Parechinus angulosus*	Feb 2012	3		3	
		Reticulated sfish	*Henricia ornata*	Feb 2012	2		3	
		Starfish		Feb 2012		1		
	Mollusca	Annulated plough shell snail	*Bullia annulata*	Feb 2012		3		3
		Purple lipped dog welk	*Nassarius speciosus*	Feb 2012		3		3
		Pustular triton	*Argobuccinum pustulosum*	Feb 2012	3		3	
		Sea hare	*Aplysia parvula*	Feb 2012	3		3	
	Porifera	Orange disc-like sponge		Feb 2012				1
		Orange finger sponge		Feb 2012		1		2
		Orange fungus sponge		Feb 2012		1		1
		Orange wall sponge	*Spirastrella spinispirulifera*	Feb 2012	2		3	
		Red encrusting sponge	*Clathria odekraalensis*	Feb 2012			2	
		Spirit of Tsitsikamma		Feb 2012				1
		Sponge covered hydroid		Feb 2012		3		3
Fish	Chordata	Blue hottentot	*Pachymetopon aeneum*	July 2011		1	1	1
				Feb 2012	3		3	
		Cape knifejaw	*Oplegnathus conwayi*	Feb 2012	2		3	
		Carpenter	*Argyrozona argyrozona*	July 2011		1		3
				Feb 2012	2	3	2	3
		Dageraad	*Chrysoblephus cristiceps*	July 2011	3		3	
		Fransmadam	*Boopsoidea inornata*	July 2011			3	
				Feb 2012	3		3	
		Hottentot	*Pachymetopon blochii*	July 2011		1		2
				Feb 2012		3		3
		Houndshark	*Mustelus mustelus*	July 2011	1		2	
		Jan Bruin	*Gymnocrotaphus curvidens*	Feb 2012	3		3	
		Kingklip	*Genypterus capensis*	Feb 2012		1		1
		Klipfish	*unkown klipfish*	Feb 2012	3		3	
		Panga	*Pterogymnus laniarius*	July 2011	2		2	
				Feb 2012	1	3	1	3
		Pyjama catshark	*Poroderma africanum*	Feb 2012		3		3
		Red Roman	*Chrysoblephus laticeps*	July 2011		1	3	3
				Feb 2012	3	3	3	3
		Red Steenbras	*Petrus rupestris*	July 2011	1	1	1	1
		Steentjie	*Spondyliosoma emarginatum*	July 2011	1		3	
				Feb 2012	2		2	
		Strepie	*Sarpa salpa*	Feb 2012	3		3	
		Twotone fingerfin	*Chirodactylus brachydactylus*	Feb 2012	3		3	
Total					104	95	110	76

Table A5.2. Similarity percentage (SIMPER) results for the macrobenthic feeding guilds. Results indicate the within group similarity, and important fatty acids that typify each feeding guild. Ave = average, sim = similarity, SD = standard deviation, - too few samples to calculate sim/SD.

Feeding guild	Ave. within group sim. (%)	Fatty acid compounds	Ave. abundance	Ave sim.	Sim/SD	Contribution (%)
Colonial						
Combined passive-active suspension feeders	56.8	22:6n-3	10.93	9.64	4.83	16.98
Upright sponges		20:4n-6	7.51	4.48	1.01	7.89
		26:1n-9	7.63	4.13	1.27	7.27
Combined passive-active suspension feeders	30.1	22:0	5.43	4.39	5.21	14.6
Massive sponges		22:6n-3	6.17	3.95	1.68	13.14
		24:1n-9	4.67	2.38	0.7	7.91
Passive suspension feeders	66.2	20:4n-6	21.77	14.87	2.4	22.32
Sea fans, hydroids		16:0	13.32	11.58	2.99	17.38
		18:0	7.67	6.17	3.63	9.26
Active massive suspension feeders	82.4	16:0	16.94	15.6	-	18.93
Bryozoans		22:6n-3	15.24	13.75	-	16.68
		18:0	6.63	5.9	-	7.16
Active upright suspension feeders	70.8	20:5n-3	15.91	12.55	1.54	17.74
Ascidians		16:0	15.55	11.17	3.94	15.78
		22:6n-3	8.87	6.56	1.53	9.27
Solitary						
Benthic scavengers	64.1	16:0	12.6	10.15	2.62	15.83
Gastropods, polycheates,		20:5n-3	12.25	9	2.34	14.03
crabs, sea stars		18:0	9.89	7.92	3.1	12.34
Detrivore/suspension feeders	86.6	20:5n-3	20.83	19.48	10.12	22.5
Amphipods & isopods		16:0	16.59	15.87	10.36	18.33
		22:6n-3	11.64	9.77	4.04	11.29
Passive suspension feeders	74.8	20:5n-3	18.19	15.54	4.39	20.77
Basket & brittles stars		20:1n-9	14.44	11.21	2.15	14.99
		18:0	9.4	8.69	7.01	11.61
Algal grazers	64	16:0	13.37	12.82	19.11	20.04
Sea hares & urchins		20:5n-3	9.08	8.52	24.57	13.32
		20:4n-6	13.91	7.01	0.77	10.96
Primary producers	80.3	16:0	43.68	36.83	6.47	45.84
Upright coralline algae		20:4n-6	19.45	15.5	3.22	19.3
		20:5n-3	10.11	7.83	2.69	9.75

Table A5.3. Similarity percentage (SIMPER) results which compare important dietary indices of each feeding guild with the remaining macrobenthic community. Results indicate the average dissimilarity, and important fatty acids that typify each feeding guild. Ave = average, Diss = dissimilarity, SD = standard deviation.

Feeding guild	Ave. diss (%)	Fatty acid compounds	Ave. abundance All	Ave. abundance Feeding guild	Ave. diss	diss/SD	Contribution (%)
Colonial							
Combined passive-active suspension feeders	28.58	∑ PUFA	40.04	52.96	5.22	1.61	18.26
Upright sponges		∑ sfa	30.59	16.62	4.4	1.25	15.39
		∑ EFA	29.39	23.31	3.63	1.26	12.7
		∑ n-3	24.09	19.51	3.02	1.61	10.56
		∑ MUFA	24.64	20.07	2.91	1.23	10.19
		∑ (22:1, 20:1)	7.52	1.37	1.89	0.9	6.6
		22:6n-3/20:5n-3	0.75	3.74	0.96	1.18	3.37
		BAFA	6.74	6.61	0.83	1.06	2.9
Combined passive-active suspension feeders	37.33	∑ EFA	29.73	9.85	6.08	2.06	16.29
Massive sponges		∑ MUFA	23.9	37.57	5.3	1.16	14.2
		∑ sfa	30.36	13.81	5.06	1.51	13.56
		∑ PUFA	40.84	39.48	4.95	1.56	13.25
		∑ n-3	24.27	11.34	4.07	1.48	10.91
		∑ (22:1, 20:1)	7.3	3.39	1.7	0.93	4.57
		BAFA	6.68	8.04	1.45	1.15	3.88
		22:6n-3/20:5n-3	0.79	4.54	1.15	2.14	3.07
Passive suspension feeders	24.42	∑ EFA	27.97	36.34	3.6	1.15	14.75
Sea fans, hydroids		∑ n-3	24.74	17.56	3.48	1.43	14.23
		∑ PUFA	39.94	46.59	3.35	1.18	13.72
		∑ MUFA	25.37	17.59	3.25	1.29	13.3
		∑ sfa	29.89	29.06	2.2	0.87	8.99
		∑ (22:1, 20:1)	7.61	4.11	1.9	1.03	7.79
		BAFA	6.43	8.78	1.08	1.46	4.43
Active massive suspension feeders	23.23	∑ PUFA	41	32.2	3.3	1.42	14.23
Bryozoans		∑ EFA	29.22	21.4	3.24	1.72	13.95
		∑ MUFA	24.31	27.07	2.73	1.32	11.75
		∑ n-3	23.83	23.3	2.7	1.7	11.62
		∑ sfa	29.69	33.35	2.39	1.04	10.31
		∑ (22:1, 20:1)	7.13	8.33	1.81	1.56	7.81
		BAFA	6.61	11.68	1.58	1.99	6.78
		22:6n-3/20:5n-3	0.87	3.16	0.73	3.68	3.15
Active upright suspension feeders	26.63	∑ PUFA	41.17	36.56	3.9	0.9	14.65
Ascidians		∑ sfa	29.04	38.15	3.89	0.77	14.61
		∑ EFA	28.92	30.39	3.65	1.06	13.72
		∑ n-3	23.61	26.15	3.64	1.35	13.69
		∑ MUFA	24.97	17.69	2.86	1.13	10.74
		∑ (22:1, 20:1)	7.75	0.54	2	0.98	7.49
		BAFA	6.41	10.41	1.49	0.97	5.58
Solitary							
Benthic scavengers	24.06	∑ n-3	22.25	29.01	3.62	1.46	15.04
Gastropods, polycheates,		∑ EFA	29.48	27.59	3.29	1.22	13.67
crabs, sea stars		∑ PUFA	40.18	42.81	3.18	1.15	13.23
		∑ MUFA	25.04	22.18	2.78	1.25	11.54
		∑ sfa	29.35	31.19	2.65	0.98	11.03
		∑ (22:1, 20:1)	6.81	8.32	2.1	1.25	8.71
		BAFA	6.82	6.43	0.92	1.05	3.82
Detrivore/suspension feeders	21.9	∑ n-3	22.09	35.64	4	1.55	18.28
Amphipods & isopods		∑ EFA	27.97	36.34	2.95	1.14	13.46
		∑ MUFA	24.01	26.83	2.51	1.46	11.45
		∑ PUFA	40.4	43.45	2.46	1.12	11.24
		∑ sfa	30.19	26.96	2.02	0.82	9.24
		∑ (22:1, 20:1)	7.93	1.94	1.73	0.95	7.91
		n-3/n-6	2.24	6.57	1.28	2.01	5.83
Passive suspension feeders	28.99	∑ MUFA	22.02	36.47	4.2	1.78	14.5
Basket & brittles stars		∑ PUFA	42.42	32.43	3.42	1.61	11.79
		∑ (22:1, 20:1)	5.47	15.83	3.13	1.81	10.8
		∑ n-3	24.19	21.94	2.84	1.57	9.8
		∑ EFA	29.3	27.73	2.54	1.36	8.76
		∑ sfa	30.45	26.33	2.08	0.86	7.16
		BAFA	6.81	6.33	0.9	1.02	3.1
Algal grazers	26.61	∑ EFA	29.48	23.18	3.74	1.25	14.06
Upright coralline algae		∑ n-3	24.69	12.26	3.69	1.52	13.86
		∑ (22:1, 20:1)	6.34	18.06	3.59	1.72	13.49
		∑ PUFA	40.97	38.51	2.65	1.18	9.97
		∑ MUFA	24.23	26.32	2.57	1.33	9.65
		∑ sfa	29.62	31.85	2.53	0.98	9.52
		BAFA	6.83	5.43	0.8	0.96	3
Primary producers	31.96	∑ sfa	28.65	48.1	5.66	1.63	17.72
Sol.Tree.Prim.AUT		∑ n-3	24.43	13.9	3.63	1.44	11.37
		∑ MUFA	25.13	12.17	3.63	1.44	11.35
		∑ PUFA	41.02	37.06	3.14	1.19	9.82
		∑ EFA	29.06	28.73	3.08	1.36	9.62
		∑ (22:1, 20:1)	7.53	1.21	1.79	0.93	5.59
		BAFA	6.89	4.09	1.03	1.11	3.24

Table A5.4. Similarity percentage (SIMPER) results which compare important dietary indices of each feeding guild with the remaining fish community. Results indicate the average dissimilarity, and important fatty acids that typify each feeding guild. Ave = average, Diss = dissimilarity, SD = standard deviation.

Feeding guild	Ave. dissimilarity (%)	Fatty acid compounds	Ave. abundance		Ave. dissimilarity	Diss/SD	Contribution (%)
			All	Feeding guild			
Herbivore	15.8	∑ n-3	24.07	19.05	2.11	1.28	13.34
Streepie		∑ EFA	23.02	21.39	2.05	1.12	12.97
		18:2n-6	0.97	1.16	0.17	0.59	1.1
Benthic omnivore	18.7	∑ n-3	24.56	16.12	2.92	1.41	15.59
Jan bruin & cape knifejaw		∑ EFA	23.53	16.95	2.65	1.26	14.17
		BAFAs	4.75	7.21	0.85	1.47	4.53
Omnivores that feed on small invertebrates	13.2	∑ SFA	44.75	41.62	2.51	1.35	18.98
Fransmadam & steentjie		∑ MUFA	22.63	25.84	1.97	1.59	14.9
		18:2n-6	0.96	1.15	0.18	0.59	1.33
Omnivores that feed on large invertebrates	15.4	∑ SFA	45.22	34.03	3.64	1.57	23.72
Hottentot		∑ MUFA	22.5	29.1	2.55	1.49	16.6
		∑ PUFA	30.06	34.35	2.13	1.19	13.88
Benthic carnivore	16	∑ SFA	44.88	38.41	2.89	1.41	18.08
Blue hottentot		∑ PUFA	30.24	32.1	2.6	1.43	16.27
		∑ n-3	23.6	26.22	2.33	1.48	14.58
Carnivores of small invertebrates	16.9	18:1n-9/18:1n-7	3.93	14.54	3.05	1.06	18.09
Klipfish		∑ SFA	44.11	49.59	2.81	1.35	16.63
		∑ EFA	23.03	21.25	1.75	1.15	10.35
Carnivores of large invertebrates	16.1	∑ SFA	43.7	45.39	2.94	1.35	18.29
Roman, dageraad, panga, juvenile red steebras		∑ PUFA	30.13	30.7	2.44	1.17	15.19
catsharks, fingerfins		∑ n-3	23.09	24.77	2.28	1.19	14.22
		∑ EFA	22.33	23.78	2.24	1.09	13.94
Carnivores of large invertebrates & fish	15.3	∑ SFA	43.71	49.21	2.37	1.35	15.54
Carpenter and kingklip		∑ n-3	23.29	27.24	2.12	1.33	13.88
		∑ EFA	22.47	26.12	2.06	1.27	13.5