Trophic transfer of metals and OCP's in organisms from a warm temperate and a subtropical intertidal rocky shore

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Summary

Globally, of all the services and functions that ecological systems provide to human welfare, ± 63 % comes from the marine domain. Pollution of the ocean by means of metals and organochlorine pesticides pose a great threat to the biodiversity of intertidal rocky shores, especially sedentary filter-feeders since they are well known to accumulate a wide range of metals in their soft tissues, as well as to consumers of these species. Past research focused on the use of bio-indicator species to determine concentrations of compounds found within the environment. This hinders the comparison of uptake and transfer between species and results in a knowledge gap in terms of most intertidal rocky shore food webs and species. By examining sites that are as near as possible to a natural ecosystem, the degree of uptake and transfer can be determined.

In this study two hypotheses were tested at Tsitsikamma and Sheffield Beach: 1) stable isotope signatures will differentiate the food web structures between the intertidal zones of two temperature based biogeographic regions; and 2) a stable isotope approach can be applied to demonstrate the transfer of inorganic (metals) and organic (OCPs) substances from one trophic level to another in the intertidal zone of these two sites. The aims of this study were: 1) to compare to the two intertidal food webs using stable isotope signatures and to determine whether there are any differences in food webs between the two biogeographic regions; 2) to determine a food chain of species that directly influence each other; 3) to determine the bio-accumulation of both metals and OCPs in selected organisms from the above mentioned intertidal zones; and 4) to determine the degree of trophic transfer of these substances in the intertidal ecosystems to allow for comparison of the biomagnification potential of metals and OCPs between the two biogeographic regions. These aims were achieved through stable isotope analysis of carbon and nitrogen isotope signatures in a variety of organisms from the intertidal rocky shores, as well as ICP-MS and GC/µECD analysis for metals and OCPs, respectively. From the stable isotope signatures, a food web was established for the species that have been collected at Tsitsikamma and Sheffield Beach, as well as a food chain for trophic transfer of compounds. The results were then compared between sites for the species that were collected from both intertidal shores, and a correlation between compounds and the trophic structures of the two regions were determined.

Tsitsikamma is located in the Eastern Cape Province on the south coast of South African and is one of the oldest Marine Protected Areas in Africa. It is located within the warm temperate biogeographic region and the Agulhas Current is the prominent ocean current

that influences it. Sheffield Beach is a small KwaZulu-Natal coastal town and a well-known tourist location. It has no formal protection but houses some of the southern-most hard corals in the southern hemisphere. Sheffield Beach falls in the Benguela Current within the subtropical biogeographic region. Both of the sites are considered to be pristine with little to no pollution in the vicinity.

Stable isotope ratios were determined for both of the locations, where the warm temperate Tsitsikamma site revealed a nutrient enriched food web and a carbon increase, compared to the subtropical Sheffield Beach site. This can be attributed to the different ocean currents associated with the specific stretch of coastline, as well as upwelling and nutrients, temperature, and freshwater input.

The metal concentrations detected in the present study correlates with past research within the Tsitsikamma section. *Ulva lactuca, Perna perna, Parvulastra exigua* and *Actinia* sp. were collected at both sites and were used to determine significant differences between the two biogeographic regions. For *P. exigua*, all of the elements indicated significant differences, while concentrations in *P. perna,* had the least significant differences. A positive regression was calculated for all of the elements at Tsitsikamma but only for Cu, Zn, As, Se and Cd at Sheffield Beach. The sea surface temperatures are predicted to have great influence on the patterns observed. From the concentrations detected at Tsitsikamma there was an obvious trend of magnification from primary producer to secondary consumer and then a decrease from secondary consumer to tertiary consumer. This can further be explained by the feeding habits of the specific species, where tertiary consumers consisted of omnivorous organisms that depict a variety of prey source concentrations rather than the direct magnification of just one secondary consumer species.

Organochlorine pesticides were also analysed in selected species. At Tsitsikamma a wider variety of compounds were detected when compared to those at Sheffield Beach. In most cases the concentrations were also higher at Tsitsikamma compared to Sheffield Beach. With regards to the trophic magnification factor calculated for OCPs, total HCH, total DD_x and total Chlordane were determined for each trophic group in order to establish the overall degree of magnification that occurs. All of the compounds did however show biodilution rather than magnification. From the results obtained during the study, magnification of compounds within the marine environment under consideration is rather the exception than a general trend.

Both of the above stated hypotheses set for the study has been accepted based on the results obtained from each chapter. The stable isotope analysis distinguished between the biogeographic regions based on species sampled, and a trophic structure for each of the regions were established. The second hypothesis was also successfully applied with regards to demonstrating the transfer of inorganic and organic substances through the established trophic structure.

Keywords: Trophic transfer, Stable Isotope Analysis, biomagnification, bio-accumulation, organochlorine pesticides, metals.

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APPENDIX B

Chapter 1: General introduction

1.1. Introduction

1.1.1. South African coastal diversity

South Africa's coastal area is considered one of the most species diverse sections on the continent, and evidently ranks third in the world considering the most number of species per unit area (Costello et al., 2010). In accordance with the 2010 Census on Marine Life (Ausubel et al., 2010), the estimated known marine species has increased from 230,000 to nearly 250,000 where South Africa is listed as having the most endemic species, together with Australia, New Zealand and Antarctica, however the knowledge of all regions are still inadequate (Ausubel et al., 2010). The coastal and marine resources of Southern Africa has an important social and economic role that provide many opportunities for the whole population regarding transport, recreation, financial gain and food supply (Attwood et al., 2002; Griffiths, 2005). Due to poor management, these resources have been degrading, overexploited and led to an overall population decline (Attwood et al., 2002; Griffiths, 2005).

Seawater surface temperature has been known to influence distribution patterns, and affect adaptations by species to survive in certain areas. In general, warm regions are found close to the equator and transports warm water away towards the poles, while the colder regions are found close to the poles and transports cold water towards the equator. This is the case with the ocean currents surrounding Southern Africa, the cold Benguela Current is located on the West coast and the warm Agulhas Current on the East coast (**Figure 1.1**). These currents are further defined according to a combination of temperature, light, oxygen, biological interactions, and geology (Lutjeharms, 2006; Turpie et al., 2000).

Southern Africa has a rich variety of coastal, marine, terrestrial and freshwater life. This diversity can, amongst other reasons, be ascribed to the two very divergent ocean currents that flow along the coast (Gibbons et al., 2010), and the coastal upwelling phenomena at numerous coastal zones. The Benguela Current is driven by the coastal upwelling phenomena resulting in high productivity and biomass but low diversity. The Agulhas Current is characteristic of high diversity but low productivity and biomass (Gibbons et al., 2010; Griffiths et al., 2010). This results in an exceedingly complex hydrographical environment (Bustamante & Branch, 1996).

The aforementioned highly diverse marine biodiversity, especially on the south and east coasts, can be attributed to these distinctive currents that flow alongside the continent on each side (Gibbons et al., 2010), resulting in a combined biotic community from the Atlantic and the Indo-Pacific (Teske et al., 2011). South Africa also falls within a variety of climate zones depending on proximity to the equator and the weather, as a result of the water temperatures, which further contribute to the diverse biodiversity (**Figure 1.1**).

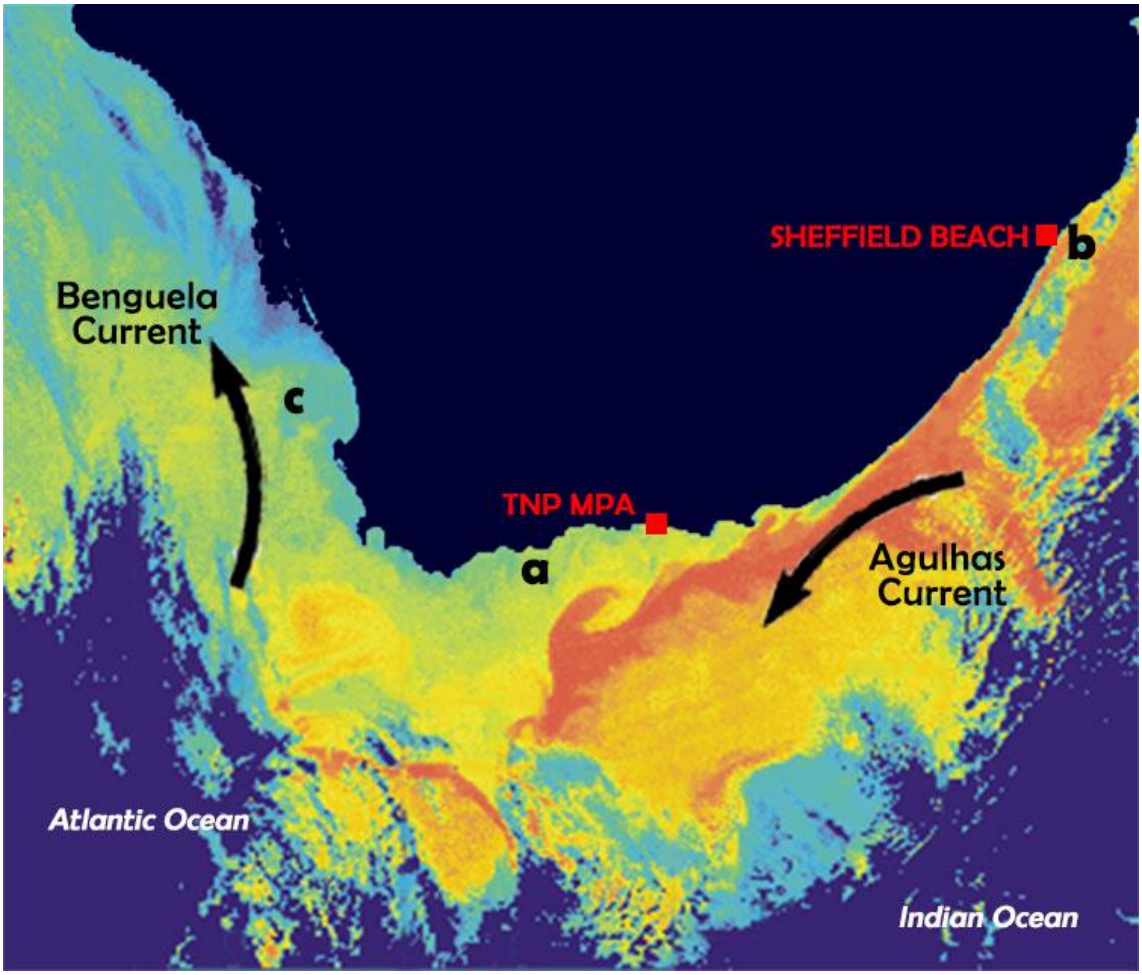


Figure 1.1: *Satellite thermal image of sea surface temperatures around South Africa's coastline, indicating the following features: a) South coast; Agulhas Bioregion; Agulhas Bank; In the warm temperate climate zone; b) East coast; Natal Bioregion; Natal Bight; In the subtropical climate zone c) West coast; Upwelling nodes; In the cold temperate climate zone (adapted from Lombard et al., 2004).*

The bioregions are based on variability in biological and biogeographical features along the coast due to the different current systems (Lombard et al., 2004). This also affects the habitat variability along the coastline. The topography of the South African coastline consists of rivers, estuaries, continental shelves, slopes and the subtidal zone.

The rocky shores addressed during the present study are located within the intertidal zone. This particular zone, i.e. intertidal zone, is exposed to a number of environmental variables including temperature fluxes, salinity, substratum, wave exposure, nutrients, freshwater inflow etc. (**Figure 1.2**) (Little et al., 2009; Sewell, 2014). Organisms that

survive here have adapted various mechanisms to withstand these extreme conditions (Little et al., 2009; Van As et al., 2012). Due to their close proximity to coastal development and anthropogenic activities, they are also susceptible to events such as pollution and exploitation.

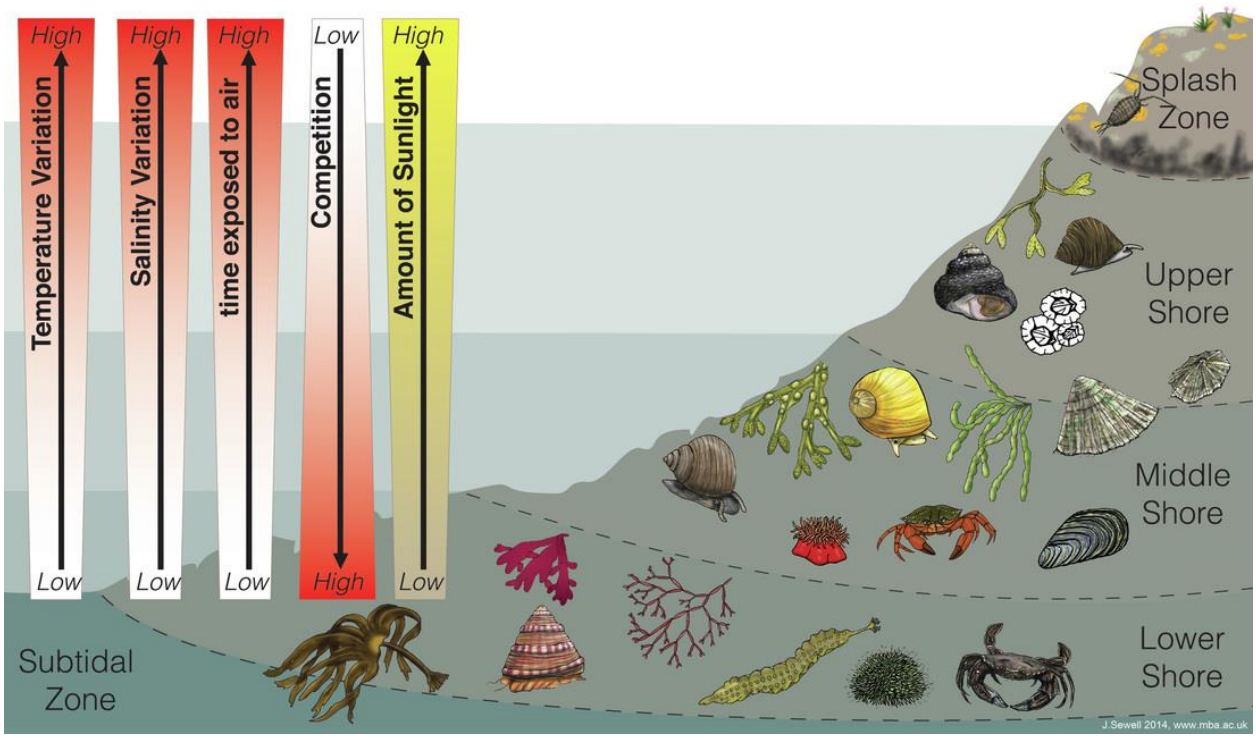


Figure 1.2: *A schematic representation of the general zonation pattern of intertidal rocky shores, depicting how temperature, salinity, air exposure, light intensity and competition varies along the vertical gradient of the shoreline (Sewell, 2014).*

The present study address the use of stable isotope analysis, metal concentrations and organochlorine pesticide extraction to better understand how contaminants are transferred through food webs. This understanding will enable optimal management of these coastal habitats and species.

1.1.2. Study rational

Intertidal zones are characterized by tough environmental conditions (Smit & Glassom, 2017), which include periodic aerial exposure, temperature and salinity fluctuations, strong wave action and periods of high sedimentation, resulting in an exceedingly dynamic environment (Smit & Glassom, 2017). The species that inhabit these ecosystems have adapted to withstand environmental pressures and predation within these dynamic spaces. The organisms found in intertidal rocky shores are also exposed to anthropogenic threats and dispersed pollution. The sites selected for this study are regarded as pristine and have been used as reference sites in past studies (Coetzee, 2015; Degger et al., 2011a; Degger et al., 2011b; Greenfield et al., 2011; Greenfield et

al., 2014; Hadfield et al., 2008; Heyns et al., 2016; Heyns-Veale et al., 2016). These sites represent an environment as near as possible to a natural ecosystem to establish a baseline for a wide range of organisms from different coastal regions.

Stable isotope studies have increased rapidly during the last few decades, focusing on predator-prey relations and trophic transfer of energy and compounds (Borgå et al., 2012). This approach creates opportunities for a variety of ecological and toxicology research. By combining stable isotope analysis with ecotoxicology, it is possible to determine to what extent pollutants and nutrients are transferred from one organism to the next. Research regarding the concentrations of metals and organochlorine pesticides (OCPs) are limited to sedentary bio-indicator species, such as mussels and barnacles (Degger et al., 2011a; Degger et al., 2011b; Greenfield et al., 2014; Rainbow & Wang, 2005). In order to establish the fate and transport of these compounds within natural ecosystems, it is important to not only consider individual bio-indicator species but to view the entire food web.

1.2. Hypotheses, aims & objectives

1.2.1. Hypotheses

In this study two hypotheses are tested:

Stable isotope signatures will differentiate the food web structures between the intertidal zones of two temperature based biogeographic regions (i.e. Tsitsikamma – representing the warm temperate, and Sheffield Beach – representing the subtropical region).

A stable isotope approach can be applied to demonstrate the transfer of inorganic (metals) and organic (OCPs) substances from one trophic level to another in the intertidal zone of two temperature based biogeographic regions.

1.2.2. Aims

To address the aforementioned hypotheses the following aims were set:

- To compare to the two intertidal food webs using stable isotope signatures and to determine whether there are any differences in food webs between the two biogeographic regions;
- To establish a food chain of species that directly influences each other;

- To determine the bio-accumulation of both metals and OCPs in selected organisms from the intertidal zones of Tsitsikamma and Sheffield Beach; and
- To determine the degree of trophic transfer of these substances in the intertidal ecosystems to allow for comparison of the biomagnification potential of metals and OCPs between the two biogeographic regions.

1.2.3. Objectives

The aims of the study were achieved through each of the following objectives:

- Determining the stable isotope signatures and related trophic positions of representative species from each of the selected biogeographic regions;
- Establish a direct food chain from each biogeographic region using significant differences among the stable isotope signatures and supporting literature;
- Analysing for metals and OCPs in selected organisms from both regions using appropriate analytical methods;
- Determining the significant differences in trophic structures and metal and OCP bio-accumulation between biogeographic regions; and
- Correlating the above mentioned compounds to the trophic structure in these two regions.

Chapter 2: Study area and species description

This chapter focuses on the nature, climate and oceanography, and significance of the two sites within different temperature based biogeographic regions. Site 1 refers to the Tsitsikamma National Park Marine Protected Area (TNP MPA), located on the south coast of South Africa within the warm temperate climate zone. Site 2 is Sheffield Beach, a recreational rocky shore on the east coast of South Africa located in the subtropical climate zone.

The species sampled at each site were subjected to distribution patterns with the focus of collecting species from different trophic levels. For each of the sites sampled, a comprehensive list of species is supplied.

2.1. Tsitsikamma National Park, Marine Protected Area

2.1.1. Background

The world famous Garden Route National Park is located on the south coast of South Africa and comprises of a few smaller parks within its borders, one of which include the Tsitsikamma Marine Protected Area, adjacent to the Tsitsikamma National Park (TNP), Storms River Mouth Rest Camp. As there are no major developments or residential areas near the Marine Protected Area (MPA) it is considered a relatively rural area. It is well known for recreational attractions such as golf, the underwater scenery, hikes and camping (SANParks, 2014). The study site falls between the Eastern and Western Cape Provinces, between the East and West Groot Rivers (Hanekom et al., 2012). Run-off from the Storms River entering the coastal section of TNP MPA, originates from the foot of the Tsitsikamma Mountains (SANParks, 2014), and any compounds released into the river will affect species on the shores.

The area was proclaimed in 1964 and aims to conserve species and habitats, maintain ecosystem function, support fishery management and provide baseline research (in terms of Section 43 of the Marine Living Resources Act (18 of 1998) ***see*** South Africa, 1998). Since its proclamation the climate and geology of the MPA has been of great importance to marine biologists due to the variety of land and coastal organisms found in the area (Kench, 1984). The MPA is a no-take protected area and is the oldest and most successful MPA in Africa (Robinson & De Graaff, 1994; SANParks, 2014). Despite scientific evidence supporting the success of this no-take MPA, the Department of

Environmental Affairs has opened 20 % of the coastline within this MPA for fishing in December 2016 (Protected Areas Act (57 of 2003) ***see*** South Africa, 2016).

This MPA conserves 7 % of the rocky shores of the Agulhas Biogeographical Region (Agulhas Bioregion; **Chapter 1**) and large populations of commercial and recreational reef fish that are vulnerable to exploitation occur here (Buxton, 1987; Buxton & Smale, 1989; Halpern, 2003; SANParks, 2014). The TNP MPA stretches over 60 km of rocky shore, covers a surface area of 340 km^2 and extends 0.5 nautical miles offshore (Tilney et al., 1996; Hanekom et al., 2012). The MPA serves as refuge for seven of South Africa's 11 endangered marine species (SANParks, 2006).

Up until 1984 at least nine species of barnacles, as well as other species including giant alikreukel, red bait (both of the latter are red data species), mussels and tubeworms could be found on this stretch of coast (Kench, 1984). These species, amongst others, rely on the coastal upwelling phenomena for the supply of food and nutrients (Kench, 1984).

2.1.2. Climate and oceanography

Weather conditions influence the coastal hydrology in a significant manner. Weather patterns within this MPA vary throughout the seasons (SANParks, 2014) and, in turn, influence the wave action and flow patterns. During summer months, intense warming originates from land and creates a shallow low pressure system (Van Zyl, 2003; SANParks, 2014). During winter the atmospheric pressure is affected by the southern subtropical high pressure belt (Van Zyl, 2003; SANParks, 2014).

The coastal area features steep inclines and is subjected to major wave action causing organisms to adapt in different ways to withstand destruction caused by the ocean (Toerien, 1976; Cowley et al., 2002; Hanekom, 2011). The direction of the different layers of surface water and deep water influence the dispersal and settlement of eggs and larvae within and outside the TNP MPA (SANParks, 2014; Tilney et al., 1996; Roberts & Van der Berg, 2005). Oceanic flow patterns within the MPA follow in an eastward direction at the surface (5 m) throughout the year, while deeper flow patterns vary during seasons (SANParks, 2014; Tilney et al., 1996; Roberts & Van der Berg, 2005). During summer (December – March), the deep flow pattern is in the opposite direction as the surface water, while in winter the direction of the currents flow pattern is similar to the pattern of the surface water (SANParks, 2014; Tilney et al., 1996; Roberts & Van der Berg, 2005).

Temperature of the ocean, biological interactions and geology are the major drivers of distribution patterns, with ocean temperatures being the most significant (Brown &

Jarman, 1978; Turpie et al., 2000; Awad et al., 2002; Heyns, 2015). Species distribution and richness decrease, as a result of sea temperatures, from the Mozambican border (tropical east coast) to the Namibia border (cool temperate coast) (Turpie et al., 2000; Awad et al., 2002; Griffiths et al., 2010; Sink et al., 2005). Tsitsikamma MPA falls within the centre of the Agulhas Bioregion, where the highest number of endemic invertebrate and fish species can be found (Turpie et al., 2000; Awad et al., 2002; Griffiths et al., 2010). Different climatic zones, local ocean conditions, habitats and topography, as well as wave action, are greatly influenced by ocean currents (Sink et al., 2012). The effects of the latter on rocky shore biodiversity are well-known in the South African context (McQuaid & Branch, 1984; Sink, 2001; Griffiths, 2000; Sink et al., 2012).

Together with the seasonal wind-driven upwelling (Gibbons et al., 2010) the current creates a kinetically driven shelf edge upwelling (Lutjeharms, 2006), resulting in nutrient rich water on the Agulhas Bank, where Tsitsikamma is situated, that prompts the Port Alfred upwelling cell (Lutjeharms, 2006). These upwelling events establish primary production within the water column (Schumann et al., 1982), and can be considered beneficial for rocky shore organisms.

2.1.3. Significance of the study area

Since areas within the MPA were recently opened for fishing, the coastal zone has been exposed to a variety of anthropogenic impacts. This calls for detailed investigation of the effects of increased human activity in and around intertidal zones to inform policy and management decisions, and will assist current and future development with regard to use and zonation of MPAs.

The site is in a pristine condition to sample a wide variety of species that will achieve the aim of a representative sampling group indicative of the warm temperate biogeographic region.

2.1.4. Map and site characteristics

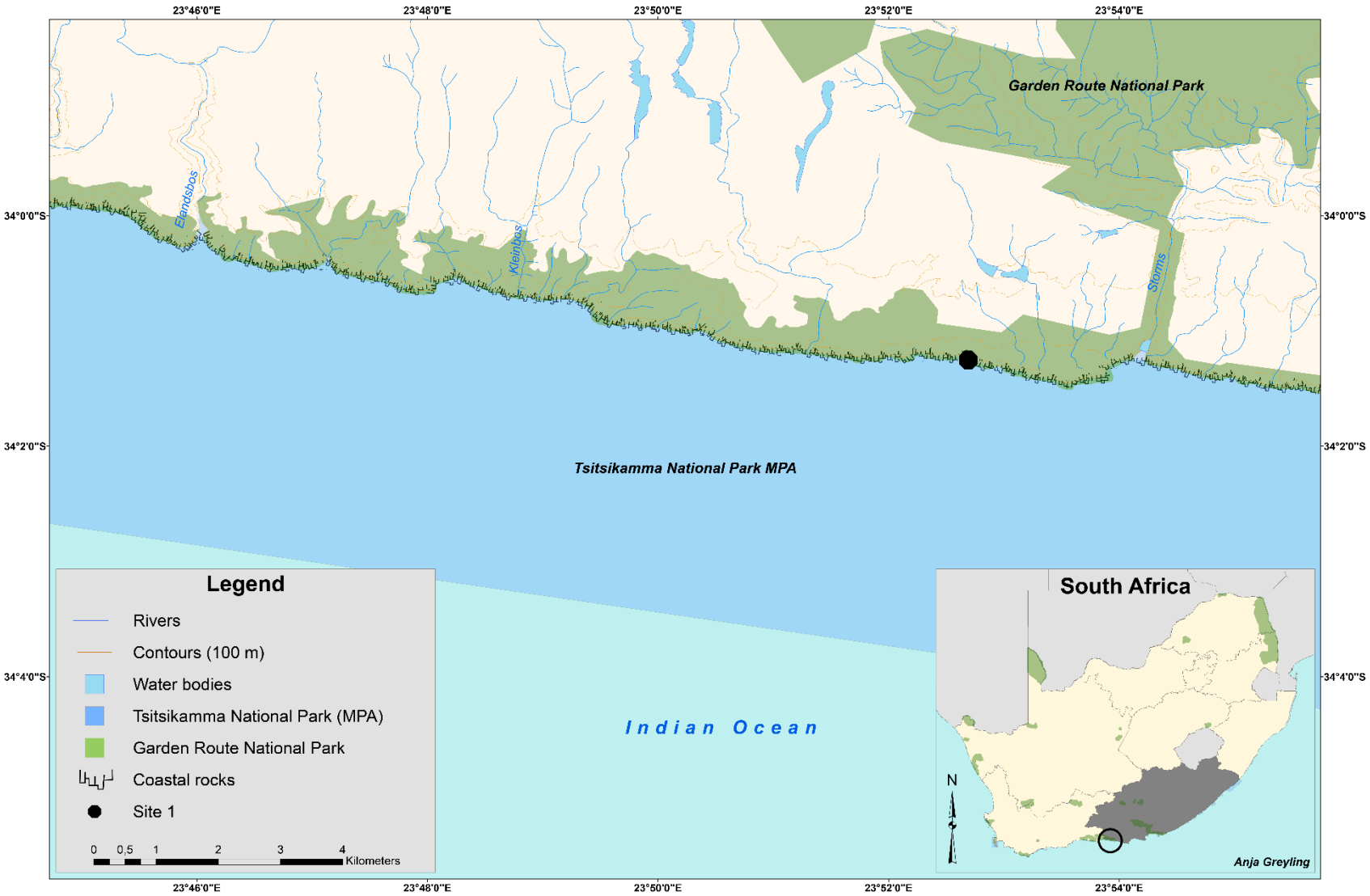


Figure 2.1: *Map of Tsitsikamma National Park Marine Protected Area (Site 1).*

Table 2.1: *Characteristics of Site 1.*

Tsitsikamma National Park Marine Protected Area

GPS coordinates	S 34°01'15.6"; E 23°52'39.4"
Average sea temperatures	15.9 °C – 19.4 °C (Hanekom, 2005)
Average rainfall	High spring rainfall (Hanekom et al., 2012) Annual rainfall of 743mm (Hanekom, 2005)
Geology	The Table Mountain geology group underlie the nearshore of TNP MPA, but yield to a strongly bent Cretaceous geology group about 2-3 km offshore (Flemming et al., 1986). Abrupt dropping quartzitic sandstone beds parallel with the coast (Buxton, 1987; Hanekom, 2011).
Shoreline type	Rock high ledge: classified as beach-rock platforms; raised 20 cm or more above sand; topographical complexities – potholes and crevices; fairly stable habitat; support rich biotic communities (Harris et al., 2012) Exposed rocky shore (Lombard et al., 2004)

2.1.5. Tsitsikamma species classification

Table 2.2: *A comprehensive list of species sampled at Site 1, Tsitsikamma National Park Marine Protected Area. Classification was done using World Register of Marine Species, FishBase, AlgaeBase and MolluscaBase.*

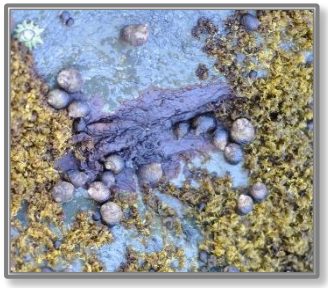
	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
1	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Mugilidae	*Liza*	*Liza richardsonii* 	Southern mullet	(Smith, 1846)
2	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Clinidae	*Clinus*	*Clinus* sp* 	Klipfish	Cuvier, 1816
3	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Gobiidae	*Caffrogobius*	*Caffrogobius* sp.* 	Goby	Smitt, 1900
4	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Sparidae	*Cymatoceps*	*Sparodon durbanensis* 	Musselcracker	(Castelnau, 1861)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
5	Animalia	Chordata	Tunicata	Ascidiacea	Stolidobranchia	Pyuridae	*Pyura*	*Pyura stolonifera** 	Red bait	(Heller, 1878)
6	Animalia	Echinodermata	Asterozoa	Asteroidea	Forcipulatida	Asteriidae	*Marthasterias*	*Marthasterias glacialis* 	Spiny starfish	(Linnaeus, 1758)
7	Animalia	Echinodermata	Asterozoa	Asteroidea	Valvatida	Asterinidae	*Parvulastra*	*Parvulastra exigua* 	Dwarf cushion-star	(Lamarck, 1816)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
8	Animalia	Echinodermata	Echinozoa	Echinoidea	Camarodonta		*Parechinus*	*Parechinus angluosus* 	Cape urchin	(Leske, 1778)
9	Animalia	Arthropoda	Crustacea	Hexanauplia	Sessilia	Tetraclitidae	*Tetraclita*	*Tetraclita serrata* 	Volcano barnacle	Darwin, 1954
10	Animalia	Arthropoda	Crustacea	Malacostraca	Decapoda	Palaemonidae	*Palaemon*	*Palaemon peringueyi** 	Sand shrimp	(Stebbing, 1915)
11	Animalia	Annelida		Polychaeta	Sabellida	Sabellariidae	*Gunnarea*	*Gunnarea gaimardi* 	Cape reef worm	(Quatrefages, 1848)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
12	Animalia	Mollusca		Bivalvia	Mytilida	Mytilidea	*Perna*	*Perna perna* 	Brown mussel	(Linnaeus, 1758)
13	Animalia	Mollusca		Gastropoda		Trochidae	*Oxystele*	*Oxystele sinensis* 	Pink-lipped topshell	(Gmelin, 1791)
14	Animalia	Mollusca		Gastropoda		Turbinidae	*Turbo*	*Turbo sarmaticus* 	Alikreukel (Giant turbo)	Linnaeus, 1758

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
15	Animalia	Mollusca		Gastropoda		Patellidae	*Scutellastra*	*Scutellastra longicosta* 	Duck's foot limpet	(Lamarck, 1819)
16	Animalia	Mollusca		Gastropoda	Neogastropoda	Buccinidae	*Burnupena*	*Burnupena cincta* 	Ridged burnupena	(Röding, 1798)
17	Animalia	Mollusca		Gastropoda	Anaspidea	Aplysiidae	*Aplysia*	*Aplysia parvula* 	Dwarf sea hare	Mörch, 1863

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
18	Animalia	Mollusca		Gastropoda		Patellidae	*Cymbula*	*Cymbula oculus* 	Goat's eye limpet	(Born, 1778)
19	Animalia	Mollusca		Gastropoda	Littorinimorpha	Littorinidae	*Afrolittorina*	*Afrolittorina knysnaensis* 	Southern periwinkle	(Krauss in Philippi, 1847)
20	Animalia	Cnidaria		Anthozoa	Actiniaria	Actiniidae	*Actinia*	*Actinia* sp. 	Plum anemone	Linnaeus, 1758

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
21	Plantea	Rhodophyta		Florideophyceae	Corallinales	Coralliniaceae	*Jania*	*Jania* sp. 	Red algae	J.V. Lamouroux, 1812
22	Plantea	Chlorophyta		Ulvophyceae	Ulvales	Ulvaceae	*Ulva*	*Ulva lactuca* 	Green macro-algae	Linnaeus, 1753
23	Chromista	Ochrophyta	Phaeista	Phaeophyceae	Ralfsiales	Ralfsiaceae	*Ralfsia*	*Ralfsia* sp.* 	Brown encrusted algae	Berkeley, 1843

* Copyright TidalTao, 2017; FishBase, 2017; AlgaeBase, 2017; AnimalBase, 2014; Femorale, 2017; WoRMS, 2017; Griffiths, 1976; and courtesy of Dr. Kerry Hadfield Malherbe, Dr. Olena Kudlai and Wentzel Pretorius.

2.2. Sheffield Beach

2.2.1. Background

The province of KwaZulu-Natal (KZN) on the east coast of Africa is characterized by high annual rainfall, warm temperatures and south flowing ocean currents originating within the Indian Ocean. The area has a lower productivity with regards to commercial fishing and invertebrate distribution than the west coast Atlantic Ocean (Harris et al., 2012), but makes up for it in terms of diversity. Coastal communities depend on these species for subsistence and recreational fishing (Harris et al., 2012), as well as harvesting of intertidal species that include oysters, crayfish, mussels and crabs. The east coast of South Africa is known for the presence of hard corals at high latitudes that have been recorded as far south as 31°04' (Smit & Glassom, 2017), however, these corals are poorly documented within intertidal zones (Smit & Glassom, 2017). Despite this, less than 10 % of the KZN coastline was under category 1 (no-take MPA) or category 2 (some extraction permitted) protection in 1997 (Griffiths, 2005). This percentage increased to 20 % by 2004 (Griffiths, 2005).

The ratio of population density to law enforcement staff in KZN was 2:1 (Griffiths, 2005), which can explain the limited protection of coastal biodiversity within this province. The aforementioned factors, as well as temperature extreme events, exploitation and other habitat degrading activities are of great concern to the rich and diverse system of the KZN intertidal rocky shores (Goble, 2014).

Sheffield Beach is located in KZN between Ballito and Christmas Bay on the subtropical east coast of South Africa. Historically the entire east coast has been poorly represented by protected areas and the associated research outputs thereof. Research conducted by Griffiths et al. (2010), indicating a reduced research focus on the east coast versus the south and west coasts of South Africa, supports the listing of the Natal Biogeographical Region (Natal Bioregion; **Chapter 1**) as a priority area for systematic biodiversity planning (Sink et al., 2012).

A pilot study conducted by Sink et al. (2005) provide more information regarding the macro-algae and invertebrate distribution of the east coast in equal sampling points to those conducted on the south and west coasts. According to Sink et al. (2005) only 6 km (i.e. the Trafalgar MPA) of the KZN shoreline is protected, with only 2 km of the total 560 km coastline identified as protected rocky shores. The Trafalgar MPA aims to conserve

fossils of intertidal species and do not represent the whole biodiversity of the KZN coastal zone, while the rest of the province is subjected to heavy exploitation of intertidal species (Sink et al., 2005). The Aliwal Shoal MPA within KZN consists of sandy shores and focuses their protection on offshore attributes regarding diving, and a variety of fishing activities such as spear fishing and surf fishing (MPA Forum, 2017). Sink et al. (2005) described the characteristics and abundance of species found in coastal habitats of different shore sections – low shore, mid shore, high shore and top shore – in the study conducted on the east coast biodiversity.

Freshwater inflow from rivers to the sea greatly contributes to the functioning of ecosystem and maintaining the health of marine resources (Sink et al., 2012). Such inflow provide nutrients to coastal habitats, and species that utilize the estuaries and coastal waters as nurseries have been reported to influence the distribution and survival of line fish up to 40 km offshore (Sink et al., 2012). According to an isotope study conducted by Porter (2009) in KZN, 8 - 33 % of the diet of filter-feeders originates from riverine sources. This can be ascribed to the fact that the Natal Bioregion is subjected to significantly high riverine input (Goble, 2014).

2.2.2. Climate and oceanography

The unique climate and oceanography of this coast give rise to the wide variety of ecosystems. KwaZulu-Natal has a subtropical climate, characterized by high annual rainfall and subsequent humidity. The weather patterns of this region are subjected to the coastal low-pressure system, which in turn creates north-easterly winds. These winds change into south-westerly winds as the coastal low-pressure systems pass along the edge of the continent (Kruger, 2014). Cold fronts follow the low-pressure system which brings clouds and rain over the area. These systems, in turn, bring high-pressure systems resulting in cool ocean air with major south-eastern winds (Kruger, 2014). While prone to infrequent tropical cyclones (KZN, 2017; Kruger, 2014), the area has a high summer rainfall and gets some rain in winter as well (KZN, 2017).

The KZN coast comprises of a number of ecosystems such as coastal dunes, estuaries, mangroves, wetlands, coastal lakes and rocky shores (Goble, 2014), creating a variety of habitats for organisms to thrive. Water temperatures are usually a mixture of nearshore and offshore waters with no prominent thermocline (Guastella, 2014). Occasional upwelling of the South Indian Central Ocean around 300 – 800 m in depth occurs and results in cooler water temperatures (Guastella, 2014). Even though the Agulhas Current transports warm water from the equator that is nutrient deprived, it often has high levels

of dissolved oxygen (Guastella, 2014). Consequently, occasional upwelling regimes are beneficial for organisms that survive in coastal habitats. The St. Lucia upwelling cell, referred to as the Natal Bight, is responsible for nutrients and is regarded as a vital regulatory mechanism of the characteristic Agulhas Current (Guastella, 2014).

2.2.3. Significance of the study area

Tourists generally prefer the climate conditions described above, making KZN one of South Africa's most popular recreational destinations. Limited research is available on this area, even though it is regarded as an area not directly affected by pollution with very little influences from industries and development (Coetzee, 2015). Sheffield Beach, in particular, is overlooked when it comes to rich natural beauty, offshore reefs, marine mammals, hard corals, turtles, and rays. Despite efforts of a few local marine enthusiasts to give the stretch of beach around Sheffield Beach some sort of conservation status, no success has been achieved. Problems with stakeholder activities stand in the way of establishing this stretch of beach as a conservation area (Harris et al., 2012). Unsustainable exploitation of resources, pollution, habitat degradation and invasive species can have lethal effects on marine systems (Leslie, 2005). Monitoring and surveying these intertidal rocky shores can help conserve and create awareness for the species and remarkable habitat within this subtropical biogeographic region.

2.2.4. Map and site characteristics

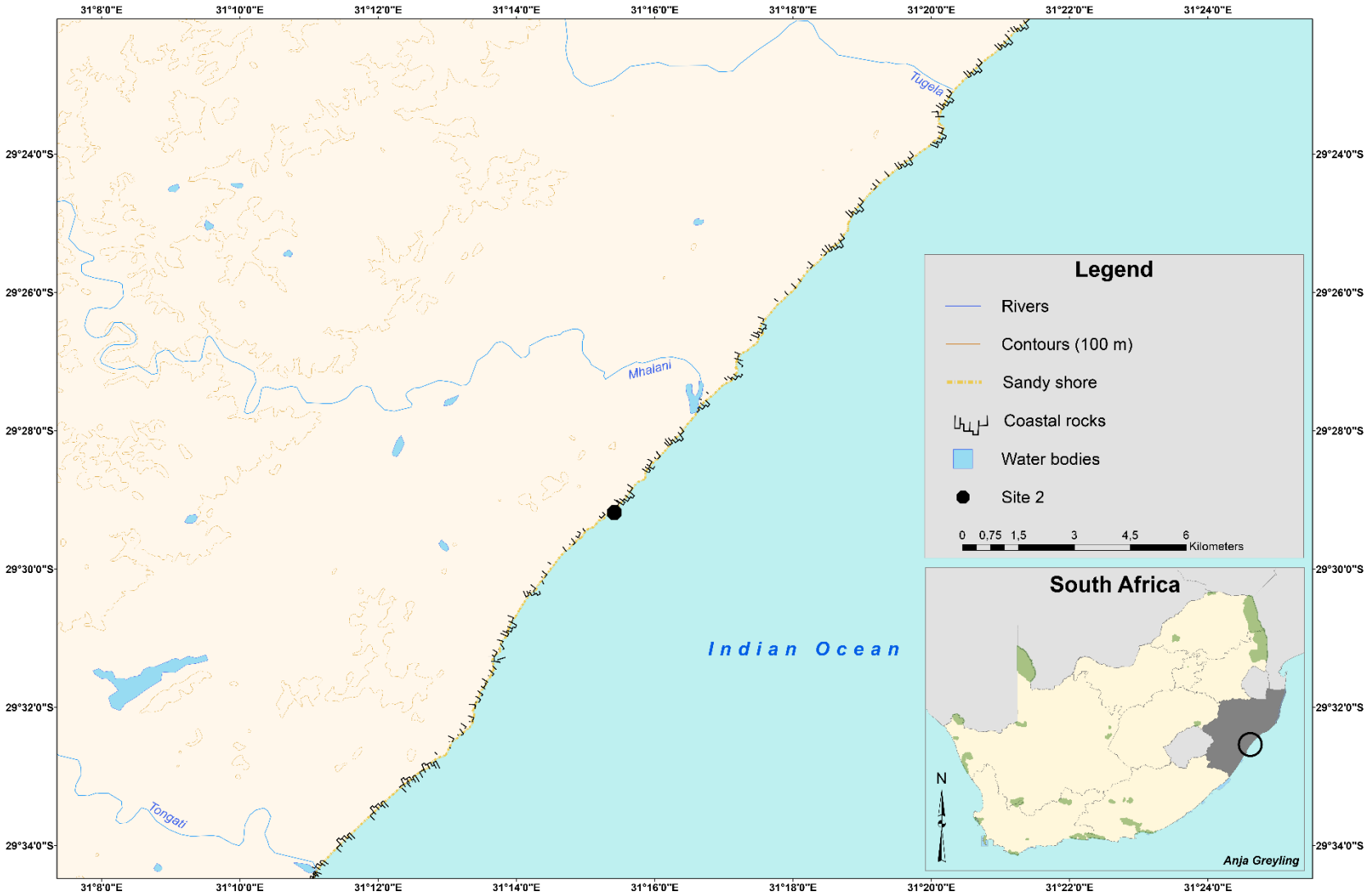


Figure 2.2: *Map of Sheffield Beach (Site 2).*

Table 2.3: *Characteristics of Site 2.*

Sheffield Beach, KwaZulu-Natal

GPS coordinates	S 29°29'10.2"; E 31°15'24.8"
Average sea temperatures	22 °C – 28 °C (Guastella, 2014; Sink, 2001)
Average rainfall	Summer rainfall of 1000 – 1200 mm per annum (Sink, 2001; KZN, 2017)
Geology	Quaternary, Ordovician and Ecca sandstone, dolerite arid granite (Sink, 2001)
Shoreline type	Broken high ledge: classified as high ledges; advanced phases of erosion; complex infratidal habitat; large crevices and gulleys (Harris et al., 2012) Exposed mixed shore (Lombard et al., 2004)

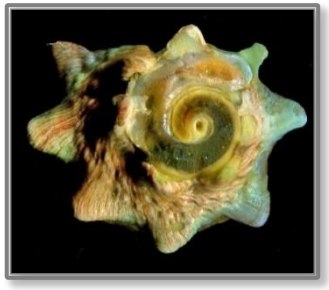
2.2.5. Sheffield Beach species classification

Table 2.4: *A comprehensive list of species sampled at Site 2, Sheffield Beach. Classification was done using World Register of Marine Species, FishBase, AlgaeBase and MolluscaBase.*

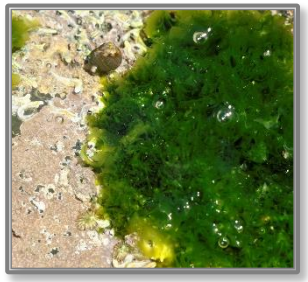
	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
1	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes			*Parablennius pilicornis** 	Ringneck blenny	(Cuvier, 1829)
2	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Gobiidae	*Psammogobius*	*Psammogobius knysnaensis#* 	Knysna sandgoby	Smith 1935
3	Animalia	Chordata	Vertebrata	Actinopterygii	Perciformes	Sparidae	*Diplodus*	*Diplodus sargus sargus** 	Blacktail	(Linnaeus, 1758)
4	Animalia	Echinodermata	Asterozoa	Asteroidea	Valvatida	Asterinidae	*Parvulastra*	*Parvulastra exigua* 	Dwarf cushion-star	(Lamarck, 1816)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
5	Animalia	Echinodermata	Echinozoa	Echinoidea	Camarodonta	Toxopneustidae	*Tripneustes*	*Tripneustes gratilla* 	Collector urchin	(Linnaeus, 1758)
6	Animalia	Arthropoda	Crustacea	Hexanauplia	Sessilia	Tetraclitidae	*Tetraclita*	*Tetraclita serrata* 	Volcano barnacle	Darwin, 1954
7	Animalia	Annelida		Polychaeta	Sabellida	Serpulidae	*Spirobranchus*	*Spirobranchus kraussii** 	Blue coral worm	(Baird, 1865)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
8	Animalia	Mollusca		Bivalvia	Mytilida	Mytilidea	*Perna*	*Perna perna* 	Brown mussel	(Linnaeus, 1758)
9	Animalia	Mollusca		Gastropoda		Patellidae	*Helcion*	*Helcion concolor** 	Variable limpet	(Krauss, 1848)
10	Animalia	Mollusca		Gastropoda	Cycloneritimorpha	Neritidae	*Nerita*	*Nerita albicilla** 	Blotched nerite	Linnaeus, 1758

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
11	Animalia	Mollusca		Gastropoda		Turbinidae	*Lunella*	*Lunella coronate** 	Crowned turban snail	(Gmelin, 1791)
12	Animalia	Mollusca		Gastropoda		Trochidae	*Monodonta*	*Monodonta australis** 	Toothed topshell	(Lamarck, 1822)
13	Animalia	Mollusca		Gastropoda		Necellidae	*Cellana*	*Cellana radiata** 	Rayed wheel limpet	(Gmelin, 1791)

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
14	Animalia	Mollusca		Gastropoda	Littorinimorpha	Littorinidae	*Afrolittorina*	*Afrolittorina Africana** 	African periwinkle	(Krauss in Philippi, 1847)
15	Animalia	Mollusca		Gastropoda	Neogastropoda	Muricidae	*Thalessa*	*Thalessa savignyi** 	Knobby dogwhelk	(Deshayes, 1844)
16	Animalia	Cnidaria		Anthozoa	Actiniaria	Actiniidae	*Actinia*	*Actinia* sp.* 	Plum anemone	Linnaeus, 1758
17	Plantae	Rhodophyta		Florideophyceae	Corallinales	Corallinaceae	*Corallina*	*Corallina* sp.* 	Red algae	Linnaeus, 1758

	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Common name	Authority
18	Plantae	Chlorophyta		Ulvophyceae	Bryopsidales	Codiaceae	*Codium*	*Codium* sp.* 	Green algae	(Suringar) Hariot, 1889
19	Plantea	Chlorophyta		Ulvophyceae	Ulvales	Ulvaceae	*Ulva*	*Ulva lactuca* 	Green macro-algae	Linnaeus, 1753

* Copyright TidalTao, 2017; FishBase, 2017; AlgaeBase, 2017; AnimalBase, 2014; Femorale, 2017; WoRMS, 2017; Griffiths, 1976; and courtesy of Dr. Kerry Hadfield Malherbe, Dr. Olena Kudlai and Wentzel Pretorius.

Chapter 3: A comparison of food web structures of the intertidal rocky shore using stable isotopes

3.1. Introduction

In general biotic communities are characterized by simple traits such as species richness, composition, and abundance (Abrantes et al., 2014). However, more recently food web structures are defined using trophic linkages between components, overall length, connectivity and movement of carbon and nutrients (Pimm, 2002; Abrantes et al., 2014). This can be achieved using naturally abundant isotope signatures. Since the costs accompanying these analyses has decreased during the past decade, and their ability to obtain trophic relationships from tissue samples, stable isotope analysis has become gradually more prevalent (MacNeil, 2008). Ratios of stable elements such as oxygen (O_2), sulphur (S), strontium (Sr), carbon (C), and nitrogen (N) can be used for a variety of applications of dietary and movement patterns within aquatic environments (Zimmo et al., 2012).

Stable isotopes have emerged as an ecological tool to determine patterns and mechanisms on organism level, in food web structures, as well as to trace nutrient cycles in systems, of both terrestrial and aquatic ecosystems (Michener & Lajtha, 2007). The distribution of these ratios is affected by biochemical properties and processes of metabolism and is the record of an organism's dietary history through its tissue (MacNeil, 2008; Hobson, 1999). Fundamental aspects in ecology can be addressed with the use of stable isotopes ratios, which include determining different processes within an ecosystem. Since the study of stable isotopes in ecology is relatively new, there is still a lot to be analysed, yet it can give rise to factors that would not have been discovered otherwise (Polunin & Pinnegar, 2002). Clarifying the food web structure and relationships of complex and unfamiliar feeding interactions, quantifying energy sources, examining mechanisms operating inside these food webs and tracking migratory species and routes through ecosystems (Zimmo et al., 2012; MacNeil, 2008) can be derived from stable isotope signatures. Stable isotopes can also be used as tracers in order to determine the aforementioned characteristics of specific organisms. Studies conducted by Wainright et al. (1993) and Satterfield & Finney (2002) concluded that stable isotopes can also be used to identify long-term changes in trophic positions. When applications of stable isotope ecology are considered, it is essential to not only consider individual species but

to also consider the whole ecosystem, including the invertebrates that inhabit intertidal rocky shores, which support commercial species such as fish (MacNeil, 2008).

Stable carbon ratios are used to determine the source and flow of carbon in food webs, as it varies between producer and consumer, and can differ between tissue types (MacNeil et al., 2005; McCutchan et al., 2003; Abrantes et al., 2014). This means that consumer signatures are largely determined by producer signatures (DeNiro & Epstein, 1978). Fry & Wainright (1991) stated that marine phytoplankton range between -18 and -25 ‰. According to a study conducted by Pinnegar & Pollunin (2000), stable carbon values in fish muscle can represent a year-long dietary average with a turn-over rate of 0.0018 d^{-1} and can be explained considerably by the lipid content of the particular species. It has been reported that stable carbon values vary substantially with latitude in marine environments (Hofmann et al., 2000) and can be attributed to temperature and phytoplankton growth differences (MacNeil, 2008). The carbon isotope signatures vary with sea surface temperature and can be determined through a time series of carbon to records of sea surface temperatures (MacKenzie et al., 2011). Carbon isotopes are used for identifying movement over major latitudes by means of primary production sources (Hill et al., 2006).

Stable nitrogen ratios are used to characterize an organism's trophic position (Zimmo et. al., 2012) where it is primarily influenced by growth and metabolism causing enrichment of ± 2 - 3 ‰ per trophic level (McCutchan et al., 2003). This is particularly true for tissues such as liver and blood that has high metabolic rates in comparison to muscle and bones that exert lower metabolic rates. The stable nitrogen value can also differ between sources, giving information regarding the organisms' diet, especially when used in combination with the stable carbon values (Vander Zanden & Rasmussen, 1999; Abrantes et al., 2014).

Stable carbon and nitrogen ratios are best known for determining the trophic structure that resembles a specific location. Studies conducted by Hill et al. (2006; 2008) show evidence of spatial and temporal variation in stable carbon and nitrogen signatures with regards to the South African coast. Another study by Puccinelli et al. 2016b indicates that signatures could be connected to a specific location based on upwelling frequency and intensity as a defining factor within biogeography. Stable isotope signatures are also affected by proximity to urban centres, where anthropogenic activities affect the diets of organisms that inhabit coastal shores (Puccinelli et al., 2016a).

Other metrics that have been introduced by Layman et al. (2007) include patterns within food web structures, niche width and other anthropogenic impacts (Cooper & Wissel, 2012; Delong et al., 2011; Jackson et al., 2012; Abrantes et al., 2014). Despite the importance of these factors, there remains a major knowledge gap in our understanding of these factors, within and amongst different ecosystems (Post, 2002). It has only recently been proposed that a study of the biogeographic variation of isotope signatures may serve as a possible tool for the composition of marine food webs (Hill et al., 2006).

This chapter provides insight to the energy transfer from two temperature based biogeographic food webs of intertidal rocky shores along the South African coastline. The hypothesis stated for this chapter is Stable isotope signatures will differentiate the food web structures between the intertidal zones of Tsitsikamma and Sheffield Beach, and will be tested by addressing the following aims: 1) to compare to the two intertidal food webs using stable isotope signatures and determine whether there are any differences in food webs between the two biogeographic regions; and 2) to establish a food chain of species that directly influences each other.

3.2. Materials & Methods

Sampling was conducted at two pre-selected sites during a single survey, in March and April 2016 during a spring low tide period (**see Chapter 2**). The appropriate permits were obtained prior to sampling at each of the sites located along the south and east coast of South Africa, respectively. Only a single survey was conducted since this study will serve as baseline data for future studies. The aim was to select sites in protected and pristine areas to obtain as wide a range of organisms in the different biogeographic coastal regions as possible. Since both sites closely represent a system in its most natural state, this could be achieved.

3.2.1. Field survey and sample collection

A variety of organisms were sampled during this survey with the intention to sample similar species at both sites. The organisms were removed from the rocky shores using a stainless steel scraper for invertebrates and fish were caught by rod and reel. The fish were caught using hand lines and euthanized according to SANS protocol, where the spinal cord was severed prior to dissection (Ethics number: NWU-00440-16-S5). A wide range of algae, representing primary producers and invertebrate species, representative of different feeding habits were carefully removed from pools in the intertidal rocky shore.

All the species collected during the survey were identified to the lowest possible taxonomic level using marine guides (Branch et al., 2010; WoRMS, 2017). To obtain suspended particulate matter (SPM), 200 litres of marine intertidal pool water was filtered through a 53µm mesh plankton net. All of the samples were placed in labelled 50 ml Falcon® tubes. The samples were then frozen for transportation to the laboratory in the North West Province, South Africa, for preparation prior to analyses.

3.2.2. Laboratory preparation

Subsamples were dried in a Labcon 5016U oven at 60 °C for 48 h, homogenized into small powder-like particles with a sterile mortar and pestle, and roughly 1 g (dry weight) of the sample was transferred into Eppendorf® tubes. All of the equipment used were pre-treated with 10 % hydrochloric acid (HCl), as well as between each sample, in order to avoid contamination. The isotopic analyses were conducted at Hokkaido University, Sapporo in Japan, using an Elementar IsoPrime100 with vario MICRO cube Isotope Ratio Mass Spectrometer (IRMS) (**Figure 3.1**b) – e)) to measure total and ratios of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The samples were treated with 1 ml 1:1 Chloroform/Methanol for 12 h to remove the lipids. Supernatant from the organic solvent was subsequently decanted and the remaining mass was oven dried at 50 °C for 24 h. Samples with non-dietary carbonates, due to the presence of an exoskeleton, i.e. shell, were treated with HCl to dissolve the excess carbon (Verhaert et al., 2013). The samples were then weighed (± 1 mg) using a Cubis® Micro Balance MSE6.6S-0CE-DM (**Figure 3.1**a)), and encapsulated in Elemental Microanalysis D1008, 5 mm x 8 mm ultrapure tin capsules before IRMS analysis.

3.2.3. Stable isotope analysis

All the samples were analysed for total carbon and nitrogen, as well as their isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) and elemental compositions. The results obtained were expressed in per mil units (‰) as a deviation from the standards, atmospheric nitrogen and PeeDee belemnite for carbon. The results were then defined according to the internationally accepted delta notation:

$$\delta X(‰)=[(R_{sample} \div R_{standard})\text{-}1]\times 1000$$

Where δX (‰) is either $\delta^{13}C$ or $\delta^{15}N$ and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the samples and standards, respectively. Replicate measurements of internal laboratory standard (L-Alanine) indicate replicate error within ± 0.02 ‰ for $\delta^{13}C$ and $\delta^{15}N$.

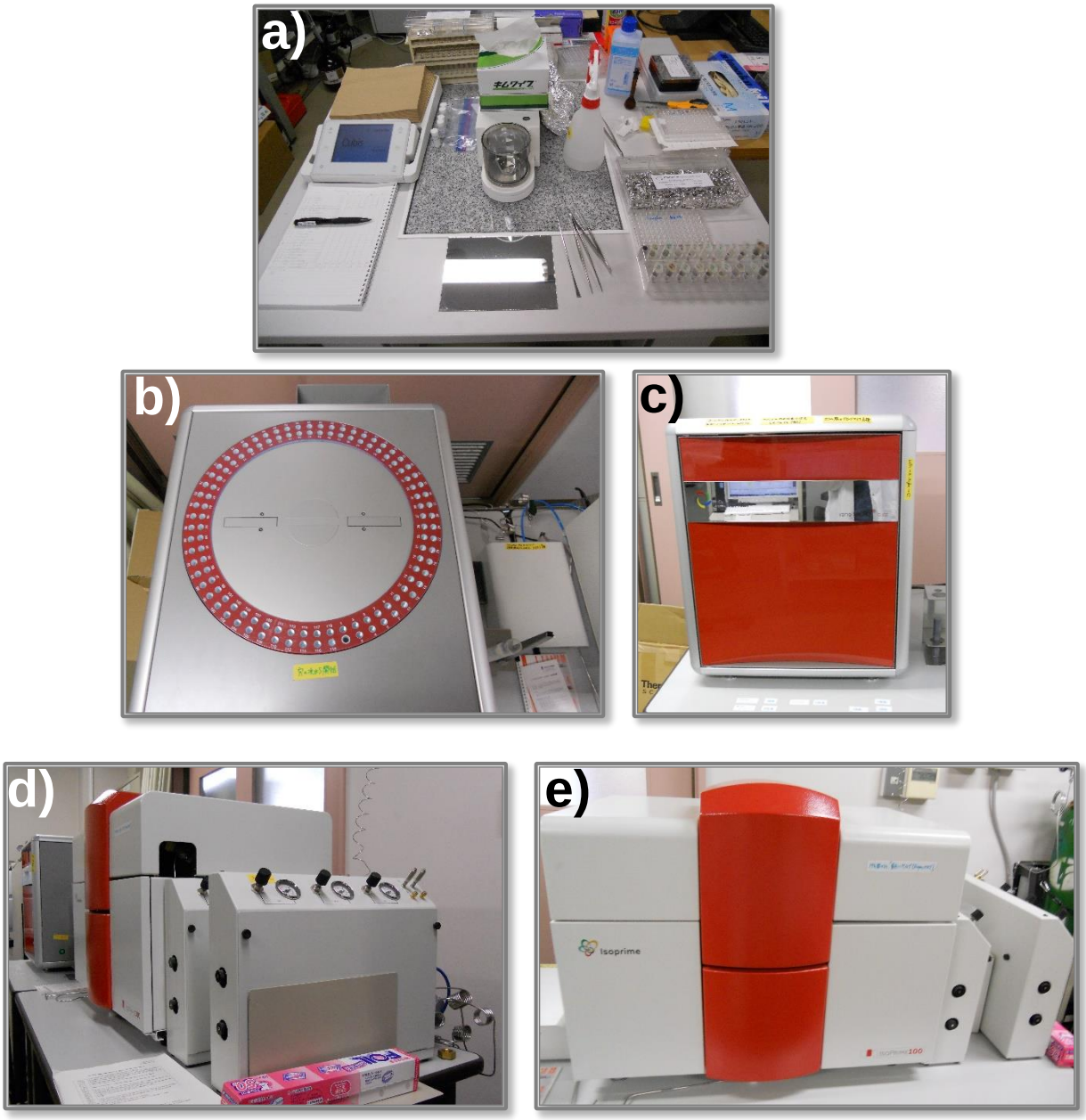


Figure 3.1: *Stable isotope analysis conducted at Hokkaido University, Japan where a) Cubis® Micro Balance MSE6.6S-0CE-DM and work station for preparing samples for isotope ratio mass spectrometry analysis, b) and c) vario MICRO cube isotope ratio mass spectrometer automatic sampler, d) and e) IsoPrime100 elemental analyser.*

3.2.4. Statistical analysis

3.2.4.1. Stable isotope signatures

Normality of the data were determined using the D'Agostino & Pearson normality test. In the case of parametric data, an unpaired two-tailed t-test was applied. Where the data were non-parametric, a Mann-Whitney test was applied. This was done to compare the isotopic values of the species listed in the pre-defined functional trophic groups, between the two temperature based biogeographic regions. A significance level of $p < 0.05$ was set and determined for each of these groups.

3.2.4.2. Trophic level and food chains

Trophic positions of each organism within the food web were calculated and compared between the sites, as well as the total length of both food chains. This was done by substituting the $\delta^{15}N$ value of each species in the following trophic level equation (Walters et al., 2016):

$$TL_{consumer} = \left[\left(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ producer}\right) \div \Delta\delta^{15}N\right] + 1$$

where $TL_{consumer}$ refers to the trophic level of the organism in question, $\delta^{15}N_{consumer}$ to the $\delta^{15}N$ value of the same organism and $\delta^{15}N_{primary\ producer}$ to the mean of the local primary producer. The value 1 was identified as the trophic level of the primary producer and $\Delta\delta^{15}N$ is the trophic enrichment factor, which is determined by the shift in $\delta^{15}N$ between two succeeding trophic levels (Post, 2002). Previous research conducted on the trophic enrichment of marine food webs indicated an enrichment factor of 3.4 ‰ (Post, 2002; Minagawa & Wada, 1984), and the same factor was used in the present study. Based on the trophic levels, each of the species were grouped according to functional trophic groups (Little et al., 2009). The species were grouped into primary producers, primary consumers, secondary consumers and tertiary consumers.

Furthermore, one-way analysis of variance (ANOVA) was performed to determine direct energy transfer between species. This was done in order to establish a food chain for possible trophic magnification. Since the $\delta^{13}C$ signature reflects the source of energy of an organism (Fry, 2006), the assumption was made that species which are not significantly different in $\delta^{13}C$ values exhibit direct energy transfer.

3.2.4.3. Group assessments according to functional trophic groups

In order to extract common principles from a wide range of species and functions, it has been known to view food webs according to functional trophic groups rather than individual species (Little et al., 2009). To establish these principles, a discriminant function analysis (DFA) was performed using SPSS version 24 (PASW Statistics, IBM, USA). This was done to determine reclassification success of the relevant functional trophic groups using the carbon and nitrogen, as well as total C:N ratios. Fisher's function coefficients were also applied to inspect the probability of membership to each of the mentioned groups (Gerber, 2012).

Canonical variates, based on Eigen values, were also established during the DFA analysis. Two variates dependent on the outcome of the analysis were plotted on a biplot to define patterns within- and between sites.

3.3. Results

3.3.1. Stable isotope signatures

During this study, a total of 187 samples from 36 species were analysed for $\delta^{13}C$ and $\delta^{15}N$ (**Appendix A**; **Table A 1**). Where possible, four replicates of each species, from the two climate zones were analysed. Samples were collected randomly without bias towards any trophic level. Samples ranged from primary producers (i.e. macro-algae), primary-, secondary- and tertiary consumers, which included a diverse range of invertebrates and small intertidal fish species. The organisms collected represent different habitats and a variety of feeding habits found within these coastal stretches.

Figure 3.2 and **Figure 3.3** are visual representations of the food web structures from Tsitsikamma and Sheffield Beach, respectively, depicting $\delta^{13}C$ on the x-axis and $\delta^{15}N$ on the y-axis. Green macro-algae, *U. lactuca*, and SPM served as δ^{15} N $_{\text{primary producer}}$ for Tsitsikamma and Sheffield Beach, respectively. Unpublished data from research conducted at the shallow reef of Tsitsikamma (**Figure 3.4**; Heyns, 2015) were used to compare different signatures of the same species within the one location. A limited number of isotope studies have been conducted on the east coast of South Africa, however studies by Puccinelli et al., (2016a) on filter feeders on the south-east coast, reported similar results for the brown mussel, *P. perna*, and volcano barnacle, *T. serrata*.

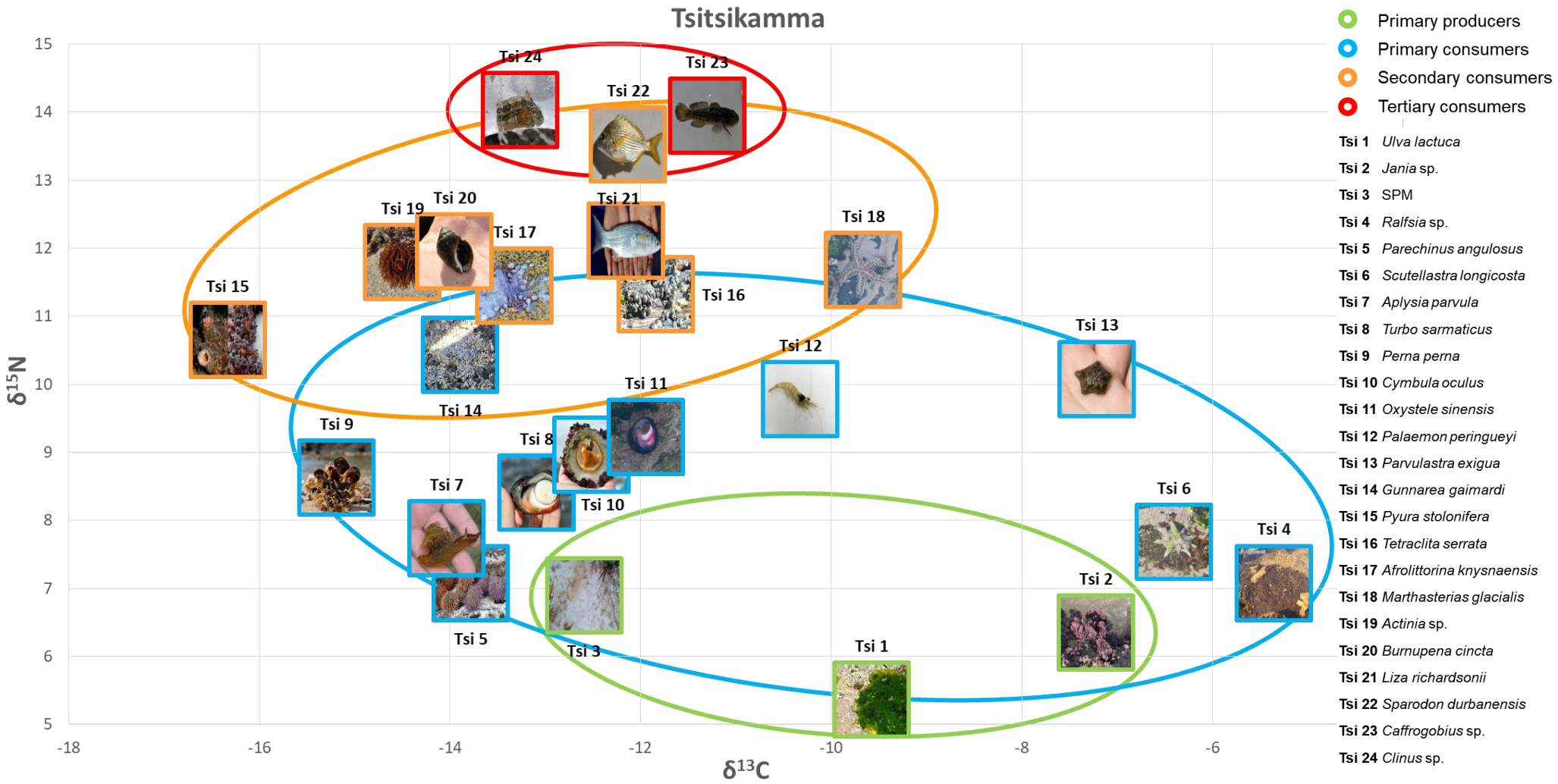


Figure 3.2: *Mean $\delta^{13}C$ and $\delta^{15}N$ signatures for species found on the Tsitsikamma rocky shore. Species are grouped according to trophic level and indicated with coloured symbols in accordance with their trophic group. Abbreviations used in the graph are listed in the legend (see **Appendix A**; **Table A 1**).*

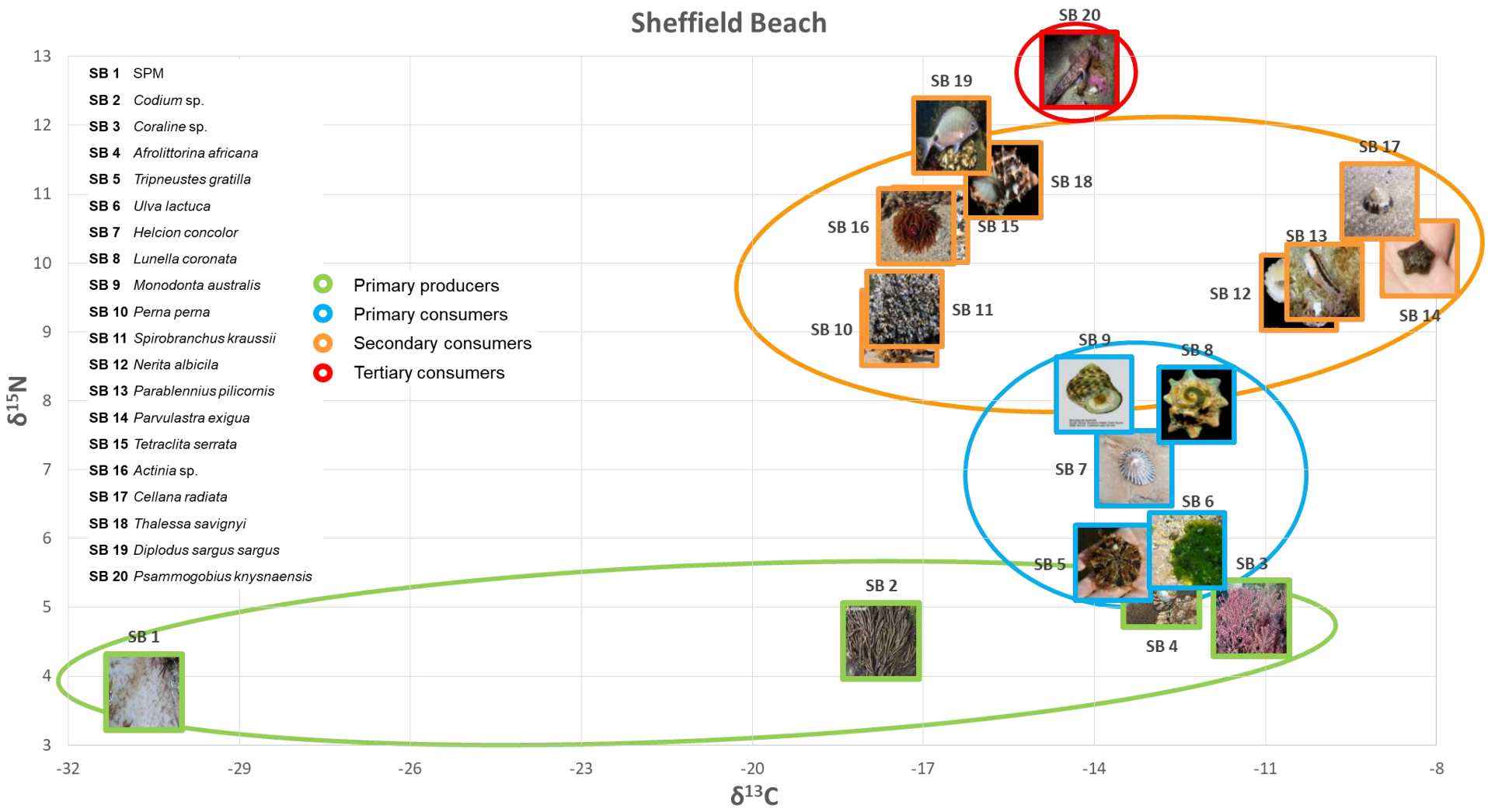


Figure 3.3: *Mean $\delta^{13}C$ and $\delta^{15}N$ signatures for species found on the Sheffield Beach rocky shore. Species are grouped according to trophic level and indicated with coloured symbols in accordance with their trophic group. Abbreviations used in the graph are listed in the legend (see **Appendix A**; **Table A 1**).*

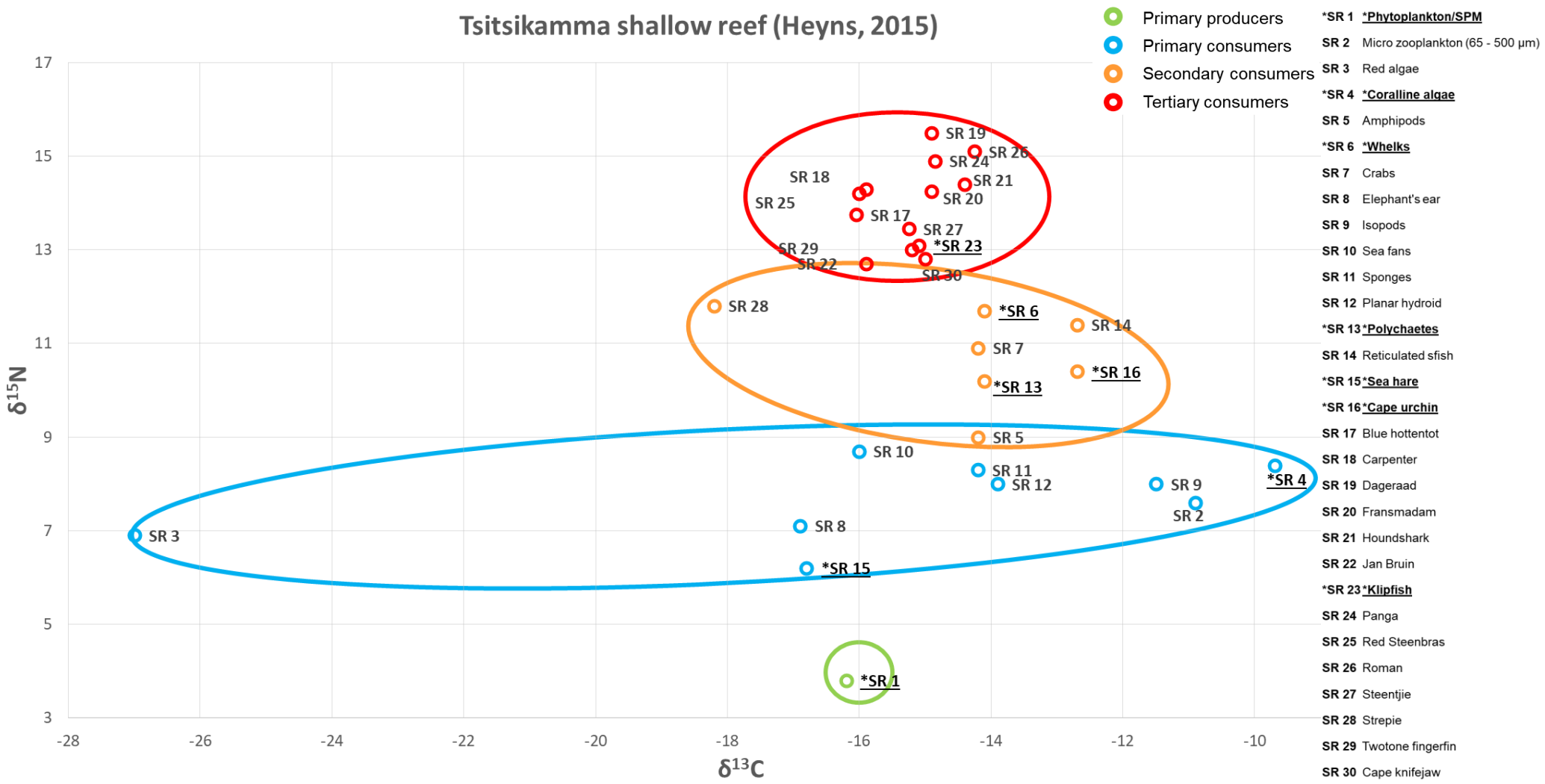


Figure 3.4: *$\delta^{13}C$ and $\delta^{15}N$ signatures adapted from Heyns (2015) for species found in the Tsitsikamma shallow reef. Species are grouped according to trophic level and indicated with coloured symbols in accordance with their trophic group. Abbreviations used in the graph are listed in the legend. The species that are indicated with an asterisks (*) represent species that were sampled in the intertidal rocky shore during the present study.*

During the present study the $\delta^{13}C$ values ranged from -30.7 ‰ in SPM to -5.35 ‰ in the encrusted brown algae, *Ralfsia* sp., where $\delta^{15}N$ values ranged from 3.77 ‰ in SPM to 14.1 ‰ in the klipfish, *Clinus* sp., at each of the sites. At Tsitsikamma, red bait (*P. stolonifera)* represented the lowest $\delta^{13}C$ signature and *Ralfsia* the highest, while at Sheffield Beach, the SPM had the lowest $\delta^{13}C$ signature and the dwarf cushion-star (*P. exigua)* the highest. As for $\delta^{15}N$, *U. lactuca* was the lowest (**Appendix A**; **Table A 1**), and *Clinus* the highest, representing the top of this particular food web at Tsitsikamma. The SPM again had the lowest $\delta^{15}N$ signature, and the Knysna sandgoby (*P. knysnaensis)* the highest, at Sheffield Beach.

For comparative purposes, unpublished data of stable isotope signatures from the shallow reef at Tsitsikamma (Heyns, 2015) is reflected in **Figure 3.4**, and were compared to the intertidal signatures. Trophic levels of the same species were compared between the intertidal rocky shore and the shallow reef of Tsitsikamma, based on the $\delta^{15}N$ values and on their feeding habits. Similarly to the intertidal region, the phytoplankton (in this study SPM) of the shallow reef was the lowest, while the dageraad, *Chrysoblephus cristiceps*, fish species was the highest species in that particular food web. The $\delta^{13}C$ values ranged from -27 ‰ to -9.7 ‰ and $\delta^{15}N$ from 3.8 ‰ to 15.5 ‰.

The different trophic levels are also indicated in **Figure 3.2**, **Figure 3.3** and **Figure 3.4**, and were derived from the $\delta^{15}N$ signatures of base species ($\delta^{15}N_{\text{primary producer}}$), *U. lactuca* and the SPM at Tsitsikamma and Sheffield Beach, respectively, and ranged from primary producers to tertiary consumers. Tsitsikamma, has a more stable intertidal food web structure within the warm temperate climate zone, with species overlapping between different trophic groups. Whereas, Sheffield Beach, located in the subtropical region, is less integrated and shows a carbon depleted structure (**Figure 3.2** and **Figure 3.3**). A total food chain length of 3.55 for Tsitsikamma and 3.66 for Sheffield Beach was recorded (**Appendix A**; **Table A 1**).

3.3.2. Trophic level and food chains

The $\delta^{15}N$ values of species assigned to a similar trophic status (derived from the TL equation), were pooled to allow for statistical comparison between the two climate zones (**Figure 3.5**). In all of the trophic groups, the $\delta^{15}N$ signature of species from the warm temperate Tsitsikamma was higher than the subtropical Sheffield Beach (**Figure 3.5**). A significant difference ($p < 0.05$) was observed between all the trophic groups from both sites (**Figure 3.5**).

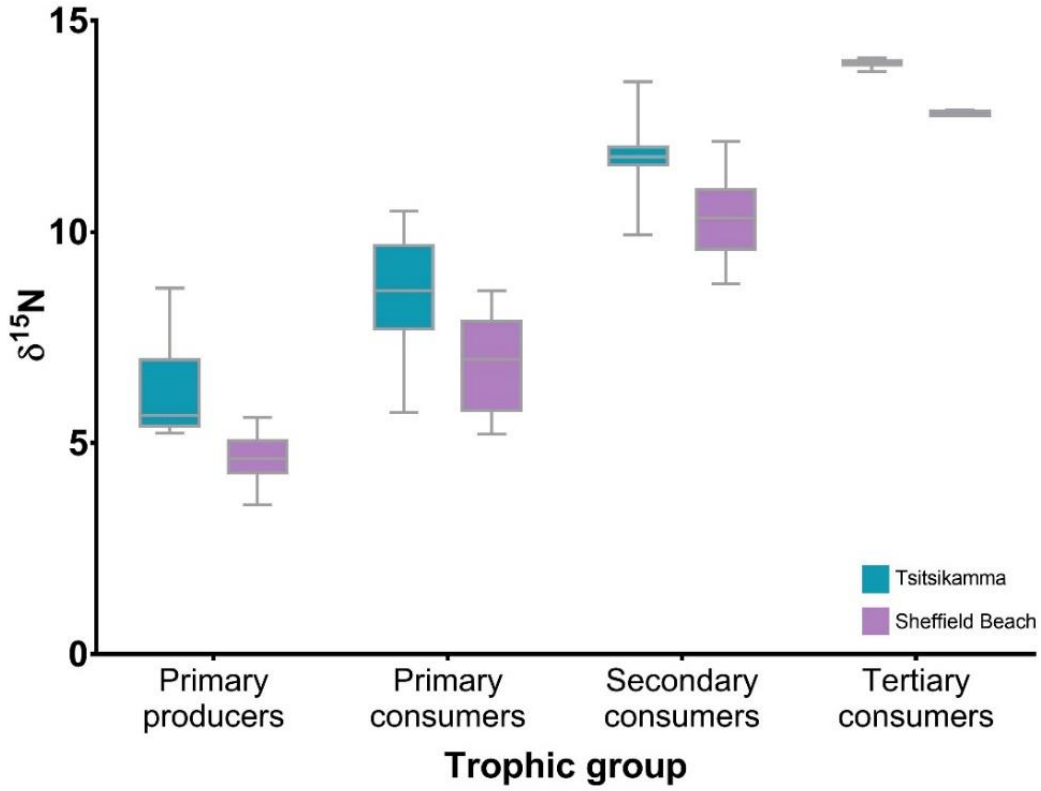


Figure 3.5: *Mean δ15N values for each trophic group from both sites, where all the trophic groups differed significantly between the two sites.*

Direct energy transfer between organisms was determined using the species that were not significantly different in $\delta^{13}C$ signatures along with literature on feeding habits of specific species. A variety of food chains were identified where one chain form each site were selected to represent a food chain for each biogeographic region (**Figure 3.6** and **Figure 3.7Error! Reference source not found.**). This chain will be used for further analyses in **Chapter 4** to determine trophic transfer of metals and organochlorine pesticides.

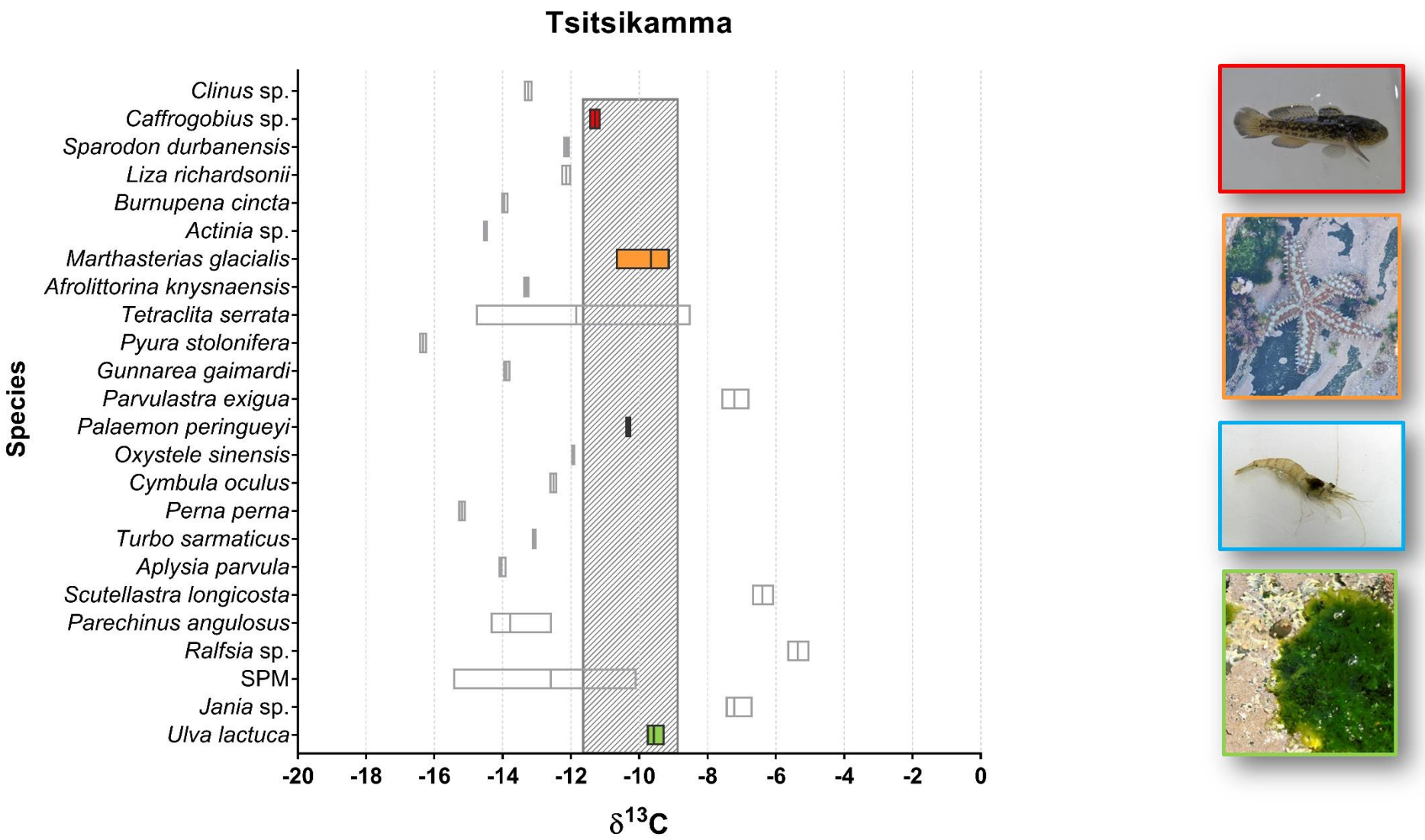


Figure 3.6: *Minimum and maximum $\delta^{13}C$ ranges of all the species collected at Tsitsikamma. The coloured bars indicate species identified as a food chain based on non-significant $\delta^{13}C$ values and supporting literature. Photographs depict the species selected from each trophic level.*

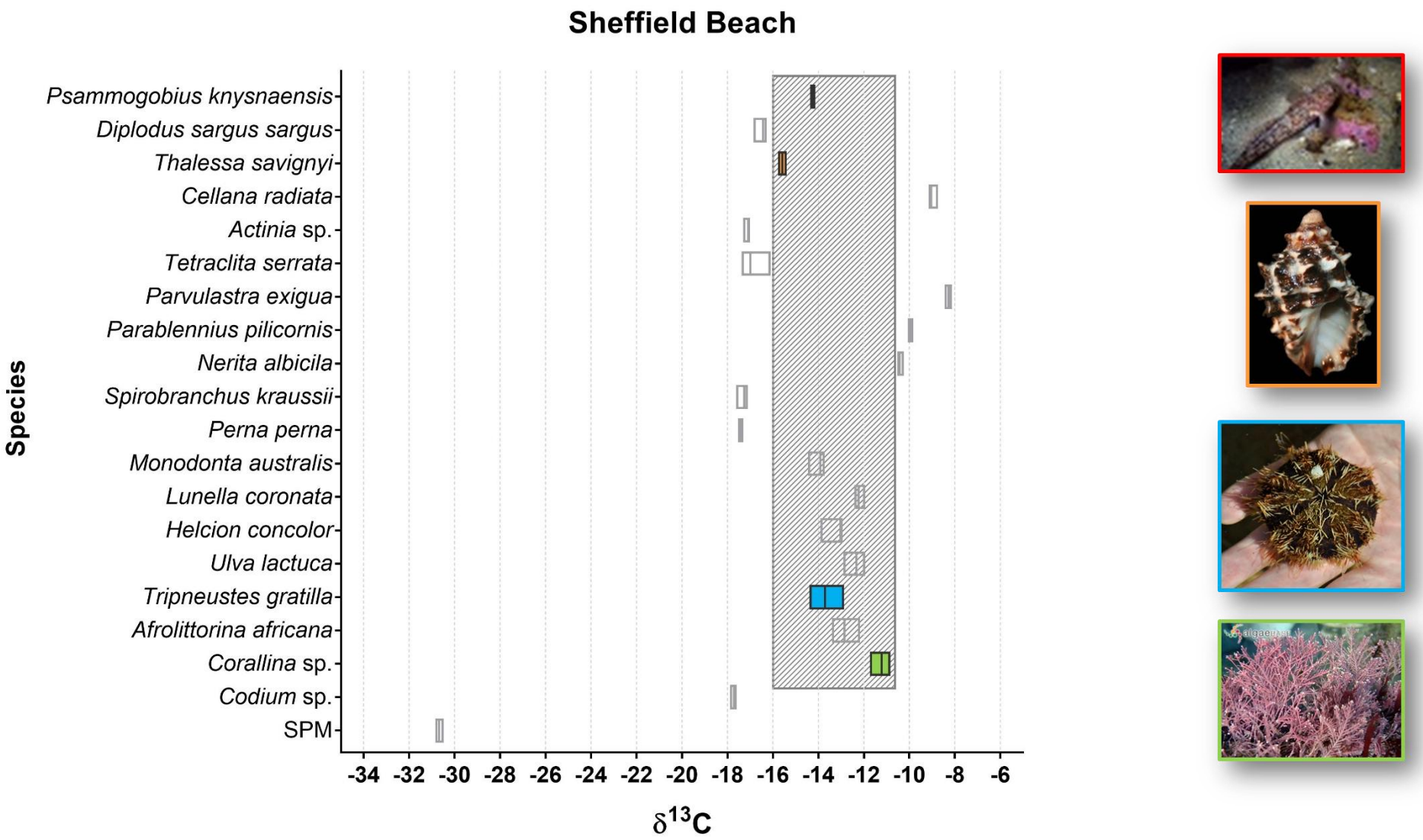


Figure 3.7: *Minimum and maximum $\delta^{13}C$ ranges of all the species collected at Sheffield Beach. The coloured bars indicate species identified as a food chain based on non-significant $\delta^{13}C$ values and supporting literature. Photographs depict the species from each trophic level.*

3.3.3. Group assessments according to functional trophic groups

A Discriminant Function Analysis (DFA) was performed in order to determine whether naturally occurring carbon and nitrogen signatures, as well as the C:N ratios of each organism, can discriminate between the four different trophic groups. The DFA reclassified 84.2 % and 86.1 % of the original groups in the Tsitsikamma and Sheffield Beach sites, respectively (**Table 3.1** and **Figure 3.8**, **Table 3.2** and **Figure 3.9**). From **Table 3.1** it is evident that the model used was able to predict 75 %, 86.4 %, 80.6 % and 100 % of the actual group memberships, for each of the trophic groups at Tsitsikamma, respectively. The Sheffield Beach site predicted 75 %, 68.4 %, 97.5 % and 100 % of the respective trophic groups' actual memberships (**Table 3.2**). Apart from some algae species categorized in the primary consumer group and invertebrates within the primary producer groups, most of the species were accurately classified.

Table 3.1: *The reclassification success as determined by discriminant function analysis for Tsitsikamma. Alphabetical superscripts indicate average classification success. Values in bold indicate percentage correctly classified.*

		Predicted Group Membership (%) [a, b]				
Group	**n**	**Primary producers**	**Primary consumers**	**Secondary consumers**	**Tertiary consumers**	**Total**
Primary producers	12	**75**	25	0	0	100
Primary consumers	44	4.5	**86.4**	9.1	0	100
Secondary consumers	31	0	6.5	**80.6**	12.9	100
Tertiary consumers	8	0	0	0	**100**	100

a. 87.4 % of original grouped cases correctly classified,
b. 84.2 % of cross-validated grouped cases correctly classified.

Table 3.2: *The reclassification success as determined by discriminant function analysis for Sheffield Beach. Alphabetical superscripts indicate average classification success. Values in bold indicate percentage correctly classified.*

		Predicted Group Membership (%) [a, b]				
Group	**n**	**Primary producers**	**Primary consumers**	**Secondary consumers**	**Tertiary consumers**	**Total**
Primary producers	16	**75**	25	0	0	100
Primary consumers	19	26.3	**68.4**	5.3	0	100
Secondary consumers	40	0	0	**97.5**	2.5	100
Tertiary consumers	4	0	0	0	**100**	100

a. 88.6 % of original grouped cases correctly classified,
b. 86.1 % of cross-validated grouped cases correctly classified.

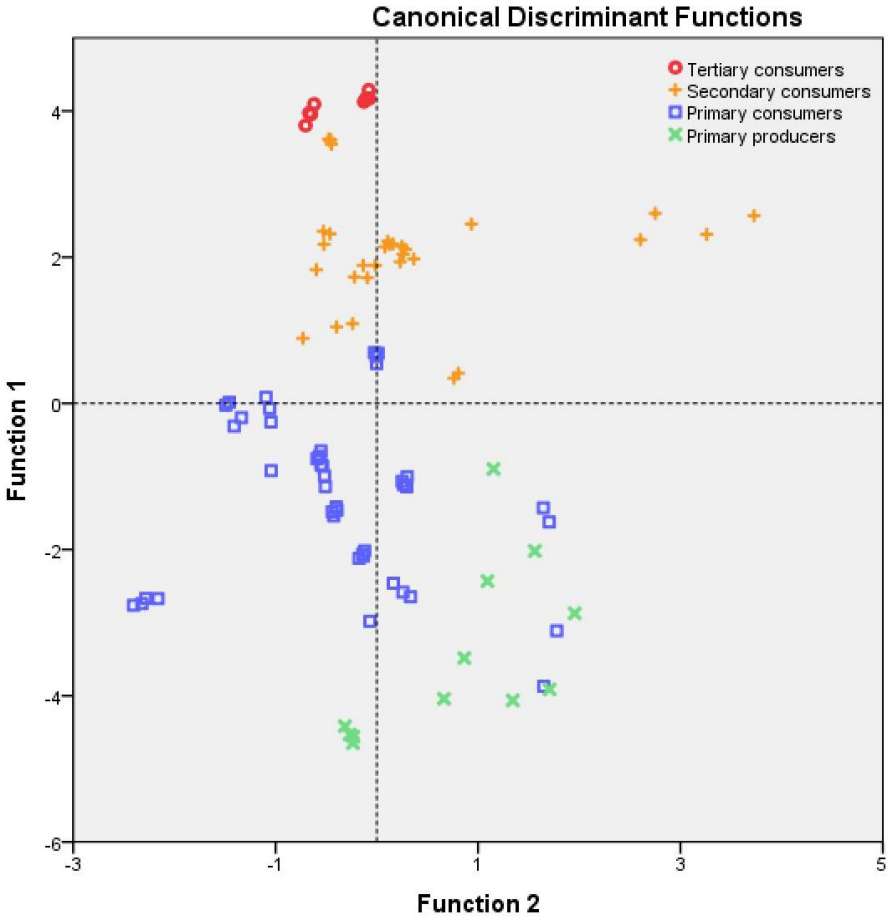


Figure 3.8: *Canonical bi-plot resulting from discriminate function analysis, using isotopic data of individual samples from different trophic groups of the warm temperate Tsitsikamma intertidal rocky shore. The graph was rotated to depict a top-down and bottom-up food web. Function 1, on the x-axis, explain 95.9 % and, Function 2, on the y-axis, explain 3.7 % of the variance in the analysis.*

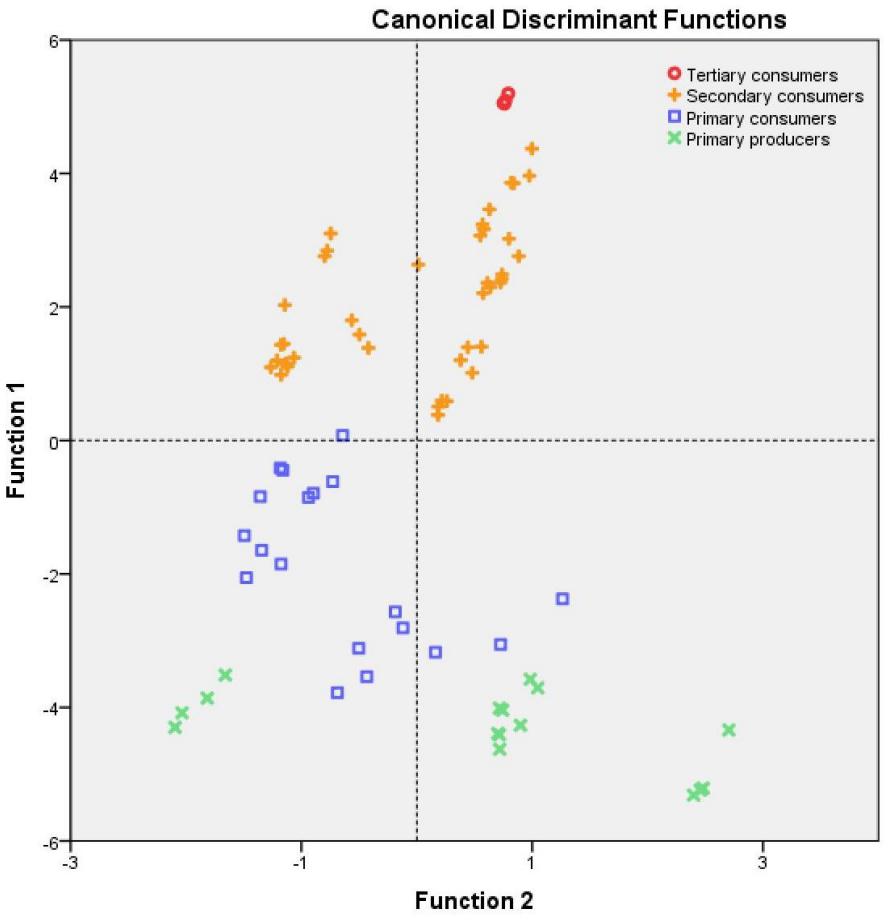


Figure 3.9: *Canonical bi-plot resulting from discriminate function analysis, using isotopic data of individual samples from different trophic groups of the subtropical Sheffield Beach intertidal rocky shore. The graph was rotated to depict a top-down and bottom-up food web. Function 1, on the x-axis, explain 97.6 % and, Function 2, on the y-axis, explain 2.3 % of the variance in the analysis.*

3.4. Discussion

When considering the food web as a whole, a trophic increase of 10 – 15 ‰ is typical for nitrogen isotope signatures (Peterson & Fry, 1987), which was also the departure for this study. In order to establish the different trophic organization of the intertidal rocky shores of the two climate zones, a wide range of species were sampled and analysed for stable isotope signatures. These trophic organizations are better known as food web structures of natural ecosystems that indicate energy transfer between species (Abrantes et al., 2014; Middelburg, 2014; Krumins et al., 2013). According to Dalling (2012) food webs are defined as quantitative frameworks that link community structure with fluctuations of energy and sources, merging ecosystem functions and the associated biodiversity. The focus is to diagrammatically present interactions of webs composed of different species (Dalling, 2012). Carbon isotopes vary temporally and spatially, as well as during photosynthesis where the degree of fractionation is influenced by light and nutrients (MacKenzie et al., 2011). These isotopic signatures of carbon and nitrogen are transferred through the food web from one species to the next (MacKenzie et al., 2011). In this case, four trophic levels were identified through functional trophic groups (Little et al., 2009) where fractionation of the isotopes takes place. As seen in **Figure 3.2**, **Figure 3.3** and **Figure 3.4**, trophic groups are clustered together, integrating a variety of carbon-based sources.

From the analyses the following conclusions are drawn in support of preferred habitats and feeding habits identified within the two intertidal shores.

3.4.1. Stable isotope signatures

The results found during this study indicate that Tsitsikamma, a MPA, showed a greater diversity in species (**Figure 3.2** and **Figure 3.3**). Tsitsikamma also had a higher mean trophic level for all the trophic groups than Sheffield Beach, which was significantly lower than Tsitsikamma (**Figure 3.4**). The species diversity can be ascribed to the distribution of the species sampled, since only some of the species collected during this study overlapped between the sites. The distribution of species on the south coast is representative of both the cooler Atlantic- and the warmer Indian Ocean, while species distribution on the east coast is only driven by the warmer Indian Ocean.

Studies conducted on SPM, macro-algae and filter feeders from the South African west to the east coast (Hill et al., 2006; Hill et al., 2008; Middelburg, 2014; Puccinelli et al.,

2016a; Puccinelli et al., 2016b; Puccinelli et al., 2016c; Abrantes et al., 2014; Kohler et al., 2011; De Lecea et al., 2013) indicate an enriched west coast with regards to carbon signatures, which decrease continuously from west to east. This is also the case in the present study with a depleted east coast increasing to the south coast. The increase in $\delta^{13}C$ has been ascribed to the deflection of the Agulhas Current by the Agulhas Bank (Lutjeharms, 2006). The $\delta^{13}C$ signatures are also depleted at upwelling sites like the St. Lucia upwelling cell (Puccinelli et al., 2016b; Gustella, 2014). Nitrogen signatures from the same studies remained constant on the west coast and also decreased from south to east (Hill et al., 2006; Hill et al., 2008; Middelburg, 2014; Puccinelli et al., 2016a; Puccinelli et al., 2016b; Puccinelli et al., 2016c; Abrantes et al., 2014; Kohler et al., 2011; De Lecea et al., 2013). The Benguela Current flows along the west coast of South Africa, bringing nutrient rich water to the coastal regions, while the Agulhas Current brings oligotrophic waters to the south and east coasts (Puccinelli et al., 2016b). Eutrophic waters are enriched with nutrients, resulting in high $\delta^{15}N$ values on the west coast decreasing to the east coast, where recycled nutrients are a result of oligotrophic waters (Puccinelli et al., 2016b; Hill et al., 2006). This result is evident in the present study, where $\delta^{15}N$ values of the primary producers from the south coast are typically higher than on the east coast.

Sheffield Beach is situated tens of kilometres from any major or permanently open estuaries and assumed not to be influenced by freshwater (Puccinelli et al., 2016c). However, Sheffield Beach is located in the Natal Bight, which receives nutrients from the St. Lucia upwelling cell. This can explain the enriched $\delta^{15}N$ values detected for *P. perna* at the KZN site compared to the lower $\delta^{15}N$ value at Tsitsikamma, since this species is a filter feeder and SPM are nitrogen enriched in areas associated with upwelling.

3.4.2. Trophic level and food chains

Parenchinus angulosus and *T. gratilla,* urchin species from Tsitsikamma and Sheffield Beach, respectively, were categorized as primary consumers feeding on benthic macro-algae and algal turf, and sometimes other organisms as well (Cabanillas-Terán et al., 2016). Urchins use specialized teeth that dig and remove encrusted coralline algae with $CaCO_3$ using their Aristotle's lanterns (Little et al., 2009). Some algae have adapted to herbivory damage by a mechanism referred to as 'activation' defence, where chemicals released by the algae can cause discouragement under urchins and other grazers (Little et al., 2009; Van Alstyne & Houser, 2003). This phenomenon occurs in temperate regions

from both hemispheres (Little et al., 2009). Species of starfish have also been known to feed on urchins.

Two species of limpet were sampled at each of the sites. At Tsitsikamma the duck's foot limpet, *S. longicosta,* and the goat's eye limpet, *C. oculus,* and at Sheffield Beach the rayed wheel limpet, *C. radiate,* and the variable limpet, *H. concolor*. At Sheffield Beach one species maintained a functional trophic group higher than the other, which can be ascribed to different feeding habits, since *C. radiate* is characterized as a predatory species (**Table 3.3**). While at Tsitsikamma, both limpet species are herbivorous and clustered within the same functional trophic group. The herbivorous limpets such as *S. longicosta,* maintain a patch of brown algae, *Ralfsia,* which they protect from other species of algae and limpets (Little et al., 2009). From **Figure 3.2** it is evident that *S. longicosta* associates with the brown algae garden.

Turbo sarmaticus, the giant alikreukel together with *P. stolonifera*, are commonly exploited species on the south and east coasts of South Africa. They are consumed and used as bait for fishing. The opercula of the former species have been known to indicate historic sea surface temperatures via the stable isotope signature of $\delta^{18}O$ (Galimberti et al., 2016).

The two species of dogwhelk sampled, *B. cincta* and *T. savignyi,* had similar $\delta^{15}N$ signatures of 11.9 ‰ and 11.2 ‰, at Tsitsikamma and Sheffield Beach, respectively. Dogwhelks are characterized as some of the most common predators on rocky shores. They are known to overcome the prey's adaptation to withstand predation, in which case the dogwhelks use their radulae to drill holes through the prey's shell or by pushing their proboscis down the cnidarians' siphon. They then inject narcotics and digestive enzymes and sucks up the tissue (Little et al., 2009; Branch et al., 2010). For this reason, the dogwhelks from both sites were classified as secondary consumers (**Figure 3.2** and **Figure 3.3** and **Appendix A**; **Table A 1**). This was also the case for the dogwhelks collected within the shallow reef of Tsitsikamma (**Figure 3.4**; Heyns, 2015). Dogwhelks feed on mussels, barnacles, oysters, limpets and anemones (Little et al., 2009; Branch et al., 2010).

A variety of fish species were collected ranging from secondary to tertiary consumers of both omnivorous and predatory/scavenger feeding habits at both of the sites. *Caffrogobius* sp. occupied a whole trophic level higher at the Tsitsikamma site than *P. knysnaensis* at the Sheffield Beach site (**Figure 3.2**, **Figure 3.3** and **Appendix A**; **Table A 1**). *Parablennius pilicornis*, at Sheffield Beach, was classified as secondary consumer

relative to the $\delta^{15}N$ signature of SPM. This species has omnivorous feeding habits (**Table 3.3**), and the isotope signature is reflective of its collective prey.

Compared to the same species sampled by Heyns (2015) in the shallow reef, there was a variation of + 3 ‰ and – 3 ‰ in $\delta^{13}C$ and $\delta^{15}N$ values. These species include cape urchin (*P. angulosus),* a species of sea hare *(Aplysia* sp.*),* cape reef-worm *(G. gaimardi),* ridged dogwhelk *(B. cincta)* and *Clinus* sp. except in the case of *P. angulosus*, all the values were higher in the intertidal zone than in the shallow reef. According to Hill et al., 2006 nearshore $\delta^{13}C$ sources are enriched, while offshore sources are depleted and remain relatively constant as mixing of the carbon sources occur.

To test the transfer within food chains, species were identified based firstly on $\delta^{13}C$ signatures and secondly as supported by literature. The chains from both Tsitsikamma and Sheffield Beach consist of one species from each trophic group. The species within the Tsitsikamma food chain can easily be concluded from **Figure 3.6**, while at the Sheffield Beach site (**Figure 3.7**), some literature input was needed. The Tsitsikamma food chain comprise of *U. lactuca* at the base, followed by *P. peringueyi*, *M. glacialis* and *Caffrogobius* sp. at the top of the chain. These findings are supported by literature listed in **Table 3.3** in which case *P. peringueyi,* a scavenger, can be classified as a detrital feeder, which represent the macrophyte they are associated with (Foreman & Henninger, 2010). *Marthasterias glacialis,* an omnivore, was included in the food chain as secondary consumer and *Caffrogobius* sp. as tertiary consumer (**Figure 3.6**, **Table 3.3**). These species can exhibit similar $\delta^{13}C$ signatures not solely because they feed on one another but since they are omnivorous, it can also be due to the fact that they feed on similar prey sources.

At Sheffield Beach the food chain consisted of *Corallina* sp., *T. gratilla, T. savignyi* and *P. knysnaensis*. Even though these conclusions could not be directly drawn from the $\delta^{13}C$ signatures, literature evidence indicates that feeding habits of these species are as follow: *T. gratilla* feeds on algae that contain high levels of calcium carbonate, i.e. *Coralline* sp., and use this to strengthen their shell and teeth (Little et al., 2009). Since the isotopic compositions of different tissues vary according to isotope routing, the tissues analysed will not necessarily reflect the composition of the diet as a whole (Gannes et al., 1997). For this reason it can be assumed that the $\delta^{13}C$ from the algae was routed to the shell and teeth of *T. gratilla,* instead of the soft tissues analysed. The secondary and tertiary consumers from this food chain are omnivorous, predatory feeders (**Table 3.3**). The same will then apply to *T. savignyi*, which is $\delta^{13}C$ depleted in comparison to *T. gratilla,* since

high levels of calcium carbonate are sequestrated for shell and teeth formation. *Monodonta australis* indicated no significant difference ($p < 0.05$) to *T. gratilla* but is a known herbivore, and can thus be assumed that they feed on similar prey. Further interpretation of the food chains will be done in **Chapter 4.**

3.4.3. Group assessments according to functional trophic groups

The classification success of both the warm temperate and the subtropical intertidal rocky shores were determined by DFA. The DFA serves to distinguish between the predefined trophic groups and to determine the overlap of species between the groups. The Tsitsikamma DFA depicts a diamond shape with *S. longicosta* and the spiny starfish (*M. glacialis)* at the widest ends of the dataset, while the species at the Sheffield Beach site depicts a pyramid shape with no distinct outliers (**Figure 3.8** and **Figure 3.9**). The different shapes and integration of the trophic groups, or the lack thereof, are indicative of the stability and variety of feeding habits of each of the food webs. Stable food webs have more integrated trophic groups, as seen at the Tsitsikamma site. Due to the fact that Tsitsikamma's shore slopes more gradually with a variety of pools at many different levels, a greater variety of habitats exist for organisms to thrive in (Little et al., 2009). Sheffield Beach has a step rocky shore with only a few pools at greater intervals (Little et al., 2009), resulting in fewer habitats. The pools at the Sheffield Beach site are not connected like the pools at the Tsitsikamma shore and can result in the distinct trophic groups seen in **Figure 3.8** vs. the integrated trophic groups in **Figure 3.9**.

From the reclassification it is evident that some of the species can be classified into different groups than what has been previously calculated. As a result some of the trophic groups show an overlap of species, which can be attributed to various factors. According to Gannes et al. (1997), through analysing only certain tissues of an organism the actual carbon and nitrogen signatures can be misconstrued and should be taken into account when food webs are analysed. From this it can be concluded that species within the overlap may not be accurately classified according to the calculated values and literature should also be consulted before any definite conclusions are drawn. Identifying the habitats and feeding modes of the organisms sampled (**Table 3.3**), can help to explain the results obtained from the stable isotope and discriminate function analysis.

Table 3.3: *Preferred habitats and feeding modes of all the species sampled according to literature. Specific dietary comments are also included, as well as the trophic levels for fish species. Numeric superscripts indicate each of the references used.*

No.	Species	Preferred habitat/zonation/habits	Feeding mode	Dietary comments	TL
			Fish		
1	South African grey mullet, *Liza richardsonii* [4]	Endemic[1] South-East Atlantic: from Walvis Bay to Natal[2] Estuaries (as nursery area), rocky and sandy bays[1,2] Extremely tolerant towards variable salinities[1]	Mainly plants/detritus[2] Variable[2]	Phytoplankton and benthic diatoms[1] Detritus, sponges and planktonic crustaceans[2] Zoobenthos[2]	2.4 ± 0.16 (based on food sources)[2]
2	Speckled klipfish, *Clinus* sp.[4]	Endemic[1] South-East Atlantic: Lüderitzbucht to Port Alfred[2] Tidal pools and subtidal reefs to ± 15 m[1]	Benthic crustaceans[2] Planktonic crustaceans[2]	Amphipods, isopods, echinoderms and mysids[2]	3.3 ± 0.48 (base on food sources)[2]
3	Banded goby, *Caffrogobius* sp.[4]	Endemic[1] Western Indian Ocean and South-East Atlantic: Delagoa Bay to Cape Peninsula[2] Epibenthic, littoral, intertidal in rock pools[2] Tidal pool occupant[1]	Omnivorous[1] Opportunistic feeder[1]	Eagerly takes bait from junior 'bent-pin' anglers[1] Zoo benthos and plants including: crabs, benthic algae/weeds, shrimps, amphipods, copepods, ostracods, molluscs and polychaetes[2]	3.0 ± 0.42 (based on food sources)[2]
4	Musselcracker, *Sparodon durbanensis*[4]	Endemic[1] Rocky shores and shallow reefs[1] Shallow, rocky coastal areas to 80 m depth[2]	Carnivorous[1,2] Omnivorous[1,2]	Juveniles feed on algae and shift to hard-shelled prey as molars develop, adults are solitary and feed on molluscs, redbait, sea urchin, crayfish, sea cucumbers, crabs and other benthic invertebrates[1, 2] Highly sought-after rocky shore angling species, takes almost any bait[1, 2]	3.6 ± 0.40 (based on food sources)[2]
5	Ringneck blenny, *Parablennius pilicornis*[4]	Western Indian Ocean: Natal to Knysna[2] Tidal pools[1] Steep walls of surf-exposed sites[2]	Omnivorous[1]	Seaweeds, crustaceans[1] Molluscs, sponges/tunicates, worms, benthic crustaceans, cnidarians and benthic algae/weeds[2]	3.2 ± 0.40 (based on food sources)[2]

No.	Species	Preferred habitat/zonation/habits	Feeding mode	Dietary comments	TL
6	Knysna sandgoby, *Psammogobius knysnaensis*[4]	Endemic[1] Sandy banks in estuaries[1]	Predatory[2] Zoobenthos[2] (benthic invertebrates) [1]	Feeds on small benthic invertebrates[1] Hunts microfauna[2]	3.3 ± 0.3 (based on food sources)[2]
7	Blacktail, *Diplodus sargus sargus*[4]	Endemic[1] Ubiquitous in range of shallow habitats[1] Juveniles are common in tidal pools and estuaries[1] Frequents the surf zone, primarily at dawn[2] Coastal rocky reef areas[2]	Omnivorous[1] Predatory[2]	Mollusc, cnidarians, echinoderms, benthic crustaceans, worms, plants, larvae fish/eggs, planktonic crustaceans, cephalopods, sponges/tunicates and other benthic invertebrates picked from the sediment[2] A fine, light-tackle angling species[1]	3.4 ± 0.1 (based on food sources)[2]
Invertebrates					
8	Spiny starfish, *Marthasterias glacialis*[4]	Low intertidal to nearly 200 m depth, on rock and gravel[4]	Predator[1, 4] Omnivorous, scavenger[4]	Mussels, winkles, limpets, urchins, barnacle and redbait[1, 12] Extrudes stomach and digests prey externally, sometimes forms large feeding aggregations and a voracious predator[1]	
9	Knobby dogwhelk, *Thalessa savignyi*[4]	Under rocks[1] Low shore pools[1] Shallow waters[1]	Predatory[1] Scavenger[1]	Sea squirts, gastropods and bivalves[1]	
10	Ridged burnupena, *Burnupena cincta*[4]	Low shore[1] Subtidally[1]	Scavenger[1]	Scavenges on dead or injured animals[1]	
11	Plum anemone, *Actinia* spp.[4]	High on shore (closing up at low tide and trapping water in cavity), also in areas up to 20 m deep [1, 5] Shady gullies and overhangs[1] Considerably versatile intertidal anemone[5]	Carnivorous[5]	Attacks using nematocysts and digests prey in central cavity and excrete through mouth[1, 5] Molluscs, benthic invertebrates, isopods, gastropods, bryozoans and chitons[5]	
12	Red bait, *Pyura stolonifera*[4]	Extensive aggregations on wave-exposed rocks from low tide to ± 10 m[1] Loose, boulder-like masses on seabed[1]	Inhalant and exhalent siphons[1] Zooplankton[6]	Commensal copepods, amphipods and pea crabs in pharynx[1] Harvested as food source[1] Diatoms, radiolarians, dinoflagellates, bacteria[6]	

No.	Species	Preferred habitat/zonation/habits	Feeding mode	Dietary comments	TL
13	Volcano barnacle, *Tetraclita serrata*[4]	Mid-intertidal zone[1] Wave-exposed locations[1]	Suspension feeder [1]	Highly modified and combs through water for food[1]	
14	Sand shrimp, *Palaemon peringueyi*[4]	Intertidal pools and shallow areas[1, 15] Bottom dwelling[1] Estuaries[15] In submerged macrophytes[15]	Scavenger[1]	Scavenges fragments of dead animals[1] Intimate relationship with some species (cleaning)[1] Detrital feeder[15]	
15	Cape reef worm, *Gunnarea gaimardi*[4]	Intertidal reefs with wave action[1] Large colonies[1] Aggressive for space[1]	Suspension feeder[1]	Feeds on particles concentrated by wave action in entrance of their tube, using feeding tentacles[1]	
16	Blue coral worm, *Spirobranchus kraussii*[4]	Abundant in moderately exposed shores[1] Fringing pools[1]	Suspension feeder [1]	Uses feeding tentacles[1]	
17	Brown mussel, *Perna perna*[4]	Crowded lower shore intertidal beds[1] Co-exists with *M. galloprovincialis* (higher on shores) on south coast[1]	Filter feeder[1]	Important resource for subsistence fishers[1] Bored into shells by mussel phoronan (prone to parasitism) [1]	
18	Spotted sea hare, *Aplysia parvula*[4]	Shallow bays[1] Intertidal rocky pools[1, 7]	Specialized herbivore[1, 7] Algivorous[5] Browser [12]	Feeds nocturnally on seaweeds, red and green algae[1] Some sea hares are specialized predators[1] Body colour changes with food consumed[5]	
19	Dwarf cushion-star *Parvulastra exigua*[4]	Southern hemisphere, intertidal [13]	Herbivorous (microscopic algae)[1]	Extrudes stomach and digest microscopic algae by plastering stomach on rocks[1]	
20	Cape urchin *Parechinus angulosus*[4]	Abundant on rocky shores and in kelp beds[1] Provides nursery for abalone[8]	Grazer[1] (kelp and algal debris)	Important for controlling survival of newly settled kelp plants[1] Use dead shells as shade[1] Prey for rock lobster [8]	

No.	Species	Preferred habitat/zonation/habits	Feeding mode	Dietary comments	TL
21	Collector urchin *Tripneustes gratilla*[4]	Rocky shore[1] Weedbeds[1] Often concealed in pieces of algae and shell overhead[1]	Grazer[1] Detrivorous (sand-dwelling)[1] Omnivorous[5]	Encrusted algae, aquatic grasses, plants, small organisms like worms, crustaceans and other marine invertebrates[5] Feeding decreases at high temperatures (summer) and increases in at low temperatures (winter)[5] Calcium carbonate shell and teeth[1]	
22	Pink-lipped topshell *Oxystele sinensis*[4]	Low on shore to ± 5 m[1]	Herbivorous[1]	Grazes on encrusted algae and micro-algae[1] Harvested as food source in Transkei[1]	
23	Giant alikreukel *Turbo sarmaticus*[4]	Pools down to ± 8 m[1] Intertidal zone (mostly in MPAs) [1] Red data species[1]	Herbivorous[1]	Consumed by humans[1] Algae from Rhodophyta, Chlorophyta, Phaeophyta [9]	
24	Blotched nerite *Nerita albicilla*[4]	Abundant aggregations in mid-shore pools[1,11] Under damp boulders[1]	Grazer[1] Herbivorous[11]	Nocturnally feeding on lichens, encrusted algae and diatoms[1] Cuts into rock-face with powerful radula to remove embedded micro-algae[1]	
25	Crown turban shell *Lunella coronata*[4]	Mid-shore pools[1] Under boulders[1]	Herbivorous[1, 7]	Small epibenthic algae and vegetable detritus[7]	
26	Toothed topshell *Monodonta australis*[4]	Very common intertidal species[1] Usually found in pools[1]	Grazer[1]	Micro-algae[1]	
27	African periwinkle *Afrolittorina africana*[4]	Dominant at top of rocky shores[1]	Herbivorous[1]	Feeds mainly on diatoms, micro-algae and lichens[1]	
28	Southern periwinkle *Afrolittorina knysnaensis*[4]	Dominant at top of rocky shores[1] (Juveniles higher up to avoid wave action) [1]	Herbivorous[1]	Nocturnally feeds on diatoms, microalgae and lichens[1]	

No.	Species	Preferred habitat/zonation/habits	Feeding mode	Dietary comments	TL
29	Duck's foot limpet *Scutellastra longicosta*[4]	Rocky shores, found in the Cochlear zone as well as the Balanoid zone[14]	Herbivorous[1] Grazer[1]	Feeds on encrusted algae, *Ralfsia,* on other shells as juveniles, move to rock-face and feed on encrusted coralline until they establish algal gardens of *Ralfsia*[1] They increase the growth rate and reduce anti-herbivore chemicals produced by *Ralfsia*[1]	
30	Goat's eye limpet *Cymbula oculus*[4]	Mid-shore zone[1]	Herbivorous[1]	Controls algae growth[1] Large individuals aggressively attacks predators, slamming shells down to damage tissue[1] Consumed in Transkei[1] Flatworms live under their shells[1]	
31	Rayed wheel limpet *Cellana radiata*[4]	Co-exists with variable limpet[1] Littoral zone[7]	Predatory Omnivorous[16]	Algae, sponge spicules, organic debris and copepods[16]	
32	Variable limpet *Helcion concolor*[4]	Mid shore[1] Co-exists with rayed wheel limpet [1]	Herbivorous[1] Generalized grazer [16]	Algae, lichen and detritus[16]	

[1]Branch et al., 2010; [2]Fishbase, 2017; [3]MarLIN, 2017; [4]WoRMS, 2017; [5]Animal Diversity Web, 2014; [6]Haris, 1990; [7]SeaLifeBase, 2017; [8]Two Oceans Aquarium, 2017; [9]Foster & Hodgson, 1998; [10]Heidt et al., 2013; [11] Day, 1969; [12]Little et al., 2009; [13]Payne et al., 2015; [14]Van As et al., 2012, [15] Foreman & Henninger, 2010; [16]Branch, 1975

3.5. Conclusions

In conclusion, the use of stable isotopes enabled us to establish organism signatures, food chains and energy flow, food web structures and comparisons between two different temperature based biogeographic regions. From the results it was evident that Tsitsikamma, on the south coast, was enriched in $\delta^{15}N$ and has a greater range of $\delta^{13}C$ sources. These results correlate with literature stating that nutrients increased from east to west along the southern Africa coastline (Puccinelli et al., 2016b; Hill et al., 2006; Lutjeharms, 2006). Using these signatures in combination with literature, food chains were established for further analysis.

This study provides a baseline for investigating future changes in isotopic signatures and food web structures, and can be used as a reference food web for other trophic related studies. By considering the hydrodynamic and geomorphological characteristics of the study areas, these results are system specific and should be adapted accordingly for every other system. By establishing the stability of a food web, it can inform the development of more efficient policies to protect species and manage ecosystems.

Chapter 4: Trophic magnification of metals and organochlorine pesticides in intertidal organisms from two rocky shores along the South African coastline

4.1. Introduction

Aquatic ecosystem health is a matter of international concern. The National Biodiversity Assessment (Driver et al., 2011) identified increased coastal development as the greatest pressure on coastal biodiversity. There is a definite lack of data, past and present, on the levels and status of organic chemical concentrations within the marine environment of South Africa (Degger et al., 2011a). While there is more information available on inorganic metal concentrations along the South African coastline, there are no formalized national marine pollution monitoring programs that provide data for implementing sustainable conservation (DEAT, 2008; Degger et al., 2011b). Inorganic metals and organochlorine pesticides (OCPs: i.e. HCHs, DD_x, Chlordane, HCBs) concentrations pose an increasing threat to the South African coastline ecosystems (DEA, 2015). Concentrations of these contaminants in the environment can originate from various sources, including geological leaching and effluent from inland sources, as well as transported from elsewhere through atmospheric deposition processes (Gregory et al., 2002; Wepener & Vermeulen, 2005). Exposure to organisms by these contaminants can be derived from dietary uptake, adsorption onto the exoskeleton and absorption through the skin. Even though some invertebrates can detoxify metals and other contaminants to some extent (Rainbow et al., 2015), high concentrations thereof may become toxic when they interfere with vital processes of these organisms (Newman, 2010).

Bio-accumulation can be defined as the net consequence of uptake, biotransformation and elimination processes within an organism, while magnification occurs when these compounds are not digested, and accumulate within the organism from the next trophic level that ingest them (Newman, 2010). The compound then becomes more concentrated as they pass along the food chain (Hall, 2004). Thus dietary absorption occurs faster than elimination (Borgå et al., 2012; Gobas & Morrison, 2000) and tend to concentrate compounds that are threatening to top predators (and possibly humans) (Borgå et al., 2012; Letcher et al., 2010). One of the main reasons these contaminants are of such great concern is that they are highly persistent in the environment, depending on their half-life, their bio-accumulative nature in animal- and human tissue, and their potential to biomagnify within food chains (ATSDR, 2002). The bio-availability and subsequent

uptake of these contaminants are influenced by a number of factors including temperature, phase association, absorption and sequestration (Wang & Fisher, 1997).

4.1.1. Metals

Metals are naturally occurring elements that are present in the Earth's crust in low concentrations, while most of the contaminants found in the environment and within organisms are a result of anthropogenic activities (Tchounwou et al., 2012). Various biochemical and physiological processes require essential metals including: cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). When these metals are not available for organisms to maintain the processes it can result in deficiency diseases (Tchounwou et al., 2012). The ability of organisms to detoxify metals varies greatly depending on the metal and the organism (Rainbow et al., 2015) and there is no single defined toxic concentration of metals for all organisms (Liu et al., 2013; Rainbow et al., 2015). Rather, detoxification ability depends on the metabolically available metal (Rainbow et al., 2015).

Past coastal studies on metal toxicity has focused mainly on mussels, oysters and barnacles (Degger et al., 2011a; Degger et al., 2011b; Coetzee, 2015; Rainbow et al., 2015; Rainbow & Wang, 2001; Rainbow & Wang, 2005). It is important for the management of coastal environments to note the transport and biological effects of these metals on all organisms that inhabit the marine environment, especially those that are easily accessible at intertidal rocky shores. Copper and Zn are metals that may be essential to organisms in small amounts, while lead (Pb) and mercury (Hg) do not seem to have any metabolic function (Cuong et al., 2008; Rainbow & Wang, 2005). Cadmium (Cd), although a non-essential metal, does have a role in enzyme carbonic anhydrase under certain conditions within diatoms (Rainbow & Wang, 2005; Cullen et al., 1999). Whether essential or not, these metals tend to be toxic when the combined rate of excretion and detoxification is less than the rate of uptake (Rainbow & Wang, 2005; Rainbow, 2002). In this chapter the focus is on the ability of metals to be biomagnified through trophic transfer rather than the extent each organism is able to sequestrate and metabolize these metals.

4.1.2. Organochlorine pesticides

Little information on the concentrations of organic compounds are available for the South African coastal environment. Some research has been done in polluted areas such as harbours (Degger et al., 2011a; Degger et al., 2011b; Greenfield et al., 2011; Greenfield

et al., 2014). Organochlorine pesticides (OCPs) are anthropogenic in origin and only one aspect of persistent organic pollutants (POPs), considered having adverse health effects on ecosystems, as well as humans (EPA, 2016). OCPs are not only toxic in the environment due to their persistent nature but they are also known to have considerably low water solubility (Walker, 2009). These compounds are also toxic to organisms, due to the fact that they are used as pesticides and herbicides to control certain processes within a system. They undergo biotransformation over long periods of time and tend to become less or more toxic depending on their chemical breakdown and mixture with other compounds (Newman, 2010). In the past the toxic effects of OCPs were not well known and even though some compounds have been banned due to harmful effects on ecosystems and species, they remain present within soils and tissues (Walker, 2009; Quinn et al., 2011).

The focus and variety of research regarding organic chemicals has increased over the past few decades. Alternative measures have been implemented to terminate the use of these compounds but they can still be detected in the environment and therefore affect ecosystems today (Newman, 2010). One of the most relevant organic contaminants in Southern Africa is the chemical used to control mosquitoes to prevent the spread of malaria. Even though this disease is responsible for many deaths, the chemical used to control it, dichlorodiphenyltrichloroethane (DDT), which inhibits the transfer of *Plasmodium* to humans, is classified as highly persistent in the environment. Chlordane is a widely applied insecticide that was in use since 1947, and is more readily being banned in many countries (WHO, 2003b). It has been reported to cause liver tumours in mice and interfere with cell exchange, and has been classified as a Group2B carcinogen in animals (WHO, 2003b).

Lindane, which includes hexachlorocyclohexane (HCH), is used for fruit and vegetable crops, to treat seeds and for forestry insecticides (WHO, 2003c). Even though it rarely leaches into groundwater it can degrade in soils (WHO, 2003c). It is important to know the fate and transport of these compounds within natural marine systems in order to maintain the management and protection of species and habitats.

4.1.3. Trophic magnification factors

By determining the background values of these contaminants, measures can be taken to prevent lethal concentrations and exposure to biota. According to recent research, TMFs are the most conclusive measure of determining biomagnification within food webs, especially in aquatic environments (Borgå et al., 2012; Walters et al., 2016). During the

present study, concentrations of metals and OCPs in aquatic biota were determined in order to establish the ability of uptake at the base of the food web and thereafter transfer to higher trophic levels (Hanna et al., 2015; Walker et al., 2006; Verhaert et al., 2017). This is calculated based on the regression of the trophic level (TL) and the log of the concentration of the contaminant (Borgå et al., 2012). The ideal would be to screen compounds that have a predisposition to magnify within natural ecosystems, before they are released into the environment. According to Walters et al. (2016) lower TMFs for freshwater are described compared to marine food webs, where freshwater TMFs are usually linked to compounds such as PCBs and breakdown products of DDT compounds. While marine TMFs exhibit low concentrations of PAHs that metabolized more rapidly due to amongst other factors, water solubility ranges. Climate changes also play a vital role on the TMF of compounds, in fact Borgå et al. (2012) states that subtropical regions will have lower TMF than temperate regions since the former has a high biomass dilution rate of these contaminants and have shorter food webs (Verhaert et al., 2017).

In this chapter, concentrations and transfer of compounds through food webs will be addressed by means of trophic magnification factors. The hypothesis states that stable isotope approach can be applied to demonstrate the transfer of inorganic (metals) and organic (OCPs) substances from one trophic level to another in the intertidal zone of two temperature based biogeographic regions. The aims of this chapter were to determine the bio-accumulation of both metals and OCPs in selected organisms from the intertidal zones of Tsitsikamma and Sheffield Beach; and to determine the degree of trophic transfer of these substances in the intertidal ecosystems to allow for comparison of the biomagnification potential of metals and OCPs between the two biogeographic regions.

4.2. Materials & Methods

4.2.1. Field survey and sample collection

Methods used for collection, preparation and transportation of organisms from the two sampling sites are described in **Section 3.2** of **Chapter 3.**

4.2.2. Laboratory analysis

Samples were pooled for all of the algal and invertebrate samples, except for the giant alikreukel, *T. sarmaticus,* and the fish samples, which were analysed individually. This was done to ensure sufficient tissue mass required for analyses (Verhaert et al., 2017). Samples were accurately weighed and noted with 0.001 g accuracy. The samples were

then freeze dried (Labconco® FreeZone 6; **Figure 4.1**a)) at 0.002 mBar and -50 °C for 72 h, where after the dry weight and moisture content were calculated, and the samples were ground for effective homogenization.

4.2.2.1. Metals

4.2.2.1.1. Laboratory preparation and sample analysis

Samples were analyzed for a standard set of metals but only the following metals were used during this study: aluminum (Al), titanium (Ti), vanadium (V), Cr, manganese (Mn), Fe, Co, Ni, Cu, Zn, arsenic (As), Se, Cd and Pb. Species selected for the metal analysis were based on the sample mass available. A sufficient number of specimens were collected to ensure that between six and ten replicate (individual, as well as pooled) samples of each species were analyzed. Acid digestion adapted from techniques described by Greenfield et al. (2011; 2014) were utilized using an Ethos Easy MAXI-44 Advanced Microwave Digestion System with Teflon vessels (Magna Analytical; **Figure 4.1**b)). Each sample was weighed up to 0.2 g and a volume of 7 ml 65 % suprapur nitric acid (HNO_3) and 1 ml peroxide (H_2O_2) were added to each of the vessels. The samples were digested on a pre-programmed method consisting of three steps with a maximum power output of 1800 W: (1) a ramping period of 15 min to reach 180 °C at an increasing pressure; (2) a holding period of 15 min at 180 °C with the pressure ranging between 80 and 100 bar; (3) a cool down period of 30 min to reach a temperature of ± 60 °C. After the cool down period, the samples were decanted and diluted to 50 ml with 1 % HNO_3, and filtered with a 0.45 µm cellulous nitrate filter (Ø 47 mm) (**Figure 4.1**c)) for inductive coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500CE) analysis to determine the metal concentrations. Concentrations obtained were converted according to the dilution factor with the following equation:

$$\frac{ICP - MS\ result \times dilution\ factor}{dry\ weight} = \mu g/g^{-1}\ dry\ weight$$

with ICP-MS results obtained in µg/g, dilution factor of the 1 % HNO_3 (50 ml) and the dry weight measured for each sample (up to 0.2 g). All further calculations were done using µg/g dry weight.

4.2.2.1.2. Quality control

For quality control purposes, blanks and certified reference material (CRM) were prepared in the same manner as the samples and analysed with every 20 samples. Reference material consisted of dried mussel tissue (ERM-CE278k). The concentrations

measured were in a 15 % range in accordance with the CRM and all of the blank samples were below the ICP-MS detection limit (DL) (**Table 4.1**).

Table 4.1: *Concentrations recovered from analysing ERM-CE278k certified reference material. Concentrations are reported in mg/kg.*

	ERM-CE278k		
	Reference	**Measured**	**% Recovery**
Mn	4.88	4.83	100.9
Fe	161.0	164.3	97.9
Cu	5.98	7.01	85.2
Zn	71.0	79.2	89.5
As	6.70	5.78	115.9
Se	1.62	1.54	104.5
Cd	0.336	0.324	103.7

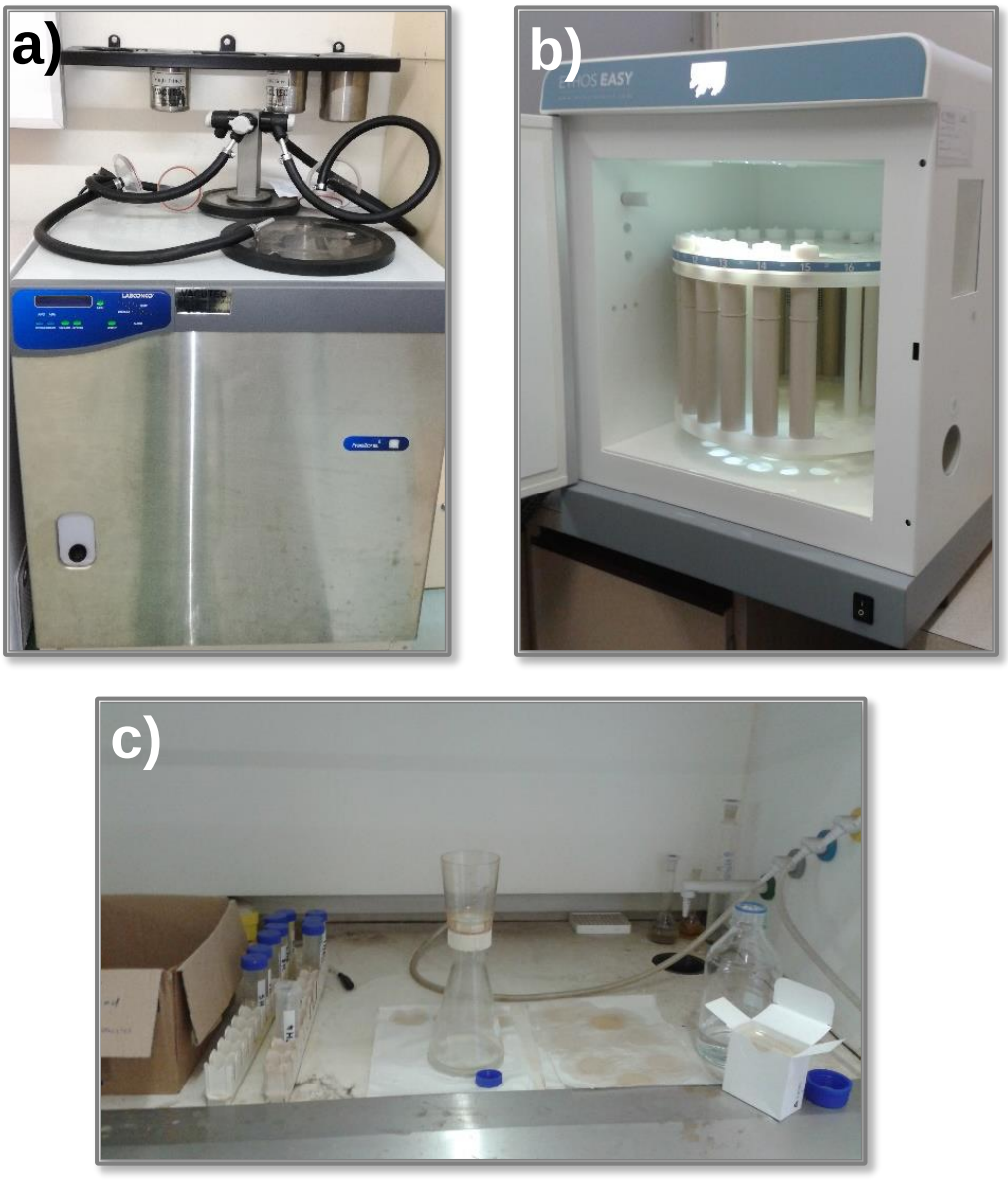


Figure 4.1: *Metal analysis were conducted using: a) Labconco® FreeZone 6 used to freeze dry samples from both sites, b) Ethos Easy MAXI-44 Advanced Microwave Digestion System used to acid digest samples and c) filtration and dilution set-up used before samples were sent for ICP-MS analysis.*

4.2.2.2. Organochlorine pesticides

4.2.2.2.1. Laboratory preparation and analysis

For the OCP analysis, only the species with sufficient mass were analysed for the following compounds: α-HCH, β-HCH, γ-HCH, δ-HCH, HCB, Aldrin, Dieldrin, Endrin, Heptachlor, *oxy*-Chlordane, *cis*-Heptachlor-epoxide, *trans*-Heptachlor-epoxide, *trans*-Chlordane, *trans*-Nonachlor, *cis*-Chlordane, *cis*-Nonachlor, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDT. The freeze dried samples were also used during this analysis. Methods were adapted from Chu et al. (2002) and Covaci et al. (2002; 2005; 2006) according to the available sample. Chemicals and solvents were used as described by Chu et al. (2002) and Covaci et al. (2002; 2005; 2006), and all of the equipment were washed with acetone and hexane prior to use and between samples. All of the samples were weighed to a minimum of 1 g dry weight and homogenized with an equal amount of Diatomaceous Earth (D.E.), and spiked with internal standards (ε-HCH and PCB 143). The samples were extracted for approximately 30 min by pressurized liquid extraction (PLE) (Dionex ASE machine) in a 66 ml cell and 3:1 hexane/acetone, using a pre-programmed method with three static cycles (**Figure 4.2**a) and b)). After PLE, samples were evaporated under nitrogen gas (± 0.5 bar) at 35 °C for approximately 30 min, washing the sides of the flask with hexane throughout, until 10 ml remained for lipid determination (20 %) from the remaining sample volume (**Figure 4.2**c) and d)). The samples were then cleaned-up with 4 g 44 % acidified silica and 4 g 5 % deactivated florisil packed in a 40 cm column, with Teflon stopcock (**Figure 4.2**e). Analytes were removed with 20 ml hexane and 15 ml dichloromethane (DCM) (**Figure 4.2**e) and were finally evaporated, until near dryness, and reconstituted in 100 µl 2,4,5,6-tetrachloro-m-xylene (TMX).

All of the OCPs were analysed using an HP 6890 Gas Chromatograph (GC) coupled with a micro Electron Capture Detector (µECD) equipped with a SGE HT8MS 30 m x 0.25 mm x 0.25 µm column (**Figure 4.2**f)) (Verhaert et al., 2017; Yohannes et al., 2013). A splitless injection of 1 µl per sample was used, with 1.5 ml / minute hydrogen carrier gas and nitrogen make-up gas. The pre-programmed oven programme consisted of four steps: (1) initial temperature of 100 °C held for 1 minute; (2) ramp period of 20 °C / minute until 200 °C was reached; (3) a further ramp period of 6 °C / minute until a temperature of 260 °C; and (4) a final holding period of 4 minutes. The inlet oven temperature was 225 °C and the detector temperature was set at 310 °C.

4.2.2.2.2. Quality control

A standard calibration curve from five concentrations (10 µg/l – 500 µg/l) was used. The identification criteria consisted of the retention times, ion chromatograms and relative abundance of the monitored ions. Quantification of the analytes was determined from the peak area of the sample to the peak area of the standard. The extraction method for determining the OCP concentrations using a small sample mass was validated in the laboratory using a spiked matrix with an average recovery of 80 % on the internal standards (ε-HCH and PCB 143). The correlation coefficient (r^2) ranged between 0.985 and 0.999 for all of the analyses.

Figure 4.2: *Organochlorine pesticide extraction was conducted by a) Pressurized liquid extraction (PLE) (Dionex ASE machine) using a 66 ml cell and 3:1 hexane/acetone to extract organic compounds from the sampled species; b) samples after pressurized liquid extraction; c) samples after nitrogen evaporation; d) 20 % of evaporated samples used for lipid determination; e) clean-up columns with 4 g 44 % acidified silica and 4 g 5 % deactivated florisil eluted with 20 ml hexane and 15 ml dichloromethane; and f) HP 6890 Gas Chromatograph with micro Electron Captured Detector.*

4.2.2.3. Trophic magnification factors

Trophic magnification factors (TMFs) for metal and OCP concentrations were calculated from the gradient of the regression of the log-transformed concentrations of each pollutant vs. the trophic level ($\delta^{15}N$ values) (Borgå, et al., 2012; Verhaert et al., 2017):

$$log[contaminant] = \text{a+b TL} \qquad TMF = 10^{b}$$

The TMFs were determined for the 14 metals used during this study based on species from a direct food chain that was identified using stable isotope signatures **(Chapter 3)**. The OCP TMFs were calculated based on concentrations that were lipid-normalized according to the trophic groups identified during the stable isotope analysis **(Chapter 3)**. Since only a few of the species that were sampled, was analysed for OCPs, there was no direct food chain from which the TMF could be determined. By using the mean concentrations of each trophic group, some extent of trophic transfer can still be determined. On average where the TMF = 1 (b = 0), no trophic magnification of the chemical occurs, TMF > 1 (b> 0), trophic magnification of the chemical occurs through the food chain or food web, and where TMF < 1 (b< 1), trophic dilution of the chemical takes place (Borgå et al., 2012).

4.2.3. Statistical analysis

Statistical analyses were performed using GraphPad Prism version7, with the significance level set at $p < 0.05$. For both the metal and OCP concentrations, a detection frequency calculation was applied for samples below detection or below limit of quantification (LOQ). Metal concentrations were tested for normality (Kolmogorov-Smirnov) followed by one-way analysis of variance (ANOVA), and Tukey's post-hoc statistical analysis test was performed for multiple comparisons to determine significant differences between sites for species that were collected at both of the locations (*U. lactuca, P. perna, P. exigua* and *Actinia* sp.).

For OCPs, the data set was tested for normality (D' Agostino & Pearson) and homogeneity (Levene's test), where after the data were log-transformed for further analysis. Multivariate comparative statistics could be applied to the metal data set since at least six replicates were analysed for each species, but the OCP data were limited to univariate statistical analysis due to the fact that species were pooled for sufficient sample mass. Trophic magnification factors were calculated for each of the metals used in the present study, using a representing food chain from each site. To determine the TMF of

total HCH, total Chlordane and total DD_x, the average concentration of each of the four trophic groups were used.

4.3. Results

A total of 14 metals and 22 OCPs were analysed for at Tsitsikamma and Sheffield Beach. At Tsitsikamma, 22 and 12 species were used for metals and OCP analyses, respectively, and at Sheffield Beach, 15 and 14 species for each of the respective analyses. The following data are indicated in **Appendix B**: 1) dry weight and the number of replicates used for both metal and OCP analyses, as well as moisture content, percentage lipid and consequent $\delta^{15}N$ signatures (**Table B 1**); 2) significant differences in metal concentrations between sites for the same species that were collected at both locations (**Table B 2**); 3) the limits of quantification for organic compound analysis (**Table B 3**); 4) and 5) the log concentrations of OCPs from Tsitsikamma and Sheffield Beach, respectively (**Figure B 1** and **Figure B 2**).

4.3.1. Metals

Of the 14 metals analysed, Al, Fe, and Zn concentrations were the highest at both of the sites, while V, Cr, Co and Pb were all detected in low concentrations (**Table 4.2** and **Table 4.3**). Average metal concentrations were higher at Tsitsikamma than at Sheffield Beach, with only Al and Mn averaging higher at Sheffield Beach, however the most of the elements were still within an acceptable range. At Tsitsikamma *M. glacialis* reported high concentrations of V, Cr, Co, Ni, Cu and As, while *P. angulosus* reported high concentrations of Al, Ti and Fe (**Table 4.2**). Sheffield Beach concentrations were highest for *T. gratilla* in Al, Ti, V and Cr, while *L. coronate* had high concentrations of Fe, Se and Cd (**Table 4.3**). Since a vast array of species were selected for metal analysis, most concentrations detected within sites differ significantly ($p < 0.05$) due to differences in feeding habits, location on the coast and wave exposure, as well as sequestration and metabolic rates associated with each species. For this reason significant differences within sites between species were not indicated during the present study.

Since the species differed between the two climatic zones, only the species that were collected at both of the locations were compared for significant differences ($p < 0.05$) between sites (**Appendix B**; **Table B 2**). These include green macro-algae, the bio-indicator mussel, *P. perna*, the dwarf cushion-star and the plum anemone, *Actinia* sp. The dwarf cushion-star was the species where the most significant differences occurred.

Table 4.2: *Chemical analysis of metal concentrations in a wide range of organisms from Tsitsikamma, indicating the mean metal concentrations (µg/g dry weight) ± standard error of the mean (SEM).*

Species name	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Ulva lactuca	21.8 ± 1.83	2.4 ± 0.12	0.98 ± 0.03	0.74 ± 0.11	4.91 ± 0.12	118.5 ± 9.87	0.12 ± 0.01	2.13 ± 0.09	1.47 ± 0.09	10.5 ± 0.54	2.37 ± 0.05	1.66 ± 0.03	0.67 ± 0.02	1.35 ± 0.19
Jania sp.	27.9 ± 2.32	2.37 ± 0.14	1.01 ± 0.04	0.19 ± 0.02	4.19 ± 0.17	314.1 ± 11.1	0.63 ± 0.01	0.65 ± 0.03	0.39 ± 0.06	3.62 ± 0.38	1.76 ± 0.07	0.74 ± 0.02	0.59 ± 0.02	0.65 ± 0.09
Ralfsia sp.	135.6 ± 23.2	4.41 ± 0.42	1.91 ± 0.04	0.99 ± 0.08	6.79 ± 0.55	402.8 ± 36.3	0.24 ± 0.02	1.56 ± 0.21	1.37 ± 0.53	9.22 ± 0.81	17.5 ± 0.45	0.59 ± 0.02	1.62 ± 0.06	0.87 ± 0.04
Parechinus angulosus	701.3 ± 43.9	129.8 ± 5.82	31.5 ± 2.41	6.56 ± 1.39	44.2 ± 3.81	4043.3 ± 431.2	5.16 ± 0.33	15.5 ± 0.29	36.3 ± 0.81	300.3 ± 13.3	68.3 ± 2.51	29.9 ± 1.01	51.2 ± 2.34	11.4 ± 1.79
Scutellastra longicosta	20.6 ± 6.03	7.61 ± 0.36	0.46 ± 0.05	1.13 ± 0.11	2.74 ± 0.32	265.6 ± 45.8	0.25 ± 0.02	0.89 ± 0.09	1.96 ± 0.16	42.7 ± 3.03	13.7 ± 1.09	1.01 ± 0.04	31.1 ± 5.54	1.32 ± 0.15
Aplysia parvula	44.8 ± 7.29	14.9 ± 0.42	4.61 ± 0.29	0.14 ± 0.01	11.9 ± 0.45	407.1 ± 31.4	0.81 ± 0.02	14.3 ± 0.21	4.57 ± 0.1	20.6 ± 0.49	15.9 ± 0.29	22.4 ± 2.22	15.2 ± 0.38	2.09 ± 0.57
Turbo sarmaticus	82.1 ± 12.7	12.1 ± 0.88	9.74 ± 0.55	1.69 ± 0.15	8.89 ± 0.93	2100.4 ± 258.3	0.72 ± 0.03	13.8 ± 1.32	23.4 ± 3.02	89.9 ± 8.28	28.2 ± 0.95	2.59 ± 0.1	99.9 ± 16.5	8.49 ± 1.54
Perna perna	109.8 ± 17.2	12.7 ± 0.86	1.31 ± 0.07	2.33 ± 0.05	3.63 ± 0.14	120.5 ± 14.5	0.39 ± 0.02	4.26 ± 0.39	4.77 ± 0.19	51.9 ± 2.03	10.1 ± 0.31	1.62 ± 0.06	4.19 ± 0.17	0.14 ± 0.02
Cymbula oculus	24.9 ± 10.3	7.06 ± 0.25	0.29 ± 0.04	1.01 ± 0.09	2.44 ± 0.33	309.2 ± 63.1	0.25 ± 0.05	0.92 ± 0.13	2.12 ± 0.25	42.4 ± 1.58	10.9 ± 0.72	1.09 ± 0.05	11.4 ± 2.26	1.44 ± 0.17
Oxystele sinensis	12.6 ± 3.41	6.45 ± 0.12	0.85 ± 0.15	1.34 ± 0.16	2.99 ± 0.21	178.3 ± 22.9	0.39 ± 0.05	2.23 ± 0.19	9.87 ± 0.98	67.6 ± 2.3	36.6 ± 3.19	1.73 ± 0.07	6.31 ± 0.98	3.14 ± 0.38
Palaemon peringueyi	35.9 ± 2.53	27.6 ± 0.48	0.39 ± 0.03	0.14 ± 0.01	9.76 ± 0.34	104.8 ± 3.09	0.47 ± 0.02	0.81 ± 0.09	70.3 ± 1.47	57.9 ± 1.05	11.6 ± 0.09	3.01 ± 0.06	1.39 ± 0.06	10.2 ± 3.05
Parvulastra exigua	140.1 ± 26.9	25.3 ± 0.84	0.98 ± 0.15	0.14 ± 0.01	49.8 ± 1.36	1460.4 ± 24.4	7.47 ± 0.12	6.06 ± 0.63	38.9 ± 9.82	56.9 ± 5.96	9.6 ± 0.59	5.96 ± 0.22	16.3 ± 0.74	17.6 ± 1.47
Gunnarea gaimardi	365.9 ± 18.3	29.2 ± 2.34	1.52 ± 0.14	0.42 ± 0.16	9.37 ± 0.41	667.7 ± 99.8	0.88 ± 0.05	4.49 ± 0.17	10.3 ± 0.39	78.3 ± 2.23	19.9 ± 0.41	7.97 ± 0.16	8.08 ± 0.26	5.18 ± 1.14

Species name	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Pyura stolonifera	151.3 ± 29.2	17.7 ± 0.6	0.75 ± 0.14	0.14 ± 0.01	4.32 ± 0.63	153.1 ± 31.9	0.38 ± 0.04	1.35 ± 0.14	3.32 ± 0.26	59.4 ± 3.78	9.95 ± 0.46	7.79 ± 0.36	0.29 ± 0.03	4.88 ± 1.68
Tetraclita serrata	610.9 ± 55.7	94.2 ± 11.2	6.33 ± 0.93	1.28 ± 0.79	48.8 ± 4.67	3657.8 ± 391.7	10.4 ± 0.21	21.7 ± 4.76	46.93 ± 13.2	445.9 ± 62.9	50.3 ± 7.82	30.1 ± 2.29	27.4 ± 4.19	3.77 ± 0.43
Marthasterias glacialis	286.8 ± 23.7	53.1 ± 7.88	27.7 ± 4.36	62.6 ± 17.9	25.4 ± 1.29	2834.5 ± 224.1	8.51 ± 0.86	41.6 ± 8.78	146.2 ± 27.5	453.9 ± 20.9	56.4 ± 11.2	15.1 ± 1.84	40.3 ± 6.97	8.86 ± 3.55
Actinia sp.	31.8 ± 3.62	14.9 ± 0.45	1.48 ± 0.15	1.18 ± 0.07	16.6 ± 0.38	219.5 ± 29.4	0.22 ± 0.02	1.39 ± 0.16	5.05 ± 0.16	89.3 ± 1.31	52.5 ± 0.34	2.81 ± 0.04	1.91 ± 1.09	11.6 ± 3.52
Burnupena cincta	30.3 ± 3.7	19.1 ± 7.66	1.75 ± 0.87	0.14 ± 0.01	5.36 ± 2.11	182.3 ± 25.8	0.89 ± 0.53	2.36 ± 1.11	29.6 ± 3.38	545.7 ± 105.8	66.9 ± 2.35	17.1 ± 7.62	61.9 ± 11.3	4.82 ± 1.89
Liza richardsonii	85.4 ± 16.6	29.9 ± 2.01	0.96 ± 0.37	1.96 ± 0.19	4.71 ± 0.26	116.3 ± 23.8	0.15 ± 0.03	1.13 ± 0.08	6.99 ± 1.26	61.7 ± 7.63	3.29 ± 0.17	0.82 ± 0.08	0.58 ± 0.19	30.7 ± 3.88
Sparodon durbanensis	63.9 ± 10.8	18.6 ± 1.82	0.01 ± 0.01	2.72 ± 0.47	1.06 ± 0.14	41.9 ± 5.58	0.03 ± 0.01	0.58 ± 0.06	4.47 ± 1.13	37.8 ± 2.19	5.15 ± 0.23	0.47 ± 0.03	0.02 ± 0.01	9.67 ± 1.78
Caffrogobius sp.	25.2 ± 5.73	13.6 ± 0.77	0.02 ± 0.01	0.44 ± 0.13	2.77 ± 0.36	34.9 ± 3.92	0.07 ± 0.02	0.44 ± 0.05	4.84 ± 1.94	33.2 ± 4.55	15.8 ± 2.47	1.11 ± 0.12	0.04 ± 0.01	0.63 ± 0.24
Clinus sp.	34.8 ± 7.08	13.4 ± 0.29	0.02 ± 0.01	0.56 ± 0.13	1.03 ± 0.16	27.4 ± 5.99	0.04 ± 0.01	0.39 ± 0.06	2.26 ± 0.29	37.8 ± 4.33	1.65 ± 0.41	0.89 ± 0.04	0.06 ± 0.03	0.33 ± 0.03

Table 4.3: *Chemical analysis of metal concentrations in a range of organisms from Sheffield Beach, indicating the mean metal concentrations (µg/g dry weight) ± standard error of the mean (SEM).*

Species name	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Codium sp.	502.3 ± 0.001	37.9 ± 2.62	4.99 ± 0.21	3.39 ± 0.25	18.5 ± 1.18	829.3 ± 41.9	0.47 ± 0.03	3.05 ± 0.07	6.64 ± 1.22	8.97 ± 0.59	19.1 ± 0.45	1.61 ± 0.17	0.17 ± 0.01	4.76 ± 0.37
Corallina sp.	422.4 ± 27.6	7.14 ± 0.87	1.16 ± 0.12	2.12 ± 0.25	12.7 ± 0.38	530.7 ± 32.2	1.06 ± 0.07	3.84 ± 1.19	1.67 ± 0.16	12.9 ± 1.38	1.69 ± 0.12	0.75 ± 0.02	0.38 ± 0.02	6.65 ± 1.08
Tripneustes gratilla	1029.4 ± 34.1a	53.5 ± 3.36	6.39 ± 0.23	5.24 ± 0.35	32.6 ± 1.24	2189.8 ± 113.6	1.06 ± 0.03	2.86 ± 0.08	3.89 ± 0.18	21.9 ± 2.27	7.13 ± 0.15	1.53 ± 0.05	0.84 ± 0.02	1.58 ± 0.09
Ulva lactuca	350.2 ± 49.1	14.1 ± 2.54	1.49 ± 0.13	4.28 ± 0.68	9.36 ± 0.39	472.7 ± 55.8	0.33 ± 0.02	1.89 ± 0.14	4.49 ± 0.31	14.9 ± 2.48	3.24 ± 0.07	1.26 ± 0.06	0.33 ± 0.02	5.26 ± 1.33
Helcion concolor	544.6 ± 25.4	27.9 ± 0.53	2.84 ± 0.11	2.27 ± 0.09	15.9 ± 0.83	2307 ± 56.5	0.58 ± 0.02	6.56 ± 0.29	5.54 ± 0.19	36.1 ± 0.55	49.7 ± 2.59	1.17 ± 0.03	8.09 ± 0.38	7.93 ± 0.53
Lunella coronata	498.6 ± 24.2	23.9 ± 1.91	3.64 ± 0.22	2.69 ± 0.21	16.1 ± 0.93	2705.3 ± 86.2	0.64 ± 0.03	4.39 ± 0.16	56.8 ± 5.62	56.6 ± 1.32	28.6 ± 0.61	2.17 ± 0.05	17.6 ± 0.55	1.99 ± 0.47
Monodonta australis	578.1 ± 26.9	22.5 ± 0.45	2.99 ± 0.16	3.08 ± 0.69	14.5 ± 0.73	1035.7 ± 49.6	1.03 ± 0.05	8.83 ± 0.53	90.2 ± 5.38	63.9 ± 1.73	34.5 ± 0.79	2.14 ± 0.07	1.28 ± 0.07	0.99 ± 0.09
Perna perna	69.4 ± 15.3	8.68 ± 0.59	1.85 ± 0.23	1.29 ± 0.17	3.52 ± 0.21	136.3 ± 18.5	0.63 ± 0.06	5.51 ± 0.46	4.81 ± 0.36	71.5 ± 6.63	13.9 ± 0.73	1.85 ± 0.07	1.15 ± 0.05	1.28 ± 0.46
Nerita albicila	104.7 ± 10.5	7.51 ± 0.32	1.14 ± 0.12	1.55 ± 0.15	11.9 ± 1.19	212.9 ± 18.9	0.34 ± 0.04	3.28 ± 0.59	15.5 ± 0.83	46.5 ± 1.89	10.3 ± 0.79	0.77 ± 0.06	4.29 ± 0.33	4.29 ± 0.42
Parablennius pilicornis	174.6 ± 47.1	25.8 ± 3.33	0.36 ± 0.06	2.06 ± 0.35	9.89 ± 2.29	153.1 ± 28.3	0.18 ± 0.02	1.06 ± 0.08	5.09 ± 0.46	76.2 ± 7.51	3.67 ± 0.44	1.37 ± 0.11	0.05 ± 0.02	0.82 ± 0.11
Parvulastra exigua	3.51 ± 0.16	0.2 ± 0.008	1.26 ± 0.12	27.6 ± 0.89	324.1 ± 13.9	0.89 ± 0.04	1.45 ± 0.13	7.96 ± 0.28	19.3 ± 1.09	3.83 ± 0.14	0.79 ± 0.03	1.19 ± 0.06	0.37 ± 0.02	0.37 ± 0.02
Actinia sp.	256.2 ± 60.3	15.2 ± 0.97	2.32 ± 0.28	2.04 ± 0.23	47.3 ± 1.65	555.8 ± 100.9	0.35 ± 0.04	0.99 ± 0.15	8.29 ± 0.72	131.7 ± 9.76	41.5 ± 1.08	4.52 ± 0.08	0.29 ± 0.01	0.33 ± 0.03
Cellana radiata	312.3 ± 71.3	8.05 ± 0.79	1.41 ± 0.27	2.22 ± 0.34	17.9 ± 1.28	493.6 ± 120.1	1.56 ± 0.19	5.69 ± 0.52	18.4 ± 1.58	43.2 ± 4.09	13.7 ± 1.62	0.95 ± 0.05	0.48 ± 0.05	1.38 ± 0.43
Thalessa savignyi	95.9 ± 6.15	11.95 ± 0.22	0.52 ± 0.03	3.51 ± 0.19	9.47 ± 0.29	198.1 ± 24.8	0.29 ± 0.06	1.79 ± 0.29	76.4 ± 3.36	348.6 ± 27.1	126.9 ± 1.72	10.3 ± 0.56	3.09 ± 0.16	5.54 ± 0.73
Psammogobius knysnaensis	51.9 ± 4.78	14.5 ± 0.38	0.09 ± 0.01	1.58 ± 0.09	4.69 ± 0.54	58.9 ± 6.61	0.08 ± 0.01	0.61 ± 0.06	3.01 ± 0.41	50.6 ± 3.67	9.39 ± 0.19	2.05 ± 0.09	0.12 ± 0.01	0.89 ± 0.34

4.3.2. Organochlorine pesticides

The different OCPs and concentrations were relatively low at both sites, with the widest variety and concentrations found at Tsitsikamma (**Table 4.4**). Of the 22 OCPs that were analysed for, 16 were detected at Tsitsikamma, and eight at Sheffield Beach (**Table 4.5**). At both Tsitsikamma and Sheffield Beach, the metabolite pp-DDE was found in the highest concentration (µg/g lipid mass) for the urchins, i.e. the cape urchin, *P. angulosus,* and the collector urchin, *T. gratilla,* respectively. Since the concentrations for the majority of the species were based on single pooled samples, only the concentrations are reported and no statistical comparisons between species could be undertaken. For the species where replicates were available, the mean ± standard error of the mean (SEM) are reported (**Table 4.4** and **Table 4.5**).

Table 4.4: *Quantification of the 16 organochlorine pesticides detected in species sampled at Tsitsikamma, indicated in µg/g lipid mass. (ND = not detected, see* ***Appendix B, Table B 3*** *for limit of quantification).*

Species name	*Jania* sp.	*Ralfsia* sp.	*Parechinus angulosus*	*Turbo sarmaticus*	*Perna perna*	*Parvulastra exigua*	*Pyura stolonifera*	*Tetraclita serrata*	*Marthasterias glacialis*	*Burnupena cincta*	*Caffrogobius* sp.	*Clinus* sp.
α-HCH	40.7	8.01	0.91	3.97 ± 1.91	2.19	2.47	2.36	5.21	3.65	1.84	2.75 ± 0.97	2.92 ± 0.48
HCB	ND	ND	ND	ND	ND	ND	ND	22.6	ND	ND	ND	ND
β-HCH	476.2	339.4	28.5	66.9 ± 37.1	20.9	30.5	13.8	41.2	106.9	44.6	104.8 ± 50.8	88.7 ± 13.9
γ-HCH	ND	ND	4.07	ND	ND	ND	ND	ND	ND	ND	ND	ND
δ-HCH	109.9	16.5	2.11	8.06 ± 3.76	3.22	7.34	2.42	26.1	9.5	3.04	10.6 ± 2.11	12.4 ± 3.47
Aldrin	ND	ND	ND	ND	3.19	ND	ND	ND	ND	ND	ND	ND
***oxy*-Chlordane**	ND	ND	ND	5.86 ± 1.98	ND	ND	ND	ND	ND	ND	ND	ND
***cis*-Heptachlor-epoxide**	ND	ND	ND	ND	ND	ND	ND	14.9	ND	ND	ND	ND
***trans*-Chlordane**	ND	57.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***trans*-Nonachlor**	ND	ND	ND	17.1 ± 3.05	ND	ND	13.9	ND	ND	6.11	ND	ND
***p,p'*-DDE**	ND	4.23	1274.3	28.6 ± 10.1	12.8	30.3	23.7	45.3	42.6	6.46	7.27 ± 0.75	19.9 ± 7.23
***o,p'*-DDD**	ND	ND	ND	ND	11.5	19.2	ND	37.7	22.4	ND	ND	ND
***o,p'*-DDT**	ND	ND	ND	4.06 ± 0.83	ND	ND	4.43	ND	ND	ND	ND	ND
***cis*-Nonachlor**	ND	ND	1.51	ND	ND	ND	ND	ND	ND	ND	ND	ND
***p,p'*-DDD**	ND	ND	ND	ND	ND	ND	3.19	ND	ND	ND	ND	ND

Species name	*Jania* sp.	*Ralfsia* sp.	*Parechinus angulosus*	*Turbo sarmaticus*	*Perna perna*	*Parvulastra exigua*	*Pyura stolonifera*	*Tetraclita serrata*	*Marthasterias glacialis*	*Burnupena cincta*	*Caffrogobius* sp.	*Clinus* sp.
***pp*-DDT**	ND	ND	ND	ND	ND	ND	8.29	ND	ND	ND	ND	ND
∑HCH	**626.8**	**363.9**	**35.6**	**78.9**	**26.3**	**40.3**	**18.5**	**72.4**	**120.1**	**49.5**	**118.1**	**104.1**
∑Chlor	**0**	**57.9**	**1.51**	**22.9**	**0**	**0**	**13.9**	**14.9**	**0**	**6.11**	**0**	**0**
∑DD_x	**0**	**4.23**	**1274.3**	**32.7**	**24.2**	**49.5**	**39.6**	**83.1**	**64.9**	**6.46**	**7.27**	**19.9**
∑OCP	**626.8**	**426.1**	**1311.4**	**134.5**	**53.7**	**89.8**	**72.1**	**193.1**	**184.9**	**62.1**	**125.3**	**123.9**

Table 4.5: *Quantification of the 8 organochlorine pesticides detected in species sampled at Sheffield Beach, indicated in µg/g lipid mass. (ND = not detected, see* ***Appendix B***, ***Table B 3*** *for limit of quantification).*

Species name	*Codium* sp.	*Corallina* sp.	*Tripneustes gratilla*	*Ulva lactuca*	*Helcion concolor*	*Lunella coronata*	*Monodonta australis*	*Perna perna*	*Nerita albicila*	*Parablennius pilicornis*	*Parvulastra exigua*	*Actinia* sp.	*Cellana radiata*	*Psammogobius knysnaensis*
α-HCH	ND	ND	ND	ND	1.19	ND	0.67	ND	ND	1.39 ± 0.36	ND	ND	ND	ND
β-HCH	49.6	89.4	116.9	166.8	63.6	5.79	43.51	30.74	37.13	40.2 ± 8.61	41.87	6.12	15.4	78.9
δ-HCH	10.3	35.3	21.9	42.6	4.09	2.77	2.82	3.79	5.73	6.25 ± 0.89	9.81	2.77	5.27	9.09
Aldrin	ND	ND	ND	ND	ND	ND	ND	1.79	ND	ND	ND	ND	ND	ND
***trans*-Chlordane**	45.8	292.1	165.9	ND	ND	ND	ND	ND	ND	ND	ND	3.73	ND	ND
***p,p'*-DDE**	32.1	16.7	364.9	ND	3.17	7.78	1.81	5.95	4.11	23.7 ± 1.81	9.16	17.4	7.85	16.8
***cis*-Nonachlor**	ND	ND	ND	ND	ND	ND	1.59	ND	ND	ND	ND	ND	ND	ND
***p,p'*-DDD**	9.37	ND	ND	ND	ND	ND	ND	2.24	ND	ND	ND	2.16	ND	ND
∑HCH	**59.9**	**124.7**	**138.8**	**209.4**	**68.9**	**8.56**	**47.1**	**34.5**	**42.9**	**47.8**	**51.7**	**8.88**	**20.7**	**88.1**
∑Chlor	**45.8**	**292.1**	**165.9**	**0**	**0**	**0**	**1.59**	**0**	**0**	**0**	**0**	**3.73**	**0**	**0**
∑DD_x	**41.5**	**16.7**	**364.9**	**0**	**3.16**	**7.78**	**1.81**	**8.19**	**4.1**	**23.8**	**9.16**	**19.6**	**7.85**	**16.8**
∑OCP	**147.2**	**433.4**	**669.6**	**209.4**	**72.1**	**16.3**	**50.4**	**44.5**	**46.9**	**71.6**	**60.9**	**32.2**	**28.5**	**104.8**

To better comprehend the ratios in which the compounds were detected, **Figure 4.3** and **Figure 4.4** depicts the percentage composition for each of the sites. From the figures below it is evident that HCHs dominated the composition followed by metabolites of DD_x.

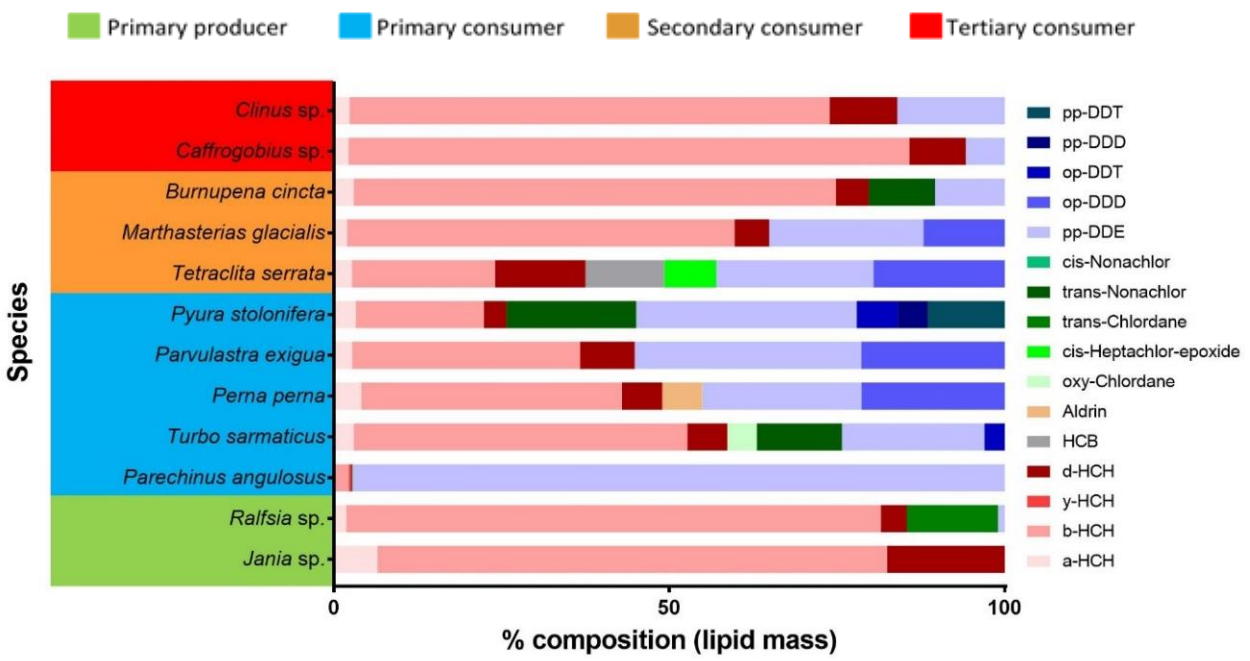


Figure 4.3: *Percentage composition of all the compounds detected in species analysed for organic compounds from Tsitsikamma. Colours allocated to the species are in accordance with the trophic groups as determined by stable isotope analysis (see Chapter 3).*

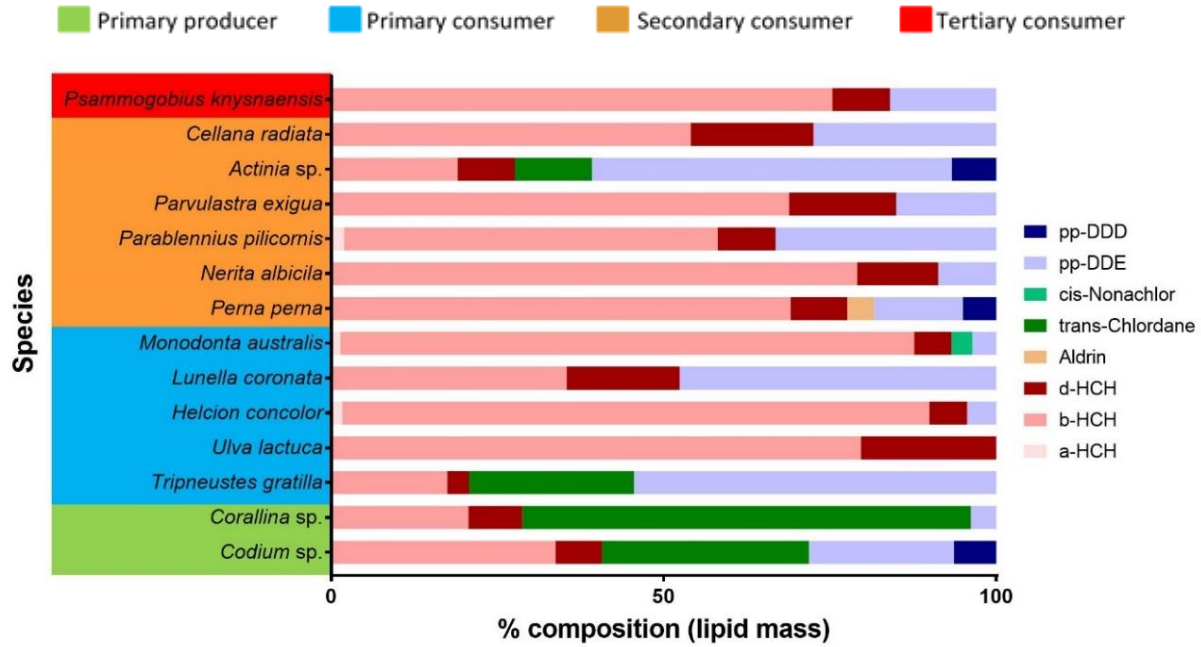


Figure 4.4: *Percentage composition of all the compounds detected in species analysed for organic compounds from Sheffield Beach. Colours allocated to the species are in accordance with the trophic groups as determined by stable isotope analysis (see Chapter 3).*

4.3.3. Trophic magnification factors

4.3.3.1. Metals

For the purposes of the trophic transfer determination, organisms that exhibit food chain interactions were selected based on stable isotope signature and supporting literature. The food webs from which the food chain was extracted are presented in **Chapter 3**. The food chain identified for Tsitsikamma involved a primary producer, green sea lettuce *(U. lactuca),* a primary consumer, sand shrimp *(P. peringueyi),* a secondary consumer, spiny starfish, *(M. glacialis),* and a tertiary consumer, a goby *(Caffrogobius* sp.*).* At Sheffield beach the chain consisted of a primary producer, red algae, *(Corallina* sp.*),* a primary consumer, collector urchin *(T. gratilla)* and secondary consumer, knobby dogwhelk *(T. savignyi)* and a tertiary consumer, the Knysna sand goby *(P. knysnaensis)*. The TL ranged from 1.00 to 3.55 and 1.31 to 3.66 at Tsitsikamma and Sheffield Beach, respectively. The food chains ranged from algae to an omnivorous and a predatory goby at both of the sites. The TMF for each of the metals were calculated based on the relationship between the log-transformed concentrations and the trophic level (**Table 4.6**). The TMF ranged from 0.99 to 2.23 at Tsitsikamma, and from 0.32 to 2.44 at Sheffield Beach. There were no significant differences ($p < 0.05$) in TMF between the subtropical and the warm temperate climatic zones (**Table 4.6**). The trophic magnification regressions of the food chains identified with stable isotope analysis are indicated in **Figure 4.5**. Only the metals that exhibited a positive slope at both sites with significant departures from zero are depicted since the other metals all indicated trophic dilution or no magnification.

Table 4.6: *Slope, R^2, p-value, and equation of the analysis of regression between the selected food chain and the log of the mean metal concentrations (µg/g dry weight) analysed, as well as the trophic magnification factors at each climate zone for each of the metals.*

	Tsitsikamma					Sheffield Beach				
	Slope	**R^2**	**P**	**Equation**	**TMF**	**Slope**	**R^2**	**P**	**Equation**	**TMF**
Al	0.15	0.09	0.70	Y = 0.15*X + 1.33	**1.40**	-0.47	0.88	0.06	Y = -0.473*X + 3.48	0.34
Ti	0.32	0.46	0.33	Y = 0.32*X + 0.44	**2.09**	-0.05	0.03	0.83	Y = -0.05*X + 1.36	0.89
V	0.06	0.009	0.91	Y = 0.06*X + 0.33	**1.14**	-0.23	0.55	0.26	Y = -0.23*X + 0.92	0.59
Cr	0.16	0.05	0.77	Y = 0.16*X + 0.13	**1.46**	-0.06	0.15	0.61	Y = -0.06*X + 0.72	0.88
Mn	0.01	0.001	0.98	Y = 0.008*X + 0.93	**1.02**	-0.22	0.64	0.20	Y = -0.22*X + 1.64	0.60
Fe	0.01	0.0001	0.99	Y = 0.009*X + 2.25	**1.02**	-0.50	0.77	0.12	Y = -0.51*X + 3.74	0.32
Co	0.11	0.07	0.74	Y = 0.11*X + 0.04	**1.28**	-0.12	0.99	0.005	Y = -0.12*X + 0.49	0.75
Ni	0.04	0.004	0.94	Y = 0.04*X + 0.53	**1.10**	-0.16	0.89	0.06	Y = -0.16*X + 0.84	0.70
Cu	0.27	0.11	0.67	Y = 0.27*X + 0.62	**1.84**	0.27	0.22	0.54	Y = 0.27*X + 0.25	1.85

	Tsitsikamma					Sheffield Beach				
	Slope	R^2	P	Equation	TMF	Slope	R^2	P	Equation	TMF
Zn	0.33	0.27	0.48	Y = 0.33*X + 0.97	**2.12**	0.39	0.53	0.27	Y = 0.39*X + 0.74	2.44
As	0.35	0.59	0.23	Y = 0.35*X + 0.29	**2.23**	0.39	0.40	0.37	Y = 0.39*X + 0.18	2.43
Se	0.06	0.03	0.84	Y = 0.06*X + 0.49	**1.14**	0.19	0.39	0.38	Y = 0.19*X + 0.09	1.54
Cd	0.10	0.02	0.85	Y = 0.11*X + 0.31	**1.26**	0.03	0.02	0.84	Y = 0.03*X + 0.18	1.08
Pb	-0.004	0.0001	0.99	Y = -0.004*X + 0.62	**0.99**	-0.10	0.15	0.62	Y = -0.09*X + 0.82	0.80

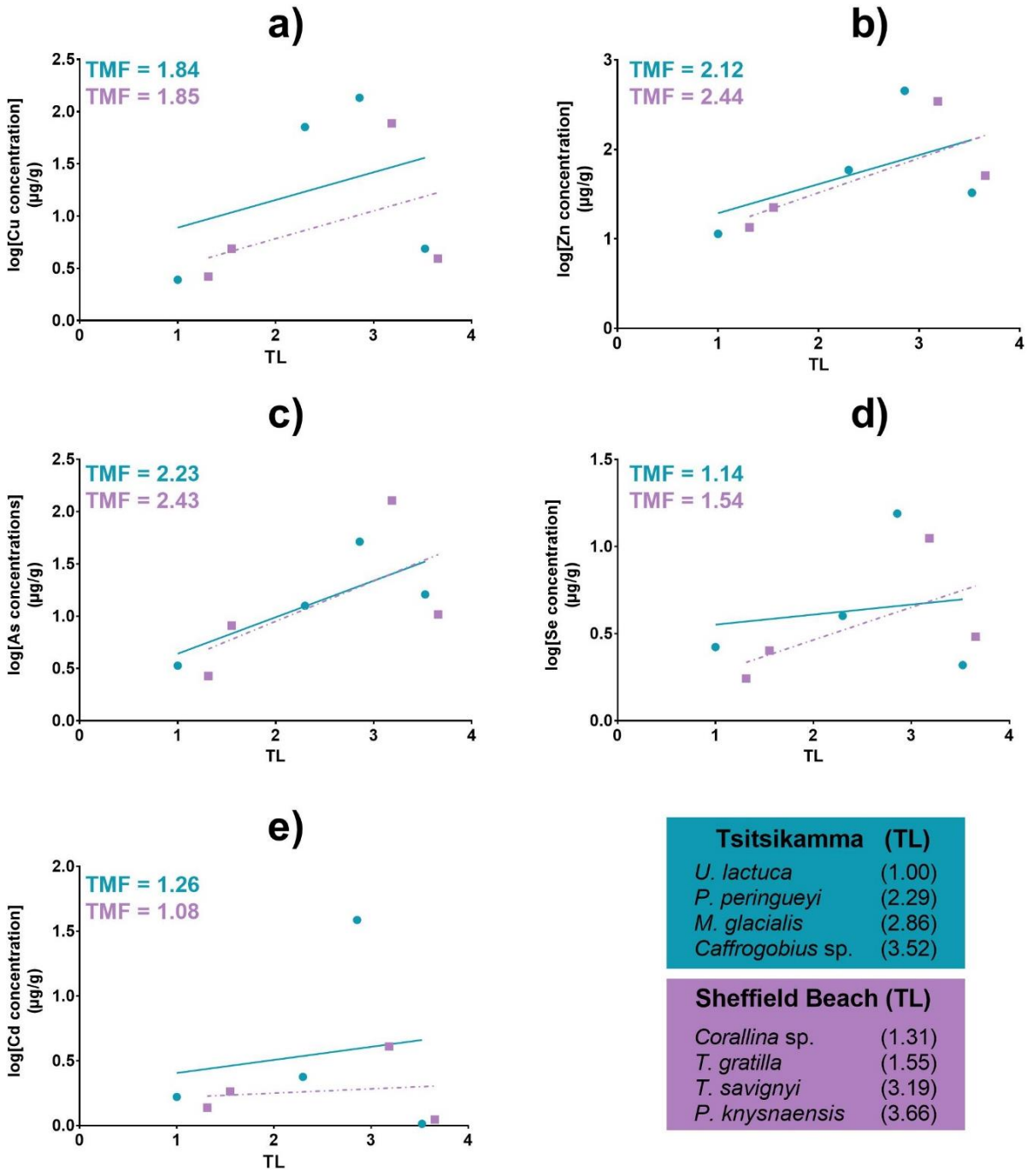


Figure 4.5: *Relation of tropic level (TL) to the logarithm mean concentrations (µg/g dry weight) of metals showing trophic magnification for (a) Cu, b) Zn, c) As, d) Se and e) Cd) through the identified food chains at Tsitsikamma (solid line) and Sheffield Beach (dotted line).Species names are presented according to the allocated TL.*

4.3.3.2. Organochlorine pesticides

Since no direct food chain could be identified for the species used for OCP analysis, the mean log concentration per trophic group were calculated for total HCH, total Chlordane and total DD_x (**Table 4.7**). A TMF was then determined for the whole food web from primary producers to tertiary consumers and indicated in **Table 4.7** and **Figure 4.6**. No significant differences ($p < 0.05$) in TMF between climate zones or compounds were detected, and all of the compounds indicated trophic dilution.

Table 4.7: *Slope, R^2, p-value, and equation of the analysis of regression between the trophic groups and the logarithm of the mean concentration (µg/g lipid mass) for total HCH, total Chlordane, and total DDx and the consequent trophic magnification factors of each compound at each climate zone.*

		Σ HCH	Σ Chlordane	Σ DD_x
TSITSIKAMMA	**Slope**	-0.18	-0.17	-0.17
	R^2	0.25	0.34	0.73
	P	0.51	0.42	0.15
	Equation	y = -0.18*X + 2.51	y = -0.17*X + 1.79	y = -0.17*X + 2.08
	TMF	**0.66**	**0.67**	**0.68**
SHEFFIELD BEACH	**Slope**	-0.03	-0.44	-0.08
	R^2	0.03	0.69	0.28
	P	0.82	0.17	0.47
	Equation	y = -0.03*X + 1.87	y = -0.44*X + 2.19	y = -0.075*X + 1.45
	TMF	**0.93**	**0.36**	**0.84**

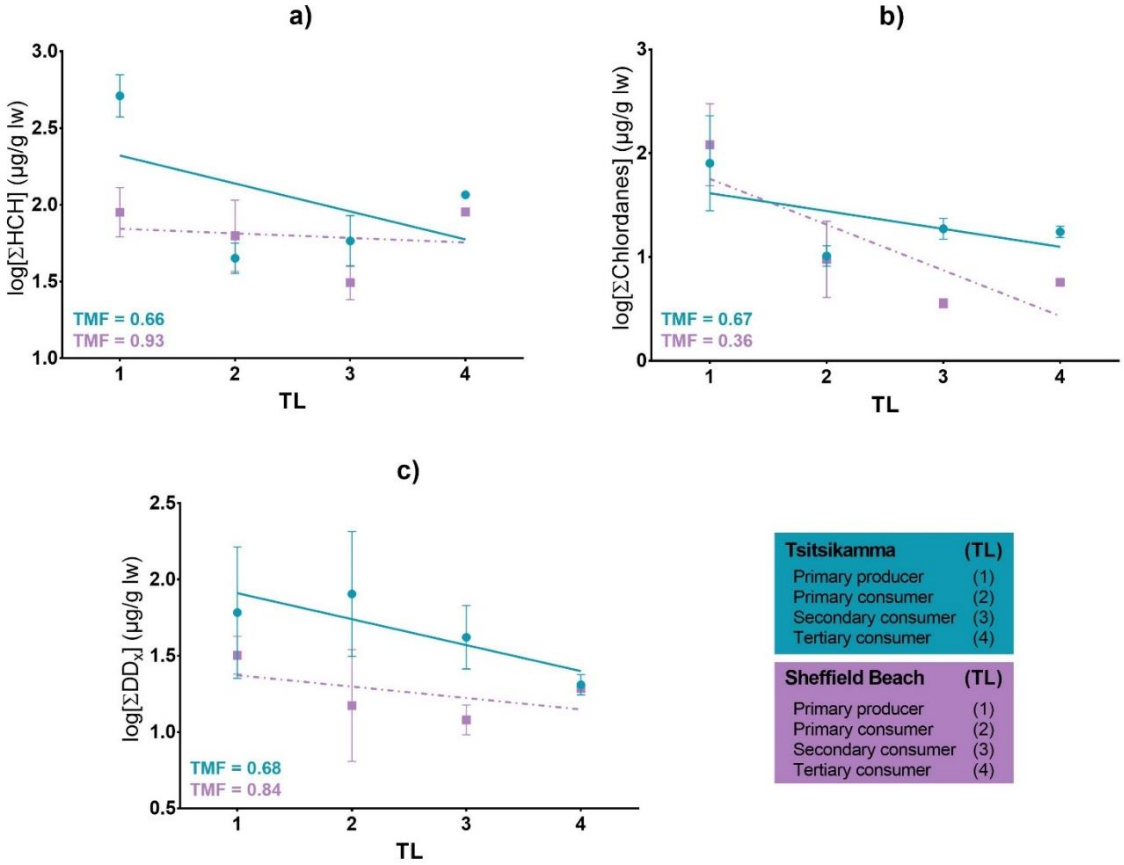


Figure 4.6: *Relationship of trophic level (TL) to the logarithm of a) total HCH, b) total Chlordane and c) total DDx (µg/g lipid mass) determined across trophic groups for Tsitsikamma (solid line) and Sheffield Beach (dotted line).*

4.4. Discussion

The results obtained during this study reflect pristine locations regarding concentrations of the analysed elements. Stable isotope results obtained from **Chapter 3** provides supporting data for further analysis of potential trophic transfer of these elements. Only the elements showing significantly higher concentrations and differences between the two climate zones are discussed below.

4.4.1. Metals

Metals are elements with a fairly high density in comparison to water, and can be toxic to consumers even at low levels in the environment (Tchounwou et al., 2014). Limited data on the comparison of metals along the South African coastline are available, while past research focused on bio-indicator species, such as *P. perna*, and polluted sites, i.e. harbours (Greenfield, et al., 2011; Greenfield et al., 2014; Degger et al., 2011a; Degger et al., 2011b). Due to the lack of past research focus, data on reference sites and food webs are even scarcer. The results obtained during this study potentially address the knowledge gap between pristine and polluted sites to enable relevant comparisons. The concentrations of metals detected during the study correlates to some extent with the results obtained by Degger et al. (2011a; 2011b) in a study comparing the concentrations of metals found in various harbours to concentrations detected at Tsitsikamma National Park, a pristine site.

Aluminium has a high affinity for solid surface of organic matter and since most of the species analysed during this study rely on SPM as food source, the elements that attach to it accumulate (Greenfield et al., 2014). Cadmium and Zn have a nutrient linked distribution which, together with Al's high affinity for solid surfaces, is associated with upwelling events (Greenfield et al., 2014; Lares et al., 2002). This is the case for As, Mn, Ni, Cu and Fe, where the elements associate with upwelling within pristine marine areas (Greenfield et al., 2014; Lares et al., 2002; Hanekom et al., 2009). The fluctuations in concentrations can possibly be ascribed to this phenomenon, since upwelling occurs occasionally and metals will not always be present at these concentrations. Manganese and Se can also be found in high concentrations in locations where upwelling regimes influence the uptake of metals (Degger 2010). A major decrease in Se was noted at Tsitsikamma since the 2010 survey, where concentrations were detected above 400 µg/g (Degger et al., 2011a; Degger et al., 2011b) and only 1.62 µg/g in 2016 for the bio-indicator species, *P. perna*. The high concentrations during the 2010 survey was also

ascribed to upwelling regimes within the area and might have been present to a lesser extent during the 2016 survey.

In most of the metals analysed at both sites, echinoderm species had the highest concentrations (Al, Ti, V, Cr and Mn at both of the sites, as well as Fe, Ni, Cu and Pb at Tsitsikamma), while limpets and topshells followed for Fe, Co, Cu, Cd and Pb at Sheffield Beach, and Zn, As and Se had the highest concentrations in dogwhelks. Concentrations of Zn were detected in similar concentrations during the 2010 and 2016 surveys, reported at 55 µg/g and 51 µg/g for *P. perna*, respectively (Degger et al., 2011a; Degget et al., 2011b). The high concentrations of Pb detected in Echinodermata can be ascribed to Pb's high affinity for calcite, especially when the exoskeleton is considered (Temara et al., 1997). According to Temara et al. (1997) the toxic effects of Pb could come directly from its absorption into the exoskeleton and influence other metabolic pathways, as well as exert an indirect lethal effect on starfish species. During the 2010 survey at Tsitsikamma (Degger et al., 2011a; Degget et al., 2011b), concentrations of Pb did not exceed 0.5 µg/g in *P. perna*, while 0.14 µg/g were detected during the present study.

Arsenic is a natural component in the Earth's crust, low levels can be found in all environmental media (WHO, 2011). Concentrations of As at Tsitsikamma during the 2010 and 2016 surveys were detected just above 10 µg/g in *P. perna*, while the concentrations at Sheffield Beach were slightly higher, at 13 µg/g (Degger et al., 2011a; Degger et al., 2011b). Arsenic has a variety of applications that include insecticides, herbicides and used as veterinary medicine to treat tropical infections such as African sleeping sickness (Tchounwou et al., 2014). According to the WHO (2011), 85 - 90% of this compound is organic, which can be found in substantially higher concentrations within seafood (fish and shellfish), and are normally less than 10 µg/l in water, except in areas of natural mineral deposits (Tchnouwou et al., 2014).

Cadmium is another toxic metal that occurs in the Earth's crust in average concentrations of 0.1 µg/g, and are used by industries in batteries, alloys and pigments (Tchnouwou et al., 2014). At Tsitsikamma, Cd was recorded in concentrations relatively higher (133.9 µg/g in *B. cincta*) than at Sheffield Beach (17.6 µg/g in *L. coronata*). However, in mussel species only a slight increase was noted since the survey conducted in 2010, where concentrations were 3.2 µg/g compared to the 4.2 µg/g recorded in the present study (Degger, et al., 2011a). This element is also associated with the upwelling phenomenon and may account for the concentrations detected (Lares et al., 2002; Clarke et al., 2009; Degger, 2010).

According to Degger et al. (2011a), natural phenomenon's such as coastal upwelling can influence the feeding of organisms, i.e. mussels, which in turn affects metal uptake by these species. This occurs due to fluctuations between the dissolved and particulate phases of the metals concerned (Newman, 2010; Degger et al., 2011; Lares et al., 2002). Another natural phenomenon that influence the uptake of metals, is temperature (Philips & Rainbow, 1993; Wu & Lau, 1996), and since the two sites are located in different biogeographic regions, it will have a major effect on the uptake patterns observed.

When considering the differences between the two climate zone, *U. lactuca, P. perna, P. exigua* and *Actinia* was used to establish the significance ($p < 0.05$). *Parvulastra exigua* exhibited significant differences in all of the metals between Tsitsikamma and Sheffield Beach. The highest concentrations were detected at Tsitsikamma, with only V, Cr, Mn and Ni higher at Sheffield Beach. The high concentrations detected in this particular species supports the finding that some elements associate with the calcite exoskeleton (Temara et al., 1997).

4.4.2. Organochlorine pesticides

Organochlorine pesticides have characteristically high solubility in lipids and low solubility in water (Degger et al., 2011a; Verhaert et al., 2017), and since they remain stable under photochemical and biological decomposition, they can accumulate in fatty tissues (Degger et al., 2011a; GESAMP, 2001). As with the case of metal accumulation, mussel and other filter feeders were used as bio-indicators because they have the ability to accumulate lipophilic compounds (Fung et al., 2004; Degger et al., 2011a).

Total OCPs detected at Sheffield Beach ranged from very low concentrations in the crowned turban shell, *L. coronata* (16.3 µg/g lipid mass) to very high concentrations in the collector urchin, *T. gratilla* (669.5 µg/g lipid mass) (**Table 4.5**). At Tsitsikamma the brown mussel, *P. perna,* showed the lowest concentrations (53.6 µg/g lipid mass) of total OCPs and the cape urchin, *P. angulosus,* showed the highest concentrations (1311.3 µg/g lipid mass) (**Table 4.4**). Overall, Tsitsikamma showed a wider variety and higher concentrations of OCPs than detected at Sheffield Beach.

Four isomers of HCH (α; β; γ; δ) were analysed in species for this study and total HCH concentrations detected ranged from 8.55 µg/g lipid mass for *L. coronata* to 209.3 µg/g lipid mass for *U. lactuca.* These four isomers are also known as technical HCH, which has been used for agricultural pest control (ATSDR, 2005; Vijgen et al., 2011). Technical HCH, together with technical DDT, has been banned in developed countries due to their

persistent nature and carcinogenic properties (ATSDR, 2005; Sibali et al., 2008; Stockholm Convention, 2012). Since these OCPs have a high affinity for fatty tissues, they tend to bio-accumulate and magnify in the environment (Ahmed et al., 2015; Degger et al., 2011a; GESAMP, 2001). Listed to be some of the most persistent compounds, OCPs are also highly toxic (Tang et al., 2013).

Concentrations of *p,p'*-DDE recorded at Sheffield Beach in 2015, ranged between 40 % to 100 % of the total OCP concentrations detected in transplanted mussels (Coetzee, 2015). Concentrations in fish from the Durban harbour were considered high for DDE (40 ng/g - 150 ng/g) and DDT (30 ng/g - 70 ng/g) (SANCOR 1985), while concentrations for these compounds at Sheffield Beach during the current survey were 364.9 µg/g *p,p'*-DDE (detected in *P. perna*) and DDT were not detected in any of the species sampled. At Tsitsikamma, on the other hand, *p,p'*-DDE were detected in concentrations as high as 1274.3 µg/g lipid mass for *P. angulosus*, and 8.29 µg/g lipid mass DDT in red bait, *P. stolonifera*. These concentrations are extremely high, indicating an increase in concentrations since the study conducted in 1985. Since these compounds metabolize in the environment, the high concentrations can be ascribed to an accumulation of historical breakdown.

High concentrations of *trans*-Chlordane were detected in *Corallina* sp. at Sheffield Beach, 292.1 µg/g, while *Ralfsia* sp. had the highest concentrations at Tsitsikamma. This compound is also used as an insecticide considered toxic to aquatic biota and expected to be carcinogenic and cause liver damage after extended periods of exposure (WHO, 2003b). *Cis*- and *trans*-chlordane are a mixture resulting in technical chlordane which is resistant to breakdown, hence unlikely to elute into groundwater (WHO, 2003b). According to literature chlordane has high potential to bio-accumulate (WHO, 2003b).

At both sites the brown mussel, *P. perna,* was the only species that accumulated levels of Aldrin. Concentrations detected at Tsitsikamma were 3.19 µg/g, while Sheffield Beach contained 1.79 µg/g lipid mass of Aldrin. Since Aldrin breaks down to Dieldrin, it can be assumed that the concentrations detected are recent exposure rather than historical. Aldrin is a poisonous insecticide used to control pest insects like termites, and was banned in the U.S. and the U.K. in 1998 (WHO, 2003a). Aldrin is also highly toxic to aquatic organisms. Since it has been banned, concentrations in water are expected to be low although some concentrations are still present in sediments and organisms. This substance has been reported to cause endocrine disruption (IPCS, 2006; Haschek et al., 2013).

4.4.3. Trophic magnification factors

According to Gray (2002) biomagnification is the result of xenobiotic transfer from a food source to an organism. In his study, he also referred to bioconcentration, as a result of direct uptake from the abiotic environment, and bio-accumulation, the uptake from the abiotic or biotic environment (Gray, 2002). Contaminants can then be sequestrated, metabolized or excreted by the organisms, resulting in a balance between intake and regulation (Gray, 2002). Isaacs (1973), on the other hand, suggests that trophic magnification does not occur in marine ecosystems, since the food chain is open compared to closed terrestrial or freshwater food chains, i.e. in ponds. Trophic magnification can also be influenced by the following factors (Borgå et al., 2012):

- Properties of chemicals and organisms
- Characteristics of ecosystems
- Seasonal and spatial variation of contaminant within and across ecosystems
- Seasonal variation in stable isotopes and
- Lipid content

In this study, only a few metals (**Figure 4.6**) showed trends of magnification, while OCPs indicated a definite dilution in concentrations from the primary producers to the tertiary consumers. It is important to note that species identified in the tertiary consumer groups are omnivorous and scavengers and tend to result in lower concentrations from a variety of food sources rather than the accumulation of just one. It is thus expected that total concentrations will be lower in higher trophic groups. Another explanation for this trend can also be ascribed to the fact that some metals are soluble in water and that exposure via the water plays a more important role in transfer then the food. The invertebrates collected during this study are classified as relatively sessile species and/or they move around in close proximity to where they were sampled. This creates a limited variety of prey resulting in magnification of any compound (Isaacs, 1973; Gray, 2002). However, the fish species collected move around as the tide rise and fall, creating an open food chain with little accumulation of compounds (Isaacs, 1973; Gray, 2002). The fish can venture to prey that are not present in the intertidal zone when it is low tide and only return during high tide.

The result of lower OCP concentrations in fish species can also be attributed to the fact that only muscle tissue was used for the analysis while for invertebrates, the whole organism (excluding shell) was analysed. It has been reported that fish store xenobiotic

chemicals in specialized organs, resulting in the lower concentrations found in muscle tissue (Gray, 2002). In an extensive review on biomagnification of metals versus organic contaminants, metals (except for mercury) showed no magnification while 67 % of studies on OCPs indicated some sort of magnification through the food chain (Gray, 2002). This is in contrast to the results obtained during the present study.

In **Figure 4.6**, mean metal concentrations of the species within each food chain are plotted with the linear regression to indicate magnification and or dilution. In all the cases, there are one species with higher concentrations than the rest, more often than not within the secondary consumer trophic group. Concentrations of the tertiary consumers are lower compared to the secondary consumers, resulting in the phenomenon of biodilution observed from the other metals.

Accumulation depends on factors like environmental setting, solubility of metals, species, lipid content, trophic status and route of uptake. Here, Fe is an example of a metal that does not magnify or dilute within the food web, since it is found in naturally high concentrations within the aquatic environment. Mason et al. (2000) states that the high concentrations found in these organisms can rather be ascribed to bioconcentration than biomagnification.

4.5. Conclusions

The determination of background concentrations of whole food webs in a natural environment can be used effectively to establish the fate and transport of compounds within ecosystems. With regard to metal concentrations, at Tsitsikamma there was a definite increase in concentrations from the primary producers to the secondary consumers, while the concentrations decreased from the secondary to the tertiary consumers. This can be explained by the fact that the species classified as tertiary consumers are omnivorous and exhibit dispersed concentrations of a variety of prey rather than direct transfer from a certain source, as well as the fact that some metals can be sequestrated and are metabolized by certain species at a faster rate than others. Sheffield Beach however, did not show the same trend for all of the metals, but in some cases had lower concentrations within the secondary consumers. The same is true with regards to trophic magnification at both of the sites, where Cu, Zn, As, Se and Cd, showed magnification within the identified food webs.

Organic compounds at Tsitsikamma consisted of a wider variety of HCHs, Chlordane and DD_x at higher concentrations than the Sheffield Beach site. Explanations include historical

and technical pesticides that degrade and break down at different rates due to compound characteristics, temperature, salinity and ocean currents. Biodilution of the compounds was evident throughout the food webs with lower concentrations detected in the tertiary consumers, i.e. small omnivorous fish species.

From this study it can thus be concluded that trophic magnification of metals and organic compounds, are the exception rather than the rule and understanding these phenomena can help maintain natural ecosystems by addressing compounds prior to their exposure to biota.

Chapter 5: Conclusions & Recommendations

5.1. General conclusions

The marine domain contributes 63 % of all the functions and services generated by ecological systems to human welfare (Costanza et al., 1997). However, there is little information available on human impact on intertidal rocky shore organisms, except for studies conducted on bio-indicator species, i.e. mussels. As stated by the Marine and Coastal Component of the National Biodiversity Assessment (Sink et al., 2012), coastal development is the greatest pressure on the biodiversity that surrounds it. By taking a closer look at the species and habitat that occur within these shores, better management can be applied to maintain natural ecosystems.

The use of stable isotope analysis to determine the food web and transfer of compounds and energy within ecosystems has changed the outlook on ecosystem research. From this study a basic food web was established for two climate zones in order to investigate the degree of transfer that occurs with regard to metals and OCPs and, in turn determine whether magnification of these compounds differ between species and ecosystems. Physical factors such as temperature, salinity, wave action, ocean currents and precipitation can influence stable isotopes, nutrient and chemical supply within these intertidal rocky shores (Little et al., 2009; Van As et al., 2012).

Based on the hypothesis stated and discussed in Chapter 3, that stable isotope signatures will differentiate the food web structures between the intertidal zones of two temperature based biogeographic regions, it was evident that each of the regions exhibit distinct isotope based food webs. This was attributed to factors that influence nutrient supply such as ocean currents, freshwater inflow and source of prey (Hill et al., 2006; Hill et al., 2008; Middelburg, 2014; Puccinelli et al., 2016a; Puccinelli et al., 2016b; Puccinelli et al., 2016c; Abrantes et al., 2014; Kohler et al., 2011; De Lecea et al., 2013). It was also noted that even though species are known omnivores and have a variety of food sources, from the $\delta^{15}N$ signatures they are still based at the top of the food web. For this reason, amongst others, the use of stable isotopes to determine trophic transfer of energy and compounds is a successful tool in ecological studies (seen in Chapter 4). It can also be applied in other research such as ecotoxicology. From the results obtained during the present study, the hypothesis was accepted.

The second hypothesis, that a stable isotope approach can be applied to demonstrate the transfer of inorganic (metals) and organic (OCPs) substances from one trophic level to another in the intertidal zone of two temperature based biogeographic regions, was tested and discussed in Chapter 4. With regards to the metal concentrations detected in each organism at the respective sites, there were definite differences between the concentrations in which the elements were detected. The concentrations, in most cases, correlate with results obtained in 2010 (Degger, 2010) with only slight increases of Cr, Cu and Cd. Organochlorine pesticides were detected in a greater variety, and in higher concentrations at Tsitsikamma than Sheffield Beach. The second hypothesis was accepted, since a transfer of substances could be established between species. However, in some species the substance was either sequestrated or metabolized. These mechanisms can be addressed in future studies. Due to the fact that the tertiary consumers are omnivorous, substances were detected in lower concentrations reflecting only a portion of their diet's concentration rather than high concentrations accumulated from only one prey group. From these results it can be concluded that biomagnification in marine species is the exception with regard to metals and OCPs, and not the rule.

The aims of the study was achieved through successfully determining the trophic structures of representative species from each biogeographic region and using stable carbon and nitrogen ratios to establish direct food chains, as well as to analyse for metal and OCP concentrations in these organisms and their correlation to the transfer of compounds through food webs.

5.2. Recommendations

To investigate the trophic transfer of compounds in future studies, it is recommended that the factors listed below should be taken into account, especially with regard to stable isotopes and OCPs:

- Sampling should be done at a variety of locations across the coast line, in order to establish baseline data for all of the species that occur on the South African coast line. An example is the west coast where the cold Benguela Current influence the species distribution and nutrient supply resulting in different sea surface temperatures, as well as a different coastal layout.
- A pilot study can also be conducted in order to determine interactions between species based on observations to support findings of stable isotope analysis.
- Isotopic tracers and seasonal surveys over 5 years should be conducted to determine the direct transfer within ecosystems under different environmental conditions, in order to establish more specific magnification or dilution regarding compounds.
- Conducting surveys after major events such as upwelling and high rainfall, can provide further insight into how species and compounds react under such pressures.
- Other compounds could also be included in future studies, i.e. poly aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc.
- Stable isotope analysis could be combined with fatty acid and biomarker analysis, and statistical analysis should be improved by using Bayesian mixing models (for example in R statistics).
- Physico-chemical parameters should be taken *in situ*, as well as water and sediment samples, to establish the influence on transfer and magnification or dilution of compounds and nutrients.
- Further laboratory experiments could be designed in order to determine the influence of different environmental conditions on the uptake of compounds through an established food web.

References

Abrantes, K. G., Barnett, A. & Bouillon, S. 2014. Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology,* 28: 270 - 282.

Ahmed, G., Anawar, H. M., Takuwa, D. T., Chibua, I. T., Singh, G. S. & Sichilongo, K. 2015. Environmental assessment of fate, transport and persistent behavior of dichlorodiphenyltrichloroethanes and hezachlorocyclohexanes in land and water systems. *Environmental Science and Technology,* 12: 2741 - 2756.

AlgaeBase. 2017. Available at: http://www.algaebase.org/search.php Date of access: 15 November 2017. [Database].

Animal Diversity Web. 2014. Browse Animalia. Available at: http://animaldiversity.org/accounts/Animalia/ Date of access: 14 November 2017. [Database].

AnimalBase. 2014. Available at: http://www.animalbase.org/search.php Date of access: 14 November 2017. [Database].

ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Toxicological Profile for DDT, DDE, and DDD. U.S. *Department of Health and Human Services.* Agency for Toxic Substances and Disease Registry. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp35.pdf Date of access: 14 November 2017.

ATSDR (Agency for Toxic Substances and Disease Registry). 2005. Toxicological profile for Alpha-, Beta-, Gamma-, and Delta- Hexachlorocyclohexane. U.S. *Department of Health and Human Services*, Atlanta.

Attwood, C., Moloney, C. L., Stenton-Dozey, J., Jackson, L. F., Heydorn, A. E. F. & Probyn, T. A. 2002. Conservation of Marine Biodiversity in South Africa. *In* Durham, B. D. & Pauw, J. C. *eds.* Summary Marine Biodiversity Status Report*, National Research Foundation Publication*, pp. 68 - 83.

Ausubel, J. H., Trew, C. & Waggoner, P. E. 2010. First Census of Marine Life 2010: Highlights of a decade of discovery. *First census of marine life 2010: highlights of a decade of discovery.* Census of Marine Life International Secretariat, 64 pp.

Awad, A. A., Griffiths, C. L. & Turpie, J. K. 2002. Distribution of South African marine benthic invertebrates applied to the selection of priority conservation areas. *Diversity and Distributions*, 8(3): 129 - 145.

Borgå, K., Kidd, K., Muir, D., Berglund, O., Conder, J., Gobas, F., Kucklick, J., Malm, O. & Powell, D. 2012. Trophic magnification factors: considerations of ecology, ecosystems, and study design. *Integrated Environmental Assessment and Management,* 8: 64 - 84.

Branch, G. 1975. Mechanisms Reducing Intraspecific Competition in Patella spp.: Migration, Differentiation and Territorial Behaviour. *Journal of Animal Ecology, 44*(2): 575 - 600. doi:10.2307/3612

Branch, G., Griffiths, C. L., Branch, M. & Beckley, L. 2010. Two oceans: a guide to the marine life of Southern Africa. *Struik Publishers*, Cape Town.

Brown, A. C. & Jarman, N. 1978. Coastal marine habitats. *In* Werger, M. J. A., Van Bruggen, A. C. *eds.* Biogeography and ecology of southern Africa, *Springer Netherlands*, pp. 1239 - 1277.

Bustamante, R. H. & Branch, G. M. 1996. Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave exposure. *Journal of Biogeography,* 23:339 - 351.

Buxton, C. D. 1987. Life history changes of two reef fish species in exploited and unexploited marine environments in South Africa. Ph.D. thesis, Rhodes University, Grahamstown.

Buxton, C. D. & Smale, M. J. 1989. Abundance and distribution patterns of three temperate marine reef fish (Teleostei: Sparidae) in exploited and unexploited area off the southern Cape coast. *Journal of Applied Ecology*, 26: 441 - 451.

Cabanillas-Terán, N., Loor-Andrade, P., Rodríguez-Barreras, R. & Cortés, J. 2016. Trophic ecology of sea urchins in coral-rocky reef systems, Ecuador. *PeerJ*, 4: 1578. doi 10.7717/peerj.1578

Chu, S. G., Covaci, A., Haraguchi, K. & Schepens, P. 2002. Optimized separation and determination of methyl sulfone metabolites of polychlorinated biphenyls (PCBs) and p, p′-DDE in biota samples. *Analyst,* 127: 1621 - 1626.

Clark, B. M., Orr, K. K., Hutchings, K., Angel, A. & Turpie, J. 2009. State of the Bay 2008: Saldanha Bay and Langebaan Lagoon. *Anchor Environmental.* Available at: http://www.anchorenvironmental.co.za Date of access: 30 October 2016.

Coetzee, A. E. 2015. The assessment of organic pollutant exposure and effects along the KwaZulu-Natal coastline. MSc. Dissertation, North-West University, Potchefstroom campus, South Africa.

Cooper, R. N. & Wissel, B. 2012. Loss of trophic complexity in saline prairie lakes as indicated by stable-isotope based community metrics. *Aquatic Biosystems*, 8: 6.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J. & Raskin, R. G. 1997. The value of the world's ecosystem services and natural capital. *Nature*, 387(6630): 253 - 260.

Costello, M. J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H. & Miloslavich, P. 2010. A census of marine biodiversity knowledge, resources, and future challenges. *PLoS ONE,* 5(8): e12110. doi:10.1371/journal.pone.0012110

Covaci, A., Ryan, J. J. & Schepens, P. 2002. Patterns of PCBs and PCDD/PCDFs in contaminated chicken and pork following a Belgian food contamination. *Chemosphere,* 47: 207 - 217.

Covaci, A., Gheorghe, A., Voorspoels, S., Maervoet, J., Steen Redeker, E., Blust, R. & Schepens, P. 2005. Polybrominated diphenyl ethers, polychlorinated biphenyls and organochlorine pesticides in sediment cores from the Western Scheldt river (Belgium): analytical aspects and depth profiles. *Environment International,* 31: 367 - 375.

Covaci, A., Gheorghe, A., Hulea, O. & Schepens, P. 2006. Levels and distribution of organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in sediments and biota from the Danube Delta, Romania. *Environmental Pollution*, 140: 136 - 149.

Cowley, P. D., Brouwer, S. L. & Tilney, R. L. 2002. The role of the Tsitsikamma National Park in the management of four important shore angling fishes along the south-east Cape Coast. *South African Journal of Marine Science,* 24: 27 - 36.

Cullen, J. T., Lane, T. W., Morel, F. M. & Sherrell, R. M. 1999. Modulation of cadmium uptake in phytoplankton by seawater CO2 concentration. *Nature,* 402(6758): 165 - 167.

Cuong, D. T., Karuppiah, S. & Obbard, J. P. 2008. Distribution of heavy metals in the dissolved and suspended phase of the sea-surface microlayer, seawater column and in sediments of Singapore's coastal environment. *Environmental Monitoring Assessment,* 138: 255 - 272.

Dalling, J. 2012. Food webs and networks: the architecture of biodiversity (Unpublished presentation). Available at: http://www.life.illinois.edu/ib/453/453lec12foodwebs.pdf Date of access: 13 November 2017. [PowerPoint presentation].

Day, J.H. 1969. A guide to marine life on South African shores. Published for the University of Cape Town by A. A. Balkema, Cape Town, 300 pp.

De Lecea, A. M., Fennessy, S. T. & Smit, A. J. 2013. Processes controlling the benthic food web of a mesotrophic bight (KwaZulu-Natal, South Africa) revealed by stable isotope analysis. *Marine Ecology Progress Series*, 484: 97 - 114. doi: 10.3354/meps10311

DEAT (Department of Environmental Affairs and Tourism). 2008. South Africa's National Programme of Action for Protection of the Marine Environment from Land-based Activities. 1st edition. Cape Town.

DEA (Department of Environmental Affairs). 2015. Spatial protection of marine habitat and biodiversity in South Africa, *In* State of the oceans and coasts around South Africa 2014. *Department of Environmental Affairs*, Pretoria.

Degger, N. 2010. The application of passive artificial devices for monitoring metallic and organic pollutants along the South African coastline. MSc dissertation, University of Johannesburg, South Africa.

Degger, N., Wepener, V., Richardson, B. J. & Wu, R. S. S. 2011a. Brown mussels (*Perna perna*) and semi-permeable membrane devices (SPMDs) as indicators of organic pollutants in the South African marine environment. *Marine Pollution Bulletin,* 63:91 - 97.

Degger, N., Wepener, V., Richardson, B. J. & Wu, R. S. S. 2011b. Application of artificial mussels (AMs) under South African marine conditions: A validation study. *Marine Pollution Bulletin,* 63:108 - 118.

Delong, M. D., Thorp, J. H., Thoms, M. C. & McIntosh, L. M. 2011. Trophic niche dimensions of fish communities as a function of historical hydrological conditions in a Plains river. *River Systems*, 19: 177 - 187.

DeNiro, M. & Epstein, S. 1978. Influence of the Diet on the Distribution of Carbon Isotopes in Animals. *Geochimica et Cosmochimica Acta*, 42: 495 - 506.

Driver, A., Sink, K. J., Nel, J. N., Holness, S., Van Niekerk, L., Daniels, F., Jonas, Z., Majiedt, P. A., Harris, L. & Maze, K. 2012. National Biodiversity Assessment 2011: An assessment of South Africa's biodiversity and ecosystems. Synthesis Report. *South African National Biodiversity Institute and Department of Environmental Affairs*, Pretoria.

EPA (Environmental Protection Agency). 2016. Persistent organic pollutants: a global issue, a global response. Available at: https://www.epa.gov/international-cooperation/persistent-organic-pollutants-global-issue-global-response Date of access: 13 November 2017.

Femorale. 2017. Available at: http://www.femorale.com Date of access: 15 November 2017. [Database].

FishBase. 2017. Available at: http://www.fishbase.org/search.php Date of access: 14 November 2017. [Database].

Flemming, B., Martin, K. & Akkers, W. 1986. Agulhas Bank studies. Marine geology off the Tsitsikamma Coast, *Agulhas Bank Symposium,* Cape Town. [Poster paper].

Foreman, P. W. & Henninger, T. O. 2010. Feeding dynamics of *Palaemon peringueyi* (Decapoda, Caridea) in the temporarily open/closed Kasouga Estuary, South Africa. *African Journal of Aquatic Science*, 32(2): 193 - 198. doi: 10.2989/16085914.2010.490982

Foster, G. G. & Hodgson, A. N. 1998. Consumption and apparent dry matter digestibility of six intertidal macroalgae by *Turbo sarmaticus* (Mollusca: Vetigastropoda: Turbinidae). *Aquaculture*, 167(3): 211 - 227.

Fry, B. & Wainright, S. C. 1991. Diatom sources of ^{13}C-rich carbon in marine food webs. *Marine Ecology Progress Series,* 76: 149 - 157.

Fry, B. 2006. Stable Isotope Ecology. *Springer Science + Business Media, LLC,* 316 pp.

Fung, C. N., Lam, J. C. W., Zheng, G. J., Connell, D. W., Monirith, I., Tanabe, S., Richardson, B. J. & Lam, P. K. S. 2004. Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using *Perna viridis* and *Mytilus edulis*. *Environmental Pollution,* 127: 203 - 216.

Galimberti, M., Loftus, E. & Sealy, J. 2016. Investigating δ^{18}O of *Turbo sarmaticus* (L. 1758) as an indicator of sea surface temperatures. *Palaeogeography, Palaeoclimatology, Palaeoecology*, in press. doi.org/10.1016/j.palaeo.2016.12.010

Gannes, L. Z., O'Brien, D. M. & Del Rio, C. M. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, 75(4): 1271 - 1276. Available at: https://pdfs.semanticscholar.org/0677/5811d595033affd19713e36edf34634b7e1d.pdf Date of access: 14 November 2017.

Gerber, R. J. L. 2012. The use tigerfish, Hydrocynus vittatus, as indicator of metal and organic contaminant exposure in the Kruger National Park. Ph. D. thesis. University of Johannesburg, Johannesburg.

GESAMP (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution). 2001. Protecting the Oceans from Land-based Activities: Land-based Sources and Activities Affecting the Quality and Uses of the Marine, Coastal and Associated Freshwater Environment (No. 71). UNEP.

Gibbons, M. J., Buecher, E., Thibault-Botha, D. & Helm, R. R. 2010. Patterns in marine hydrozoan richness and biogeography around southern Africa: Implications of life cycle strategy. *Journal of Biogeography,* 37:606 - 616.

Gobas, F. A. & Morrison, H. A. 2000. Bioconcentration and biomagnification in the aquatic environment. *In* Boethling, R. S. & Mackay, D. *eds*. Handbook of property estimation methods for chemicals, environmental and health sciences. *Boca Raton, Florida (FL): CRC Press,* pp. 189 - 231.

Goble, B. J. 2014. Introduction. *In* Goble, B. J., Van der Elst, R. P. & Oellermann, L. K. *eds.* Ugu Lwethu - Our Coast. *A Profile of Coastal KwaZulu-Natal. KwaZulu-Natal Department of Agriculture and Environmental Affairs and the Oceanographic Research Institute, Cedara*, 202 pp.

Gray, J. S. 2002. Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin,* 45(1): 46 - 52.

Greenfield, R., Wepener, V., Degger, N. & Brink, K. 2011. Richards Bay Harbour: Metal exposure monitoring over the last 34 years. *Marine Pollution Bulletin,* 62:1926 - 1931.

Greenfield, R., Brink, K., Degger, N. & Wepener, V. 2014. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. *Marine Pollution Bulletin,* 85:566 - 573.

Gregory, M. A., Marshall, D. J., George, R. C., Anandraj, A. & McClurg, T. P. 2002. Correlations between metal uptake in the soft tissue of *Perna perna* and gill filament pathology after exposure to mercury. *Marine Pollution Bulletin,* 45: 114 - 125.

Griffiths, R. J. 1976. The larval development of *Pyura stolonifera* (Tunicata). *Transactions of the Royal Society of South Africa*, 42(1): 1 - 9. doi:10.1080/00359197609519899

Griffiths, M. H. 2000. Long-term trends in catch and effort of commercial linefish off South Africa's Cape Province: snapshots of the 20th century. *African Journal of Marine Science*, 22: 81 - 110.

Griffiths, C. L. 2005. Coastal marine biodiversity in East Africa. *Indian Journal of Marine Sciences*, 34(1): 35 - 41.

Griffiths, C. L., Robinson, T. B., Lange, L. & Mead, A. 2010. Marine biodiversity in South Africa: an evaluation of current states of knowledge. *PLoS ONE* 5(8): e12008. doi:10.1371/journal.pone.0012008

Guastella, L. 2014. Physical Oceanography. *In* Goble, B. J., Van der Elst, R. P. & Oellermann, L. K. *eds.* Ugu Lwethu - Our Coast. *A Profile of Coastal KwaZulu-Natal. KwaZulu-Natal Department of Agriculture and Environmental Affairs and the Oceanographic Research Institute, Cedara*, pp. 20 - 26.

Hadfield, K. A., Smit, N. J. & Avenant-Oldewage, A. 2008. *Gnathia pilosus* sp. nov. (Crustacea, Isopoda, Gnathiidae) from the east coast of South Africa. *Zootaxa*, 1894: 23 - 41.

Hall, J. E. 2004. Bioconcentration, Bioaccumulation, and the Biomagnification in Puget Sound Biota: Assessing the Ecological Risk of Chemical Contaminants in Puget Sound. *Tahoma West Literary Arts Magazine*, 8(1): 40 - 51.

Halpern, B. S. 2003. The impact of marine reserves: do reserves work and does size matter? *Ecological Applications,* 13: 117 - 137.

Hanekom, N. 2005. Weather and sea temperature patterns occurring in the Tsitsikamma National Park. *Progress report for SANParks*. Pp. 1 - 2.

Hanekom, N., Randall, R.M., Bower, D., Riley, A. & Kruger, N. 2009. Garden Route National Park: The Tsitsikamma Area – State of Knowledge. South African National Parks Publication Series, Pretoria.

Hanekom, N. 2011. Trophic structure and biomass distribution of macrobenthos on sheltered and semi-exposed rocky shores of Tsitsikamma Marine Protected Area. *African Zoology,* 46: 224 - 238.

Hanekom, N., Randall, R. M., Bower, D., Riley, A. & Kruger, N. 2012. Garden Route National Park: The Tsitsikamma Section – State of Knowledge. South African National Parks Publication Series, Pretoria.

Hanna, D. E. L., Solomon, C. T., Poste, A. E., Buck, D. G. & Chapman, L. J. 2015. A review of mercury concentrations in freshwater fishes of Africa: patterns and predictors. *Environmental Toxicology and Chemistry,* 34: 215 - 223.

Haris, V. 1990. Sessile animals of the sea shore.*Springer Science & Business Media,* 381 pp.

Harris, J. M., Livingstone, T., Lombard, A. T., Lagabrielle, E., Haupt, P., Sink, K., Schleyer, M. & Mann, B. Q. 2012. Coastal and Marine Biodiversity Plan for KwaZulu-Natal. *Spatial priorities for the conservation of coastal and marine biodiversity in KwaZulu-Natal.* Ezemvelo KZN Wildlife Scientific Services Technical Report.

Haschek, W. M., Rousseaux, C. G. & Wallig, M. A. (*eds.).* 2013. Haschek and Rousseaux's Handbook of Toxicologic Pathology, 3rd edition. *Academic Press, Elsevier,* London

Heidt, A., Khalsa, A., Myers, S., Trinh, T. & Wade, V. 2013. Territoriality in the South African intertidal limpet *Scutellastra longicosta*. Available at:https://courses.pbsci.ucsc.edu/eeb/bioe159/wp-content/uploads/2013/06/S.-longicosta-Final-Paper-Amrita-Khalsa-Amanda-Heidt-Victoria-Wade-Shannon-Myers-Travis-Trin_final_edit.pdf Date of access: 14 November 2017. [PDF].

Heyns, E. R. 2015. Community structure and trophic ecology of shallow and deep rocky reefs in a well-established Marine Protected Area. Ph.D. thesis, Rhodes University, Grahamstown.

Heyns, E. R., Bernard, A. T. F., Richoux, N. B. & Götz, A. 2016. Depth-related distribution patterns of subtidal macrobenthos in a well-established marine protected area. *Marine Biology*, 163(39): 1 - 15.

Heyns-Veale, E. R., Bernard, A. T. F., Richoux, N. B., Parker, D., Langlols, T. J. & Götz, A. 2016. Depth and habitat determine assemblage structure of South Africa's warm-temperate reef fish. *Marine Biology*, 163(158): 1 - 17.

Hill, J. M., McQuaid, C. D. & Kaehler, S. 2006. Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. *Marine Ecology Progress Series,* 318: 63 - 73. doi: 10.3354/meps318063.

Hill, J. M., McQuaid, C. D. & Kaehler, S. 2008. Temporal and spatial variability in stable isotope ratios of SPM link to local hydrography and longer term SPM averages suggest heavy dependence of mussels on nearshore production. *Marine Biology*, 154(5): 899 - 909. doi: 10.1007/s00227-008-0983-2

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, 120: 314 - 326.

Hofmann, M., Wolf-Gladrow, D. A., Takahashi, T., Sutherland, S. C., Six, K. D. & Maier-Reimer, E. 2000. Stable carbon isotope distribution of particulate organic matter in the ocean: a model study. *Marine Chemistry,* 72: 131 - 150.

IPCS (International Program for Chemical Safety). 2006. Concise International Chemical Assessment Document 70, Heptachlor. International Programme on Chemical Safety. *WHO press, World Health Organization*, Geneva

Isaacs, J. D. 1973. Potential trophic biomasses and trace-substance concentrations in unstructured marine food webs. *Marine Biology,* 22: 97 - 104.

Jackson, M. C., Donohue, I., Jackson, A. L., Britton, J. R., Harper, D. M. & Gray, J. 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS ONE,* 7(2): e31757. doi:10.1371/journal.pone.0031757

Kench, J. 1984. The coast of southern Africa. *C. Struik Publishers*, 171 pp.

Kohler, S. A., Connan, M., Hill, J. M., Mablouké, C., Bonnevie, B., Ludynia, K., Kemper, J., Huisamen, J., Underhill, L. G., Cherel, Y., McQuaid, C. D. & Jaquemet, S. 2011. Geographic variation in the trophic ecology of an avian rocky shore predator, the African black oystercatcher, along the southern African coastline. *Marine Ecology Progress Series,* 435: 235 - 249. doi: 10.3354/meps09215

Kruger, A. 2014. Climate. *In* Goble, B. J., Van der Elst, R. P. & Oellermann, L. K. *eds.* Ugu Lwethu - Our Coast. *A Profile of Coastal KwaZulu-Natal. KwaZulu-Natal Department of Agriculture and Environmental Affairs and the Oceanographic Research Institute, Cedara*, pp. 15 - 16.

Krumins, J. A., Van Oevelen, D., Bezemer, T. M., De Deyn, G. B., Hol, W. H. G., Van Donk, E., De Boer, W., De Ruiter, P. C., Middelburg, J. J., Monroy, F., Soetaert, K., Thebault, E., Van de Koppel, J., Van Veen, J. A., Viketoft, M., & Van der Putten, W. H. 2013. Soil and Freshwater and Marine Sediment Food Webs: Their Structure and Function. *Bioscience,* 63: 35 - 42. doi:10.1525/bio.2013.63.1.8

KZN (KwaZulu-Natal Department of Economic Development, Tourism and Environmental Affairs). 2017. KwaZulu-Natal Coastal Management Programme.*KwaZulu-Natal Department of Economic Development, Tourism and Environmental Affairs*, Pietermaritzburg.

Lares, M. L., Flores-Munoz, G. & Lara-Lara, R. 2002. Temporal variability of bioavailable Cd, Hg, Zn, Mn and Al in an upwelling regime. *Environmental Pollution,* 120: 595 - 508.

Layman, C. A., Arrington, D. A., Montaña, C. G. & Post, D. M. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology,* 88(1): 42 - 48.

Leslie, H. M. 2005. A synthesis of marine conservation planning approaches. *Conservation Biology*, 19(6): 1701 - 1713.

Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jorgensen, E.H., Sonne, C., Verreault, J., Vijayan, M. M. & Gabrielsen, G. W. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment,* 408: 2995 - 3043.

Little, C., Williams, G. A. & Trowbridge, C. D.2009. The biology of rocky shores, 2nd edition, *Oxford: Oxford University Press,* 352 pp.

Liu, F., Rainbow, P. S. & Wang, W.-X. 2013. Inter-site differences of zinc susceptibility of the oyster *Crassostrea hongkongensis*. *Aquatic Toxicology,* 132(133): 26 - 33.

Lombard, A. T., Strauss, T., Harris, J., Sink, K., Attwood, C. & Hutchings, L. 2004. South African National Spatial Biodiversity Assessment 2004: Technical Report. *Volume 4: Marine Component.* Pretoria, South African National Biodiversity Institute.

Lutjeharms, J. R. E. 2006. The coastal oceans of south-eastern Africa. *In* Robinson, A. R. & Brink, K. H. *eds.* The Sea, Volume 14B, pp. 783 - 834.

MacKenzie, K. M., Palmer, M. R., Moore, A., Ibbotson, A. T., Beaumont, W. R. C., Poulter, D. J. S. & Trueman, C. N. 2011. Locations of marine animals revealed by carbon isotopes. *Scientific reports*, 1(21): 1 - 6. doi.org/10.1038/srep00021

MacNeil, M., Skomal, G. & Fisk, A. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. *Marine Ecology Progress Series,* 302: 199 - 206.

MacNeil, M. A. 2008. Scientific Basis for Ecosystem-Based Management in the Lesser Antilles Including Interactions with Marine Mammals and Other Top Predators: The application of stable isotope analysis in marine ecosystems. FAO, Barbados, FI:GCP/RLA/140/JPN. Technical Document No. 8

MarLIN (The Marine Life Information Network). 2017. Species list. Available at: http://www.marlin.ac.uk/species Date of access: 14 November 2017. [Database].

Mason, R. P., Laporte, J. M. & Andres, S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology,* 38(3): 283 - 297.

McCutchan, J. H., Lewis, W. M., Kendall, C. & McGrath, C. C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos,* 102: 378 - 390. doi:10.1034/j.1600-0706.2003.12098.x

McQuaid, C. D. & Branch, G. M. 1984. Influence of sea temperature, substratum and wave exposure on rocky intertidal communities: an analysis of faunal and floral biomass. *Marine Ecology Progress Series,* 19: 145 - 151.

Michener, R. H. & Kaufman, L. 2007. Stable isotope ratios as tracers in marine food webs: an update. *In* Michener, R & Lajtha, K. *eds.* Stable Isotopes in Ecology and Environmental Science, 2nd edition, *Blackwell Publishing Ltd*, Oxford, UK. doi: 10.1002/9780470691854.ch12

Middelburg, J. J. 2014. Stable isotope dissect aquatic food webs from the top to the bottom. *Biogeosciences,* 11: 2357 - 2371. doi:10.5194/bg-11-2357-2014

Minagawa, M. & Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta,* 48: 1135 - 1140.

MPA Forum. 2017. Aliwal Shoal Marine Protected Area Management Plan. Available at: http://mpaforum.org.za/wp-content/uploads/2016/08/Management-Plan-ALIWAL-SHOAL-MPA-PLAN-31-01-06.pdf Date of access: 14 November 2017.

Newman, M. C. 2010. Fundamentals of Ecotoxicology, 3rd edition, *Boca Raton: CRC Press,* Taylor & Francis Group.

Payne, R. P., Griffiths, C. L., Von der Heyden, S. & Koch, E. 2015. The cushion–star *Parvulastra exigua* in South Africa: one species or more? *ZooKeys*, 524: 1 - 16.

Peterson, B. J. & Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics,* 18: 293 - 320.

Philips, D. J. H. & Rainbow, P. S. 1994. Biomonitoring of trace aquatic contaminants. *Springer, Dordrecht*: Chapman & Hall, New York.

Pimm, S. L. 2002. Food Webs. 2nd edition,*The University of Chicago Press*, Chicago, 258 pp.

Pinnegar, J. K. & Polunin, N. V. C. 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia,* 122(3): 399 - 409.

Polunin, N. & Pinnegar, J. 2002. Chapter 14: Trophic ecology and the structure of marine food webs. *In* Hart, P. & Reynolds, J. *eds*. Handbook of Fish and Fisheries, Volume 1,*Blackwell Science Ltd., Oxford*, pp. 301 - 320.

Porter, S. N. 2009. Biogeography and potential factors regulating shallow subtidal reef communities in the western Indian Ocean. Ph. D. thesis, University of Cape Town, Cape Town.

Post, D. L. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* 83(3): 703 - 718.

Puccinelli, E., Noyon, M. & McQuaid, C. D. 2016a. Does proximity to urban centres affect the dietary regime of marine benthic filter feeders? *Estuarine, Coastal and Shelf Science,* 169: 147 - 157.

Puccinelli, E., Noyon, M. & McQuaid, C. D. 2016b. Hierarchical effects of biogeography and upwelling shape the dietary signatures of benthic filter feeders. *Marine Ecology Progress Series,* 534: 37 - 54.

Puccinelli, E., McQuaid, C. D. & Noyon, M. 2016c. Absence of an effect of freshwater input on the stable isotope and fatty acid signatures of intertidal filter-feeders. *African Journal of Marine Science,* 38(4): 481 - 492. doi: 10.2989/1814232X.2016.1227277

Quinn, L. P., De Vos, B. J., Fernandes-Whaley, M., Roos, C., Bouwman, H., Kylin, H., Pieters, R. & Van den Berg, J. 2011. Pesticide use in South Africa: one of the largest importers of pesticides in Africa, Pesticides in the Modern World – Pesticides use and management. Available at:http://www.intechopen.com/articles/show/title/pesticide-use-in-south-africa-one-of-the-largest-importers-of-pesticides-in-africa Date of access: 13 November 2017.

Rainbow, P.S. 1993. The significance of trace metal concentrations in marine invertebrates. *In* Dallinger, R. & Rainbow, P. S. *eds.* Ecotoxicology of metals in invertebrates. *Lewis Publishers, Boca Raton.* pp. 120-131.

Rainbow, P. S. & Wang, W.-X. 2001. Comparative assimilation of Cd, Cr, Se, and Zn by the barnacle *Elminius modestus* from phytoplankton and zooplankton diets. *Marine Ecology Progress Series,* 218: 239 - 248.

Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution,* 120: 497 - 507.

Rainbow, P. S. & Wang, W.-X. 2005. Trace metals in barnacles: the significance of trophic transfer. *Science in China Series C: Life Sciences*, *48*(1): 110 - 117.

Rainbow, P. S., Liu, F. & Wang, W.-X. 2015. Metal accumulation and toxicity: the critical accumulated concentration of metabolically available zinc in an oyster model. *Aquatic toxicology,* 162: 102 - 108.

Roberts, M. J. & Van den Berg, M. 2005. Currents along the Tsitsikamma coast, South Africa, and potential transport of squid larvae and ichthyoplankton. *African Journal of Marine Science,* 27(2): 375 - 388.

Robinson, G. A. & De Graaff, G. 1994. Marine protected areas of the Republic of South Africa. *Council for the Environment*. IUCN: National Parks Board, Pretoria (South Africa), 232 pp.

SANCOR (South African Network for Coastal and Oceanic Research). 1985. The SANCOR Marine Pollution Research Programme 1986-1990. *South African National Scientific Programmes report 112*.

SANParks (South African National Parks). 2006. Tsitsikamma National Park: Park management plan October 2006.

SANParks (South African National Parks). 2014. Garden Route National Park: State of Knowledge. South African National Parks unpublished report.

Satterfield, F. R. & Finney, B. P. 2002. Stable isotope analysis of Pacific salmon: insight into trophic status and oceanographic conditions over the last 30 years. *Progress in Oceanography,* 53: 231 - 246.

Schumann, E. H., Perrins, L. A. & Hunter, I. T. 1982. Upwelling along the South Coast of the Cape Province. *South African Journal of Science,* 78: 238 - 242.

SeaLifeBase. 2017. Available at: http://www.sealifebase.org/search.php Date of access: 14 November 2017. [Database].

Sewell, J. 2014. Fact sheet: Rocky shore. Available from: http://www.mba.ac.uk/fact-sheet-rocky-shore Date of access: 31 October 2017.

Sibali, L. L., Okwonkwo, J. O. & McCrindle, R. L. 2008. Determination of selected organochlorine pesticide (OCP) compounds from the Jukskei catchment area in Gauteng, South Africa. *Water SA,* 34: 611 - 622.

Sink, K. J. 2001. A hierarchical analysis of abiotic determinants and harvesting impacts in the rocky intertidal communities of KwaZulu-Natal. Ph.D. thesis, University of Cape Town, Cape Town.

Sink, K. J., Branch, G. M. & Harris, J. M. 2005. Biogeographic patterns in rocky intertidal communities in KwaZulu-Natal. *South Africa. African Journal of Marine Science,* 27(1): 81 - 96.

Sink, K., Holness, S., Harris, L., Majiedt, P., Atkinson, L., Robinson, T., Kirkman, S., Hutchings, L., Leslie, R., Lamberth, S., Kerwath, S., Von der Heyden, S., Lombard, A., Attwood, C., Branch, G., Fairweather, T., Taljaard, S., Weerts, S., Cowley, P., Awad, A., Halpern, B., Grantham, H. & Wolf, T. 2012. National Biodiversity Assessment 2011: Technical Report. *Volume 4: Marine and Coastal Component*. South African National Biodiversity Institute, Pretoria, 325 pp.

Smit, K. & Glassom, D. 2017. Large fluctuations but constant mean temperatures allow corals to persist in intertidal rock pools on the east coast of South Africa. *Helgoland Marine Research*, 71(3): 1 - 9.

South Africa. 1998. Marine Living Resources Act, 18 of 1998.

South Africa. 2016. Protected Areas Act 2003 (Act no. 57 of 2003): Regulations for the Management of the Tsitsikamma National Park Marine Protected Area. (Government notice no. 40511). *Regulation Gazette,* 10676, 19 Dec.

Stockholm Convention. 2012. Report of the DDT expert group on the assessment of the production and use of DDT and its alternatives for disease vector control. Available at: http://chm.pops.int/Implementation/DDT/DDTMeetings/DDTEG42012/tabid/2942/mctl/ViewDetails/EventModID/874/EventID/338/xmid/9462/Default.aspx Date of access: 14 November 2017.

Tang, Z., Huang, Q., Yang, Y., Zhu, X. & Fu, H. 2013. Organochlorine pesticides in lower reaches of Yangtze River: Occurrence, ecological risk and temporal trends. *Ecotoxicology and Environmental Safety* 87: 89 - 97.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. 2012. Heavy Metals Toxicity and the Environment. *Experientia Supplementum*, 101: 133 - 164. Available at: http://doi.org/10.1007/978-3-7643-8340-4_6 Date of access: 13 November 2017.

Temara, A., Warnau, M., Jangoux, M. & Dubois, P. 1997. Factors influencing the concentrations of heavy metals in the asteroid *Asterias rubens L.* (Echinodermata). *Science of the total environment,* 203(1): 51 - 63.

Teske, P. R., Von der Heyden, S., McQuaid, C. D. & Barker, N. P. 2011. A review of marine phylogeography in southern Africa. *South African Journal of Sciences,* 107(5):1 - 11.

TidalTao. 2016. Your guide to common critters of the KwaZulu-Natal rocky shores.Available at: http://www.tidaltao.com/attachments/category/104/ID-Guide-TidalTao.pdf Date of access: 14 November 2017. [Pamphlet].

Tilney, R. L., Nelson, G., Radloff, S. E. & Buxton, C. D. 1996. Ichthyoplankton distribution and dispersal in the Tsitsikamma National Park marine reserve, South Africa. *South African Journal of Marine Science,* 17: 1 - 14.

Toerien, D. K. 1976. Geologie van die Tsitsikammakusstrook. *Koedoe,* 19: 31 - 41.

Turpie, J. K., Beckley, L. E. & Katua, S. M. 2000. Biogeography and the selection of priority areas for conservation of South African coastal fishes. *Biology of Conservation,* 92: 59 - 72.

Two Oceans Aquarium. 2017. Species explorer. Available at: https://www.aquarium.co.za/species?category=4 Date of access: 14 November 2017. [Database].

Van Alstyne, K. L. & Houser, L. T. 2003. Dimethylsulfide release during macroinvertebrate grazing and its role as activated chemical defence. *Marine Ecology Progress Series,* 250: 179 - 185.

Van As, J., De Preez, J., Brown, L. & Smit, N. 2012. The story of Life & the Environment: an African perspective. *Random House Struik, Struik Nature*, 456 pp.

Van Zyl, D. 2003. The climate of South Africa. *In* Perryer, F. *ed*. South African weather and atmospheric phenomena, *Briza Publications, Cape Town*, pp. 36 - 53.

Vander Zanden, M. J. & Rasmussen, J. B. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecology,* 80(4): 1395 - 1404.

Verhaert, V., Covaci, A., Bouillon, S., Abrantes, K., Musibono, D., Bervoets, L., Verheyen, E. & Blust, R. 2013. Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (DR Congo). *Environment International,* 59: 290 - 302.

Verhaert, V., Newmark N., D'Hollander, W., Covaci, A., Vlok, W., Wepener, V., Addo-Bediako, A., Jooste, A., Teuchies, J., Blust, R. & Bervoets, L. 2017. Persistent organic pollutants in the Olifants River Basin, South Africa: Bioaccumulation and trophic transfer through a subtropical aquatic food web. *Science of the Total Environment,* 586: 792 - 806.

Vijgen, J., Abhilash, P. C., Li, Y. F., Lal, R., Forter, M., Torres, J., Singh, N., Yunus, M., Tian, C., Schäffer, A. & Weber, R. 2011. Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs – a global perspective on the management of Lindane and its waste isomers. *Environmental Science and Pollution Research,* 18: 152 - 162.

Wainright, S. C., Fogarty, M. J., Greenfield, R. C. & Fry, B. 1993. Long-term changes in the Georges Bank food web: trends in stable isotopic compositions of fish scales. *Marine Biology,* 115: 481 - 493.

Walker, C. H. 2009. Organic pollutants: An ecotoxicological approach, 2nd edition, *Boca Raton*: Taylor & Frances Group.

Walker, C. H., Hopkin, S. P., Sibly, R. M. & Peakall, D. B. 2006. Principles of Ecotoxicology, 3rd edition, *Boca Raton, Florida (FL)*: Taylor & Francis Group.

Walters, D. M., Jardine, T. D., Cade, B. S., Kidd, K. A., Muir, D. C. G. & Leipzig-Scott, P. 2016. Trophic magnification of organic chemicals: a global synthesis. *Environmental Sciences and Technology,* 50: 4650 - 4658.

Wang, W.-X. & Fisher, N. S. 1997. Modeling metal bioavailability for marine mussels. *Reviews of Environmental Contamination and Toxicology,* 151: 39 - 65.

Wepener, V., Bervoets, L., Mubiana, V. & Blust, R. 2008. Metal exposure and biological responses in resident and transplanted blue mussels (*Mytilus edulis*) from the Scheldt estuary. *Marine pollution bulletin,* 57(6): 624 - 631.

WHO (World Health Organization). 2003a. Aldrin and Dieldrin in Drinking-water. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/adrindieldrin.pdf Date of access: 14 November 2017.

WHO (World Health Organization). 2003b. Chlordane in drinking-water. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/chlordane.pdf Date of access: 14 November 2017.

WHO (World Health Organization). 2003c. Lindane in drinking-water. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/lindane.pdf Date of access: 14 November 2017.

WHO (World Health Organization. 2011. Guidelines for drinking-water quality, 4th edition, WHO Press, Switzerland, Geneva. Available at: http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf Date of access: 14 November 2017.

WoRMS (World Register of Marine Species). 2017. Available at: http://www.marinespecies.org Date of access: 14 November 2017. [Database].

Wu, R. S. S. & Lau, T. C. 1996. Polymer-ligands: A novel chemical device for monitoring heavy metals in the aquatic environments. *Marine Pollution Bulletin,* 32: 391 - 396.

Yohannes, Y. B., Ikenaka, Y., Nakayama, S. M. M., Saengtienchai, A., Watanabe, K. & Ishizuka, M. 2013. Organochlorine Pesticides and Heavy Metals in Fish from Lake Awassa, Ethiopia: Insights from Stable Isotope Analysis. *Chemosphere,* 91: 857 - 863.

Zimmo, S., Blanco, J. & Nebel, S. 2012. The Use of Stable Isotopes in the Study of Animal Migration. *Nature Education Knowledge,* 3(12): 3.

Appendix A

Table A 1: *Species names with reference numbers (Ref. no.) from each of the sites sampled with the number of replicates (n), stable nitrogen signature ($\delta^{15}N$), stable carbon signature ($\delta^{13}C$), elemental composition (C:N), trophic level (TL) and trophic groups.*

Ref. no.	Common name	Species name	n	Weight (mg)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C:N	TL	Trophic group
Tsitsikamma									
Tsi_1	Green macro-algae	*Ulva lactuca*	4	1.13	5.36	-9.57	8.25	1.00	Primary producer
Tsi_2	Red algae	*Jania* sp.	4	1.15	6.34	-7.22	17.66	1.29	Primary producer
Tsi_3	SPM	Suspended particulate matter	4	1.10	6.89	-12.6	9.26	1.45	Primary producer
Tsi_4	Brown encrusted algae	*Ralfsia* sp.	4	1.10	7.07	-5.35	19.68	1.50	Primary consumer
Tsi_5	Cape urchin	*Parechinus angulosus*	4	1.20	7.07	-13.8	5.08	1.50	Primary consumer
Tsi_6	Duck's foot limpet	*Scutellastra longicosta*	4	1.11	7.67	-6.40	3.56	1.68	Primary consumer
Tsi_7	Spotted sea hare	*Aplysia parvula*	4	1.13	7.73	-14.0	3.51	1.70	Primary consumer
Tsi_8	Alikreukel	*Turbo sarmaticus*	4	1.11	8.40	-13.1	3.35	1.89	Primary consumer
Tsi_9	Brown mussel	*Perna perna*	4	1.09	8.62	-15.2	3.67	1.96	Primary consumer
Tsi_10	Goat's eye limpet	*Cymbula oculus*	4	1.13	8.96	-12.5	3.43	2.06	Primary consumer
Tsi_11	Pink-lipped topshell	*Oxystele sinensis*	4	1.12	9.22	-11.9	3.82	2.14	Primary consumer
Tsi_12	Sand shrimp	*Palaemon peringueyi*	4	1.06	9.77	-10.3	3.58	2.30	Primary consumer
Tsi_13	Dwarf cushion-star	*Parvulastra exigua*	4	1.15	10.1	-7.21	5.46	2.39	Primary consumer
Tsi_14	Cape reef worm	*Gunnarea gaimardi*	4	1.14	10.4	-13.9	3.62	2.49	Primary consumer
Tsi_15	Red bait	*Pyura stolonifera*	3	1.16	10.6	-16.3	4.14	2.56	Secondary consumer
Tsi_16	Volcano barnacle	*Tetraclita serrata*	4	1.16	11.3	-11.8	4.91	2.75	Secondary consumer
Tsi_17	Southern periwinkle	*Afrolittorina knysnaensis*	4	1.15	11.4	-13.3	3.40	2.79	Secondary consumer
Tsi_18	Spiny starfish	*Marthasterias glacialis*	4	1.08	11.7	-9.66	3.55	2.86	Secondary consumer
Tsi_19	Plum anemone	*Actinia* sp.	4	0.99	11.8	-14.5	3.68	2.89	Secondary consumer
Tsi_20	Ridged burnupena	*Burnupena cincta*	4	1.11	11.9	-13.9	3.72	2.94	Secondary consumer
Tsi_21	Southern mullet	*Liza richardsonii*	4	1.13	12.1	-12.1	3.17	2.98	Secondary consumer
Tsi_22	Musselcracker	*Sparodon durbanensis*	4	1.05	13.5	-12.1	3.15	3.40	Secondary consumer

Ref. no.	Common name	Species name	n	Weight (mg)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C:N	TL	Trophic group
Tsi_23	Goby	*Caffrogobius* sp.	4	1.07	13.9	-11.3	3.20	3.52	Tertiary consumer
Tsi_24	Klipfish	*Clinus* sp.	4	1.20	14.0	-13.3	3.22	3.55	Tertiary consumer
Sheffield Beach									
SB_1	SPM	Suspended particulate matter	4	1.16	3.77	-30.7	8.87	1.00	Primary producer
SB_2	Green algae	*Codium* sp.	4	1.14	4.51	-17.7	12.21	1.22	Primary producer
SB_3	Red algae	*Corallina* sp.	4	1.14	4.83	-11.2	18.78	1.31	Primary producer
SB_4	African periwinkle	*Afrolittorina africana*	4	1.14	5.26	-12.8	4.16	1.44	Primary producer
SB_5	Collector urchin	*Tripneustes gratilla*	4	1.21	5.64	-13.7	9.18	1.55	Primary consumer
SB_6	Green macro-algae	*Ulva lactuca*	4	1.08	5.82	-12.4	14.41	1.60	Primary consumer
SB_7	Variable limpet	*Helcion concolor*	3	1.22	7.01	-13.3	3.59	1.95	Primary consumer
SB_8	Crowned turban shell	*Lunella coronata*	4	1.14	7.93	-12.2	3.37	2.23	Primary consumer
SB_9	Toothed topshell	*Monodonta australis*	4	1.16	8.08	-14.0	3.64	2.27	Primary consumer
SB_10	Brown mussel	*Perna perna*	4	1.09	9.05	-17.4	3.77	2.55	Secondary consumer
SB_11	Blue coral worm	*Spirobranchus kraussii*	4	1.12	9.33	-17.3	3.81	2.64	Secondary consumer
SB_12	Blotched nerite	*Nerita albicila*	4	1.16	9.57	-10.4	3.40	2.71	Secondary consumer
SB_13	Ringneck blenny	*Parablennius pilicornis*	4	1.15	9.72	-9.95	3.25	2.75	Secondary consumer
SB_14	Dwarf cushion-star	*Parvulastra exigua*	4	1.22	10.1	-8.28	8.04	2.85	Secondary consumer
SB_15	Volcano barnacle	*Tetraclita serrata*	4	1.08	10.5	-16.9	4.00	2.99	Secondary consumer
SB_16	Plum anemone	*Actinia* sp.	4	1.12	10.5	-17.1	3.52	2.99	Secondary consumer
SB_17	Rayed wheel limpet	*Cellana radiata*	4	1.11	10.9	-8.99	3.90	3.09	Secondary consumer
SB_18	Knobby dogwhelk	*Thalessa savignyi*	4	1.15	11.2	-15.6	3.53	3.18	Secondary consumer
SB_19	Blacktail	*Diplodus sargus sargus*	4	1.17	11.8	-16.5	3.12	3.38	Secondary consumer
SB_20	Knysna sandgoby	*Psammogobius knysnaensis*	4	1.13	12.8	-14.2	3.22	3.66	Tertiary consumer

Appendix B

Table B 1: *Species, δ15N, moisture content, percentage lipid, as well as number of replicate (n) and mass of species analysed for both metal and organochlorine pesticide analyses at each site ((-) indicate species that were not analysed, the values were not determined).*

Species name	$\delta^{15}N$	Moisture content	% lipid	Metals		OCPs	
				n	Mean mass (mg)	n	Mean mass (g)
Tsitsikamma							
Ulva lactuca	5.36	-	-	10	0.121	-	-
Jania sp.	6.34	0.84	0.34	10	0.206	1	1.16
Ralfsia sp.	7.07	0.81	3.21	10	0.203	1	1.06
Parechinus angulosus	7.07	0.75	16.9	6	0.210	1	1.03
Scutellastra longicosta	7.67	-	-	10	0.201	-	-
Aplysia parvula	7.73	-	-	6	0.204	-	-
Turbo sarmaticus	8.40	1.06	10.8	5	0.205	5	1.06
Perna perna	8.62	1.04	14.8	6	0.154	1	1.04
Cymbula oculus	8.96	-	-	10	0.203	-	-
Oxystele sinensis	9.22	-	-	10	0.204	-	-
Palaemon peringueyi	9.77	-	-	6	0.204	-	-
Parvulastra exigua	10.1	0.64	7.81	6	0.205	1	1.00
Gunnarea gaimardi	10.4	-	-	6	0.208	-	-
Pyura stolonifera	10.6	0.79	17.2	6	0.205	1	1.04
Tetraclita serrata	11.3	0.59	3.63	6	0.208	1	1.02
Marthasterias glacialis	11.7	0.67	4.87	6	0.210	1	1.15
Actinia sp.	11.8	-	-	6	0.204	-	-
Burnupena cincta	11.9	0.68	16.5	6	0.205	1	1.04
Liza richardsonii	12.1	-	-	6	0.104	-	-
Sparodon durbanensis	13.5	-	-	6	0.103	-	-
Caffrogobius sp.	13.9	0.79	5.88	5	0.152	2	1.04
Clinus sp.	14.0	0.77	4.94	5	0.157	4	1.08
Sheffield Beach							
Codium sp.	4.51	0.88	4.68	6	0.205	1	1.24
Corallina sp.	4.83	0.86	0.86	6	0.204	1	1.20
Tripneustes gratilla	5.64	0.79	4.68	6	0.153	1	1.09
Ulva lactuca	5.82	0.86	0.86	6	0.205	1	1.28
Helcion concolor	7.01	0.77	15.2	6	0.205	1	1.07
Lunella coronata	7.93	0.71	24.1	6	0.207	1	1.04
Monodonta australis	8.08	0.72	20.1	6	0.153	1	1.13
Perna perna	9.05	0.78	15.8	6	0.205	1	1.04
Nerita albicila	9.57	0.57	11.6	6	0.205	1	1.05
Parablennius pilicornis	9.72	0.76	11.4	6	0.152	3	1.04
Parvulastra exigua	10.1	0.66	6.92	6	0.154	1	1.17
Actinia sp.	10.5	0.85	18.3	6	0.204	1	1.06
Cellana radiata	10.9	0.75	11.1	6	0.203	1	1.07
Thalessa savignyi	11.2	-	-	6	0.103	-	-
Psammogobius knysnaensis	12.8	0.79	5.53	6	0.154	1	1.03

Table B 2: Matrix indicating significant differences ($p < 0.05$) as detected for the same species collected at both of the sites.

Sites	Species	Sheffield Beach			
		Ulva lactuca	*Perna perna*	*Parvulastra exigua*	*Actinia* sp.
Tsitsikamma	*Ulva lactuca*	Al, Ti, V, Cr, Mn, Fe, Co, Se			
	Perna perna		Ti, Cr, Co, Ni, Zn, As, Cd		
	Parvulastra exigua			Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Pb	
	Actinia sp.				Al, V, Mn, Fe, Zn, As, Se, Pb

Table B 3: The limit of quantification was determined by the standard calibration curve, based on internal standards (ε-HCH and PCB 143).

Compound	LOQ (µg/g)
α-HCH	0.010
HCB	0.068
β-HCH	0.006
γ-HCH	0.061
δ-HCH	0.011
Heptachlor	0.018
Aldrin	0.029
***oxy*-Chlordane**	0.066
***cis*-Heptachlor-epoxide**	0.050
***trans*-Heptachlor-epoxide**	0.046
***o,p*-DDE**	0.070
***trans*-Chlordane**	0.033
***trans*-Nonachlor**	0.047
***cis*-Chlordane**	0.042
***p,p*-DDE**	0.012
Dieldrin	0.019
***o,p*-DDD**	0.050
Endrin	0.037
***o,p*-DDT**	0.025
***cis*-Nonachlor**	0.021
***p,p*-DDD**	0.018
***p,p*-DDT**	0.025

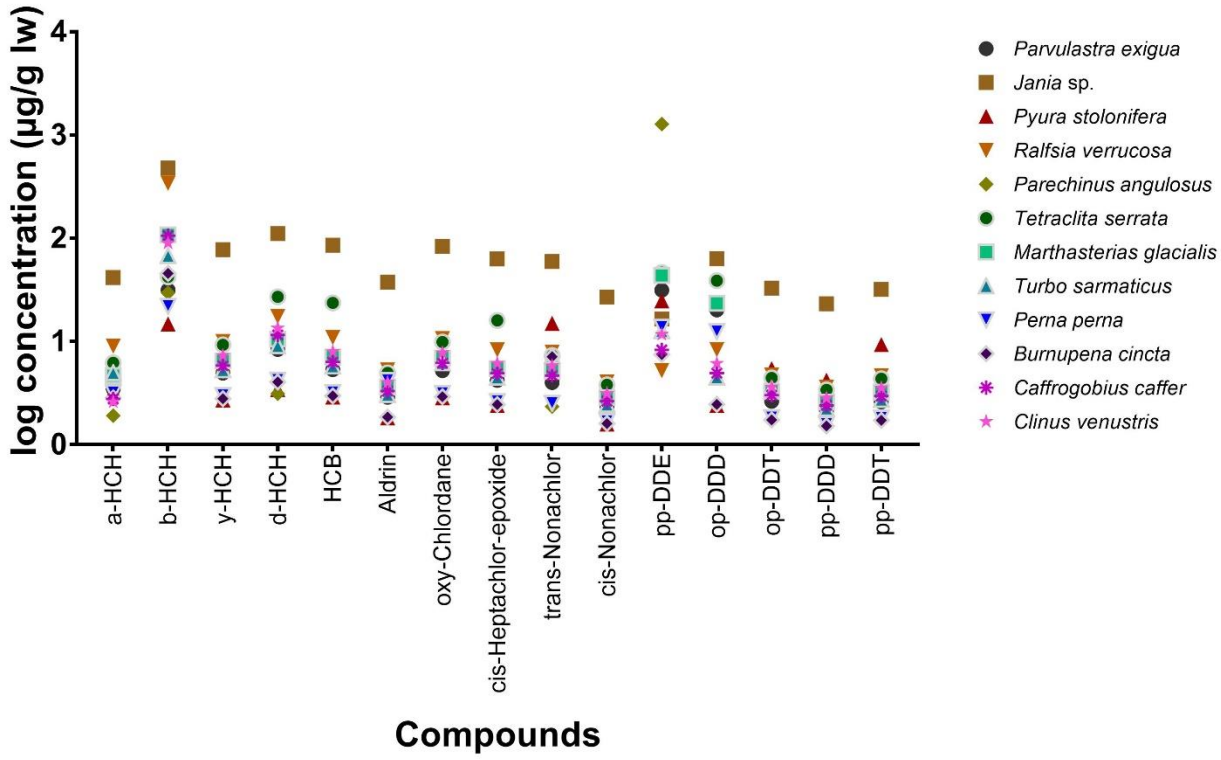


Figure B 1: *Log of the organochlorine pesticide concentrations (µg/g lipid mass) for each of the species analysed at Tsitsikamma.*

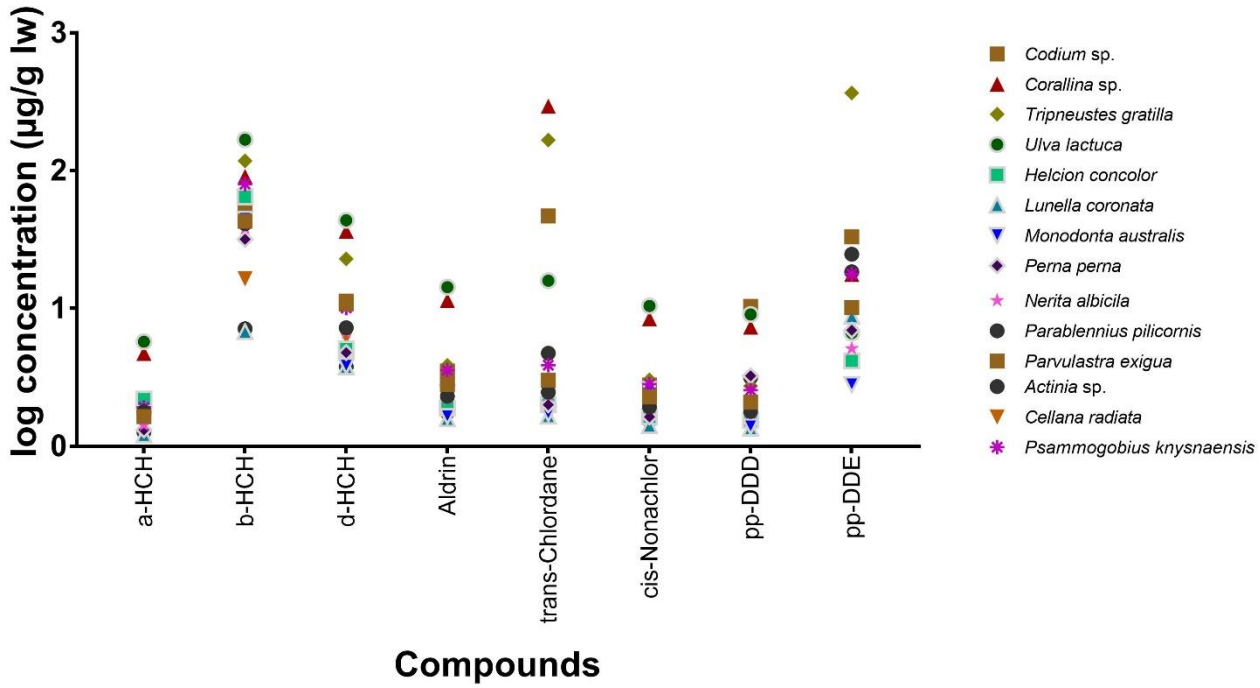


Figure B 2: *Log of the organochlorine pesticide concentrations (µg/g lipid mass) for each of the species analysed at Sheffield Beach.*