THE TROPHIC ECOLOGY OF PARROTFISH OF ZANZIBAR: APPLICATION OF STABLE ISOTOPE ANALYSIS

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ABSTRACT

Parrotfish are a critical component of the herbivore functional group on tropical coral reefs around the world because they mediate competition that occurs between algae and scleractinian corals. Also, because of their feeding technique, which consists of rasping at the substratum with their beak-like teeth, they play an important role in carbonate turnover and the clearing of reef surface area for the settlement of new sessile organisms. Because of these roles, parrotfishes are an important structuring component of coral reef communities. However, individual species can play different roles depending on their physiology, behaviour and ecology. Despite the possible ecological differences that may exist amongst species, specific roles of the fishes remain unclear as the group is most often studied at higher community levels.

This thesis applied stable isotope analysis to differing levels of organisation within a parrotfish community to help elucidate their trophic ecology on coral reefs in Zanzibar. Firstly, blood and muscle tissues were compared to identify differences in their isotope signatures. In other organisms, blood turns over faster than muscle tissue so that muscle tissue represents the diet as integrated over a longer period of time. In most species of parrotfish the blood and muscle $\delta^{13}C$ signatures were not found to be significantly different, but the $\delta^{15}N$ signatures were significantly different between tissues. This indicated that the $\delta^{13}C$ signature of both tissues would reveal similar dietary information. Conversely, differences in the $\delta^{15}N$ signature indicated that the nitrogen relationship between tissues was more complicated.

Secondly, spatial variability in parrotfish, coral, detritus and macroalgae isotope signatures was assessed at different scales. In macroalgae and coral tissues (zooxanthellae and polyp treated separately), the $\delta^{13}C$ signatures were shown to differ with depth, presumably because of changes in photosynthetic processes related to depth-associated changes in light. While $\delta^{15}N$ signatures were not affected by depth, all organisms showed enrichment at the Nyange reef, the closest reef to the capital of Zanzibar, Stone Town, presumably reflecting the effects of sewage outfall. These results show that processes that impact the $\delta^{15}N$ signatures of primary producers (macroalgae and zooxanthellae) can be traced to higher trophic levels (coral polyps and fish).

Lastly, $\delta^{13}C$ and $\delta^{15}N$ signatures were used to identify ontogenetic dietary changes in multiple species of parrotfish. Four of the species showed stages that varied from the

diets that are normally assumed on the basis of their dentition and feeding technique. This indicates that functional roles based on taxonomy or morphology may fail to include possible ontogenetic dietary changes, and may also fail to elucidate the full impact a species could have on coral reef communities.

The conclusions from these studies indicate that the species-specific ecological role of parrotfish in coral reef communities can be complex within and between species, and may differ amongst reefs. In light of the natural and anthropogenic pressures that affect coral reef systems, management decisions based on a more complete understanding of the role of these fish in coral reef communities will help decisions that maintain resilience in these fragile systems.

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Chapter One
Introduction and overview

Coral reefs are frequently described as the marine equivalent of tropical rain forests because of their high species diversity and the fact that structuring processes rely on sessile organisms (Connell 1978, Jackson 1991, Sebens 1994). Despite occupying less than 0.1% of the ocean, coral reefs are the most biodiverse of marine ecosystems and provide habitat for tens of thousands of species, including a third of the world's fish species (Sebens 1994, Reaka-Kudla 1996, Jackson *et al.* 2001). They are found mainly in oligotrophic, tropical, shallow waters but a few species are also found in deeper cold water systems. Coral reef systems are immensely important to human populations, and their services and resources are worth an estimated US$375 billion per year (Constanza *et al.* 1997). Despite their economic value to humans, anthropogenic disturbances are the main threats to coral reefs.

In the absence of human influences, coral reefs are strongly affected by normal environmental systemic variability such as storms, thermal stress events and changes to water chemistry, that contribute to the maintenance of their biodiversity (Rogers 1993, Aronson and Precht 1995, Brown 1997, Green *et al.* 1999). Primary anthropogenic influences include overexploitation of coral reef resources, coastal development and effluents from farms and cities (Richmond 1993, Jackson *et al.* 2001, Bellwood *et al.* 2004, Fabricus 2005). Although the significance of anthropogenic influences is debated in the context of climate change, it is also associated with multiple impacts on coral reefs that include a rise in water temperature and changes in the chemical make-up of the oceans. These can cause coral bleaching, when the polyp host expels its symbiotic zooxanthellae (Buddemeier and Fautin 1993), and a reduction in the ability of corals to deposit their calcium carbonate skeletons (Smith and Buddemeier 1992, Hoegh-Gulber 1999, Hoegh-Guldberh *et al.* 2007). Also, weather pattern changes may influence the frequency and intensity of tropical storms meaning recovery times between disturbances will be limited (Hughes *et al.* 2003, IPCC 2007). The scale and frequency of these impacts continually increases (Hughes *et al.* 2003, Wilkinson 2008), signalling rising pressures on a system that is already believed to live near its upper threshold in terms of environmental conditions (Knowlton 1992, Graham *et al.* 2006, Hoegh-Guldberh *et al.* 2007).

In a recent review of coral reefs around the world, Wilkinson (2008) reported that an estimated 19% of the world's coral has been lost in the last century and another 35% will be threatened in the next 30 to 40 years. Most of this loss has occurred in the Caribbean and Indian Ocean, while Australia and the Pacific Islands have seen fewer impacts. Southeast Asia is the area of greatest decline with an upper prediction of 50% loss in reefs in the last 40 years (Bruno and Selig 2007). The cause of coral loss, and a reef's ability to withstand impacts and/or recover is area specific and can be affected by intrinsic spatial and temporal variables such as distance from other reefs and timing between impacting events. Although, further loss is compounded by humans through destructive fishing practices and high rates of exploitation for protein resources.

In the absence of severe human impacts, coral reefs are able to regenerate after natural disturbances. However, the compounding effects of human and natural processes have rendered some coral reefs incapable of regeneration, sometimes leading to phase shifts to alternate stable states (Done 1992, Hughes 1994, Bellwood *et al.* 2004). In coral reefs, the most common phase shift is from an environment that is predominantly coral to a habitat dominated by macroalgae. Until recently, the drivers of this phenomenon were poorly understood until, in the 1970's, Jamaican reefs showed systematic declines. Through overexploitation of fishes, Jamaican coral reefs had lost almost all of their larger predatory species such as carangids (jacks), serranids (groupers) and sharks. Subsequently, fishers began to target the herbivorous fishes such as labrids (parrotfishes and wrasses) and acanthurids (surgeonfish); because fishermen were non-selective in size, they effectively removed the majority of reproductive individuals. Recruitment of juveniles was minimised, overall size classes and abundances were drastically reduced and fishers increased effort despite declines in catch (Koslow *et al.* 1988, Hughes 1994). The effects were not immediately noticed as hard coral cover remained constant and macroalgal growth was still low mainly due to the increasing abundance of the sea urchin, *Diadema antillarum* (Ogden *et al.* 1973, Sammarco 1982), which took the role of dominant herbivore. Urchin numbers increased because their main predators, balistids (triggerfish), and their main competitor for algal resources, parrotfishes, were already overfished (Hay 1984). In 1984, the spread of disease throughout the Caribbean caused mass mortality of *Diadema* populations, resulting in increasing biomass of algae standing stocks and reduced the health of scleractinian corals that compete with algae for space and light (Bak *et al.* 1984, Lessios *et al.* 1984, Hughes 1994). Furthermore, subsequent hurricanes destroyed the

structurally compromised corals leaving only species with more robust morphologies. Coral recruitment was impeded by dense algal stands on the remaining rubble, lowering fecundity, settlement rates and survival (Hughes 1989, Birrell *et al.* 2008). Nearly 30 years later, some Jamaican reefs have become stable, algal dominated systems because fish stocks and corals have not been able to recover (Wilkinson 2008). Although the evidence of a reefs ability to recover after phase shifts is still being evaluated and their susceptibility has been questioned (Bruno *et al.* 2009), the causal relationship between coral reef degredation and the loss of herbivorous species indicates the importance of maintaining biological diversity both prior to and after disturbances (Bellwood *et al.* 2004, Paddack *et al.* 2009).

It is now accepted that control of algal communities plays an important role in the resilience of coral reefs and reducing the chances of phase shifts. Factors such as herbivory, nutrient input and algal recruitment all help control algal stocks on coral reefs, with herbivory being of particular importance (Steneck 1988, Lirman 2001, Fabricus 2005, Mumby *et al*. 2006, Hughes *et al.* 2007, Mumby 2009). There are large taxonomic differences among coral reefs around the world, mostly attributed to different evolutionary histories. In the Caribbean, herbivory is attributable to fishes and echinoids while in the Indo-Pacific, it is largely dominated by fishes (Klumpp *et al.* 1987, Choat 1991, Done *et al.* 1996). These groups of organisms fulfil complementary functions in the processes removing algae and have been of increasing interest in research because of their ability to influence competitive interactions between corals and macroalgae (Lirman 2001). Thus, a complete understanding of the ecological role of herbivorous coral reef fish species and their functional role is a priority in assessing the resilience and recovery of coral reefs.

Demonstrations of the negative relationship between fish grazing pressures and macroalgal cover have shown fish to be of particular importance within the herbivorous functional group (Lewis 1986, Williams and Polunin 2001, Williams *et al.* 2001, Mumby *et al.* 2006, Hughes *et al.* 2007, Wismer *et al*. 2009). Because of the differing methods of feeding among the herbivorous fishes, not all groups exert the same effects on the system. For example, kyphosids and acanthurids crop off pieces of macroalgae, but certain species of labrids (parrotfish) feed directly off the substratum and remove part of the calcium carbonate structure. Using their distinct, fused plate-like teeth, parrotfish act as omnivores, and will feed off almost all coral reef substratum types making them not only important to in the removal of algae, but also in the carbonate

turnover of the reef (bioerosion) and the predation of live corals (Lewis and Wainwright 1985, Bellwood 1994, Bellwood *et al*. 2003, McClanahan *et al.* 2005, Rotjan and Lewis 2005, Paddack *et al.* 2006, Hughes *et al.* 2007, Cole *et al.* 2008, Alwany *et al.* 2009). Furthermore, parrotfishes are often among the most abundant fishes on reefs (Russ 1984a,b, Floeter *et al.* 2005, Fox and Bellwood 2007), contributing significantly to their impact on the system.

Because of their important role in algae removal, parrotfish have been increasingly scrutinised to identify their exact ecological impact as herbivores. Recent studies have shown that functional differences can exist amongst groups or between and within species of parrotfishes. Three different groups have been identified on the basis of these feeding modes. These are excavators, scrapers and browsers, defined according to the amount of substratum that is removed during feeding (Bellwood and Choat 1990, Bellwood 1994). Certain species such as *Bolbometopon muricatum* and *Sparisoma amplum* play disproportionately important roles in their regions as live coral feeders (Bellwood *et al.* 2003, Francini-Filho *et al.* 2008). Furthermore, the impact of parrotfish through scraping increases markedly once they reach a size of 20 cm (Lokrantz *et al.* 2008). All of these facts indicate that functional roles of the parrotfish group may vary among reefs depending on the parrotfish size distribution, species diversity and total species available on a given reef. As a consequence, and considering their diverse diet, a better understanding of the species-specific roles of parrotfishes in algae removal on coral reefs will clarify ecological complexities that structure coral reefs, and may allow for better management of these systems.

Although there has been increased interest in the species-specific roles of herbivores, relatively few studies on this topic have been conducted with nearly none in the western Indian Ocean (McClanahan *et al.* 2005). Most studies have focused on herbivores as a group and have not considered the individual roles of particular species, how they can differ among species, or how these roles can differ spatially. The examination of ecological differences amongst species can identify both individually important groups and also the existence of functional redundancy. Functional redundancy, once thought to be high in high diversity systems, has been shown to be lower than originally thought and even the loss of a single key species can have important impacts on the equilibrium or resilience of a system (Estes and Palmisano 1974, Bak *et al*. 1984, Fonseca and Ganade 2001).

Although examining the role of species-specific ecological processes on coral reefs can elucidate similarities and differences among species, coral reef communities include processes that can operate over spatial scales from microscopic to global (Jackson 1991, Hatcher 1997). This indicates that the scale of measurement must be adjusted to best answer the question being tested (Chase and Leibold 2002). Therefore, measuring the variability in trophic processes on coral reefs will help better understand how species-specific roles can change spatially and may uncover seemingly unrelated processes operating at different levels of organisation (Menge and Olson 1990). Studies of trophic interactions on coral reefs at different spatial scales may provide direct information about the factors that affect the distribution and abundance of parrotfishes, and their benthic prey (Hay *et al.* 1983, Hay 1985, Murdoch and Aronson 1999).

The overall goal of this study will help increase understanding of species-specific roles of parrotfishes in coral reef systems of Zanzibar through the study of their feeding ecology. The study of feeding ecology can allow better understanding of what foods were consumed, food habits, foraging habits, or how the food is acquired (Gerking 1994). Given the diversity of foods consumed by parrotfishes, and the important role they play in structuring coral reef communities, greater understanding of feeding, including what foods are fed upon, how the foods are procured, processed and assimilated, and where and when feeding can change will allow for more accurate interpretation of the influence of parrotfish on the structure and dynamics of these systems. Also, detailed understanding of this group may provide critical information regarding coral reef resilience and diversity.

AIMS AND THESIS OUTLINE

The different components of this thesis are addressed in a series of three separate studies, following the chapters outlined below. Chapter 2 addresses the materials and methods used in the collection of data for the three subsequent chapters. This includes a description of reefs around Zanzibar, the methodology of fish and food collection, sample processing and the background of stable isotope analysis. Chapter 3 examines the isotopic differences in tissues of species of parrotfish to understand if two tissues (blood and muscle) differ in their stable isotope values. Chapter 4 describes spatial differences in isotope signatures of parrotfishes and their foods at different scales. Chapter 5 explores ontogenetic differences in feeding of multiple species of parrotfish and provides a comparison of their functional roles. Chapter 6 discusses the relative

importance of parrotfish identified in the present study in relation to their ecological roles on coral reefs in the light of existing work and makes suggestions for future studies.

Chapter Two
Materials and methods

2.1 STUDY SPECIES

Globally, there are approximately 96 species of parrotfishes found within ten genera, occupying the tropical and sub-tropical waters of the Atlantic, Pacific and Indian Oceans. They are generally associated with shallow coral reefs where their greatest abundance can be found at less than 10 m (Russ 1984b). They have gained their common name from their bright colouration and their fused dental plates that resemble a parrot's beak.

A monophyletic grouping of parrotfishes is supported by derived characteristics, with a diet based on the procuring of algae from a calcareous substratum. The occurrence of the beak-like dental plates, pharyngeal jaw and the intermandibular joint are morphological traits that allow parrotfishes access to benthic resources not obtainable by other reef associated teleosts. Additionally, parrotfish intestines are shorter than those of other herbivorous fish, but longer than non-herbivores and include a sacculated section with high levels of carbonic anhydrase enzymes, allowing a diet that is high in carbonates (Al-Hussaini 1947, Smith and Paulson 1974, Smith and Paulson 1975).

Despite most species sharing similar morphological traits, there exist three different functional groups based on their feeding method: scrapers, excavators and browsers (Bellwood and Choat 1990). Excavators have a powerful jaw that, during the procurement process, removes large amounts of the calcareous substratum. Scrapers have a less powerful jaw that barely scars the surface. The presence of each group can be implied from the feeding scars that are left on coral and rubble, with the excavators leaving a much more pronounced scar than the scraper (Bonaldo and Bellwood 2009). Browsers feed as traditional herbivores by removing parts of macroalgae. Excavators include all species found within the genera *Chlorurus*, *Bolbometopon* and *Cetoscarus* and are also represented by a few species of *Sparisoma*. Scrapers are represented in *Sparisoma* and all species within *Scarus* and *Hipposcarus* and browsers include *Calotomus*, *Leptoscarus* and a few *Sparisoma.* Due to the large amount of inorganic material that is removed by parrotfishes during feeding, they have been recognised as important bioeroders in coral reef systems (Frydl and Stern 1978, Bellwood and Choat 1990, Bellwood 1995, Bruggemann *et al.* 1996) and subsequently they are important in

clearing space for new benthic sessile organisms (Lewis and Wainwright 1985, Hughes 1994, Nyström *et al.* 2000, Bellwood *et al.* 2004).

The sexual behaviour of parrotfishes is complex, with most species being protogynous hermaphrodites. Their sexual ontogenetic stage is often closely related to dramatic colour changes with the first adult form, termed the initial phase (IP), usually being drab in red, browns or greys. The second stage, termed the terminal phase (TP), is usually associated with blues and greens. In addition, the juvenile stage has a colouration that is distinctly different from adults. IP are often female and TP males but, in some species males can exist in the IP and are termed primary males. Males resulting from sex inversion are termed secondary males. Species with only secondary males are monandric and species with both primary and secondary are termed diandric (Table 2.1). Diandry is dominant in parrotfishes (Table 2.1), but primary males rarely make up a large proportion of the IP population (Choat and Robertson 1975, Randall and Bruce 1983, Page 1998, Gust 2004, McIlwain and Taylor, 2009). Parrotfishes display a wide variation in the size to which they can grow and subsequently, the associated size at maturity and sexual inversion can vary between species and within species by region (Table 2.1).

In the western Indian Ocean there are approximately 24 species of parrotfishes that are found on coral reefs and in seagrass beds (Randall and Bruce 1983, Parenti and Randall 2000). Many of these species are endemic to the region (*Chlorurus atrilunula*, *C. cyanescens*, *C. enneacanthus*, *C. strongylocephalus*, *Hipposcarus harid*, *Scarus caudofasciatus*, *S. falcipinnis*, *S. rubroviolaceus*, *S. russelii*, *S. scaber* and *S. viridifucatus*), while a few are distributed throughout the rest of the Indo-Pacific (*Bolbometopon muricatum*, *Calotomus carolinus*, *C. spinidens*, *Cetoscarus bicolor*, *Chlorurus sordidus*, *Leptoscarus vaigiensis*, *Scarus frenatus*, *S. ghobban*, *S. globiceps*, *S. niger*, *S. psittacus* and *S. tricolor*) (Parenti and Randall 2000). During at least one of their life stages, most species can be found associated with coral reef communities but *Leptoscarus* is exceptional in that it is a strictly seagrass genus.

2.2 A NOTE ON TAXONOMY AND NOMENCLATURE

Since the development of molecular phylogenetic tools, species-rich families of fishes have come under increased scrutiny in order to answer evolutionary, taxonomic and ecological questions (Helfman *et al.* 1997). Both diversity in parrotfishes (Bellwood 1994, Streelman *et al.* 2002, Smith *et al.* 2008) and their relatedness to other

Table 2.1 Literature cited for length at which each species transitions from sexually immature (IM) to sexually mature initial phase (IP) fishes for parrotfish species sampled within this study. Method of measurement (TL is total length or from snout to end of caudal fin, FL if fork length or from snout to the caudal peduncle) and region where the study occurred (GBR is Great Barrier Reef, WIO is Western Indian Ocean), whether the length was based on actual observation (Obs.) or the calculation of a Von Bertalanffy growth curve (VB). No study concerning length at maturity exists for the endemic Western Indian Ocean *Chlorurus strongylocephalus*, so estimates were added concerning the sympatric sister taxa, *C. gibbus* and *C. microrhinos* (Randall and Bruce 1983). No references were found for *C. atrilunula*, *S. falcipinnis, S. russelii, S. scaber, S. tricolor* and *S. viridifucatus* so stage was inferred from Randall and Bruce (1983). References include (1) Randall and Bruce (1983), (2) Choat and Robertson (1975), (3) Robertson *et al.* (1982), (4) Choat and Robertson (2002), (5) Page (1998), (6) Gust *et al.* (2002), (7) Gust (2004), (8) McIlwain and Taylor (2009), (9) Venkataramani and Jayakumar (Ukn.), (10) Randall and Choat (1980), (11) Grandcourt (2002). Functional group is also indicated (B: browser, S: scraper, E: excavator).

Species	FG	Monandric / Diandric	Observed length (cm)	Obs. or VB	Region
Calotomus carolinus	B	Monandric[3]	17-18 (TL)	Obs.	Heron Island, GBR[2]
			16-18 (TL)	Obs.	Aldabra Atoll, WIO[3]
Cetoscarus bicolor	E	Diandric[1]	30 (TL)	VB	GBR[4]
Chlorurus atrilunula	E	Diandric[1]			WIO[1]
Chlorurus sordidus	E	Diandric[1]	10-14 (TL)	Obs.	Heron Island, GBR[2]
			10-18 (FL)	Obs.	Northern GBR[7]
			16-19 (TL)	VB	Northern GBR[6]
			14.8 (TL)	VB & Obs.	Guam[8]
			17 (FL)	VB & Obs.	Am. Samoa[5]
			10 (TL)	Obs.	GBR[4]
				VB	
Chlorurus strongylocephalus	E	Diandric[1]	30 (FL)	VB & Obs.	Am. Samoa[5]
			25 (TL)	Obs.	Gulf o. Mannar[9]
			30 (TL)	VB	GBR[4]
Scarus falcipinnis	S	Diandric[1]			WIO[1]
Scarus frenatus	S	Diandric[1]	18 (TL)	VB	GBR[4]
Scarus ghobban	S	Diandric[11]	30 (TL)	Obs.	Gulf o. Mannar[9]
Scarus niger	S	Monandric[2]	18-24 (TL)	VB	Northern GBR[6]
			19 (FL)	VB & Obs.	Am. Samoa[5]
			17.5 (TL)	VB	GBR[4]
Scarus psittacus	S	Diandric[1, 2]	11	VB	GBR[4]
Scarus russelii	S	Diandric[1]			WIO[1]
Scarus scaber	S	Diandric[1]			WIO[1]
Scarus tricolor	S	Diandric[10]			WIO[1]
Scarus viridifucatus	S	Diandric[1]			WIO[1]

labrid, perciform groups have been widely examined (Westneat and Alfaro 2005). Due to the changing situation of their taxonomic status, this thesis will refer to the group by their common nomenclature "parrotfish", as this has been a historically unique identifier. Furthermore, this study recognises the most recent molecular phylogenetic work by Westneat and Alfaro (2005) and Cowman *et al.* (2009) who place them in the family Labridae within the tribe Scarinae. It should be noted that in much of the earlier literature and even in recent literature, they can be referred to as the family Scaridae.

2.3 General Description of Study Area

Zanzibar has a wet and dry tropical climate with two distinct wet seasons. Heavy rainfall occurs during April and May and lighter rainfall occurs between October and November. The island receives between 1000 and 1500 mm of rainfall per annum. Air temperatures range from 27° to 30°C. Predominantly north-easterly winds occur between October and March, changing to south-easterly from March to October (McClanahan 1988). The western side of the island is protected from the dominant easterly swell that occurs through the year and water temperatures within coral reefs are reasonably consistent. For example, during the study period, temperatures ranged between 25°C in August 2010 and 28°C in February 2011. Visibility in the water, however, was more variable, ranging from 3 m on some occasions to more than 20 m on others but, tended more often toward the latter.

Zanzibar was selected because of access to a research station (Institute of Marine Science) and the relatively consistent environmental conditions. This reduced both seasonal and within season (daily to weekly) variability, reducing the need to take this into account within the study.

2.4 Study Sites

Data were collected between June 2010 and February 2011 at reefs on the western coastline of Unguja Island, Zanzibar (United Republic of Tanzania) in the western Indian Ocean (Fig 2.1). Three reefs were selected with visually similar coral cover, bathometry and topographic complexity in order to standardise physical environmental conditions among reefs; these were Ukombe (6° 20' 06" S, 39° 14' 32" E), Pwakuu (6° 14' 56" S, 39° 04' 41" E) and Nyange (6° 13' 23" S, 39° 08' 52" E). Within each reef, three sites were chosen, approximately 500 m apart, to allow the investigation of scale-

dependent variability in feeding patterns in fishes. Each site had relatively high coral cover (> 40%) at the reef crest that declined down a steep slope, to a predominately sandy base (Fig 2.2). At all reefs, the reef crest at high tide was at approximately 3 m depth and the base at ~15 m. Limited sampling of seagrasses and fishes also occurred at Chwaka Bay (6° 09' 53" S, 39° 26' 26" E). Fishes were also obtained from a fish landing site within Stone Town and a second 10 km farther south (Mizingani). Multiple landing sites along the west coast of the island allow fishers to reduce petrol costs by bringing fish to the closest occurring site (Personal communication with local fisherman).

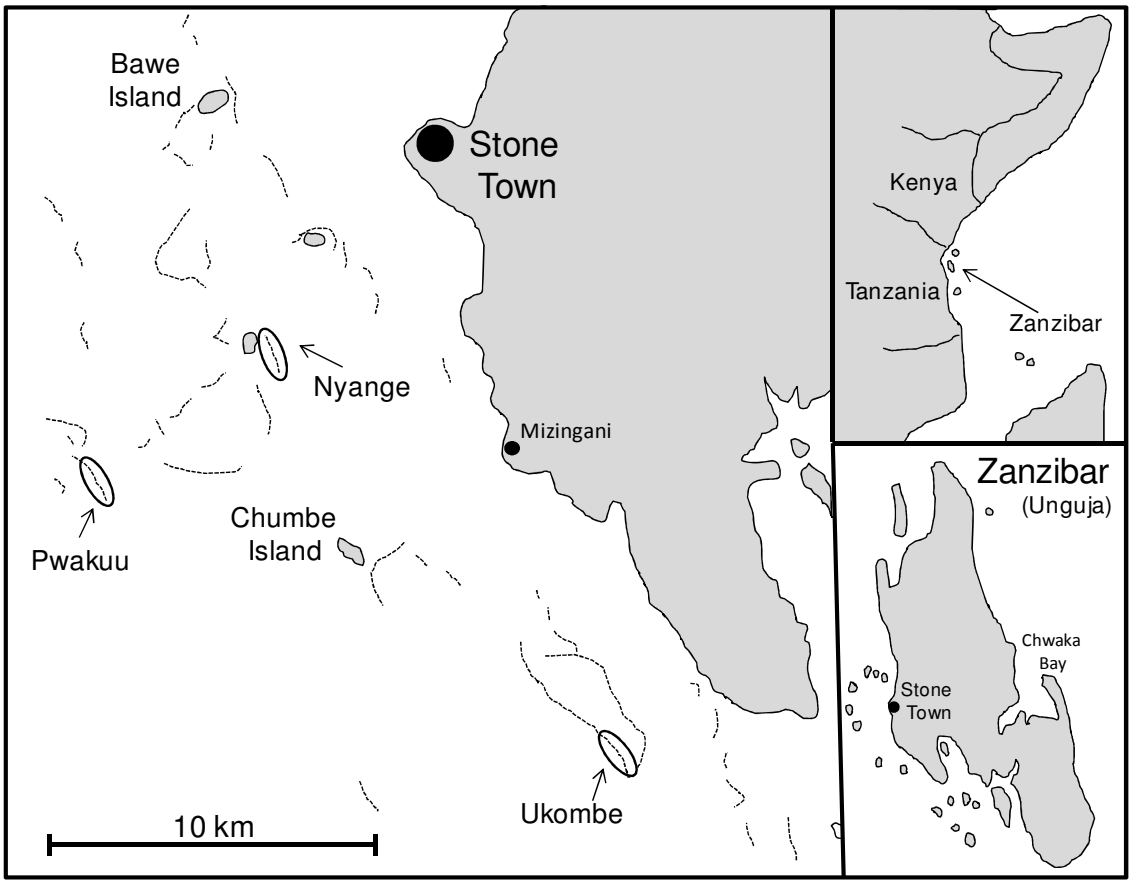


Figure 2.2 Overview of study area and location of Nyange, Pwakuu and Ukombe reefs where sampling occurred. Reefs are indicated by a dashed line. Three sites were located within the sampling area of each reef (circled area). In-sets show the location of Zanzibar in relation to east Africa and an overall map of Unguja Island, Zanzibar.

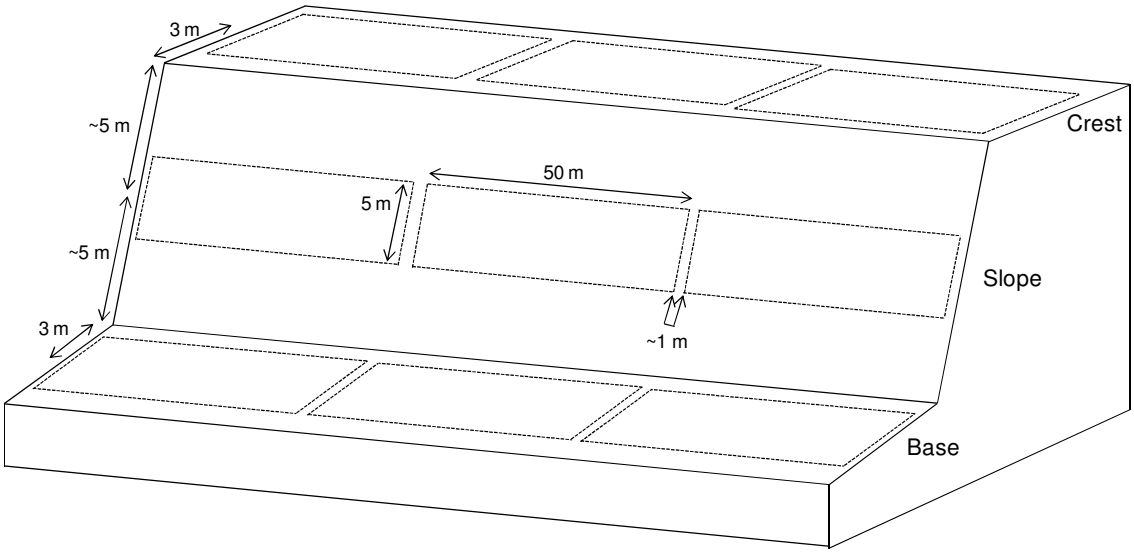


Figure 2.2 Overlay showing general shape and depths of reefs around Zanzibar where sampling occurred. Food sources were collected within 250 m^2 areas of either the crest, slope or base of each "site", or 50 m length of reef. Foods from either the base or crest were collected within 3 m of the start of the slope.

2.5 FOOD SOURCE COLLECTION

The identified food sources for parrotfishes included live coral, macroalgae, turfing algae and detritus (Choat *et al.* 2002, Choat *et al.* 2004). Collections were made in triplicate and included: two species of live coral, two species of macroalgae, coral rubble with growth of turfing algae and detritus vacuumed from the substratum. For live coral, individual coral heads of the genus *Porites* that showed previous feeding scars by parrotfish were selected (Bonaldo and Bellwood 2009). Using a chisel and hammer, pieces of coral were removed that contained approximately 225 cm^2 of surface area (15 × 15 cm). Previous experimentation showed this to be suitable tissue for stable isotope analysis (See appendix 2.5). Furthermore, because isotope signatures can vary on coral due to differing light attenuation conditions affecting photosynthesis, the top-most, horizontal lying part of the colony was sampled (Maier *et al.* 2010). Selection of macroalgae was opportunistic and based on availability. Pieces of coral rubble and intact coral heads with turfing algae were selected after observation of feeding by individual parrotfish. If no parrotfish were available, rubble was selected that showed feeding scars similar to live coral. Detritus (defined as dead organic/inorganic matter, microorganisms, microalgae and associated meiofauna as per Bowen 1987) was vacuumed (Texsport Double Action Hand Pump, 2.0 l volume) from areas of rubble, dead and live coral heads after feeding was observed. After collection, coral, rubble and

algae were rinsed with distilled water and then placed in individually marked freezer bags. Detritus samples were expelled from the pump into marked 1.5 L plastic bottles after vacuuming from benthic surfaces. The pump was filled and evacuated to remove residual detritus between each sample.

All samples from each dive were put on ice upon return to the surface (boat travel from the farthest reef (Ukombe) took approximately two hours) and frozen at the field station (University of Dar es Salaam's Institute of Marine Science, Stone Town) until further processing.

Thawed coral tissue was removed from the skeleton with an airbrush, connected to a diving regulator and tank, using approximately 20 ml of distilled water (Szmant *et al.* 1989). Coral homogenate was allocated to Eppendorf tubes (2 ml) and centrifuged (Eppendorf Centrifuge 5402 R) for 15 minutes at 14,000 rpms (at 4°C) to separate zooxanthellae from tissue. The supernatant was decanted and centrifuged a second time to ensure separation of all zooxanthellae. All supernatant was decanted and passed through glass-fibre filters (GFFs; Advantec 47 mm, GF-75, pre-combusted) with a hand vacuum/pump (MityVac MV8010) and then both the filters and remaining precipitate (i.e. zooxanthellae pellet) were oven-dried. Dried filter paper with coral tissue was randomly checked for residual zooxanthellae using a dissecting microscope. Identification of coral species was based on Veron (2000).

Thawed rubble with turfing algae was rinsed in distilled water multiple times to remove epiphytes and detritus. The surface of the rubble was scraped with a stainless steel scalpel and scrapings were oven-dried in Eppendorf tubes. Samples were ground with a mortar and pestle and half of the sample was acidified using 1N HCL with the drop by drop method described by Ng *et al*. (2007) and Jacob *et al*. (2005) and re-dried (Kolasinski *et al.* 2008). After stable isotope analysis, acidified samples were compared with untreated ones to identify any changes in nitrogen (ANOVA: n = 4, $F_{1,6} = 0.16$, $p = 0.71$).

Detritus samples were thawed, re-suspended through mechanical shaking and then passed through pre-combusted GFFs using a hand pump until the filter was blocked and did not allow water to pass through. Filter papers were oven-dried and acidified in the manner described above. After isotope analysis they were compared to untreated samples to identify any changes in nitrogen (ANOVA: n = 5, $F_{1,8} = 1.52$, $p = 0.25$).

Macroalgae were thawed and rinsed with distilled water and identified based on Oliveira *et al.* (2005).

All GFFs (coral tissues & detritus), zooxanthellae, macroalgae, coral rubble and turf algae samples were oven-dried (fan forced) at 60°C for 48 hours, then ground to fine powder for allocation to tin capsules for stable isotope analysis.

2.6 FISH COLLECTION

2.6.1 Coral Reefs

Parrotfish were collected at each site opportunistically using SCUBA with a speargun (see appendix, Permits 2.6.1). Where possible, approximately 0.5 ml of blood was drawn from the caudal vein with a 21 gauge hypodermic needle and added to 1 ml of 70% ethanol in Eppendorf tubes for storage (Hobson *et al.* 1997). Fish were then photographed and bagged in individually marked freezer bags. Fish and blood were immediately put on ice for transport back to the field station and frozen until further processing. In the lab, fish were re-photographed and length, weight, species and life history stages were recorded. Length at maturity was based on published literature (Table 2.1), while IP and TP were distinguished by either colouration or the gonad. IP primary males were rare and were grouped with IP when present. Due to their low occurrence, differences in diet from female IP could not be established. Dorsal white muscle samples were collected for isotope analysis (oven dried, fan forced, at 60°C for 48 hours). Fish were sexed based on the presence of either eggs or sperm. Species identification was based on Randall and Bruce (1983) and Parenti and Randall (2000). Whole blood samples (red cells and plasma not separated) for isotope analysis were spread over glass microscope slides and oven-dried.

2.6.2 Fisherman Landing Sites

Further fish samples were obtained from fish landing sites where fish were sold immediately after coming from the boats. For parrotfishes, the traditional method used by artisanal fishers of Zanzibar included a "dema," or fish trap made of stiff reeds. They were constructed as a hexagon of approximately 80 × 80 × 20 cm, with an opening measuring 20 × 10 cm (Fig 2.3). *Ulva spp*. are generally used as bait targeting "pono", or parrotfishes and the traps are generally checked daily for catches. Often fish arrive at the market alive, but samples that were not alive were confirmed to have been caught that day. The gills were checked to still be red, and eyes were checked for “cloudiness”. These methods provided a general idea to the sample’s level of decomposition, reducing

the chances of bacterial onset affecting stable isotope analysis. The fish were put on ice until return to the field station where they were processed as described above. Blood was drawn from areas seeping after otolith removal. The blood and muscle tissue sampled were processed as described above.

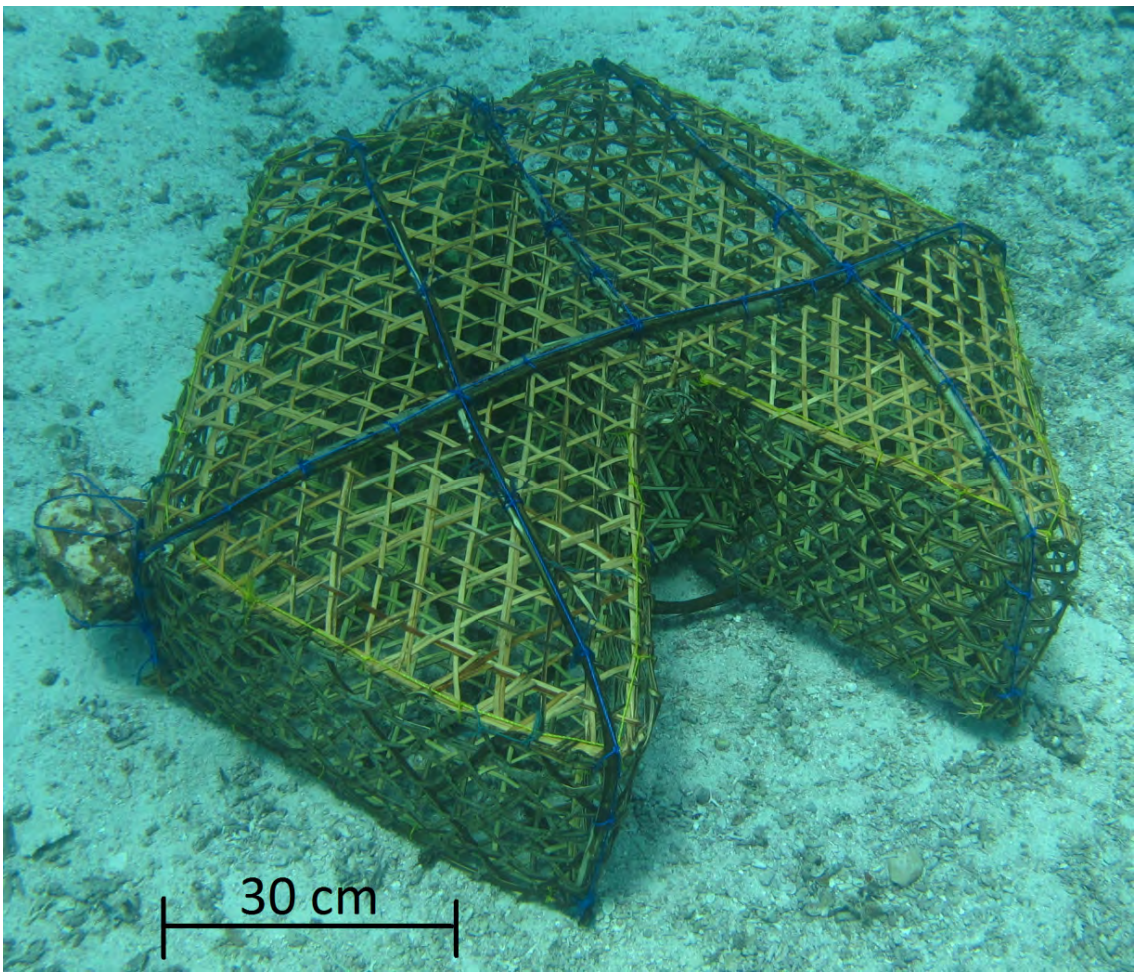


Figure 2.3 Picture of "dema" fish trap used by artisanal fishers of Zanzibar. Fish are able to enter the trap trough an opening in the middle. Traps are lined with *Ulva spp.* to target parrotfish.

2.6.3 Chwaka Bay Samples

Additional fish and diet material samples were gathered from a seagrass dominated habitat in Chwaka Bay. Parrotfish were obtained by means of a beach sine net and stored on ice after blood samples and photographs were taken. Food sources included the dominant seagrasses and detritus which were manually removed from the substratum and then put on ice. Fish and food samples were processed as described above on return to the field station.

2.7 STABLE ISOTOPE ANALYSIS

Lipids were not removed from parrotfish tissues because C:N ratios were always < 4, which indicates a minimal presence of lipids and thus will not affect stable isotope analyses (Post *et al*. 2007). Samples were weighed and placed in tin capsules (OEA Laboratories, C11470.250P, pressed tin capsules 8 x 5 mm) and analysed for stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) using a continuous flow Isotopic Ratio Mass Spectrometer (Europa Scientific Integra IRMS), at the IsoEnvironmental Laboratory, Rhodes University, Grahamstown, South Africa. Results are expressed in standard δ unit notation as:

$$\delta X(‰) = [(R_{sample}/R_{reference}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen. Ammonium sulphate, beat sugar and casein were used as standards, calibrated against the International Atomic Energy reference standards of Vienna Pee Dee Belemnite (PDB) for carbon and atmospheric air for nitrogen. Precision of replicate determinations for carbon was in a range of ±0.03-0.25‰ and nitrogen was ±0.08-0.28‰.

Chapter Three
Inter- and intra-species tissue comparisons of stable isotope signatures

3.1 INTRODUCTION

Numerous studies on trophic interactions in herbivorous fish have utilised stomach content analysis, observation and/or experimental work in aquaria (for examples see: Bruggemann 1994a,b,c, 1996, Choat *et al.* 2002, 2004, Bellwood *et al.* 2004). These techniques, however, have inherent shortcomings that include the need for destructive sampling and large sample sizes for stomach contents, "spot" or “instantaneous” observations and difficulties in separating ingestion versus assimilation in controlled experiments. Furthermore, stomach contents of many reef herbivores are often highly mechanically processed due to a pharyngeal jaw, rendering the identification of contents problematic (Cocheret de la Morinieré 2003, Choat *et al.* 2002, 2004). Stable isotope analysis, therefore, offers an alternate and/or complementary method of sampling, gaining insight into the trophic interactions of herbivorous fishes (Perga and Gerdeaux 2005, McIntyre and Flecker 2006, Kurle *et al.* 2011). This type of analysis is based on the principle that, isotopically, a consumer's tissue signature will resemble that of its prey based on known fractionation during metabolic assimilation (Post 2002). An animal's stable isotope signature provides an index of assimilation over integrated time periods and is a product of growth and metabolic tissue replacement (Tieszen *et al.* 1983, MacAvoy *et al.* 2001, Olive *et al*. 2003); although in some cases, isotopic routing and growth associated changes in metabolism can cause assimilation into different tissues at different rates and may affect the interpretation of data (Schwarcz 1991, Hill and McQuaid 2009, Martínez del Rio *et al.* 2009).

Strangely, the understanding of isotopic dynamics in coral reef herbivores has lagged far behind that of other taxa. For example, isotopic assimilation and turnover rates have been widely explored in birds (Hobson and Clark 1992a,b, Mizutani *et al.* 1992, Bearhop *et al*. 2002, Cherel *et al.* 2005, Wunder *et al.* 2005, Inger and Bearhop 2008), terrestrial (Tieszen *et al.* 1983, Hildebrand *et al.* 1996, Hobson 1999, Miller *et al.* 2008) and marine (Hobson *et al.* 1996, Kurle 2011) mammals, elasmobranchs (MacNeil *et al.* 2006, Logan and Lutcavage 2010, Matich *et al.* 2010) and temperate bony fishes (Perga and Gerdeaux 2005, Sakano *et al.* 2005, German and Miles 2010). However, studies on

tropical teleosts are limited to one freshwater catfish, *Ancistrus triradiatus*, from the Andean piedmont of Venezuela (McIntyre and Flecker 2006). This study reported high muscle turnover rates attributed to the high metabolic replacement of nitrogen and high growth rates found in tropical species, resulting in non-significant differences in signatures between various tissues (muscle, fin and blood). Additionally, $\delta^{15}N$ fractionation was investigated in three coral reef herbivores (*Acanthurus sohal, Zebrasoma xanthurum*, and *Pomacentrus arabicus*) in Oman (Mill *et al.* 2007). This study found trophic-step $^{15}N/^{14}N$ to be much lower than the commonly cited 3.4‰ because of high consumption rates of N-rich diets. Physiological processes can be significantly different between tropical and temperate organisms as well as between freshwater and marine animals. Understanding the variability in isotopic signatures for different tissue types within tropical marine herbivorous fish is a step towards comparing information between taxa and environments (del Rio *et al.* 2009) to better understand the autecology of the system.

Parrotfish are a dominant and important herbivorous group of fishes on many coral reefs (Lewis and Wainwright 1985, Choat 1991, Hughes *et al.* 2003, Hughes *et al.* 2007) and they display behavioural and mechanical feeding differences amongst species. Most commonly they are protogynous hermaphrodites, the majority being diandric, though monandric species also exist (Nelson 2006) and their gut is relatively short for a herbivore. The relatively short gut has been attributed to a diet that is high in detritus (Choat *et al.* 2002, 2004), requiring a shorter digestion period than needed for more structurally complex plant material, but may also include macro- and turfing algae and coral tissue (Bonaldo *et al*. 2006, Mantyka and Bellwood 2007b, Francini-Filho *et al.* 2009). The ratio of these three major food constituents differs amongst species and is largely based on mechanical feeding modes that are differentiated by jaw structure and ontogenetic stage (see chapter 5). Parrotfish have been included in very few isotope studies and when present, are mostly part of community meta-analyses (Cocheret de la Morinière *et al*. 2003, Lugendo *et al.* 2006). As such, very little is understood about the physiology and ecological isotope dynamics at the species level within parrotfishes.

The purposes of this study were (1) to determine if there is a relationship between the $\delta^{13}C$ and $\delta^{15}N$ signatures of muscle and blood from multiple parrotfish species, (2) to understand if differences between tissues within individuals vary with body size and (3) to identify commonalities between 1 and 2 amongst parrotfish species. Confirming that stable isotope analysis provides relatively consistent dietary information between tissue

types and across size classes will provide insight into ecological drivers, physiological processes and dietary variation.

3.2 MATERIALS AND METHODS

Details on the study sites, sample collection and stable isotope analysis are given in Chapter 2.

3.2.1 Data Analysis

(1) To determine the relationship between blood and muscle tissues for $\delta^{13}C$ and $\delta^{15}N$, species-specific linear regressions were applied to each of the paired tissues. Each significant linear regression was then compared to a theoretical slope of one. (2) Linear regressions were also applied to the isotopic signature of each tissue and compared to total length (TL). These linear regressions were compared between tissues to identify if relationships between tissues and TL differed. Analyses were applied only to species that had a large sample size, spanning a wide spectrum of body size (Table 2.1). (3) Pair-wise comparisons were then made between the slopes of length vs. isotope signature regressions for species where the relationship was significant. All comparisons were completed with a homogeneity of slopes test. Muscle signature was the covariate in tissue comparisons and TL was the covariate when tissues were compared. Type-III sums of squares were used in the homogeneity of slopes test between species due to unequal sample sizes. Each linear regression was tested to see if slope $\neq 0$, after eliminating outliers identified using Cook's test. Means (±SE) and ranges were calculated for the isotope signature for each tissue for each species. Differences were represented as the absolute difference between means. Statistical tests were completed in StatSoft Statistica 6 and plots were constructed with SigmaPlot 10.

It should be noted that sampling of *Hipposcarus harid* was limited to a single excursion, occurring in a seagrass-dominated bay. This is the only obligate seagrass species. TL was not recorded for individuals, but individuals differed in TL by only a few centimetres.

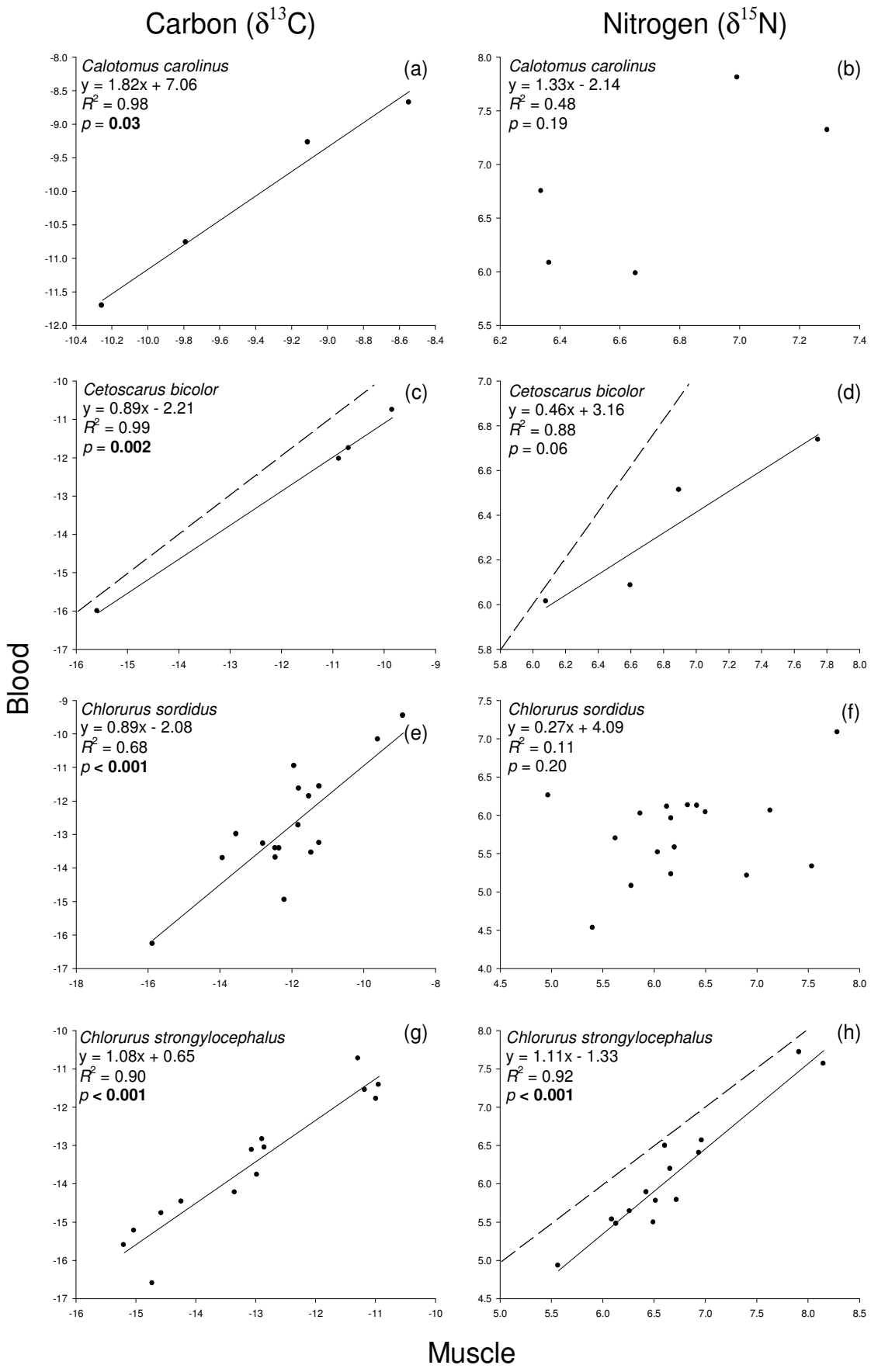
3.3 RESULTS

Comparison of $\delta^{13}C$ signatures revealed a significant, positive relationship between muscle tissue and blood in seven out of nine species (Figures 3.1a, c, e, g, i, m, o). For six of these seven, the slopes for $\delta^{13}C$ did not differ significantly from a ratio of 1:1 ($p >$

0.05). All R^2 values were > 0.67, with five being > 0.90. Blood $\delta^{13}C$ was always lower than that muscle tissue and ranged from 0.2±0.2‰ to 0.9±0.2‰ (Table 3.1). The $\delta^{15}N$ signatures for blood and muscle were significantly correlated for five species (Figures 3.1h, j, n, p, r) and slightly non-significant for a sixth (Figure 3.1d). All R^2 values were > 0.58. Of the five species with a significant $\delta^{15}N$ slope, two differed from a 1:1 ratio (*Chlorurus strongylocephalus*: $F_{14,1} = 4.19$, $p = 0.05$; *Scarus russelii*: $F_{6,1} = 5.18$, $p = 0.04$). The slope for *C. strongylocephalus* was > 1 and *S. russelii* was < 1. For all but one species (*Calotomus carolinus*), blood was depleted compared to muscle in $\delta^{15}N$ and ranged from 0.1±0.3‰ to 0.9±0.2‰. *Hipposcarus harid* was the only species with a negative $\delta^{15}N$ relationship between tissues, although this was not significantly different from a negative 1:1 ratio ($p > 0.05$). *S. psittacus* was the only species with no significant relationship between tissues for either $\delta^{13}C$ or $\delta^{15}N$ (Figure 3.1k, l). *Cetoscarus bicolor* was the only species with a slope for $\delta^{13}C$ that was significantly different from a 1:1 ratio ($F_{4,1} = 20.05$, $p = 0.01$), while the relationship for $\delta^{15}N$ was nearly significant ($p = 0.06$).

In general, tissues were more closely related in $\delta^{13}C$ than $\delta^{15}N$. Particularly strong relationships were seen in *Calotomus carolinus* ($R^2 = 0.98$), *Cetoscarus bicolor* ($R^2 = 0.99$), *Chlorurus strongylocephalus* ($R^2 = 0.90$), *S. niger* ($R^2 = 0.91$) and *Scarus tricolor* ($R^2 = 0.97$). All species showed a large range of $\delta^{13}C$ signatures (despite a relatively small sample size for the first two species) indicating a wide selection of food sources or possible trophic specialisation amongst individuals (Figure 2.1a, c, g, i, o and Table 3.1). For $\delta^{15}N$, *C. strongylocephalus, S. niger* and *H. harid* also had a close relationship between tissues ($R^2 = 0.92$, 0.81 and 0.88, respectively). *C. strongylocephalus* and *S. niger* spanned a wide range of signatures, roughly equivalent to one trophic level step (~3.0‰; Peterson and Fry 1987, France and Peters 1997, Post 2002) (Figures 3.1h, p and Table 3.1). Interestingly, there were no clear similarities in tissue relationships amongst species of similar functional group (Table 3.1).

There were significant relationships between tissue $\delta^{13}C$ signatures and TL. For the species analysed (*C. sordidus*, *C. strongylocephalus*, *S. niger* and *S. russelii*; Table 3.1), most of the relationships between blood $\delta^{13}C$ and TL or muscle $\delta^{13}C$ and TL were significant (Figure 3.2), with the exception of the *C. sordidus* blood $\delta^{13}C$ vs. TL regression ($p = 0.056$, Figure 3.2a). Coefficients of determination for most tissue relationships with TL were in the region of $R^2 = 0.60$, again with the exception of *C. sordidus*, which had lower coefficients for both blood and muscle ($R^2 = 0.22$ and 0.39,


Carbon ($\delta^{13}C$)
Nitrogen ($\delta^{15}N$)
Blood
Muscle
(a)
Calotomus carolinus
y = 1.82x + 7.06
R^2 = 0.98
p = **0.03**
(b)
Calotomus carolinus
y = 1.33x - 2.14
R^2 = 0.48
p = 0.19
(c)
Cetoscarus bicolor
y = 0.89x - 2.21
R^2 = 0.99
p = **0.002**
(d)
Cetoscarus bicolor
y = 0.46x + 3.16
R^2 = 0.88
p = 0.06
(e)
Chlorurus sordidus
y = 0.89x - 2.08
R^2 = 0.68
p < **0.001**
(f)
Chlorurus sordidus
y = 0.27x + 4.09
R^2 = 0.11
p = 0.20
(g)
Chlorurus strongylocephalus
y = 1.08x + 0.65
R^2 = 0.90
p < **0.001**
(h)
Chlorurus strongylocephalus
y = 1.11x - 1.33
R^2 = 0.92
p < **0.001**

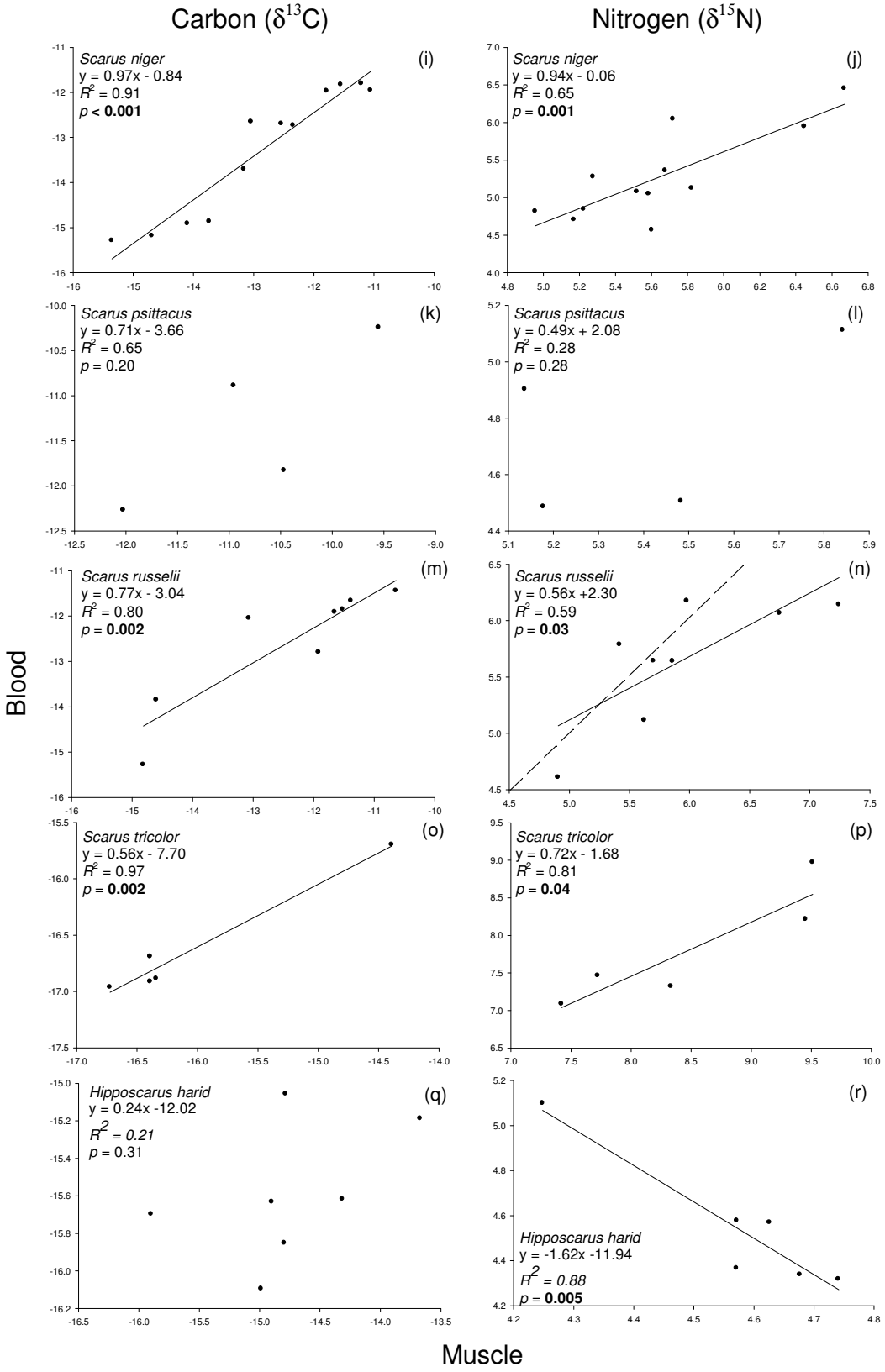


Figure 3.1 Comparisons of δ^{13}C and δ^{15}N for blood and muscle tissue of nine species of parrotfishes. Significant relationships between tissues are indicated with a solid regression line and a dotted line represents a theoretical 1:1 ratio (only present in plots that were significantly different from this ratio). Species and regression details are provided within individual plots and significant relationships are indicated in **bold**.

Table 3.1 Species sampled for comparison of blood and muscle $\delta^{15}N$ and $\delta^{13}C$ signatures. Sample size (n), functional group (FG; B = browser, E = excavator, S = scraper) and total range of lengths (cm) are indicated for each species and means (±SE) and ranges are given for each isotope. Differences are the absolute difference between blood and muscle. Length ranges in **bold** indicate species that were used for comparison between the isotope signature of tissues and total length.

	n	FG	TL	Nitrogen ($\delta^{15}N$)		Carbon ($\delta^{13}C$)	
			Range	Range	Mean	Range	Mean
Calotomus carolinus	5	B	18.7:27.0				
Blood				6.0:7.8	6.8±0.4	-11.7:-8.7	-10.1±0.7
Muscle				6.3:7.3	6.7±0.2	-10.3:-8.5	-9.4±0.4
Average Difference					0.1±0.3		0.6±0.3
Cetoscarus bicolor	4	E	20.5:38.4				
Blood				6.0:6.7	6.3±0.2	-16.0:-10.8	-12.6±1.2
Muscle				6.1:7.7	6.8±0.3	-15.6:-9.8	-11.7±1.3
Average difference					0.5±0.2		0.9±0.2
Chlorurus sordidus	17	E	**14.2:25.6**				
Blood				4.5:7.1	5.8±0.1	-16.3:-9.5	-12.8±0.4
Muscle				5.0:7.8	6.3±0.2	-13.9:-8.9	-12.1±0.4
Average Difference					0.5±0.2		0.7±0.2
C. strongylocephalus	14	E	**14.1:31.9**				
Blood				4.9:7.7	6.1±0.2	-16.6:-10.7	-13.5±0.5
Muscle				5.6:8.2	6.7±0.2	-15.2:-10.9	-13.1±0.4
Average Difference					0.6±0.1		0.4±0.2
Scarus niger	12	S	**11.8:34.8**				
Blood				4.6:6.4	5.3±0.2	-15.3:-11.8	-13.3±0.4
Muscle				5.0:6.7	5.6±0.1	-15.4:-11.1	-12.9±0.4
Average Difference					0.4±0.1		0.4±0.1
Scarus psittacus	4	S	19.5:28.5				
Blood				4.5:5.1	4.8±0.2	-12.3:-10.2	-11.3±0.5
Muscle				5.1:5.8	5.4±0.2	-12.0:-9.5	-10.7±0.5
Average Difference					0.7±0.2		0.6±0.3
Scarus russelii	8	S	**18.3:29.5**				
Blood				4.6:6.2	5.6±0.2	-15.3:-11.4	-12.6±0.5
Muscle				4.9:7.2	5.9±0.3	-14.8:-10.6	-12.4±0.5
Average Difference					0.3±0.2		0.2±0.2
Scarus tricolor	6	S	14.6:26.4				
Blood				7.1:9.0	7.8±0.3	-17.0:-15.7	-16.6±0.2
Muscle				7.4:9.5	8.5±0.4	-16.7:-14.4	-16.0±0.4
Average Difference					0.9±0.2		0.8±0.3
Hipposcarus harid	7	B	NA				
Blood				4.3:5.1	4.6±0.1	-16.1:-15.1:	-15.6±-0.1
Muscle				4.3:4.8	4.6±0.1	-15.9:-13.7	-14.8±0.3
Average Difference					0.0±0.2		0.8±0.2

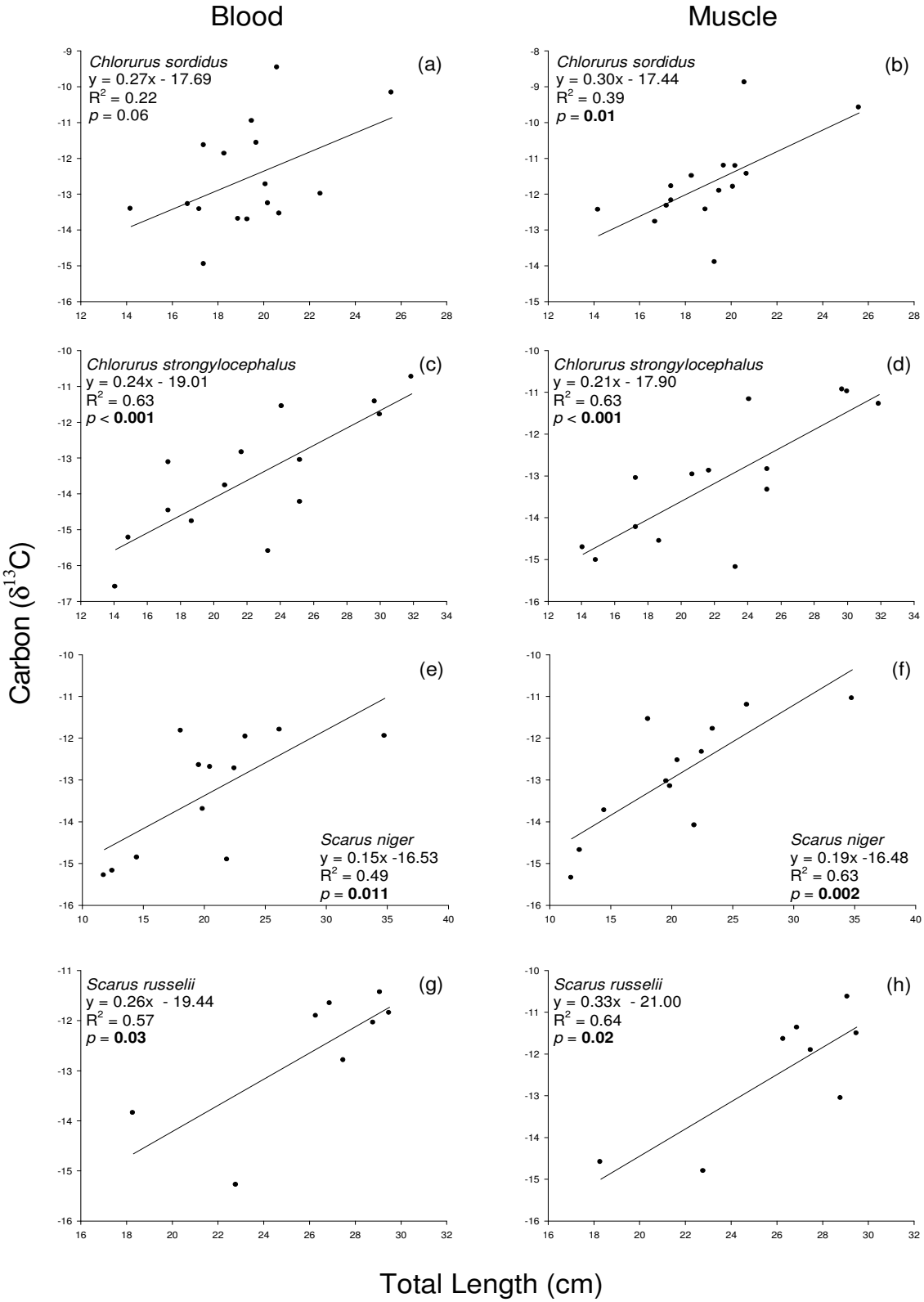


Figure 3.2 Comparisons of total length (cm) with blood and muscle δ^{13}C signatures for species that had a high sample size across a range of lengths (see Table 3.1). **Bold** type indicates significant relationship between an isotope signature and TL.

respectively). Overall, $\delta^{13}C$ samples became enriched with an increase in TL for both blood and muscle and inter-specific comparisons of slopes for the $\delta^{13}C$ vs. TL relationships revealed no significant differences ($p > 0.05$). Conversely, no tissue $\delta^{15}N$ signatures were significantly correlated with TL, though again with the exception of *C. sordidus* muscle ($m = 0.16$, y-int. = 3.21, $R^2 = 0.31$, $p = 0.02$). Pair-wise comparisons of slopes between species indicated no difference in slopes for either tissue in any species.

3.4 DISCUSSION

Studying multiple parrotfish species from similar coral reefs, across a broad range of lengths and ontogenetic stages has important implications for isotopic studies on tropical marine, herbivorous fishes. Changes in environment, physiology and behaviour can cause variation in isotopic signatures within and amongst individuals of a population (Vanderklift and Ponsard 2003). Identifying these patterns of variability provides understanding of trophic ecology both within individuals, amongst individuals of similar species and amongst individuals of a population or community. The understanding of inter-tissue isotopic variability can also help to elucidate temporal patterns in diet that may provide insights into the degree of specialisation and help to understand how environmental influences can shape consumer diets.

Previous literature on isotopic relationships between blood and muscle in birds (Hobson and Clark 1992a,b) and fishes (MacAvoy *et al*. 2001, Buchheister and Latour 2010) suggest that isotopic signatures of blood would show different values to those of muscle, as blood tissue turns over faster than muscle and thus, represents a shorter period of time integration. This, however, does not appear to be the case in parrotfish, as only one species showed a blood vs. muscle relationship slope significantly different from 1:1 for $\delta^{13}C$ and only three for $\delta^{15}N$ (see Figure 3.1). McIntyre and Flecker (2006) reported that in a tropical catfish, turnover rates in muscle, fin and blood were statistically equivalent (26, 18 and 24 days, respectively). They postulated that this was largely due to increased respiration and metabolic rates found in organisms of high temperature environments (Clarke and Johnston 1999) and the high growth rate of the catfish (Solomon *et al*. 2004). Although tissue turnover time has not been quantified in parrotfishes, these conditions are applicable because they have been shown to have a relatively high assimilation rate when compared to other herbivorous temperate fish

species (Edwards and Horn 1982, Bruggemann *et al.* 1994b) and can accumulate mass at a rate greater than that of the catfish (> 0.9-1.1% d^{-1}, Clifton 1995). Furthermore, under laboratory conditions, increased rates of isotopic turnover in fish tissues are seen with increases in temperature (Herkza and Holt 2000, Bosley *et al.* 2002). As parrotfishes are primarily found in tropical waters, they would be at the high-end of this thermally-affected metabolic spectrum. Alternatively, spatially and temporally consistent diets would cause both tissues to reflect similar isotopic signatures. This was falsified as diet can vary ontogenetically amongst multiple food sources (Chapter 5).

The lack of large individuals in the samples may help explain the close relationship between $\delta^{13}C$ signatures and TL, because growth within our sampled range would have mostly been continuous (van Rooij *et al.* 1995a). The close relationship between blood and muscle carbon signatures and the presence of an ontogenetic shift in tissue $\delta^{13}C$ signature (see Chapter 5) suggests that metabolic and assimilation processes occur at a constant rate throughout the sampled size classes. This is surprising, as parrotfishes undergo change in sex, which in some species, is associated with behavioural and dietary shifts. Most species start out as females with a diet of low quality foods and high feeding rates and change sex at a later stage with a diet higher in proteins and a behaviour that includes territorial defence (Hanley 1984, Bruggemann *et al.* 1994a,b, van Rooij *et al*. 1995b, 1996). It is also possible that, within tropical species, the observed correlation between TL and isotopic signatures is more closely related to ontogenetic changes in diet (see Chapter 5). Additionally, parrotfish have been shown to have three different functional groups defined by their modes of feeding (scraping, excavating and browsing). Generally these are associated with different diets, but somewhat unexpectedly; these functional groups were not reflected in blood or muscle isotopic signatures. This lack of inter-tissue, size-based variation in $\delta^{13}C$ signatures is important because, despite the large differences in signatures between species, significant differences did not occur between tissues. Consequently, species differences in isotopic signatures more likely reflect changes such as differences in habitat use or body condition, rather than inter- and intra- specific differences in metabolism. It should be noted that these inter-tissue and sized based trends must be assessed within the capacity of the empirical data, and thus, variability may be hard to qualify as some of the species had small sample sizes. This may lead to situations where the data do not represent all variability in ecological processes among parrotfish.

Generally, different dietary integration rates amongst consumers' tissues can help to clarify different temporal processes associated with isotopic signatures. However, in parrotfishes, a strong positive correlation of tissues in species with a large sample size suggests that either blood or muscle may be sampled when constructing trophic profiles of parrotfishes, with the disclaimer that tissues may not reflect dietary changes on different temporal scales. A simple 1:1 substitution of blood values for muscle or vice versa may be applicable for some species but tissue signatures are not interchangeable for all parrotfishes because of significant inter-species variation. The cause of this variability was not directly tested but may represent ecological differences in isotopic assimilation within individuals of the same taxa. Also, the single browser *Calotomus carolinus* did not show a ratio different from 1:1, but given its high R^2 and highly different slope (m = 1.82), an increase in sample size might indicate a variance from this ratio. This may mean that correction factors, or standardised substitution between tissues, generated for one taxon within a single location may differ from those generated in a different area; currently, it may be more appropriate to generate correction factors within species and populations.

In comparison to $\delta^{13}C$, relationships were relatively weak between tissues for $\delta^{15}N$ signatures and this should be taken into account when considering correction factors. This relationship did not become stronger with an increase in sample size as seen in *Chlorurus sordidus*, which had the largest n (17), but lowest R^2 (0.11). In contrast, although displaying opposite trends, *C. strongylocephalus* and *H. harid* showed a tight relationship between tissues, indicating that even across taxonomically similar species, processes contributing to $\delta^{15}N$ signatures may not be similar but may instead be species-specific. The lack of correlation of $\delta^{15}N$ signature with TL also indicates that signature is not related linearly to TL and may cause increased confusion when trying to compare ontogenetic dietary changes between tissues. For $\delta^{15}N$, the relatively weak isotopic relationship between blood and muscle tissues, the isotopic differences amongst species and the lack of isotopic relationship with TL, indicate that while in some cases muscle tissue $\delta^{13}C$ signatures can be swapped for blood, the same is not true for $\delta^{15}N$. Further ecological and physiological studies on $\delta^{15}N$ tissue fractionation need to be conducted before trying to determine correction factors.

The published literature concerning tissue-turnover rates in tropical teleosts and the close isotopic relationship between blood and muscle tissue found in the current study

suggest that both tissues will reveal a similar story for $\delta^{13}C$. This permits for the use of multiple tissues of parrotfishes to answer specific trophic questions. The same is not true for $\delta^{15}N$. Considering the degree of inter-specific variability in signatures in tissues, it is probable that the processes that cause isotopic signature differences occur at the species level and generalisations across parrotfish as a group should be conservative, if carried out at all. The lack of variability between tissues of many of the studied parrotfishes indicates that both blood and muscle may reflect similar time scales of integrated diet, allowing for either tissue to be applied to studies concerned about diets based on a longer time scale. Stable isotope analysis can be considered a powerful tool, especially when accompanied by analyses of stomach content, although stomach content analyses may be redundant if ontogenetic shifts occur on a time scale greater than the tissue turnover time. Also, stomach content analysis provides exact identification of dietary constituents, but may fall short when species display a high level of mechanical processing, as often occurs in herbivores. When taken from the caudal vain, sampling of fish blood for isotope analysis is a non-destructive alternative to dorsal or caudal muscle sampling and would be especially useful in protected areas such as marine reserves. Further studies exploring species-specific tissue turnover rates in parrotfishes would help to elucidate the current study's applicability amongst differing species. The identification of isotopic relationships between blood and muscle tissues provides a better understanding of variability in signatures within individuals and at present, this is one of the first studies to investigate these relationships in coral reef fish. The interpretation of trophic webs is dependent on isotopic fractionation at the species level which must first be identified before interpretation is conducted at larger scales. Within coral reefs, trophic studies are an essential tool in understanding species roles before, during and after ecological perturbations such as storms, phase shifts and the impact of anthropogenic effluents. The application of stable isotopic analysis is a powerful tool in all of these circumstances, but understanding how elements are transferred both within and amongst individuals must be understood before larger scale interpretations can be made.

Chapter Four
Spatial variation in the isotopic signatures of coral reef producers and consumers

4.1 INTRODUCTION

If we are to understand an ecosystem's ecology, understanding energy flow through a system is as critical as elucidating the trophic interactions between different groups of consumers (Hairston and Hairston 1993). Following nutrient patterns within a system can be difficult because spatial heterogeneity exists within both the biotic and the abiotic processes that determine energy transfer and flow. For instance, coastal marine ecosystems can be affected by external sources such as nutrients from terrestrial or marine sources, but these depend on the acting climatic and transportation processes (Martinetto *et al.* 2006, Bowen *et al.* 2007). Furthermore, the processes within a coastal marine ecosystem depend on the abundance and diversity of primary producers, herbivores and secondary consumers (Simenstad and Wissmer 1985, Harrigan *et al.* 1989). Because of such possible heterogeneity, the processes that affect productivity and nutrient transport within and between coastal marine systems must be studied at the appropriate spatial scale to understand how coastal marine communities are structured.

In comparison with other marine and terrestrial ecosystems, coral reefs are highly complex ecological communities that are characterised by relatively high biodiversity (Knowlton *et al.* 2010). They are often located on the continental shelf and thus, are affected by both terrestrial and oceanic processes (Hearn *et al.* 2001). Additionally, their relative closeness to the shore subjects them to anthropogenic stresses such as sewage effluent and resource use (Hughes 1994, Jackson 1997, Jackson *et al.* 2001). Anthropogenic impacts can be especially high in developing countries where fishing and waste regulations are minimal and/or poorly enforced (Moberg and Folke 1999, Cinner *et al.* 2009). As a result, coral reefs can be exposed to differing levels of anthropogenic pressure depending on their accessibility and the productivity of natural resources (Cinner *et al.* 2009).

In Zanzibar, coral reef organisms have long been a primary source of protein and minerals for the island's population (Jiddawi and Öhman 2002). Anthropogenic inputs are loosely managed by authorities and effluents can come from multiple sources including cities and plantations (Kulekana 1998). On the west coast near the capital,

Stone Town, fringing reefs are common and are often surrounded by intertidal sand flats. These reefs occur at different distances from the city and may be separated from one another by kilometres of relatively deep, sandy bottoms, creating semi-isolated systems. Limited exchange between reefs and spatially different fishing and nutrification pressures suggest that within-reef energy flow may vary amongst reefs. One common method for identifying patterns of nutrient exchange within systems is through the application of stable isotope analysis. Using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values, organisms with isotopically distinct signatures can be placed within the food web as each trophic level is enriched by roughly 0.5‰ and 3.0‰, respectively (DeNiro and Epstein 1978, 1981, Fry and Sherr 1984, Post 2002). Different primary producers such as mangrove trees (-27‰), phytoplankton (-22‰) and benthic algae (-17‰) (Atkinson and Smith 1983, France 1995, Muzuka and Shunula 2006) have distinct carbon isotope values, allowing the signatures to be differentiated in the tissues of a consumer and for identification of the food sources (Fry and Sherr 1984, Peterson and Fry 1987). Because $\delta^{15}N$ is enriched to a greater degree than $\delta^{13}C$ between prey and consumer, it is often used to assess consumer trophic level (Minagawa and Wada 1984, Owens 1987). However, $\delta^{15}N$ is also used for information pertaining to the source of nitrogen in primary producers. Atmospheric nitrogen has a $\delta^{15}N$ signature near 0‰, whereas human and animal wastes tend to have an enriched value (≥ 5‰) (Mendes *et al.* 1997, Gartner *et al.* 2002). For example, water with septic effluents has a $\delta^{15}N$ of 5.5‰ and the watershed around cattle grazing land can have a signature of 12.5‰ (Souza and Wasserman 1997, Scharffenberger *et al.* 1999, Cole *et al.* 2004).

In order to capture long-term variation between different ecosystems, or to compare areas within an ecosystem, long-lived primary consumers are often sampled to identify isotopic differences (Post 2002, Nadon and Himmelman 2006). In temperate areas, bivalve molluscs are often used because their method of resource procurement, filter feeding, means their tissue will reflect changes in the planktonic community (Mallela and Harrod 2008). On coral reefs, scleractinian corals behave in a similar fashion, with allochthonous resources procured via the tentacles of the polyp (Sebens *et al.* 1996, Ferrier-Pagès *et al.* 1998, Palardy *et al.* 2006), but alternatively, with autochthonous sources obtained from endosymbiotic zooxanthellae (Davies 1984, Muscatine 1990). Energy requisition between these two methods for the polyp is balanced depending on the level of photosynthetic radiation available (Porter 1976, Muscatine and Porter 1977), often correlating with depth (Kaiser et *al.* 1993, Muscatine and Kaplan 1994,

Palardy *et al.* 2006). The isotopic signatures of the two tissues can be used to identify spatial differences in environmental processes because the zooxanthellae acquire nutrients primarily from the water's dissolved organic nitrogen and the polyp will reflect the zooxanthellar and planktonic sources (Muscatine *et al*. 1989, Muscatine and Kaplan 1994).

To determine if these spatial differences in isotope signatures are passed through trophic levels, it is necessary to sample consumers that use corals as a food source. On coral reefs, there are multiple corallivores, but one of the most abundant is the parrotfish (Lewis and Wainwright 1985, Choat 1991, Hughes *et al.* 2003, Hughes *et la.* 2007). These fish have been shown to prey on hard corals but are largely herbivorous, feeding on foliose and turfing algae, as well as the associated epiphytic material (Bellwood and Choat 1990, Bellwood 1994, Choat *et al.* 2002, 2004). Therefore, the isotopic signature of parrotfish tissue will reflect the signatures of the corals, algae and detritus of the coral reef and indicate the associated transition of energy through trophic levels.

Aside from resource use and energy transfers within a system, it is becoming increasingly important to consider spatial variation in isotopic signatures to address the issue of foraging ecology. There has been increasing interest in the application of "isoscapes", or maps quantifying regional variation in isotope signatures (Jennings *et al*. 1997, Jennings and Warr 2003, Bowen 2010, Grahams *et al.* 2010). These may help in tracing the movement of marine consumers without the intensive sampling and costs required for other methods such as satellite tracking. However, data are lacking for isotopic variation on coral reefs and without some understanding of potential variation, isotope data may not be extrapolated between ecologically similar, but spatially separate, habitats (Greenwood *et al.* 2010).

This study used the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of organisms spanning multiple trophic levels including primary producers (macroalgae), detritus, scleractinian corals and parrotfish to explore the differences in signatures between multiple reefs and depths off Zanzibar. The objectives of this study were to: (1) examine the $\delta^{13}C$ and $\delta^{15}N$ isotopic variation in four food sources and consumers (detritus, macroalgae, corals and parrotfishes) to provide baseline information for the evaluation of foodweb and trophic level structure and (2) assess levels of spatial variability in $\delta^{13}C$ and $\delta^{15}N$ signatures across three reefs differing in distance from Stone Town.

4.2 METHODS AND MATERIALS

For information concerning the study site, sample collection and preparation and stable isotope analysis, please see Chapter 2.

4.2.1 Variability in parrotfish

Data for fish were a subset of the complete sampling effort and reflected a range of sizes and ontogenetic stages that were available for all reefs being compared. Not all species of fish were present at each reef. A detailed description of individual species used in the analysis is given in Table 4.1. Differences among reefs were tested with a one-way ANOVA for each species and element. Homogeneity of variance was tested using Levene's test and post-hoc comparisons were conducted with Tukey's HSD.

4.2.2 Variability in macroalgae

Dictyota spp. was used to analyse differences amongst reefs and depths, as it was the most dominant primary producer in terms of biomass. The comparison was only completed between Ukombe and Nyange reefs because the Pwakuu sample size was not large enough to include the effect of depth. To compare differences between reefs, a two-way ANOVA was performed with the fixed factors, reef and depth, for each signature individually. A type-III sums of squares was used because sample size was not equal across depths. Depth was orthogonal and categorical because similar depths were sampled at all reefs. Homogeneity of variance was tested with Levene's test and post-hoc comparisons were conducted with Tukey's HSD.

4.2.3 Variability in detritus

Detritus was sampled to allow for testing the effects of reef, site and depth. This was tested with a three-way, mixed-model ANOVA on each signature individually. Site was nested within reef and depth was orthogonal. Homogeneity of variance was tested with Levene's test and post-hoc comparisons were performed with Tukey's HSD.

4.2.4 Variability in corals

4.2.4.1 Spatial Comparison

To identify spatial trends in corals for each isotope, a three-way nested ANOVA was applied to the factors reef, depth and site. Site was nested within reef and depth was orthogonal. Data from *Porites lobata* were used because it was the only species that

was represented within all levels of all factors. Data for polyps and zooxanthellae were analysed separately. A Newman-Keuls post-hoc test was used to identify pair wise specific differences between factors and levels within the polyp and zooxanthellae $\delta^{13}C$ signatures. Tukey's HSD post-hoc comparisons were used for the polyp and zooxanthellae $\delta^{15}N$ signatures.

4.2.4.2 Effects of Depth

Depth was analysed with a three-way full factorial ANOVA with factors, tissue, reef and depth. This was completed with data from *Porites lobata, P. solida* and *Acropora divaricata* pooled to make broader tests about the effects of depth on scleractinian corals. Tukey's HSD post-hoc comparisons were used for $\delta^{13}C$ signatures and Newman-Keuls post-hoc for $\delta^{15}N$ signatures.

4.2.4.3 Inter-specific Isotopic Signature Comparison

To test the inter-specific differences in polyp and zooxanthellae signatures, a two-way ANOVA was performed on $\delta^{15}N$ and $\delta^{13}C$ signatures with the fixed factors tissue and species. This was done for *Porites lobata, P. solida, Acropora digitifera, A. formosa, A. nasuta* and *A. valenciennesi* from the reef crest at Nyange, Site 2. Tukey's HSD post-hoc comparisons were used to identify homogeneous subgroups for $\delta^{13}C$ and $\delta^{15}N$ signatures between tissues and species.

All data were checked for homogeneity of variance with Levene's test; normality was checked with frequency histograms and no transformations were required. The use of either Tukey's HSD or Newman-Keuls post-hoc depended on the number of comparisons being made. Newman-Keuls allows pair-wise comparisons and was applied when interactions were significant. Tukey's HSD was used to identify homogeneous subgroups when the main effects were significant. All statistical analyses were completed in Statistica 6 and figures were made with SigmaPlot 10.

4.3 RESULTS

4.3.1 Variability in parrotfish

The different fish species analysed were *Scarus tricolor*, *S. niger*, *S. viridifucatus*, *Chlorurus sordidus* and *C. strongylocephalus*. For each species, sample size and the range of total lengths from each reef are provided in Table 4.1. In all significant

Table 4.1 Detailed list of individual fish species including sample size at each reef. Stage (initial phase = I, terminal phase = T), total length (T.L.) range (cm) of samples from each reef and results from one-way ANOVA testing the significance of reef. Each element was tested separately. For each species, degrees of freedom are total sample size for all individuals amongst reefs minus number of reefs and number of reefs minus one. Significance was interpreted at alpha < 0.05and is indicated in **bold**. Results from post-hoc are displayed below significant results.

		St. (n)		T.L.	Nitrogen		Carbon	
Species	Reef	I	T	Rng (cm)	*F*	*p*	*F*	*p*
Scarus niger	Nyange	4	3	14.8:19.9	8.21	**0.014**	0.07	0.792
	Ukombe	5	2	12.0:20.5		N>U		
Scarus tricolor	Nyange	4	1	13.9:16.7	3.98	0.081	0.16	0.703
	Ukombe	4	1	15.3:19.4				
Scarus viridifucatus	Nyange	3	2	14.9:15.8	6.63	**0.033**	9.25	**0.016**
	Pwakuu	2	3	14.7:16.5		N>P		N<P
Chlorurus sordidus	Nyange	7	0	16.7:19.8	10.39	**0.001**	7.73	**0.004**
	Pwakuu	7	0	17.1:19.6		N=P>U		N<U
	Ukombe	7	0	17.3:20.2				
Chlorurus strongylocephalus	Pwakuu	4	4	11.5:23.2	0.01	0.927	0.69	0.421
	Nyange	5	3	10.8:20.7				

Table 4.2 ANOVA results for effect of reef and depth on carbon and nitrogen signature of *Dictyota spp*. Reefs sampled were Nyange and Ukombe.

	SS	df	MS	*F*	*p*
Nitrogen ($\delta^{15}N$)					
Reef	5.41	1	5.41	10.01	**0.004**
Depth	0.48	2	0.24	0.44	0.64
Reef × Depth	0.02	2	0.01	0.02	0.98
Error	12.98	24	0.54		
Carbon ($\delta^{13}C$)					
Reef	1.67	1	1.67	0.48	0.49
Depth	28.15	2	14.07	4.06	**0.03**
Reef × Depth	0.42	2	0.21	0.06	0.94
Error	83.14	24	3.46		

Table 4.3 Mean (±SE) of macro algae (*Dictyota spp.*) and detritus for variables that were significant in their ANOVA's. Tukey's post-hoc results are also provided.

	Nitrogen ($\delta^{15}N$)	Carbon ($\delta^{13}C$)		Nitrogen ($\delta^{15}N$)	
Depth	Detritus	*Dictyota spp.*	Reef	*Dictyota spp.*	Detritus
Crest	2.9±0.2	-13.7±0.5	Nyange	4.6±0.1	2.8±0.2
Slope	3.5±0.2	-14.4±0.8	Pwakuu		3.3±0.2
Base	3.6±0.2	-16.1±0.1	Ukombe	3.8±0.2	3.9±0.1
Post-hoc	C<S,B	C<B		N>U	N<U

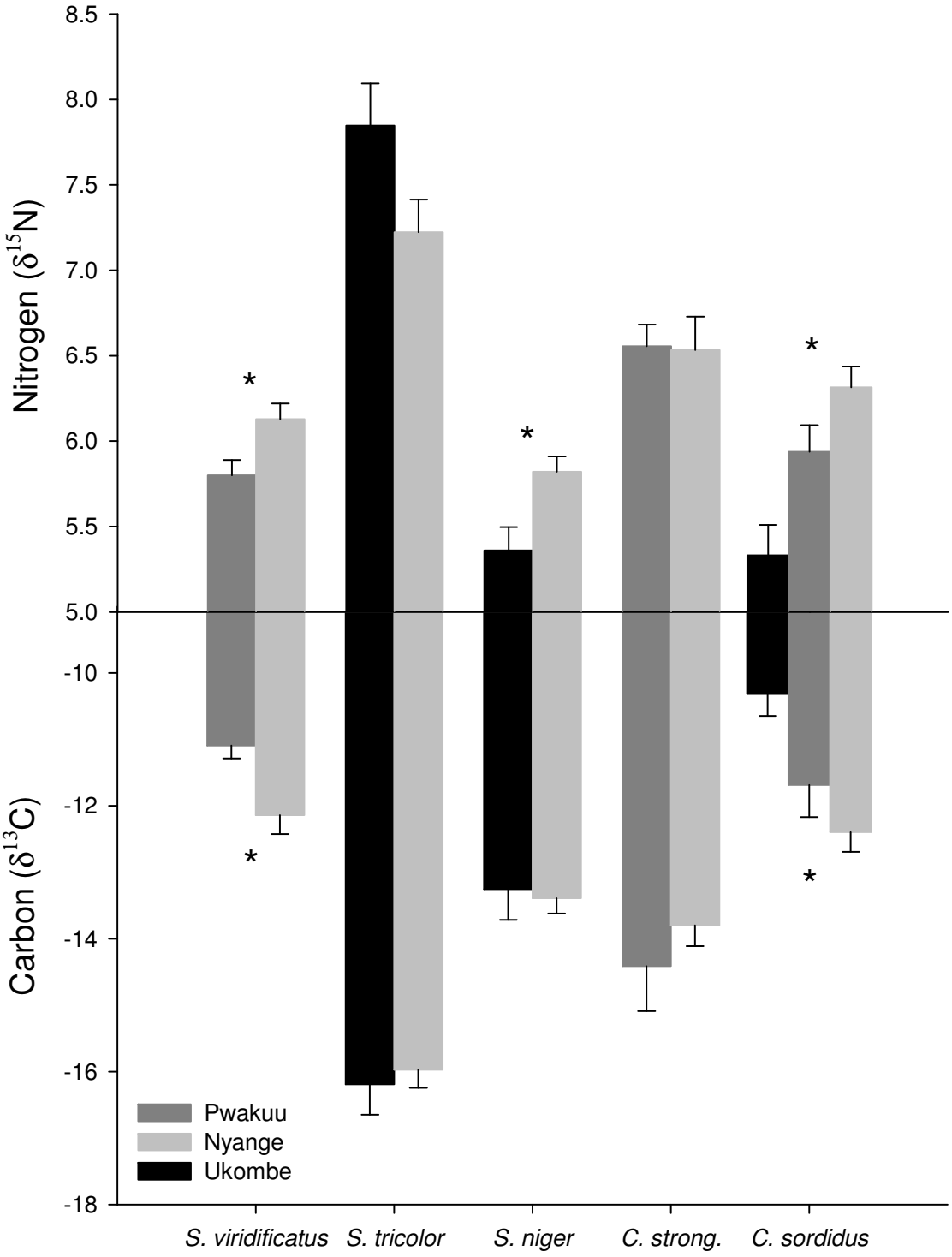


Figure 4.1 Carbon and nitrogen signatures for five species of parrotfish compared in a one-way ANOVA for differences amongst reefs. Stars indicate species that showed significant differences between reefs as indicated by the Tukey's HSD post-hoc.

comparisons of $\delta^{15}N$, Nyange was always enriched. Nyange was more enriched than Pwakuu and Ukombe for *S. viridifucatus* and *S. niger*, respectively, and more enriched than Ukombe, but not different from Pwakuu for *C. sordidus* (Fig 4.1, $\delta^{15}N$). *S. tricolor* and *C. strongylocephalus* showed no differences in nitrogen signatures between reefs (Fig 4.1, nitrogen). Nyange $\delta^{13}C$ was significantly lower compared to Pwakuu in *S. viridifucatus* and significantly different to Ukombe but not from Pwakuu in *C. sordidus* (Fig 4.1, carbon). *S. tricolor*, *C. strongylocephalus* and *S. niger* were not different in carbon signature for their compared reefs (Fig 4.1, $\delta^{13}C$).

4.3.2 Variability in Macroalgae

For nitrogen, *Dictyota spp*. was significantly different between reefs, but not among depths, nor was there a significant interaction (Table 4.2). Nyange was significantly higher in $\delta^{15}N$ than Ukombe. For carbon, there were no differences between reefs, but there was an effect of depth (Table 4.2). The base was depleted in comparison to the crest, but slope was not different from either crest or base (Table 4.3).

Table 4.4 Results for a three-way mixed ANOVA to test the effects of reef, site and depth on each signature of detritus. Site was nested within reef and depth was orthogonal. Significance was at an alpha level < 0.05 and indicated in **bold**.

	SS	df	MS	*F*	*p*
Nitrogen ($\delta^{15}N$)					
Reef	14.26	2	7.13	8.68	**<0.001**
Site (Reef)	5.64	6	0.94	1.15	0.35
Depth	7.38	2	3.69	4.49	**0.016**
Reef × Depth	0.53	4	0.13	0.16	0.96
Site (Reef) × Depth	18.20	12	1.52	1.85	0.06
Error	44.37	54	0.82		
Carbon ($\delta^{13}C$)					
Reef	24.91	2	12.46	0.84	0.44
Site (Reef)	170.96	6	28.49	1.92	0.09
Depth	2.44	2	1.22	0.08	0.92
Reef × Depth	36.72	4	9.18	0.62	0.65
Site (Reef) × Depth	141.34	12	11.78	0.79	0.66
Error	801.72	54	14.85		

4.3.3 Variability in detritus

Detrital $\delta^{15}N$ was lowest at Nyange and highest at Ukombe (Table 4.3). Pwakuu was not different from either of the other reefs. Also, the crest was depleted compared to slope, but the base was not different from either of the other depths. There were no differences found between sites or for either of the interactions in nitrogen. The three-way ANOVA found no differences amongst reefs, sites or depths for carbon (Table 4.4).

4.3.4 Variability in corals

4.3.4.1 Spatial Comparison

$\delta^{13}C$ of *Porites lobata* polyps showed significant effects of all factors within the nested ANOVA (Table 4.5). The site × depth interaction was largely driven by sites within Ukombe. In Site 1, the Ukombe base was different from all other sites, depths and reefs (Fig 4.2). In Ukombe Site 1 and Site 2, the crests were different from most other sites, depths and Pwakuu and Nyange reefs. It was not different from Pwakuu Site 1, slope or any Pwakuu bases. Pwakuu Site 3, crest was different from its Site 1, 2 and 3 bases and Site 1, slope (Fig 4.2). Interpretation of results is considered at the reef/depth levels because the differences were mostly caused by a few site/depths within Ukombe, or only three of the total 27 possible comparisons. Within all three reefs, mean signatures showed similar patterns of increased enrichment with increasing depth (Fig 4.3). Overall, Nyange was more enriched than both other reefs, while Ukombe was the most depleted. Within Pwakuu, base and slope were different from the crest, but not from each other. There was no significant difference in depths at Nyange. At Ukombe, base was depleted when compared to the slope and crest; however slope and crest were not different. All three depths at Nyange were significantly more enriched than all of Ukombe and the base or slope of Pwakuu. Ukombe base was significantly depleted compared with everything, while its slope and crest were not different from Pwakuu base or slope.

Nyange $\delta^{15}N$ (mean 5.49‰) was significantly more enriched than Pwakuu and Ukombe (4.63 and 4.34‰, respectively) by nearly 1‰, the other two reefs were not different from each other. Nyange Site 2 was the most enriched site (6.56‰) and was different from all Ukombe sites (Site 1: 4.00, 2: 4.19, 3: 4.81‰) and Pwakuu Site 2 (3.77‰). All other sites were not different from each other.

Zooxanthellae $\delta^{13}C$ data were significant for all factors except Site (Table 4.2), including both interactions. Nyange crest was significantly more enriched from all

other depths and reefs (Fig 4.2). Ukombe base was the most depleted and was significantly different compared to Pwakuu crest and Nyange slope (Fig 4.2). The enrichment of the Nyange crest was due to the enrichment found at Site 1 and 3 (Figure 4.3). These were higher than all other sites and depths besides for Pwakuu Site 2, all depths and other Nyange sites. Also, Ukombe Site 3, base was lower than crests of Nyange and Pwakuu (Fig 4.2).

Table 4.5 Results from the three-way nested ANOVA testing the effect of differing spatial treatments on *Porites lobata* isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) of polyp and zooxanthellae tissues. Site tested differences at a medium scale (100s of metres) and reef was at a large scale (1000s of metres). Depth was orthogonal and samples were taken from the crest (3 m depth), slope (~7m) and base (~13m). Significance was inferred at $\alpha < 0.05$ and is indicated in **bold**.

	Effect	SS	df	MS	*F*	p
Polyp	Depth	37.48	2	18.74	19.73	**<<0.001**
Carbon ($\delta^{13}C$)	Reef	72.00	2	36.00	37.90	**<<0.001**
	Site (Reef)	26.82	6	4.47	4.71	**<0.001**
	Reef × Depth	9.57	4	2.39	2.63	**0.044**
	Site (Reef) × Depth	50.73	12	4.23	4.45	**<<0.001**
	Error	49.05	54	0.91		
Polyp	Depth	1.32	2	0.66	0.56	0.574
Nitrogen ($\delta^{15}N$)	Reef	19.90	2	9.95	8.43	**<0.001**
	Site (Reef)	28.25	6	4.71	3.99	**0.002**
	Reef × Depth	11.64	4	2.91	2.46	0.056
	Site (Reef) × Depth	18.49	12	1.54	1.31	0.243
	Error	63.77	54	1.18		
Zooxanthellae	Depth	18.42	2	9.21	10.98	**<0.001**
Carbon ($\delta^{13}C$)	Reef	17.28	2	8.64	10.29	**<0.001**
	Site (Reef)	3.72	6	0.62	0.74	0.620
	Reef × Depth	10.76	4	2.69	3.20	**0.020**
	Site (Reef) × Depth	30.45	12	2.54	3.02	**0.003**
	Error	45.31	54	0.84		
Zooxanthellae	Depth	0.79	2	0.40	2.16	0.125
Nitrogen ($\delta^{15}N$)	Reef	0.46	2	0.23	1.26	0.291
	Site (Reef)	4.14	6	0.69	3.77	**0.003**
	Reef × Depth	1.41	4	0.35	1.92	0.120
	Site (Reef) × Depth	2.89	12	0.24	1.32	0.236
	Error	9.89	54	0.18		

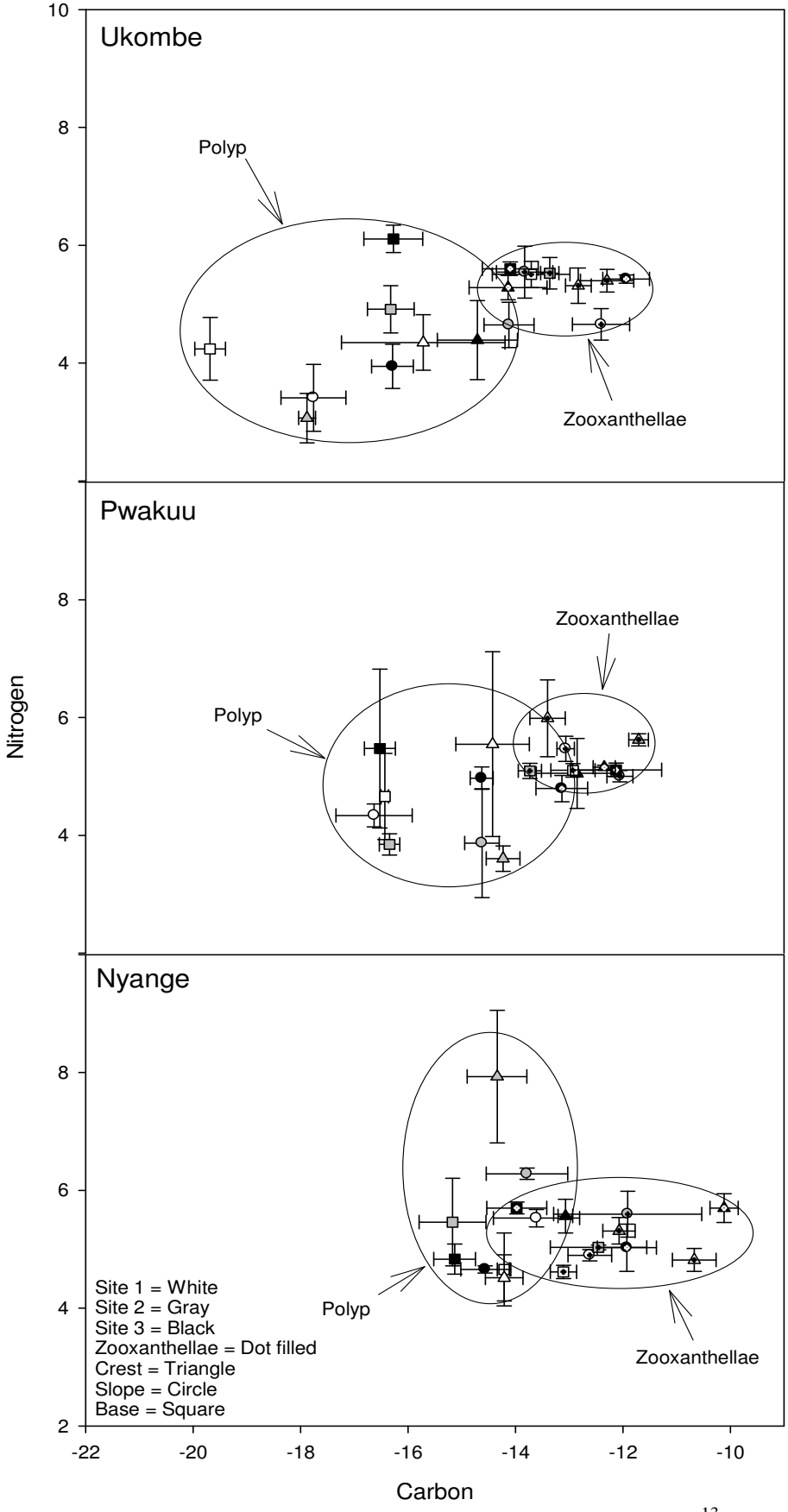


Figure 4.2 Isotopic bi-plot of mean (± SE) *Porites lobata* carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures. Sites were 100s of metres apart while reefs were 1000s of metres.

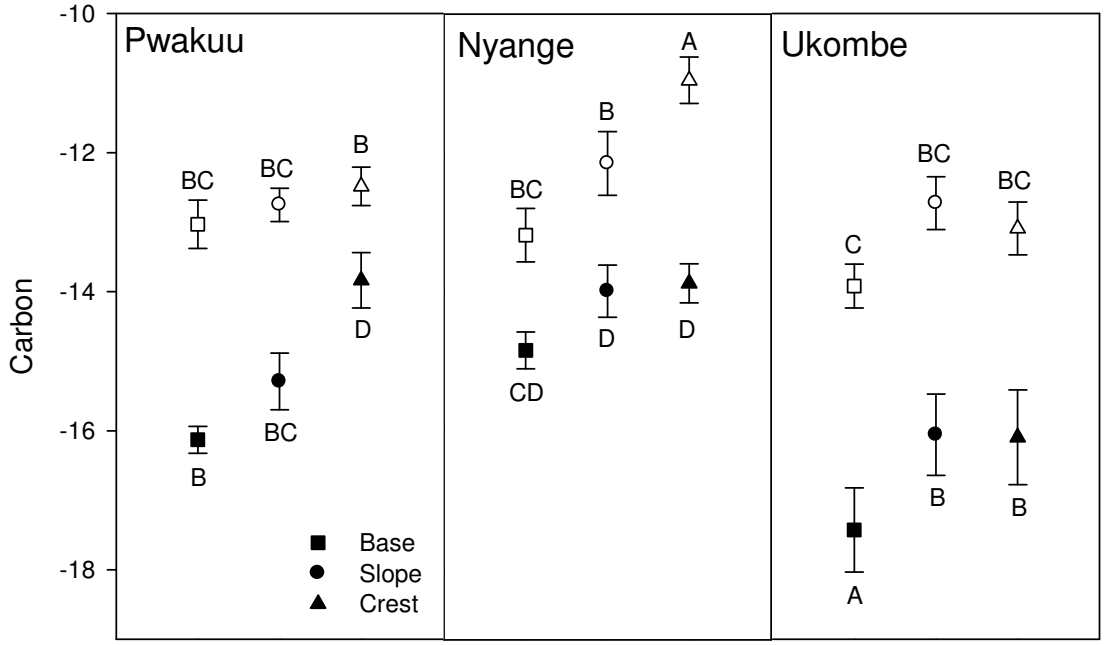


Figure 4.3 $\delta^{13}C$ signatures (mean ± SE) of tissue (polyp: black, zooxanthellae: white) for each reef and depth for *Porites lobata*. The effect of the site by depth interaction was largely caused by the depleted signatures in Ukombe for the polyp and for the enriched zooxanthellae crest signature at Nyange (Newman-Keuls post-hoc). Lettering indicates homogeneous subgroups. Subgroups for each tissue are mutually exclusive.

Zooxanthellae $\delta^{15}N$ signatures at Nyange Site 1 were the most depleted (4.78‰) and were different from Nyange Site 3, Ukombe Site 2 and 3 and Pwakuu Site 1. Pwakuu Site 1 was the most enriched (5.73‰) and also higher than Pwakuu Site 3 (5.02‰). There were no differences between reefs or depths (Table 4.5).

4.3.4.2 Effects of Depth

The three-way ANOVA for carbon ($\delta^{13}C$) signatures of grouped *Porites lobata, P. solida* and *Acropora divaricata* tissues resulted in a significant effect of the main factors (depth, reef and tissue) but not the interactions (Table 4.6). Post-hoc comparisons (Tukey's HSD) found zooxanthellae to be enriched by nearly 2‰ compared to the polyp (Table 4.7). The $\delta^{13}C$ signatures became more depleted with depth, also by roughly 2‰. Nyange was the most enriched and Ukombe was the most depleted by nearly 3‰ of Nyange (Table 4.7). For nitrogen ($\delta^{15}N$), all factors were significant ($p < 0.05$) and only the interactions of Tissue × Reef and Reef × Depth were significant (Table 4.6). For Tissue × Reef, the post-hoc comparisons indicated that the Ukombe polyp signature was significantly lower than all other tissues and reefs (Fig 4.4). The Reef × Depth was

significant because Nyange crest was more enriched than all other reefs and depths, including all other crests (Fig 4.4).

4.3.4.3 Effect of Species

The carbon ($\delta^{13}C$) isotope signatures were significantly affected by both factors (tissue and species), but not the interaction (Table 4.8). The polyp (-14.82±0.32‰) tissue was depleted compared to the zooxanthellae (-14.03±0.33‰) and *P. lobata* was depleted compared to *A. valenciennesi,* but all other species were not different to either of these species (Fig 4.5; $\delta^{13}C$).

Nitrogen ($\delta^{15}N$) signatures were also significant at tissue and species levels, but not at the interaction level (Table 4.7). The polyp signature (4.21±0.12‰) was significantly higher than the zooxanthellae (4.58±0.11‰). *Porites* species were significantly lower than all *Acropora* species, with the exception of *A. nasuta*. *A. nasuta* was not different from *A. digitifera*, but was different from the other *Acropora* species. The other *Acropora* species were not different from each other (Fig 4.5; $\delta^{15}N$).

4.5 DISCUSSION

Coral reefs are good systems in which to examine spatial differences in the transfer of energy between trophic levels because their high species diversity and their proximity to differing anthropogenic and environmental processes means that there are many opportunities to observe dynamic changes. In this study, the marked isotopic differences at Nyange suggest that the processes affecting isotopic signatures can vary at small scales (tens of kilometres). Previously, the spatial relation of coral reefs to terrestrial effluents has been shown to cause a change in isotopic signatures of corals (Risk *et al.* 1994, Sammarco *et al.* 1999, Swart *et al.* 2005), macroalgae (Derse *et al.* 2007) phytoplankton (Montoya 2007) and gorgonians (Sherwood *et al.* 2010). This study, however, shows that multiple trophic levels, from detritus, macroalgae, scleractinian corals to herbivorous fishes show distinct differences in isotopic signatures between reefs. These results indicate that processes causing changes in primary production signatures can be transferred and retained through trophic levels within a single system.

The symbiotic zooxanthellae living in corals assimilate dissolved inorganic nitrogen (DIN) from the external environment (Burris 1983, Wilkerson and Trench 1986, Heikoop *et al.* 1998), but also from the nitrogenous waste excreted by the coral polyp.

Table 4.6 Results of 3-way ANOVA testing the effect of spatial differences on tissue (polyp and zooxanthellae) isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$). Tissues of *Porites lobata, P. solida* and *Acropora divaricata* were grouped to make inferences about factors on scleractinian corals. Significance was at $\alpha < 0.05$ and indicated in **bold**.

	Factor	SS	df	MS	*F*	*p*
Carbon ($\delta^{13}C$)	Tissue	341.58	1	341.58	182.55	**<<0.001**
	Depth	164.11	2	82.06	43.85	**<<0.001**
	Reef	141.20	2	70.60	37.73	**<<0.001**
	Tissue × Depth	5.25	2	2.62	1.44	0.238
	Tissue × Reef	0.10	2	0.05	0.03	0.973
	Reef × Depth	12.39	4	3.10	1.70	0.150
	T × R × D	8.65	4	2.16	1.19	0.316
	Error	662.40	354	1.87		
Nitrogen ($\delta^{15}N$)	Tissue	5.31	1	5.31	6.31	**0.012**
	Depth	6.72	2	3.36	3.99	**0.020**
	Reef	15.19	2	7.59	9.01	**<0.001**
	Tissue × Depth	2.25	2	1.12	1.33	0.265
	Tissue × Reef	8.66	2	4.33	5.14	**0.006**
	Reef × Depth	9.11	4	2.28	2.71	**0.030**
	T × R × D	4.25	4	1.06	1.26	0.285
	Error	298.16	354	0.84		

Table 4.7 Mean (± SE) of carbon ($\delta^{13}C$) signatures of grouped *Porites lobata, P. solida* and *Acropora divaricata* tissues. All levels are significantly different (Tukey's HSD).

Factor	Levels and mean $\delta^{13}C$ (±SE) signature		
Tissue	Polyp	Zooxanthellae	
(n = 186)	-15.32 ± 0.11	-13.37 ± 0.13	
Depth	Base	Slope	Crest
(n = 372)	-15.01 ± 0.16	-14.39 ± 0.14	-13.44 ± 0.18
Reef	Ukombe	Pwakuu	Nyange
(n = 372)	-15.06 ± 0.17	-14.38 ± 0.16	-13.75 ± 0.16

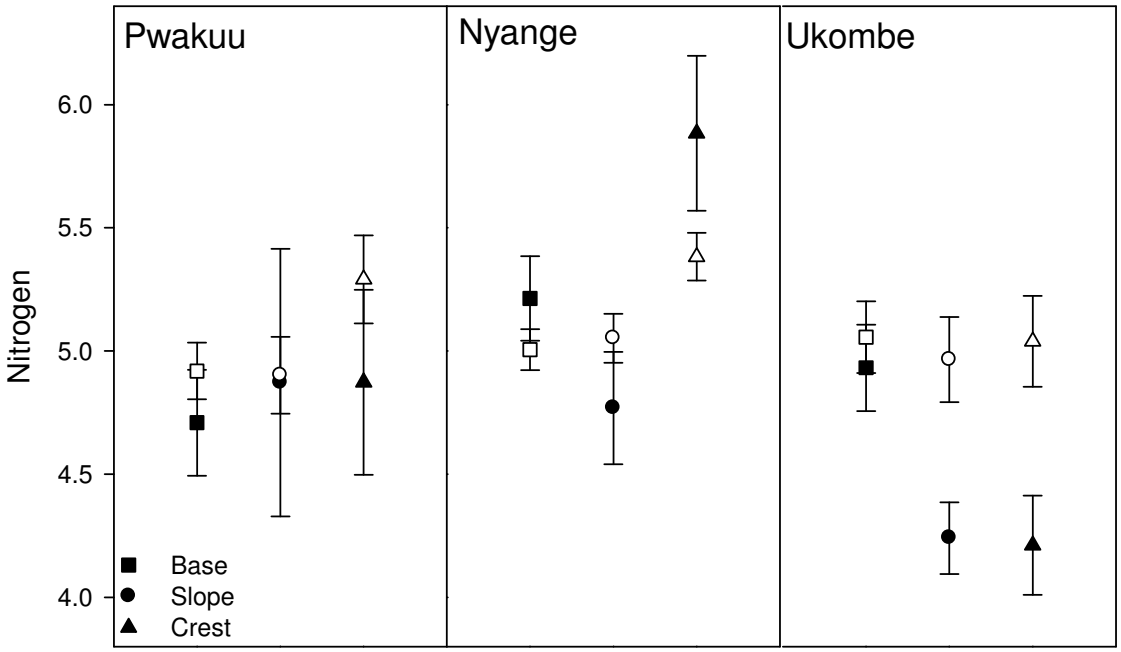


Figure 4.4 $\delta^{15}N$ signatures (mean ± SE) of grouped *Porites lobata, P. solida* and *Acropora divaricata* polyp (black) and zooxanthellae (white) tissues by depth and reef.

Table 4.8 Results of 2-way factorial ANOVA comparing nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope signatures of polyp and zooxanthellae tissues and species. Species included *Porites lobata, P. solida, Acropora digitifera, A. formosa, A. nasuta* and *A. valenciennesi.* All samples were from Nyange Site 2, crest. Significance was at $\alpha < 0.05$ and indicated in **bold**.

	Factor	SS	df	MS	*F*	p
Carbon	Tissue	5.55	1	5.55	10.64	**0.003**
($\delta^{13}C$)	Species	50.61	5	10.12	19.41	**<<0.001**
	Ti. x Sp.	2.38	5	0.48	0.91	0.490
	Error	12.51	24	0.52		
Nitrogen	Tissue	1.18	1	1.18	7.32	**0.012**
($\delta^{15}N$)	Species	2.75	5	0.55	3.40	**0.018**
	Ti. x Sp.	1.94	5	0.39	2.39	0.068
	Error	3.89	24	0.16		

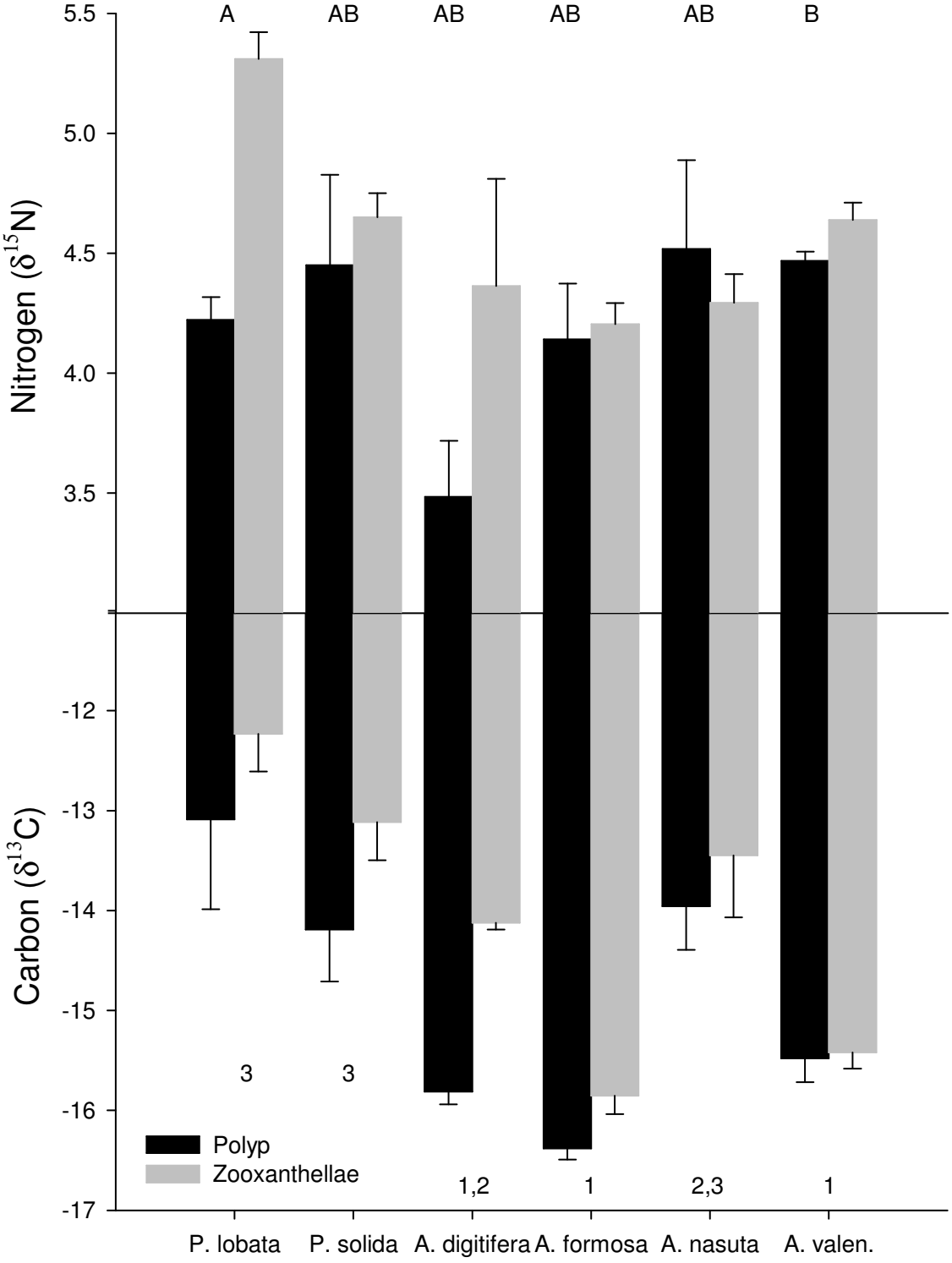


Figure 4.5 Mean (±SE) carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope signatures of polyp and zooxanthellae between species. Homogeneous subgroups for differences in species are indicated by subscripts and were identified with Tukey's HSD with significance at $\alpha < 0.05$. Letters indicate homogeneous subgroups for nitrogen (δ^{15}N) and numbers are for carbon (δ^{13}C).

This dual assimilation may complicate interpretations of zooxanthellar $\delta^{15}N$ signatures, but is likely standardised when analysed amongst corals of different reefs and depths.

On this assumption, the enrichment seen in the zooxanthellae would represent an enrichment of the DIN that is recycled within the coral and might be used as a comparison amongst reefs. However, the nutrient cycling within corals may confound trophic level interpretations between the zooxanthellae and polyps and would then be unlikely to conform to the widely accepted 3.5‰ fractionation step in nitrogen (Post 2002, Reynaud *et al.* 2009).

Despite the confounding of interpretation of trophic levels between zooxanthellae and polyp, clear isotopic trends were visible. Regardless of the reef or depth, zooxanthellae were generally enriched in $\delta^{13}C$ and $\delta^{15}N$ in comparison to the polyp (see Fig 4.3). This enrichment was consistently seen at each reef, which supports past literature showing that isotopic signatures of zooxanthellae and polyps reflect differences in assimilated nitrogen such as dissolved inorganic nitrogen (Muscatine and Kaplan 1994) and polyp waste (Muscatine *et al.* 1989).

Upon closer examination of this general trend, it became apparent that the change in coral $\delta^{13}C$ signature was associated with depth but was not different amongst reefs. This is likely due to the photosynthetic processes of the zooxanthellae (Muscatine *et al.* 1989, Maier *et al.* 2010). At shallow depths, zooxanthellae translocate up to 95% of their photosynthetic products to the coral host, which is believed to be sufficient for the polyps' daily carbon demand (Muscatine 1990, McClosky and Muscatine 1984). Under lower light conditions, however, zooxanthellae increase their photosynthetic efficiency (Titlyanov *et al.* 2001), but less product is passed on to the host. Consequently, the host compensates by increasing its intake of allochthonous carbon sources (McCloskey and Muscatine 1984, Titlyanov *et al*. 2000), leading to a greater difference in $\delta^{13}C$ signatures between the tissues at greater depth and polyp signatures that reflect alternative carbon sources (Muscatine *et al.* 1989). Across the Great Barrier Reef (GBR) for example, Risk *et al.* (1994) found a positive linear relationship of enrichment of polyp and zooxanthellae $\delta^{13}C$ signature in *P. lobata* and *A. formosa* with increasing distance from the Australian shore. This inter-reef difference was attributed to a decreasing level of terrigenous inputs, with trends continuing 100 km off shore. Heikoop *et al.* (2000) found markedly different $\delta^{13}C$ signatures in *P. lobata* from reefs in the western Indian Ocean, Maldives, Indonesian archipelago and the GBR. Both of these examples represent sampling that occurred on a scale much greater than the

current study and provide comparisons to show the scales at which isotopic signatures can vary.

Rather than responding to depth, however, $\delta^{15}N$ signatures of coral appeared to be more associated with reefs. The $\delta^{15}N$ of coral tissue has been used to identify the influence of sewage and other pollutants on reefs around the world (McClelland *et al.* 1997, Sammarco *et al.* 1999, Heikoop *et al.* 2000, Swart *et al.* 2005) and in controlled experimental situations (Hoegh-Guldberg *et al.* 2004). Generally the eutrophic sites were enriched by up to 5‰ (Risk *et al.* 1994, Mendes *et al.* 1997), which supports the data from this study, where corals at Nyange were enriched by approximately 2‰ compared to Ukombe, the difference perhaps reflecting the relative distance to the most probable source of pollution, Stone Town. Waters of the Zanzibar channel have a predominantly northward flow (Shagude *et al.* 2003), but it is possible that small scale eddies exist off Stone Town because of the bathymetry (C Anderson pers. comm.). In absence of empirical evidence for water flow in the region, it is hard to speculate about the possible effects on nutrient distribution.

Similarly to coral signatures, macroalgae showed variation in $\delta^{15}N$ related to reefs and $\delta^{13}C$ related to depth. Possible explanations for variation in $\delta^{13}C$ have been attributed to changes in water temperature, water motion, biochemical fractionation associated with seasonal variability, isotopic variability in the dissolved inorganic carbon pool, differential use of bicarbonate and dissolved carbon dioxide and variability in light intensities affecting photosynthetic process (Degens *et al.* 1968, Andrews and Abel 1977, McMillan and Smith 1982, Raven *et al.* 1982, Cooper and DeNiro 1989, Purcell and Bellwood 2001). A review is provided by Cooper and DeNiro (1989) covering the changes needed in these variables to cause changes in the $\delta^{13}C$ signature of macrophytes. Given the relatively stable physical environment on the reefs in the current study (Plass-Johnson unpublished data) and the distance from significant freshwater inputs; changes in the light intensity over the depth gradient is the most likely factor (Fry and Sherr 1984) that is responsible for the pattern of depletion in the $\delta^{13}C$ signatures seen with depth in the current study. Similar patterns have been described for *Posidonia oceanica* (Cooper and McRoy 1988, Cooper and DeNiro 1989), *Halimeda incrassata* (Wefer and Killingley 1986) and zooxanthellae in corals (present study, Muscatine *et al.* 1989, Swart *et al.* 2005). In contrast, variability in $\delta^{15}N$ signatures may be related to inorganic nitrogen incorporation by macrophytes and/or to sediment and water column geochemistry, but is not as well understood (Fourqurean *et*

al. 1997). This would indicate that changes in the water chemistry between reefs could be the cause of the differences seen in *Dictyota spp.* signatures between reefs.

Detritus showed patterns that were different to coral and macroalgae, but other studies have shown that coral reef detritus can have signatures that range from 7-30% (Lugendo *et al.* 2006, Kolasinski *et al.* in review). Patterns in this respect are unclear, principally because detrital sources could not be clearly identified. If sources are unknown, or signatures do not reflect known sources (e.g. estuarine or mangrove material, Marconi *et al.* 2011), interpretation of variability is difficult. Nyange's more enriched detrital signature may represent terrigenous inputs, as it is closer to Zanzibar.

Macroalgae and corals represent possible food sources for parrotfishes, which have previously been documented to feed on both (Rotjan and Lewis 2005, Francini-Filho *et al.* 2008, Bonaldo and Bellwood 2011a). Along with turfing algae and detritus, the proportion of these constituents in parrotfish diets can change among and within species (see Chapter 5). The enrichment of both prey and consumer in the current study suggests that changes in nutrient input can be transferred to higher trophic levels. Enrichment in nitrogen has been correlated with eutrophication and can be traced through trophic levels, signifying that anthropogenic inputs can impact the complete length of a food web (Hadwen and Arthington 2007). Similar results were also found in studies that were not affected by anthropogenic effects, rather large scale oceanographic processes affected signatures of primary producers on differing coasts of southern Africa that were followed through subsequent trophic levels (Hill *et al.* 2006, Kohler *et al*. 2011). Although the changes in the ecological processes associated with the variation in signatures were not quantified in this study, nitrogen enrichment may indicate an impacted reef. Gaston and Suthers (2004) found fish tissues to be depleted in $\delta^{15}N$ but enriched with $\delta^{13}C$ at a site in Sydney that received sewage waste. This was analogous to the particulate organic matter (POM) signature from the waste, but dissolved organic matter (DOM) was found to be enriched in both signatures, implying that the fishes sampled were using the POM as a food source. This may help to explain some of the patterns seen in the current study; Nyange (the reef closest to Stone Town) is likely far enough away to avoid direct anthropogenic inputs but close enough to interact with DOM carried offshore. Dilution of that same DOM however may have been too great to affect signatures in the food webs of Pwakuu and Ukombe. Alternatively, in invertebrates a number of other studies show direct $\delta^{15}N$ enrichment because of sewage outfalls (Spies *et al.* 1989, van Dover *et al.* 1992) suggesting that

understanding the nature of the impact is necessary to interpret its effect on the local marine community.

The interpretation of stable isotopes ratios relies on the assumption that isotopic composition of tissues reflects that of the diet (Gannes *et al.* 1997, 1998). Our results show that signatures in the muscle tissue of fishes can differ at the scale of tens of kilometres. Sampling of ontogenetic stages of fishes in this study was standardised between reefs, yet only three and two out of five species showed statistically different $\delta^{15}N$ and $\delta^{13}C$ signatures amongst reefs, respectively. It is therefore possible that enrichment factors and/or factors affecting muscle tissue signatures are species-specific. This needs serious consideration when interpreting coral reef fish isotope data from different areas, as, depending on the question, some species may need more extensive sampling to identify spatial differences in isotopic trends. It is also important to note that minimal sampling of some species may not represent signatures in spatially separated populations. For example, if the total area of collection used in the current study had been treated as a single site, spatial variability in the $\delta^{13}C$ and $\delta^{15}N$ would have been masked and the evidence for localised differences in tissues and the associated causal processes would not have been identified. Both Kurle *et al.* (2011) and Gaston and Suthers (2004) found spatial variation in fish isotopic signatures in temperate systems, at scales much larger than the present study (10 -100s km).

The basis for most of the differences in nitrogen seen between reefs might be linked to spatially different nutrient inputs, resulting in organisms from Nyange being enriched in $\delta^{15}N$ compared to those from Pwakuu and Ukombe. Other studies have shown that stable isotopes can be used to identify geographic differences in nutrient sources that are transferred through multiple trophic levels. In South Africa, changes in isotope signatures were used to show that trophic patterns were consistent from primary producers (macroalgae and phytoplankton), to primary consumers (limpets and mussels, barnacles and polychaetes) to secondary consumers (African black oystercatchers and Cape gannets) (Hill *et al*. 2006, Hill and McQuaid 2008, Jaquemet and McQuaid 2008, Kohler *et al.* 2011). These studies showed that nutrient input differed between the western, southern and eastern coasts, reflecting large-scale oceanographic differences between the oligotrophic east coast and the more eutrophic, upwelling influenced, west coast. On a smaller spatial scale, the current study demonstrates that isotopes can be used to show that different nutrient sources for producers can be transferred and traced through multiple trophic levels. The differences seen between reefs within this study

may support this as Nyange is the reef closest to Stone Town, the largest city in Zanzibar, and it was significantly more enriched with a pattern of depletion moving away from this source. Furthermore, other studies showed that the highest levels of benaldehyde, limonene, salicylic acid and coprostanol were found at Nyange and declined with distance from Stone Town (C. Anderson unpublished data). These substances may not have a direct effect on $\delta^{15}N$ signatures, but were used as proxies for the level of anthropogenic influence across the waters off west Unguja Island. This enrichment was significant in the macroalgae and corals and showed similar trends in the fishes. The $\delta^{13}C$ signatures were not as clear, but appeared to be associated with more localised effects such as depth (algae and corals). This indicates that photosynthetic processes affect $\delta^{13}C$ patterns, while $\delta^{15}N$ indicated differing sources of nutrient inputs.

Field studies have shown that isotopic signatures can vary spatially on coral reefs and may be affected by different environmental nutrient sources. These differences can be traced through multiple trophic levels, implying that the changes in primary producers are conserved through the food web within reefs. The variability in $\delta^{13}C$ signature in photosynthesising organisms can be largely attributed to processes affected by light (i.e. depth) but changes in $\delta^{15}N$ may be an indication of a differing source of nutrient inputs. This study highlights the need for rigorous sampling at the proper scale when trying to interpret isotope data, as spatial variability can occur between coral reefs and to a limited extent, even within reefs.

Chapter Five
Stable isotope analysis of parrotfish diet: the effects of ontogenetic stage and functional group

5.1 INTRODUCTION

The recent loss and degradation of coral reef systems has drawn into focus the need for a better and more thorough understanding of all processes that occur on coral reefs to reduce the possibility of their shift to a less desirable, macroalgal-dominated state (McClanahan *et al.* 2001, Bellwood *et al*. 2004, Hughes 1994, Hughes *et al*. 2007). In light of recent studies showing variability in the functional role of herbivores and the limited functional redundancy (the ability of one group to substitute for another in a given ecological role) amongst fishes found on coral reefs (Bellwood *et al.* 2006, Lefèvre and Bellwood 2011), there is a need to study herbivory at the level of individual species, in order to understand the processes and interactions by which they are able to keep coral reef systems in equilibrium (Bellwood *et al.* 2006).

The functional role of an individual species can vary ontogenetically throughout its life due to morphological, environmental and physiological changes; for example juvenile settlement from planktonic to benthic communities (Sale 1980), development of jaw structure (Wimberger 1991) and sexual maturation (Johnsson and Johnsson 1993). Size related morphological changes in foraging technique can alter the prey-specific foraging ability of an individual and result in variation of the exploitation of food resources (e.g. Luczkovich *et al*. 1995, Mullaney and Gale 1996). Additionally, because of ontogenetic changes in diet requirements, inter- and intra- specific competition (e.g. Holbrook and Schmitt 1992) and predation vulnerability (e.g. Holbrook and Schmitt 1988), ontogeny can lead to changes in microhabitat use (Lirman 1994, McCormick 1998a). Furthermore, ontogeny can correlate with changes in activity schedules, which may lead to differential access to prey (Clements and Choat 1993). For instance, during the wet season, the Neotropical fish *Roeboides affinis* is able to take advantage of a wide range of food sources such as insects and crustacea. When sources become limited during the dry season, it changes to lepidophagy (scale eating) because other fishes are still widely available. The degree of allometric snout differentiation between juveniles and adults means the former perform more as insectivores and the latter lepidophagovores (Peterson and McIntyre 1998). Thus, the

ecological function of *R. affinis* is a product of both environmental conditions and ontogenetic morphology. From an ecological perspective, this means that individuals of the same species can, at different life stages, undergo dramatic niche shifts and can function more similarly to species of alternate functional groups. Uncovering ontogenetic change and trophic interactions amongst and within dominant herbivorous fishes will lead to a better understanding of species-specific functional roles and consequently, a better understanding of the processes that structure coral reef communities.

In coral reef fishes, ontogenetic changes can be complex; the habitat for a single fish species can change from an open ocean planktonic habitat to a benthic coral reef habitat (Sale 1980); many are sequential hermaphrodites (Nakamura *et al.* 2005) and can show changes in diet related to gamete production (Wainwright and Richard 1995). Many also go through significant changes in body shape and colouration throughout their life history (Lorenz 1962). Parrotfishes (Labridae) are a conspicuous component of tropical and subtropical coral reefs worldwide (Parenti and Randall 2000). They have a planktonic larval stage and, after recruitment, show an increase in habitat area with an increase in size, related to foraging area and reproduction (Bruggemann *et al.* 1996). Many parrotfish are protogynous hermaphrodites (Nelson 2006). They have been identified as playing dominant roles as herbivores in these systems (e.g. Choat 1991, Choat *et al*. 2002, Hughes *et al.* 2003, Mumby *et al.* 2006, DeMartini *et al.* 2008, Jayewardene 2009) and their specific function within coral reef communities has largely been related to their feeding techniques.

Feeding techniques are not uniform amongst all parrotfishes, which display three feeding modes: scrapers, excavators and browsers. Each of these modes of feeding is associated with specific morphological and behavioural adaptations (Bellwood and Choat 1990, Bellwood 1994). Browsers remove algae and the associated epiphytic material, while scrapers and excavators also remove portions of the substratum. The unusual feeding mode of the latter two groups, with their beak-like, fused oral plates, consists of rasping the substratum and ingesting proportions of carbonate reef matrix along with algae (Lewis and Wainwright 1985, Paddack *et al.* 2006, Hughes *et al.* 2007). Excavators have powerful jaws that remove the substratum leaving deep scars (Bonaldo and Bellwood 2009). Scrapers however, are characterised by reduced musculature and jaw dentition in comparison to excavators (Bellwood 1994), resulting in shallower bites. In addition to herbivory, this makes scrapers and excavators

important factors in coral predation (McClanahan *et al.* 2005, Rotjan and Lewis 2006, Cole *et al.* 2008), in the turnover of reef carbonate (Bellwood 1995, Bruggemann *et al.* 1996, Alwany *et al.* 2009) and also means they play a role in clearing areas for the colonisation of new sessile organisms (Bellwood and Choat 1990, Choat 1991, Bonaldo and Bellwood 2009).

Some parrotfishes grow continuously throughout their life (van Rooij *et al.* 1995a) and go through significant ontogenetic changes that include juvenile (IM; sexually immature), initial (IP; usually female) and terminal (TP; male) phases (Choat and Robertson 1975, Bellwood and Choat 1990). The transition from the pelagic-juvenile to demersal sub-adult is dramatic and has been well discussed in the literature (e.g. Bellwood 1986, Bellwood 1988, Chen 2002). Briefly, this includes a proportional decrease in orbital size compared to overall body, a change from canniform to characteristic fused teeth, a proportional increase in the length of the intestine and the development of a sacculated gut. These changes are concurrent with a shift in diet from carnivory, which benefits growth, to herbivory which also includes the ingestion of a large amount of carbonate. Ontogenetic changes in adult parrotfishes have also been well documented and are more closely related to behaviour and the development of reproductive organs. For example IP and TP fish have different energy needs for the development of eggs and sperm, respectively. Also, individual TP fish spend more time defending potential IP mates and territory against other conspecifics, while IP fish spend much of their time feeding in schools within the territory of selected TP individuals (Hanley 1984, van Rooij *et al.* 1995b, van Rooij *et al.* 1996). IP fish are generally smaller, grow slower and spend more time foraging on lower quality foods than TP fish (Bruggemann *et al.* 1994b, van Rooij *et al.* 1995a), which is also a consequence of gape size (Bruggemann *et al.* 1994b, Bruggerman *et al*. 1996). Furthermore, phase change in most species is associated with a colour change from "drab" to "gaudy" and some species also show a change in body shape (Robertson and Choat 1973, Choat and Robertson 1975). Many of the ontogenetic changes seen within parrotfishes are either directly associated with diet (food choice and growth) or are indirectly affected by diet (defence of reproductive mates).

Although classified functionally as herbivores, studies looking at the biology of parrotfishes have identified alternate choices during food selection. While algae appear to be the targeted food choice, termed the epilithic algal matrix (EAM), this food also contains detritus, invertebrates and microbes (Crossman *et al*. 2001), representing other

possible sources of nutrition (Hatcher 1983, Choat 1991, Wilson and Bellwood 1997). Therefore, the trophic status of fishes feeding on the EAM cannot be defined as strictly herbivorous and in fact, some fishes have been found to farm and defend the rich resources of the EAM (Wilson and Bellwood 1997). In a comprehensive study on the trophic status of parrotfishes, Choat *et al.* (2002, 2004) found large portions of organic matter and/or detritus, with very little identifiable algal material in the alimentary tract. The presence of isovalerate, a metabolite often found in organisms with a high protein diet, also indicated the dietary importance of detritus, although a large proportion of the material was unidentifiable or labelled organic matter because of problems with identifying highly processed, parrotfish gut content.

Many studies have investigated ontogeny or function within parrotfishes; few however, have compared diet at different life stages in relation to function. Furthermore, studies looking at function and ontogeny often only consider a single species within a functional group. A comparison of inter- and intra-specific differences in diet in relation to ontogeny and function may help to clarify species-specific roles within the herbivorous functional group and how different species/stages may collectively interact with coral reef systems.

The combination of distinct ontogenetic stages and differing functional roles within parrotfishes makes them a good candidate to explore inter- and intra-specific differences in diet. Inherent problems in the identification of gut contents within parrotfishes (Choat *et al*. 2002, Cocheret de la Morinière *et al.* 2003, Choat *et al*. 2004) can be overcome by using trophic chemical tracers such as stable isotopes, which can be used to elucidate trophic pathways and relationships between organisms and their environment due to predictable fractionation between trophic levels (DeNiro and Epstein 1978, Fry and Sherr 1984). An animal's stable isotope signature provides an index of assimilation over integrated time periods (DeNiro and Epstein 1978, Rau *et al*. 1983, Post 2002), avoiding the problems inherent with observational data (e.g. ingestion vs. assimilation and "spot" observations). Interpretation of isotopic data depends on the degree to which isotopic signatures of different dietary constituents mix. This in turn depends on a number of factors, including for herbivores, the degree of fermentation and urea cycling in the gut (Smith and Paulson 1975, Gannes *et al*. 1997). Parrotfishes, however, have a relatively short gut in relation to other herbivores and therefore a fast digestion period and reduced fermentation and urea cycling (Smith and Paulson 1974, Choat *et al.* 2004), simplifying dietary reconstruction. Additionally, through observational and gut content

studies, the diet composition of parrotfishes has been identified as including algae, detritus and coral tissue (see Bruggemann *et al.* 1994a,b,c, Choat *et al*. 2004), all of which have differing isotopic signatures. This is an important condition for reconstructing diets in isotope ecology.

Using stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), this study investigated the diets of parrotfishes found in the tropical waters of Zanzibar. A wide range of species and sizes were sampled across reefs in order to identify ontogenetic variation amongst and within species. The present study was designed to address the following questions: (1) Do the diets of parrotfishes change with respect to ontogenetic stages and between functional groups? And if so (2) how do these diets compare (a) between species and (b) within species?

5.2 Materials And Methods

For information concerning the study site, sample collection and stable isotope analysis, please see Chapter 2.

5.2.1 Data Analysis

5.2.1.1 Isotope Signature × Total Length Relationship

To identify the rate of change of diet with size in parrotfish, linear regression was applied to the total length (TL) and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures separately, of each species and a two-tailed t-test was performed to test the null hypothesis that slope $\neq 0$. Only species with more than five samples were used to ensure the fitted model would be representative of the sampled population. For species with significant regressions, homogeneity of slopes based on covariance was tested to compare slopes of species within and between functional groups. Species with a significant slope and R^2 were pooled within functional groups and checked for differences of slopes amongst groups. TL was the continuous independent variable. Because of unbalanced sample sizes, the test for homogeneity of slopes was performed with a type-III sums-of-square. Homogeneity of variance was confirmed with an *F* statistic defined as:

$$F = \frac{\sum_{i=1}^{n_1} n_1 (x_{1i} - \bar{x}_1)^2}{\sum_{j=1}^{n_2} n_2 (x_{2j} - \bar{x}_2)^2}$$

where x_{1i} and x_{2j} denote the i^{th} and j^{th} observations of group 1 and 2, respectively. $\bar{x}_1$ and $\bar{x}_2$ are the mean for group 1 and 2, respectively. Degrees of freedom are: $F(N_1 - 1, N_2 - 1)$, where N is the number of observations in groups 1 or 2 (Zar 1996). The ratio was compared with the 0.95 percentile F statistic to test H_0: that variance does not differ between groups. If heteroscedasticity existed, the results were interpreted conservatively or not at all depending on the alpha level.

5.2.1.2 Comparison of Ontogenetic Isotope Signatures Amongst and Within Species

To compare the diets of ontogenetic stages (IM, IP and TP) within species, stages were plotted on an isotopic bi-plot. Stages with less than three individuals, a $\delta^{15}N$ standard error (SE) > 0.5 or a $\delta^{13}C$ SE >1.0 were removed due to lack of replication and to be conservative when interpreting isotopic diet positions. These thresholds were arbitrarily defined and based on sample sizes. On the bi-plots, individual stages were compared with functional groups by defining a functional group's envelope by the means and SE of each constituent species across all life stages. *Scarus tricolor* and *S. ghobban* means and SE were not used in the definition of the scraper functional group envelope because of their exceptionally different isotopic signatures when compared to other scrapers.

5.2.1.3 Food Source Contribution and Comparison

Stable Isotope Analysis in the R environment (SIAR; Parnell and Jackson 2010, available at http://cran.r-project.org/web/packages/siar/index.html) was run as a nine source mixing model to estimate proportional source contributions to parrotfish diets (for constituents see Table 5.3). A 10% random sub-sample of data was taken from total source samples for coral tissue, detritus and turfing algae. Macroalgae were not sub-sampled due to relatively small sample size, with the exception of *Dictyota spp.* which was a 10% sub-sample of the original sample. Although *Halimeda* has been recognised as a preferred food source of parrotfish (Mantyka and Bellwood 2007a,b), it was not sampled in the field. Alternatively, values were added to the model taken from studies in comparable environments (Yamamuro *et al.* 1995, Cocheret de la Morinière *et al.* 2003, Lugendo *et al.* 2006). No C:N ratio was provided for *Halimeda spp.* in those studies and subsequently has not been provided here. A one-way MANOVA with

type-III sums of squares was performed on $\delta^{13}C$ and $\delta^{15}N$ signatures of all food sources, to identify possible confounding input values in the mixing model. A Fisher's LSD posthoc was used to identify homogenous sub-groups. For parrotfish, the same groups were used as in the isotopic bi-plot. For herbivorous fishes, Mill *et al.* (2007) presented the possibility of adopting an enrichment value of 4-5‰. Considering the possible differences in diets, differences in regional environmental factors (water temperature), and differences in physiology (proportional gut length) between the fish groups in the current study and those of Mills *et al.* (2007), a $\delta^{15}N$ enrichment value of 2.3‰ was adopted following Kolasinski *et al.* (2009). Their study was done in Mauritius on *Mulloidichthys flavolineatus*, a species that, like parrotfish, has a diet consisting of both high and low protein foods. This was a mean value for aquatic organisms feeding on either high (animal, microbial) or low (algal) protein content diets (Post 2002). An enrichment value of 0.5‰ was used for $\delta^{13}C$. The model was based on 3000 iterations for each food source by fish stage. To compare the relatedness of each fish stage based on their diet proportions (model output), a cluster analysis was performed on the means of the food source iterations. The cluster analysis was based on Euclidean distance and clusters were created using group averages. For clarity, SE values are not shown on the figures, but were ±~0.001‰ for all sources..

All statistical tests were completed in StatSoft Satatistica 6, R (v. 2.12.2) and Primer 6 and plots and graphs were made with SigmaPlot 10.

5.3 RESULTS

Muscle tissue of 174 individual fish from 14 different species was analysed for both carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios (Table 5.1) with a large size range within and between species (Table 5.1). Few species showed overlap in length between IP and TP stages (including: *Chlorurus sordidus*, *C. strongylocephalus*, *Scarus niger*, *S. tricolor* and *S. viridifucatus*). No overlap occured between IM and IP stages because length threshold for sexual maturity for each species was inferred from the literature (see section 2.6.1 for relevant reference material). In most species, all three stages (IM, IP and TP) were collected, with the exception of the less abundant (*C. atrilunula*, *S. falcipinnis*, *S. frenatus*) and larger growing species (*Cetoscarus bicolor* and *S. ghobban*) for which TP fish were not obtained. Browsers were only represented by one species (*Calotomus carolinus*), while excavators and scrapers had four (*Cetoscarus bicolor*, *Chlorurus atrilunula, C. sordidus* and *C. strongylocephalus*) and

Table 5.1 Mean (±SE) and range of nitrogen and carbon stable isotope values of each ontogenetic life stage (IM = immature, IP = initial phase, TP = terminal phase) of each species. Functional groups (F): B = browsers, E = excavators, S = scrapers. Sample size (n) and their length range (TL = for total length) are also given. **Bold** type indicates that the stage (Stg.) was used in the isotopic biplots and mixing model.

Species	F	Stg.	n	TL	Nitrogen ($\delta^{15}N$)		Carbon ($\delta^{13}C$)	
				Range (cm)	Mean	Range	Mean	Range
Calotomus carolinus	B	IM	2	15.5:16.3	6.3±0.2	6.1:6.5	-12.5±2.1	-14.6:-10.4
		IP	2	21.4:23.1	6.9±0.4	6.5:7.3	-9.8±0.5	-10.3:-9.3
		TP	2	26.5:27.0	6.8±0.2	6.7:7.0	-9.5±0.3	-9.8:-9.1
Cetoscarus bicolor	**E**	**IM**	**5**	**10.7:19.3**	**6.4±0.7**	**5.9:7.3**	**-12.7±0.3**	**-13.8:-12.1**
		IP	2	20.6:32.5	6.3±0.2	6.1:6.5	-10.6±0.1	-10.7:-10.4
Chlorurus atrilunula	E	IP	2	12.6:23.1	6.9±0.3	6.6:7.2	-14.4±1.5	-15.8:-12.8
Chlorurus sordidus	**E**	**IM**	**10**	**9.5:15.6**	**6.0±0.1**	**5.6:6.4**	**-13.2±0.3**	**-14.7:-11.6**
		IP	**34**	**16.1:23.0**	**6.0±0.1**	**4.9:7.1**	**-11.6±0.2**	**-14.1:-8.9**
		TP	**5**	**22.3:25.6**	**6.6±0.3**	**6.0:7.5**	**-12.6±0.8**	**-13.6:-9.6**
Chlorurus	E	IM	4	10.8:13.7	6.2±0.3	5.5:6.8	-13.3±1.4	-15.9:-9.8
strongylocephalus		**IP**	**9**	**14.1:25.8**	**6.7±0.2**	**5.6:7.6**	**-13.3±0.5**	**-14.7:-10.3**
		TP	**13**	**14.9:30.7**	**6.4±0.1**	**5.6:6.8**	**-13.9±0.4**	**-15.2:-11.2**
Scarus falcipinnis	S	IP	2	17.7:20.2	5.1±0.1	5.0:5.1	-12.2±0.8	-13.0:-11.4
Scarus frenatus	S	IP	2	12.7:13.2	5.7±0.3	5.4:6.0	-13.5±0.3	-13.8:-13.2
Scarus ghobban	**S**	**IM**	**6**	**18.8:30.6**	**7.5±0.2**	**6.6:8.3**	**-13.0±0.7**	**-15.9:-11.1**
		IP	2	32.8:44.6	5.6±0.5	5.0:6.1	-12.5±3.4	-15.9:-9.1
Scarus niger	**S**	**IM**	**10**	**10.2:16.4**	**5.8±0.1**	**4.8:6.5**	**-14.5±0.2**	**-15.1:-13.4**
		IP	**7**	**17.4:23.4**	**5.5±0.1**	**5.0:6.2**	**-12.4±0.3**	**-13.6:-11.6**
		TP	**7**	**19.6:34.8**	**5.5±0.2**	**5.0:6.5**	**-12.1±0.3**	**-13.0:-11.0**
Scarus psittacus	S	IP	1	19.3	5.7	5.7	-12.1	5.7
		TP	**10**	**19.5:28.5**	**5.3±0.1**	**5.1:5.8**	**-11.1±0.2**	**-12.0:-10.9**
Scarus russelii	**S**	**IM**	**5**	**15.6:22.4**	**5.3±0.2**	**5.0:5.9**	**-13.5±0.4**	**-14.4:-12.2**
		IP	3	22.5:27.5	6.2±0.2	5.6:7.4	-13.3±1.7	-16.6:-11.4
		TP	1	29.5	6.0	6.0	-11.5	-11.5
Scarus scaber	S	IP	2	15.7:17.7	6.1±0.0	6.0:6.1	-12.1±0.2	-12.3:-11.9
Scarus tricolor	**S**	**IM**	**3**	**12.9:13.9**	**7.5±0.3**	**7.0:7.9**	**-16.8±0.3**	**-17.3:-16.3**
		IP	**9**	**14.5:20.2**	**7.5±0.2**	**6.5:8.3**	**-16.4±0.2**	**-16.9:-15.0**
		TP	**4**	**16.7:22.8**	**7.0±0.1**	**6.8:7.4**	**-15.5±0.4**	**-15.9:-14.4**
Scarus viridifucatus	**S**	**IP**	**8**	**14.7:16.5**	**5.6±0.3**	**4.0:6.4**	**-11.7:0.3**	**-13.1:-10.4**
		TP	2	15.9:16.7	5.6±0.2	5.4:5.7	-10.7:0.6	-11.3:-10.1

nine (*S. falcipinnis*, *S. frenatus*, *S. ghobban*, *S. niger*, *S. psittacus*, *S. russelii, S. scaber*, *S. tricolor* and *S. viridifucatus*) species, respectively.

5.3.1 Isotope Signature × Total Length Relationship

There was a strong positive linear relationship between total length and $\delta^{13}C$ for seven of the ten species including all of the excavators and four of the scrapers. The

relationship was non-significant for the scrapers, *S. russelii* and *S. viridifucatus*, and the browser, *Calotomus carolinus* (Table 5.2). The models had particularly strong correlations for *Cetoscarus bicolor, S. ghobban*, *S. niger* and *S. psittacus*, with R^2 values near or above 0.50. No species showed a significant relationship between $\delta^{15}N$ and length (Table 5.2).

There were no significant differences among the $\delta^{13}C$ signatures of excavator species ($F_{2,1} = 0.98$, $p = 0.381$). Among the scrapers with slopes significantly different from zero (Table 5.2), there were no significant differences ($F_{3,1} = 1.18$, $p = 0.325$) in $\delta^{13}C$ slopes. When slopes of individual scraper species were compared to the slopes of excavators, only *S. tricolor's* slope was not significant ($F_{3,1} = 1.55$, $p = 0.208$), whilst all other scrapers showed a significant difference ($p < 0.05$). Conversely, all individual excavators were significantly different when compared with scrapers ($p < 0.05$). When $\delta^{13}C$ slopes of all relevant species were categorised by functional group (browsers excluded) their independent slopes were significantly different from zero (scrapers: $t_{56} = 7.22$, $p < 0.001$; excavators: $t_{77} = 3.37$, $p = 0.001$) and there was a significant difference between their slopes ($F_{1,1} = 12.61$, $p < 0.001$). There is clearly a positive relationship between TL and $\delta^{13}C$ signatures for both groups, with the scraper slope nearly double that of the excavator's (Fig 5.1).

5.3.2 Comparison of Ontogenetic Isotope Signatures Among and Within Species

Isotopic bi-plots included *Cetoscarus bicolor* (IM), *Chlorurus sordidus* (IM, IP and TP), *C. strongylocephalus* (IP and TP), *Scarus ghobban* (IM), *S. niger* (IM, IP and TP), *S. psittacus* (TP), *S. russelii* (IM), *S. tricolor* (IM, IP and TP) and *S. viridifucatus* (IP). Most individual ontogenetic stages grouped with their appointed functional group (Fig 5.2) with the exception of the IM and IP stages of *C. sordidus* (excavator), which were positioned more closely to the browser group. Interestingly, the TP stage of *S. tricolor* fell within the excavator group boundaries, while it's conspecifics were depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$ than all other species, aside from IM *S. ghobban* individuals. Furthermore, *S. ghobban*'s enriched $\delta^{15}N$ also separated it from all other stages and groups.

Table 5.2 Results from regression analyses applied to carbon and nitrogen stable isotopic signatures of individual species with life stages (IM, IP and TP) grouped. A significant value ($p < 0.05$; in ***bold***) indicated the slope ≠ 0, tested with a Student t-test. Functional group (F.G.): B = browser, E = excavators and S = scrapers.

Species	F.G.	Stable Isotope	Slope	y-Intercept	R^2	p
Calotomus carolinus	B	$\delta^{13}C$	0.27	-16.5	0.44	0.151
(n = 6)		$\delta^{15}N$	0.04	0.8	0.05	0.335
Cetoscarus bicolor	E	$\delta^{13}C$	0.15	-15.0	0.75	***0.012***
(n = 7)		$\delta^{15}N$	-0.03	6.9	0.15	0.392
Chlorurus sordidus	E	$\delta^{13}C$	0.15	-15.0	0.21	***0.003***
(n = 49)		$\delta^{15}N$	0.02	0.4	0.02	0.174
Chlorurus strongylocephalus	E	$\delta^{13}C$	0.14	-16.4	0.26	***0.009***
(n = 26)		$\delta^{15}N$	-0.00	6.6	0.00	0.802
Scarus ghobban	S	$\delta^{13}C$	0.21	-18.7	0.61	***0.038***
(n = 8)		$\delta^{15}N$	-0.04	8.4	0.18	0.350
Scarus niger	S	$\delta^{13}C$	0.21	-16.9	0.78	***0.001***
(n = 24)		$\delta^{15}N$	-0.01	5.9	0.03	0.455
Scarus psittacus	S	$\delta^{13}C$	0.17	-15.2	0.49	***0.025***
(n = 11)		$\delta^{15}N$	0.01	5.1	0.03	0.643
Scarus. russelii	S	$\delta^{13}C$	0.19	-17.4	0.28	0.144
(n = 9)		$\delta^{15}N$	0.03	4.9	0.05	0.568
Scarus. tricolor	S	$\delta^{13}C$	0.13	-18.4	0.23	***0.015***
(n = 16)		$\delta^{15}N$	0.09	5.9	0.18	0.115
Scarus viridifucatus	S	$\delta^{13}C$	0.12	-13.7	0.01	0.846
(n = 8)		$\delta^{15}N$	-0.22	9.1	0.03	0.700

5.3.3 Food Source Contribution and Comparison

The nine food sources used in the mixing model covered a wide range of ratios for both elements (Table 5.3). Besides coral tissue and *Dictyota spp.*, the MANOVA for both elements show significant differences among all food sources ($F_{2,16} = 15.69$, $p < 0.001$) so that interpretation of food sources in the mixing model is not confounded. *Halimeda spp*. was the most depleted in both $\delta^{13}C$ and $\delta^{15}N$. Coral tissue had the highest enrichment in $\delta^{15}N$ and *Dictyosphaeria cavernosa* in $\delta^{13}C$. Coral tissue and detritus had the lowest C:N ratio while *Sargassum spp.* had the highest and was double that of the next highest, *D. cavernosa.* Macro and turfing algae had similar C:N ratios.

Cluster analysis based on the nine-source mixing model identified four different groups of fish (A, B, C and D in Fig 5.3) based on their diets. Overall, it identified fish within their functional groups with a few exceptions: group A consisted of a single species (*S. tricolor* (IP)), group B represented excavators (apart from *Chlorurus sordidus* (IP)

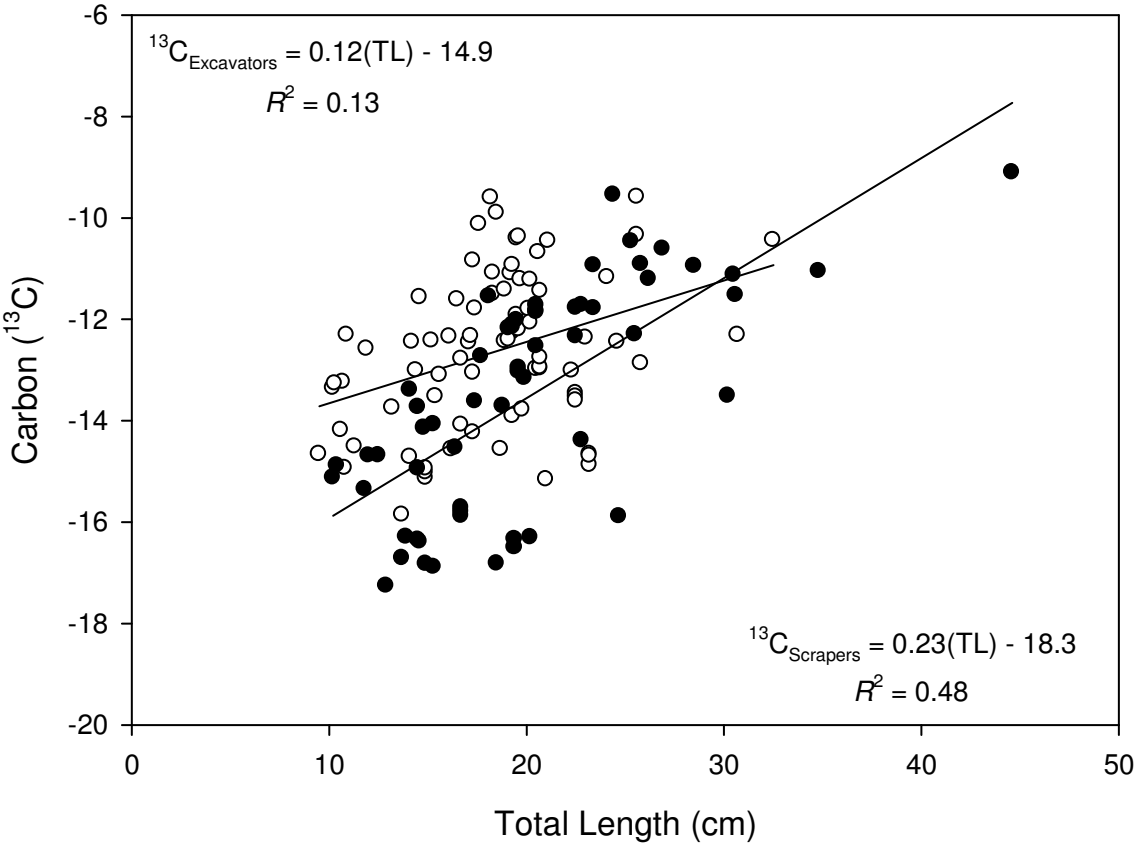


Figure 5.1 Carbon isotope signatures scrapers (black filled) and excavators (white filled) with species that had a significant linear relationship between length and carbon (Table 5.2). Their individual regression equation is given within the graph. TL = total length of the parrotfish.

which was included with group D) and the scrapers divided into two groups, C (closer to excavators) and D (apart from *S. ghobban* (IM) which was in group B).

Consider the groups identified by the cluster analysis, the mixing model identified coral tissue, detritus, green algae and *Dictyota spp.* as showing similar trends for all members of each group. These four food sources all occurred in high proportions in the diets of groups A, B and C (Fig 5.4, c), but were lower for D. Green algae formed a particularly high proportion for group A (*S. tricolor* (IP)) and detritus was less variable amongst the groups, with a mean of around 0.10 for groups A, B and C and approximately 0.08 for group C. *Dictyosphaeria cavernosa* and turfing algae showed similar trends amongst the groups (Fig 5.4, b); low for groups A and C, and intermediate for group B and D (Fig 5.4, b). *Halimeda spp*. and *Padina sp*. were more

variable than the other sources within and between groups (Fig 5.4, a). *Padina sp.* formed a relatively high proportion for the single species group A and for group C, lower for group B, although *C. sordidus* (IM) was higher and highly variable within D (Fig 5.4, a). The contribution of *Halimeda spp*. was low for group A and for *C. strongylocephalus* (IP and TP) but relatively high for the other three species in group B. *Sargassum spp*. was the least variable amongst and within groups, varying from ~0.09 to 0.11 and contributed minimally to group A (Fig 5.4, a).

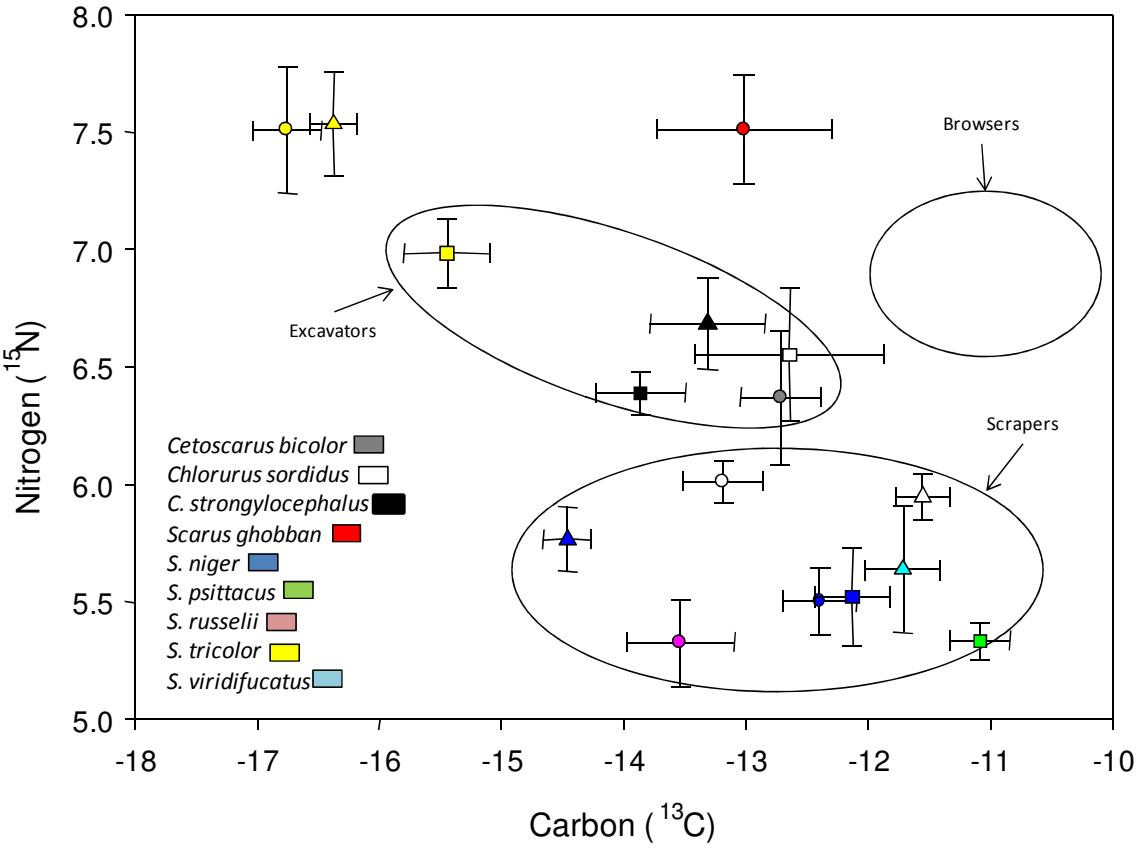


Figure 5.2 Isotopic bi-plot ($\delta^{13}C$ and $\delta^{15}N$) of selected ontogenetic stages of parrotfish (stages with n < 3, $\delta^{15}N$ SE > 0.5 or $\delta^{13}C$ SE > 1.0 were not included). Stages are compared to functional group envelopes (Functional groups defined by the means ± SE of each constituent species across all life stages). *Scarus tricolor* and *S. ghobban* were excluded from defining the envelope for scrapers because of their extremely different signatures. Ontogenetic stages include IM (circle), IP (triangle) and TP (square). Functional group envelopes are indicated in the graph. The browser envelope is provided based on all stages of *Calotomus carolinus*, but none of its stages met the requirements to be added to the analysis.

Table 5.3 The nine food sources, including sample size (n), mean (±SE), range and carbon to nitrogen ratio, used in the mixing model to reconstruct the diet of parrotfishes in Fig 5.2. Homogeneous subgroups (H.S.) were identified with Fisher's LSD posthoc test and were exclusive between isotopes.

Food Source	n	Nitrogen ($\delta^{15}N$)			Carbon ($\delta^{13}C$)			C:N±SE
		Mean	Range	H.S.	Mean	Range	H.S.	
Coral tissue	18	4.8±0.2	2.1:6.2	D	-15.0±0.3	-18.4:-12.9	C	6.3±0.3
Detritus	9	3.5±0.2	2.5:4.3	B,C	-16.1±0.8	-19.5:-12.8	C	8.3±0.5
Green algae	14	4.4±0.3	2.7:6.3	D	-18.0±0.4	-20.4:-14.8	B	13.2±0.8
Padina sp.	6	2.6±0.6	0.7:5.4	A,B	-11.6±0.5	-13.9:-10.9	D	15.6±1.2
Turfing algae	9	3.4±0.3	2.0:5.1	B,C	-10.1±0.9	-13.3:-6.2	D	16.8±1.1
Dictyota spp.	10	4.7±0.1	4.1:5.5	D	-15.8±0.6	-20.7:-13.3	C	17.5±0.8
Dictyosphaeria cavernosa	3	4.1±0.2	3.8:4.4	C,D	-5.6±1.3	-7.6:-3.1	E	18.3±1.7
Sargassum spp.	9	3.7±0.4	1.8:6.5	A,B,C	-14.1±1.2	-19.4:-10.9	C	35.9±3.2
Halimeda sp.	4	2.1±0.1	1.8:2.3	A	-20.5±1.3	-24.2:-17.3	A	NA

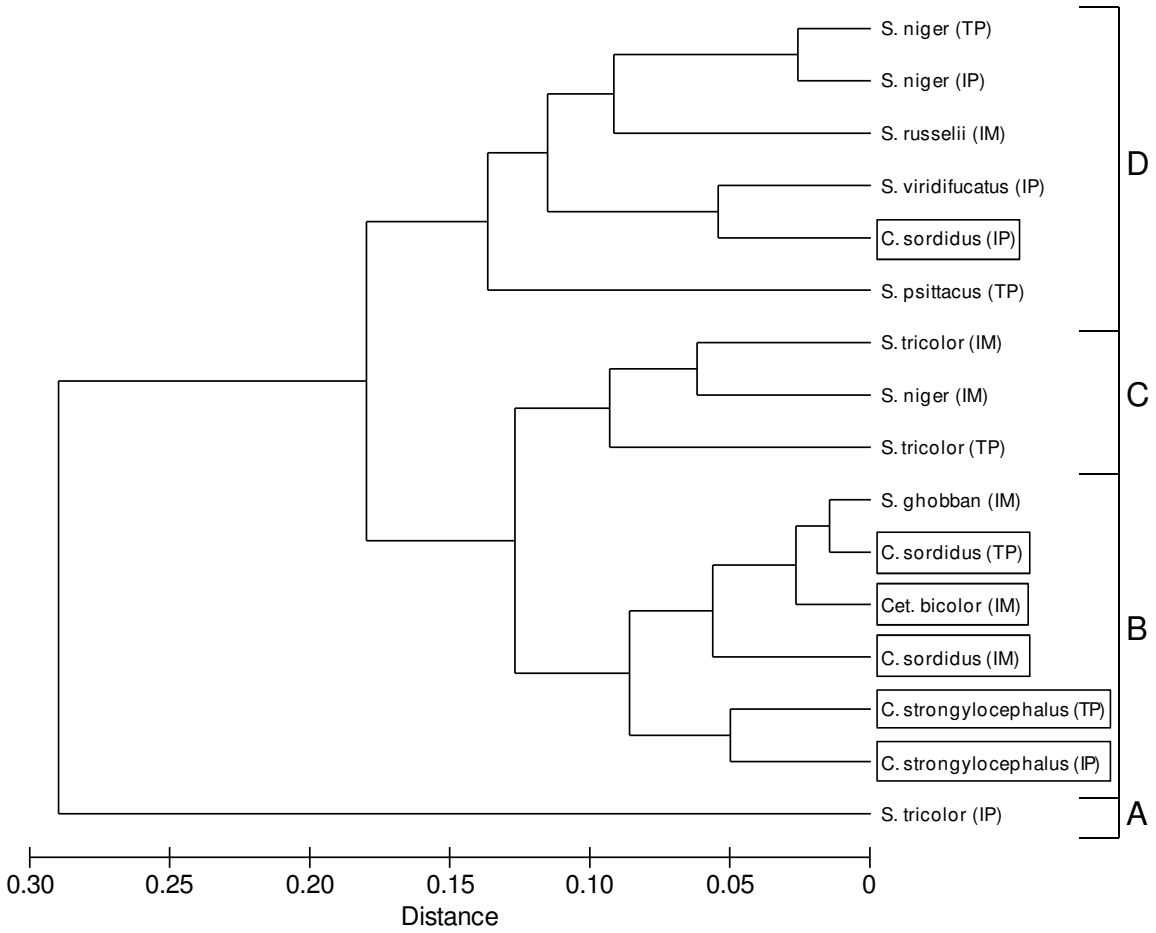


Figure 5.3 Dendrogram of the cluster analysis based on the nine-source mixing model. Food sources and signatures are listed in Table 5.3 and fish groups are given in Table 5.1. Black borders represent the excavator functional group and no border represent the scraper functional group. Lettering indicates groups of fishes with similar diets as identified by the cluster analysis.

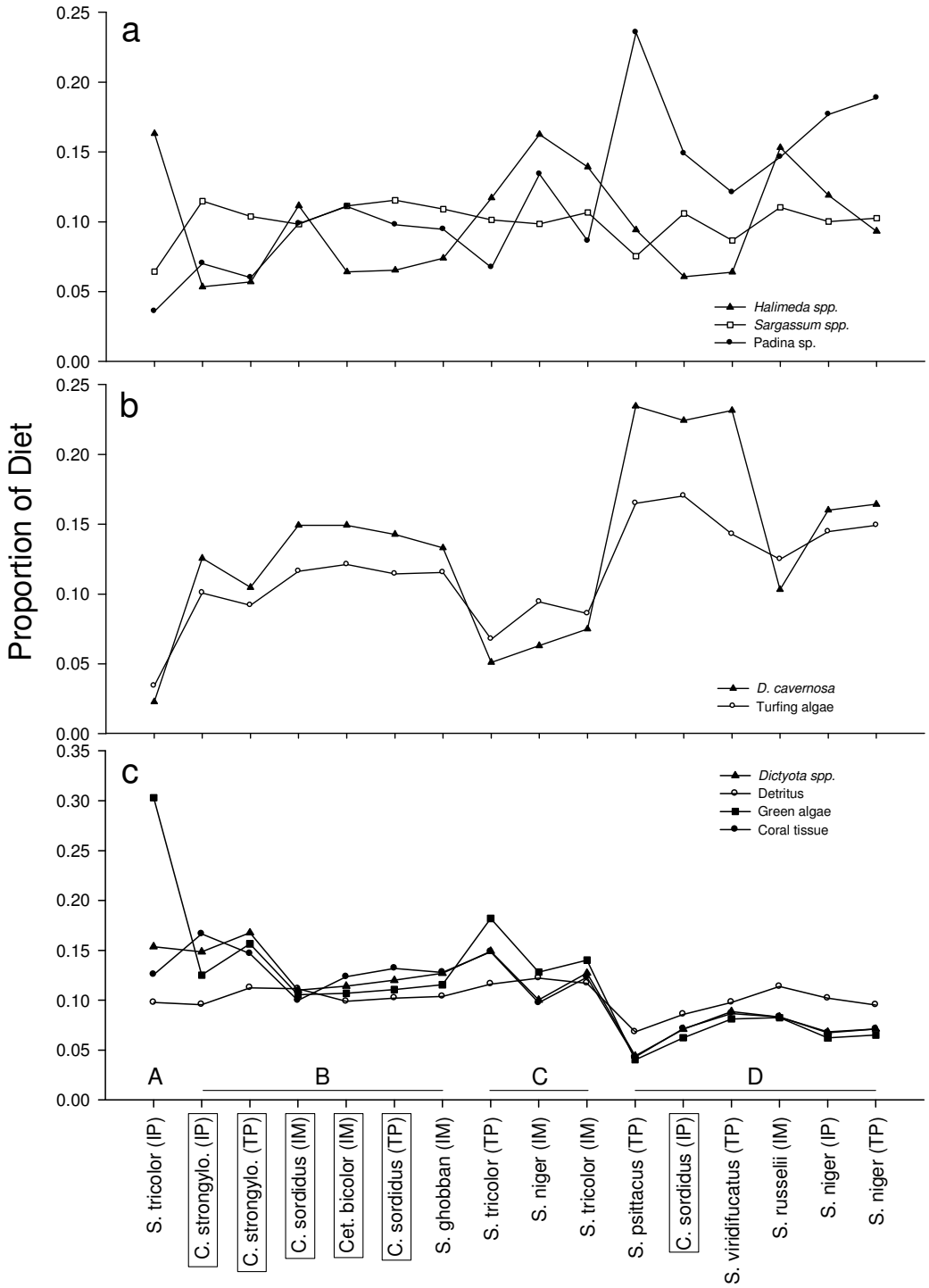


Figure 5.4 Mean SIAR results for the dietary proportion of each food source and fish stage. Boxed fish stages belong to the excavating functional group and no box represents the scraper group. Lettering indicates similar sub-groups identified in the cluster analysis (Fig 5.3). Sources were subjectively divided into three graphs to better display common trends in dietary proportions of source amongst groups. For visual purposes, SE was not plotted but was ~±0.001‰ for each source.

5.4 DISCUSSION

Before interpreting stable isotope signatures of muscle tissue with relation to ontogenetic changes in fish, a few assumptions must first be clarified. Changes in tissue signatures can be the result of changes in diet, tissue growth or tissue repair (Tieszen *et al.* 1983). Because metabolism is directly related to growth (Kerrigan 1994, McCormick 1998b), changes in isotopic signature will be greatest at times of fastest growth (Fry and Arnold 1982). Despite the lack of information on fractionation in parrotfish, studies investigating isotopic signatures in juvenile coral reef fishes (i.e. fastest growth rate) have shown little relationship between growth and isotopic signature (Kolasinski *et al*. 2009). Furthermore, most parrotfishes show continuous growth throughout the length ranges sampled(Choat *et al.* 1996), indicating that metabolism has minimal effects on isotopic signatures and as such, we may safely assume that isotopic change here indicates a dilution of the original isotopic pool by newly deposited biomass.

Unlike studies using gut contents, which in coral reef herbivores are complicated by high levels of mechanical food processing (Choat *et al*. 2002, Cocheret de la Morinière 2003, Choat *et al*. 2004), stable isotope analysis does not require abundance estimates or identification of food within the stomach for dietary interpretation. Instead, stable isotope analysis of consumer tissues describes a time integrated diet based on assimilation rather than ingestion. Stable isotope signatures of consumers will reflect their dietary constituents due to documented predictable trophic-level enrichment of isotope signatures via fractionation during diet assimilation (DeNiro and Epstein 1977, 1978, Fry and Sherr 1984). However, such techniques are only useful when knowledge of consumer diet exists and representative sampling can be conducted. Parrotfish are well studied teleosts and likely components of their diet have been well described in the literature (Lewis and Wainwright 1985, Choat *et al*. 2004, Bonaldo *et al*. 2006, Mantyka and Bellwood 2007b, Francini-Filho *et al.* 2009, Jayewardene 2009), even to a point where the original hypotheses concerning the diet of herbivorous fishes being strictly plant material has been questioned (Crossman *et al*. 2001, Wilson *et al.* 2003, Choat *et al*. 2004). Furthermore, the current study was able to construct a representative parrotfish diet based on general $\delta^{13}C$ and $\delta^{15}N$ signatures of basic preferred food groups, and help answer questions concerning inter- and intra- specific differences. It is important to note that the algal food sources used in this study's mixing model only represent a portion of the available algal sources (Choat *et al*. 2002, Bruggemann

1994a,b,c, Mantyka and Bellwood 2007b), although it is believed that the chosen sources would represent food sources accessible to these groups of consumers. Given the inherent restrictions of stable isotope mixing models, where their output is only as good as the source signatures used to construct them, the results presented here need to be interpreted conservatively because important information may have been missed in sampling for my data.

Many other studies have used stable isotope signatures to identify ontogenetic dietary shifts in teleosts. Most of these studies used signatures of food sources from differing habitats to show large scale ontogenetic habitat shift between juveniles and adult fishes. Kolasinski *et al*. (2009) found that $\delta^{13}C$ signature of *Mulloidichthys flavolineaus* became more enriched in relation to total length, which was interpreted as representing a change from their pre-settlement, planktonic, juvenile stage to their post-settlement adult stage. As such, the change in $\delta^{13}C$ identified a change in diet from pelagic particulate organic matter (POM) to reef associated invertebrate infauna. Wells *et al*. (2008) found that *Lutjanus campechanus* $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ signatures were more closely related to ontogenetic dietary shifts, but also indicated that signatures were also related to a change in habitat use. This relationship was described as representing differences between artificial and natural reefs and areas open or closed to trawling. A study completed by Cocheret de la Morinière *et al*. (2003) provides another example, reporting $\delta^{13}C$ and $\delta^{15}N$ enrichment with size, interpreted as a change from mangrove and seagrass associated juveniles to coral reef associated adults. Because the current study did not look at pre- and early post-settlement of individual fishes, which in parrotfish is associated with a carnivorous planktonic diet (Bellwood 1988, Chen 2002), it is one of few studies not relating dietary shifts with changes in macro-habitat and stable isotope signatures (Post 2003). In general, most species of adult parrotfishes are closely associated with the coral reef habitat through their post-settlement life (Randall *et al*. 1997), therefore allowing ontogenetic changes in isotopic signatures to identify other environmental or physiological changes.

5.4.1 Isotope Signature, Length and Functional Relationships

The relationship seen between total length (TL) and $\delta^{13}C$ of fish muscle tissue found within most sampled species as well as within the overall scraper and excavator functional groups in this study indicates a dietary shift in resource selection related to ontogeny. The lack of relationship between $\delta^{15}N$ and TL suggests that the link between

trophic position and size is not linear and is more complicated than originally thought. Although most food sources used in the mixing model fell within the designated $\delta^{15}N$ fractionation range (i.e. 2.3‰) per trophic level, they separated clearly into homogeneous subgroups. Consequently, the change in consumer $\delta^{13}C$ but not $\delta^{15}N$ signatures suggests that ontogenetic shifts may not represent an overall change in food sources, but rather a proportional change in their assimilation.

The nature of the linear relationship between $\delta^{13}C$ and TL was consistent amongst species within functional groups, suggesting that the change in food sources is similar amongst functionally similar species. The greater slope in the scraper group seems reasonable as excavators with the more robust jaw morphology (Bellwood 1994, Wainwright *et al*. 2004) would have access to a wider variety of food sources from an early stage effectively inundating their $\delta^{13}C$ pool with a wider variety of source signatures. An increase in TL (and hence size) would mean that scrapers would experience increased food accessibility because of increases in gape and muscle mass. The regression lines of the two groups only converged at a large TL (~30 cm), suggesting that use of scraper and excavator food sources becomes similar at this size. One scraper species, *Scarus ghobban*, had a diet more consistent with excavators, but also had the largest average TL, suggesting this possible size-based interaction seen in the functional group regressions is likely realistic.

5.4.2 Inter- and Intra- Specific Dietary Comparisons

Of the three species with all three life stages (IM, IP and TP) represented in the mixing model (*Chlorurus sordidus*, *S. tricolor* and *S. niger*) there were no consistent ontogenetic dietary trends. Both *C. sordidus* and *S. tricolor* had IM and TP stages that fed on similar sources, but completely different diets at the IP stage. The IP and TP stages of *S. niger* had comparatively similar diets, but both differed from the IM stage. The comparable IM and TP diets may reflect a similar nutritional requirement during increased growth (greatest at the IM stage) and/or for sperm production. Bruggemann *et al.* (1994b) and van Rooij *et al.* (1995a) reported differing diets for *Sparisoma viride* IP individuals compared with other stages, where diets were lower in energy and protein, but this required much longer foraging times. They suggested this was a result of behavioural differences, where small fish spend a significant amount of time avoiding predators, while larger TP fish spend more time in territorial defence against conspecifics. In this study both IM and TP stage *C. sordidus* relied proportionally more

on coral tissue and detritus, the foods with the lowest C:N ratios, than the IP stage individuals. The IP stage had a very high reliance on *Dictyosphaeria cavernosa*, the source with the second highest C:N ratio. In agreement with the studies by Bruggemann *et al.* (1994b) and van Rooij *et al.* (1995a), as *C. sordidus* has other similar documented behaviours to *S. viride* (Cook 1999), it is likely that the fish in this study consisted of a group of foraging IP and a group of highly territorial TP individuals.

Such trends were more complicated with the scraper *Scarus tricolor*, for which green algae and *Halimeda spp.* were important diet contributors across all stages, although the IP fish had a very high reliance on the former. The $\delta^{13}C$ and $\delta^{15}N$ signatures in all stages of *S. tricolor* were very different to those of both scrapers and excavators, implying an ability to access resources unavailable to other scrapers. As nearly all of the food source signatures were significantly different from each other (with the exceptions of coral tissue and *Dictyota spp.*), variability in consumer signatures represents variability in food sources. Given similar morphology to other scrapers, the distinctly different signature in *S. tricolor* may represent resource partitioning based on alternate sources, effectively reducing competition with other species that share similar dietary needs. This may also be a strategy utilised by the scraper *S. ghobban*, which had $\delta^{15}N$ signatures similar to those of *S. tricolor*, suggesting these fish feed at a similar trophic level not shared with other scraper species. Although behavioural studies on *S. tricolor* are lacking, behaviour witnessed on Zanzibar reefs suggests that they do not have the same territorial behaviour seen in *C. sordidus* (pers. obs.) and isotopic differences may be a product of differing resource use. The elucidation of two distinct scraper groups, one that utilises sources with a relatively high $\delta^{15}N$ signature (i.e. *S. tricolor* and *S. ghobban*) and another with lower signatures (all other scrapers in Fig 5.2), would require further identification of more scraper species that share similar signatures with *S. ghobban* and *S. tricolor*, but the presence of two groups could help in describing resource partitioning in functionally similar species.

In contrast, the shift in diet seen in *S. niger* may be associated with a different life strategy, as they are one of the few parrotfishes that are monandric. Mixing models showed that the IM stage may be more reliant on detritus and coral tissue than later stages, perhaps assisting growth. The later stages had high proportions of *D. cavernosa* and turfing algae, suggesting similar dietary needs of males and females. Alternatively, if the ecology of *S. niger* (IM stage) and the two stages of *S. tricolor* followed the feeding results predicted by the dendrogram (Fig 5.3) and cluster analysis, it could

represent a strategy for reduction in resource competition from other scrapers. Clearly it would be energetically beneficial to compete with a single species rather than the four scrapers in group D.

Herbivorous fishes that feed over the epilithic algal matrix (EAM) can consume turfing algae, macroaglae, detrital aggregates and epiphytic material (Wilson and Bellwood 1997, Wilson 2002, Choat *et al*. 2002). When determining the dietary selectivity of parrotfishes, the presence of low levels of short-chain-fatty-acids and high levels of the amino acid, isovalerate indicate an important role of detritus and more specifically bacteria and meiofauna (Choat and Clements 1998, Choat *et al.* 2002, Choat *et al*. 2004, Crossman *et al*. 2005). Although the patterns differed between species, overall, the current study did not find detritus to be proportionally more significant in diet relative to other algal material such as green algae and *Sargassum spp*., but it was less important than *D. cavernosa* and very similar to coral tissue. Corallivory is well documented within this group of fish (Randall 1974, Bellwood and Choat 1990, Bruggemann *et al*. 1994a, Miller and Hay 1998, McClanahan *et al*. 2005, Rotjan and Lewis 2005, Francini-Filho *et al.* 2008, Bonaldo and Bellwood 2011a) which may add to the high levels of isovalerate, which is produced by a species when it feeds on animal material. Isovalerate is a metabolite of leucine catabolism and is therefore directly dependent on the amount of available leucine. Leucine plays an important role in the metabolism of cnidaria (Schlichter 1982), also potentially adding to the levels of isovalerate seen in parrotfish hindgut after the consumption of coral tissue. After passing the pharyngeal jaw, identification of coral polyps is difficult and this food is rarely identified in gut content studies (Choat *et al*. 2002).

Historically, the separation of the scrapers and excavators was largely based on differences in jaw morphology and biting strategies (Bellwood and Choat 1990, Bellwood 1994). Accordingly, this was the definition used to separate the *Chlorurus* group from *Scarus* (Bellwood 1994). It was also believed that this morphological difference allowed ecological diversification by allowing access to different resources. However, *Chlorurus* and *Scarus* may have similar diets, indicating the different mechanical feeding strategies may have little impact on their respective diets (Choat *et al*. 2002, Crossman *et al*. 2005). Through stable isotope analysis, this study was able to identify broad scale differences in the diets of the scraper and excavator functional groups where coral tissue, along with *Dictyota spp.* and green algae, comprised the main diet of the former and *Dictyosphaeria cavernosa* and turfing algae comprised that

of the latter. However, this becomes more complicated when the scale of analysis is decreased to include ontogenetic dietary differences within and amongst species. The presence of two species' stages within opposing functional groups (*C. sordidus* (IP) within the scraper group and *S. ghobban* (IM) within the excavator group, see Fig 5.3) and the occurrence of an intermediate group of scrapers reveals that diet cannot be determined based solely on morphological differences. Indeed this agrees with other studies in the Caribbean where scrapers, browsers and excavators can all be present within the morphologically similar *Sparisoma* genus (Randall 1967, Robertson and Warner 1978, McAfee and Morgan 1996, Bruggerman *et al*. 1994a,b). In fact, diet may exist on a proportional continuum depending on the requirements of the ontogenetic stage, size and resource partitioning. This is likely an important consideration when constructing trophodynamic models of a coral reef system. The IP stage of *C. sordidus* was one of the most abundant fishes within this study (Plass-Johnson unpublished data) and has been found elsewhere (Russ 1984a,b). Estimates based on a similar diet of conspecifics would severely over and underestimate *C. sordidus'* roles as excavators and scrapers, respectively. Similarly, the role of large and/or distinct groups may be confounded. Given the complex, yet tractable life cycle of parrotfishes, this study brings to light the need to better understand a species' biology before making broad generalisations about its systemic role.

It is important to note that while diet can often be a proxy for functional role, the analysis of fish muscle tissue does not clarify mechanical interactions that occur with the environment. One of the most important functional roles attributed to parrotfish is that of a bioeroder (Bellwood 1995, Bruggemann *et al.* 1996, Alwany *et al.* 2009). Although stable isotope analysis can help to examine the food source selections of parrotfish, which may help to better define the role of a bioeroder, it does not make quantifiable distinctions within this role. Furthermore, variability in consumer isotopic signatures can be a result of variability in the food signatures, variability within the sample population and/or within the consumer itself (Gannes *et al*. 1997, Vanderklift and Ponsard 2003). This variability makes it hard to identify the influence of resource use but generalisations about the variability in parrotfish populations can be identified, leading to a better understanding of inter- and intra-specific resource use. Further studies clarifying the variability of source isotopic signatures will allow more specific interpretation. Additionally, stable isotopes are a powerful tool for the identification of time integrated diets; as a result, their utilisation combined with behavioural and shorter

term diet trends (gut contents) may help to disseminate the exact ecological role of fishes with complex ontogenies.

A few observations are warranted regarding the functional role of a fish throughout its life. Firstly, within a functional group, it may not be justified to assign a single role to individual species. Access to foods will change with growth because of physiological and ecological transitions that can occur. Within this study the dietary $\delta^{13}C$ signature in parrotfish had a strong positive relationship with TL, indicating a change in dietary constituents through the lifetime of these fish. When considering dietary utilisation amongst ontogenetic stages, both within and between species, it is hard to make ubiquitous generalisations about the functional role of a fish through the entirety of its life. Ontogenetic changes can make the diets of specific stages of a fish resemble that of other species more so than other stages within the same species. Furthermore, these diets can be so different that they do not resemble any other species or life stage within the functional group. Making individual generalisations about the functional role of a species based on the entirety of its life may draw conclusions that over- or underestimate its impact within that believed role. These observations should be taken into account when creating hypotheses about the role of a species within a coral reef system and lend further evidence for the need to better understand these systems at a species level. Finally, the use of stable isotopes in the clarification of an organism's diet can be a useful tool when observational studies fail to identify highly processed gut material as seen in herbivores and may be a necessary tool in assisting and understanding of species-specific roles within coral reefs.

Chapter Six
General discussion

The identification of ontogenetic changes in patterns associated with diet in herbivorous fishes, and subsequently how these are transmitted up through the trophic levels of coral reef food webs is central to understanding the processes that structure these communities. Such information would also be useful for prevention and/or reversal of reef degradation (Dahlgren and Eggleston 2000, Loreau *et al.* 2001, Bellwood *et al.* 2004). Through the use of stable isotope analysis, the current thesis focussed on understanding the trophic ecology of parrotfish species on the coral reefs of Zanzibar. Firstly it identified a lack of isotopic differentiation between parrotfish muscle and blood (Chapter 3), secondly it investigated isotopic changes associated with spatial scales in prey and herbivorous consumers amongst three different reefs in Zanzibar (Chapter 4) and finally it identified ontogenetic changes in the diets of multiple parrotfish species, providing a detailed overview of assimilation of ten possible dietary constituents (Chapter 5).

The resilience of coral reefs has become a topical issue of late and describes the ability of a reef to absorb natural and human induced perturbations while still being able to retain the same ecological state or function. Studies that examine biodiversity in the context of ecological function suggest that species diversity can contribute to ecosystem resilience (Chapin *et al.* 2000, Bellwood *et al*. 2004). Functional groups, defined as groups of species that perform a similar ecological task despite taxonomic differences (Steneck and Dethier 1994, Bellwood *et al.* 2004) are key to the maintenance and resilience in these ecological systems. The presence of multiple species within these groups, termed functional redundancy, allows for one species to compensate for the loss of another (Elmqvist *et al.* 2003). Furthermore, within these functional groups, varied responses to ecological disturbances or perturbations exist that further help maintain ecosystem health, increasing the capacity of this group to maintain its functional role. Response diversity is influenced by species distribution and the scales at which these species operate (Peterson *et al.* 1998, Elmqvist *et al.* 2003). Species that operate at different spatial and temporal scales but perform the same functional role help to maintain group dynamics while competition is minimised (Elmqvist *et al.* 2003).

Herbivory underpins the maintenance of coral-dominated states during times of succession and preconditions reefs for possible ecological perturbations. Compared with other functional groups of reef fish, the species diversity of herbivores is relatively low (Harmelin-Vivien 2002); this group however, incorporates a wide variety of feeding strategies, food preferences and behaviours. Most herbivores aid in the control of macroalgae but individuals can have different impacts on the system depending on species and size (Bellwood and Wainwright 2002, Jayewardene 2009), implying that a fish's full role within an ecosystem is best understood when examined at the species level.

Chapter 5 investigated the diets of multiple species of parrotfishes considering ontogenetic dietary changes and comparing these amongst functional groups. In this study, stable isotope analysis was applied to fourteen species of parrotfish as well as all the major potential food items. The results identified multiple species and stages that had diets that differed from their previously, taxonomically assigned functional groups (*Chlorurus sordidus* (IP), *Scarus tricolor* (IM, IP, TP), *S. ghobban* (IM) and *S. niger* (IM)) which could have important implications for our understanding of the ecological roles of these functional groups. *C. sordidus* (IP) for example, is one of the most abundant parrotfish on Zanzibar reefs (Plass-Johnson unpublished material) and has been assumed to be an excavator (Bellwood 1994). In this study however, the IP stage was found to have a diet more consistent with that of a scraper (Fig 5.2 and 5.3). The genus *Chlorurus* is seen as an important component in the turnover of carbonate on coral reefs (Bellwood *et al.* 2003), a role that is important in removing old, dead corals, allowing new settlement and growth. Misidentifying the role of a species as an excavator could cause significant under- or overestimations of the bioerosion or carbonate turnover of reef matrices. Another species, the one with the largest average total length in the study, *S. ghobban*, has been shown in other studies to be a scraper, but in terms of isotope signature, it demonstrated a diet closer to that of the excavators when it reached larger sizes (Fig 5.3 and 5.4).

Results from these studies and indeed worldwide show that the grazing impact of fish on benthic substratum can markedly change with an increase in body size. In Hawaii (Jayewardene 2009), the Great Barrier Reef (Bonaldo and Bellwood 2008) and Zanzibar (Lokrantz *et al.* 2008), the effects of larger fish were shown to be more dramatic than those of smaller individuals. These studies, however neglected to explore the change in dietary constituents in relation to total fish length. The previous studies considered the

impact of the fishes as grazers on algal turfs, but the present study found algal turfs to be a proportionally important dietary component for only a couple of species (Group D, Fig. 5.4), forming only a low component of the diet for several groups that are thought to be significant turf grazers; *S. tricolor* (all stages) and *S. niger* (IM) (Lokrantz *et al.* 2008, Jayewardene 2009). However, the current study did not quantify individual consumption of dietary sources and so cannot evaluate their impact on these foods, or how this might alter their functional role or ecological effects within these reef systems.

Previous studies often limited the role of parrotfish to consuming turfs, but failed to identify other potential foods, whereas this study found that macroalgae were important dietary components for multiple species that were originally classified as turf grazers (Group A and C, Fig. 5.4). Additionally, coral tissue was found to be as important as turfs, detritus and macroalgae for the excavator group (Group B, Fig. 5.4). Such results indicate that studies that look solely at the role of parrotfishes as stenotrophic consumers may not correctly identify their roles or influence within a coral reef system. For example, a species present within the excavator group may conform at one ontogenetic stage, but over its entire lifetime, the occurrence of ontogenetic dietary transitions may alter its impacts on food sources and possibly the entire reef community. The use of stable isotopes does not quantify a species' role in a system, but dietary patterns might be used as a proxy to better understand other functional roles, or at least highlight the importance of examining these roles more carefully. These findings emphasise the importance of identifying both, inter- and intra-specific, functional roles of parrotfish species, as their functional role may not be consistent through the life of an individual.

Understanding the diet of parrotfishes also helps to elucidate how this group of morphologically similar species are able to coexist. Ontogenetic changes in diet help to reduce competition not only amongst species, but also with other individuals of the same species. For example, *Scarus niger* TP and IP stages had diets most similar to other scrapers, but the IM stage was most similar to *S. tricolor*. This would mean that this early life stage would only have to contend with one taxonomically similar competitor as opposed to four. Furthermore, isotopic dietary analyses were able to identify species that had diets that were completely different to other species. All ontogenetic stages of *S. tricolor* for example did not group with any other species (with the exception of *S. niger* (IM)), due to its primary reliance (as determined by SIAR mixing models) on macroalgae rather than turfs, perhaps indicating a feeding strategy

more akin to browsing. Original functional groupings of parrotfishes are largely based on morphology (Bellwood 1994), and consequently, species were essentially allocated to functional groups based on taxonomic affinities. Findings from this study suggest that this should be more of a guide rather than a rule and is supported by recent literature from the Caribbean. The Caribbean genus *Sparisoma* was originally identified as a scraping group, but further research found species that fit an excavating role (e.g. *Sparisoma amplum* and *S. viride,* Miller and Hay 1998, Francini-Filho *et al.* 2008). Taxonomic relationships then may not be the best proxy for estimating the ecological function of a species.

In most marine habitats, pelagic consumers can have strong impacts on the structure of benthic communities (Chabanet *et al.* 1997, Bonaldo *et al.* 2006, Burkepile and Hay 2008, Plass-Johnson *et al.* 2010). Similarly for coral reef communities, parrotfishes apply strong grazing pressure on benthic algae as well as predation pressure on hard coral communities (Rotjan and Lewis 2005, Bonaldo and Bellwood 2011b), indicating that they play an important role in the transfer of energy. Chapter 4 showed that parrotfish can have different signatures amongst reefs at a relatively small spatial scale (tens of kilometres). This may represent differing food sources amongst reefs, or an indication of anthropogenic processes affecting the signatures of their food sources, or a combination of both. For example, nutrient inputs affecting primary producers can be transferred up through trophic levels and can be seen as far up as parrotfish consumers (see Chapter 4), underlining the importance of choosing an appropriate scale as well as understanding the processes acting upon and within a reef (Weins 1989).

There is a large body of evidence to suggest that stable isotopes in primary producers can be used to track nutrient impacts in marine systems (Reviewed by Lepoint *et al.* 2004 and Marconi *et al.* 2011). Alternatively, Cabana and Rasmussen (1996) showed that, when adjusted for baseline $\delta^{15}N$ signatures in aquatic systems, higher trophic consumers can also be used to understand spatial differences in impacted systems. To apply this methodology most effectively, it is important to understand the bottom-up transfer of energy in the affected system (Cabana and Rasmussen 1996). Through the application of stable isotope analysis, the current study determined that the processes that affect baseline producers can be traced through trophic levels. Results showed that the reef closest to Zanzibar, Nyange, demonstrated enriched nitrogen signatures at all levels and may be linked to variation in source nutrients taken on by primary producers. In this case, possible sources of nutrient enrichment are caused by its relatively close

proximity to Stone Town and the effluents associated with the city. Enrichment in the $\delta^{15}N$ signatures of fish tissues indicate that it is possible that the signature of organic pollution is transferred to higher trophic levels. This $\delta^{15}N$ enrichment was not seen amongst all fishes however, suggesting processes of isotopic trophic transfer may not be systematically shared amongst all individuals and/or species. Furthermore, corals, including both the polyps and the zooxanthellae, showed systematic $\delta^{15}N$ enrichment at all depths and reefs further indicating impacts can be assimilated into multiple trophic levels on a reef despite differing metabolic pathways or spatial gradients. Although this may not conform to Cabana and Rasmussen's predictions of sampling higher trophic levels to observe nutrient impacts at lower trophic levels, it does suggest that processes that affect fish signatures may vary amongst reefs and when studying isotopic signature changes in fish one must account for possible spatial variation.

Chapter 3 of this thesis is one of the first studies to examine the isotopic tissue differentiation in tropical teleosts. Understanding the differences between blood and muscle allows for the interpretation of temporal differences between tissues because, in many aquatic and terrestrial species, blood turns over faster than muscle (Hobson and Clark 1992a, Buchheister and Latour 2010), suggesting that feeding patterns on two different time scales can be assessed. The statistical equivalence between the tissues found in this study indicates that either, diets never vary in parrotfishes, tissue turnover times do not differ or inter-individual variability overrides species signatures at different temporal scales. Given the findings of ontogenetic changes of diets (see Chapter 5), the second is supported because signatures between tissues did not vary at any body size (Fig 3.2). Although unexpected, these results are not the first to identify this trend but are supported by only one other equivalent study where isotopic turnover times were found to similar for studied tissues in a tropical freshwater catfish in Venezuela (McIntyre and Flecker 2006). These findings are contrary to studies of tissue turnover time in other organisms which show that blood usually displays dietary changes over short periods and muscle will represent the accumulation of longer dietary trends (Hobson and Clark 1992a, b, MacAvoy *et al*. 2001). Understanding isotopic signature differences between tissues is important to prevent misinterpretation of dietary data leading to wrong assumptions based on tissue turnover.

Stable isotope ecology has become an increasingly important approach for the evaluation of ecological communities, as it helps to elucidate trophic relationships amongst organisms (Post 2002). Stable isotopes can provide a time integrated

measurement of trophic position by accounting for possible trophic pathways. In the complex case of omnivory, variability in signatures can be derived from time patterns of the sampled tissue including time needed for turnover and equilibration (Sweeting *et al.* 2003). Thus, to better understand how variability in different tissue occurs, studies of spatial (Chapter 4) and ontogenetic changes (Chapter 5) in a species must be combined with physiological studies (Chapter 3). The isotopic signature of a consumer provides insights into the possible food sources and can be used to describe trophic differences in taxonomically similar species. This in turn can be used to describe the functional roles of an organism within its community. Such methods were applied in this thesis where stable isotopes were used to describe physiological (Chapter 3) and ontogenetic dietary changes (Chapter 5) in parrotfishes and their changing signature in coral reef systems indicating possible anthropogenic influences (Chapter 4). Underpinning stable isotope analysis is an understanding of the variability inherent with the system in question, including the variability of the organism's signatures themselves (Post 2002). This study increased the understanding of sources of variability in populations of parrotfishes that can be seen at multiple levels of organisation. When describing patterns of variability in these fishes one must account for the scale, the community, the species and the life stage within a species being sampled as well as the differential isotopic tissue turnover rates within a single individual. To neglect this potential variability could lead to erroneous interpretations.

When comparing overall systems, the isotopic signatures of higher trophic levels provide little information because of the potential variability in the ultimate source of carbon and nitrogen. In coral reef systems, nutrients are taken in by primary producers, algae and corals, which in turn are used by primary consumers. In the current study, isotopic signatures showed not only that signatures can vary between reefs at the primary producer level but, that these differences can be transmitted up to higher trophic level consumers such as parrotfishes.

On coral reefs, the understanding and identification of critical functional groups and their constituent species is vital to understanding the processes shaping these systems if we hope to manage for system resilience (Tilman *et al.* 1997, Loreau *et al.* 2001). Parrotfishes are considered an important part of the herbivorous functional group, and a few species have been shown to have limited functional redundancy (Bellwood *et al.* 2003, Bonaldo and Bellwood 2008, Jayewardene 2009). This thesis offers an alternate view, exploring the diets of different ontogenetic stages of multiple species, and

providing comparisons. The groupings of fishes determined in Chapter 5 (Fig 5.3 and 5.4) show how diets compare when ontogenetic dietary changes are considered. If the functional role of a species was solely based on diet, these findings might indicate a level of redundancy within each group. Although this study identified diets of multiple species through the use of stable isotopes, it did not quantify the physical impacts of the parrotfishes on the benthic coral reef community. Given the differing signatures in fishes amongst reefs (see Chapter 4), it is possible that the diet and thus, functional role of species may differ in a spatial context. Given the differences in diets that occur in parrotfish within and between species as well as between reefs, these data suggest that if we are fully to understand the functional roles of coral reef fishes, studies must be conducted at the species level and at the relevant scale.

Parrotfish remain one of the few fish groups able to feed on the majority of substrata found in coral reef systems, and because of this they will play an important role in structuring reef communities in an environmentally changing world. Given the spatial and temporal environmental gradients of coral reefs, the present study provides further information pertaining to the physiological and dietary dynamics in parrotfishes, thus providing further insight into their ecological roles. Uncovering the exact role these fish play will help to maintain resilience, and perhaps assist in the recovery of affected or degrading reef systems.

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APPENDIX

2.5 BASELINE EXPERIMENTATION FOR REQUIRED CORAL TISSUE

In February 2010, *Porites* and *Acropora* samples were collected from a bay in Punta do Ouro, Mozambique. Samples were frozen and transported to Rhodes University, Grahamstown, where they were later processed as per section 2.5. Samples were analysed for isotope signatures as per section 2.7, but with emphasis on the strength and variability of $\delta^{13}C$ and $\delta^{15}N$ signatures. Acceptable signatures were identified to the physical sample that identified relative surface area, and later applied to Zanzibar coral sampling.

2.6.1 PERMITS

This study was approved by the ethics committee of the Department of Zoology and Entomology, Rhodes University, South Africa on 15 June, 2010. Tracking number: ZOOL-12-2010.

This study was approved by the Revolutionary Government of Zanzibar. Name of Authorising officer: Mohamed H. Rajab. Issued on 18/06/2010. Permit number: ZRP/98.