Studies in the stable isotope ecology of coral-reef fish food-webs

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Abstract

Coral-reef fish food-webs are complex and few studies have explored the range of production sources or how these support the higher trophic levels of coral-reef food-webs. I collected muscle tissue samples of abundant coral-reef fish at Bahamian and Maldivian sites and used bulk stable isotope ($\delta^{13}C$ and $\delta^{15}N$) data in bi-plot to analyse their isotopic niches in relation to their putative trophic guilds; some species (e.g. some corallivorous Chaetodontidae) are evidently confined to their guild, but others seem to be utilizing multiple food sources.

I also analysed $\delta^{15}N$ (as a proxy for trophic position) to body size relationships among all individuals, individuals of the same species, individuals sharing the same trophic pathway and of the community as a whole. At species, some fishes had relatively flat relationships whereas others varied greatly with size. Individuals relying on the same production source type (e.g. planktivorous Chaetodontidae and Acanthuridae, Maldivian data only) had similar body size-$\delta^{15}N$ trends with variation potentially due to at least family-related traits. At community level, there was a positive linear relationship in the Bahamas sites but a parabolic relationship in the Maldives, suggesting trophic function changes at different size classes in different ways in the two locations.

I further tested whether the putative trophic guilds precisely portrayed the strict feeding patterns among fishes and whether location affected source partitioning using both bulk (SIA) and compound-specific stable isotope data (CSIA). The results suggested that certain consumers (e.g. corallivores [SIA] and detritivores [SIA and CSIA]) were confined to the trophic guild to which they have typically been assigned, while others show some evidence of relying on multiple sources (e.g. diurnal planktivores [SIA and CSIA]) indicating that it is imprecise to assign them to the single guilds to which they have commonly been assigned. At outer-atoll sites, some

fish fed more on diurnal plankton than those in the inner-atoll suggesting some geographic characteristics could affect the feeding preferences.

I have applied stable isotope data to elucidate the importance of body size and geographic location in determining the feeding strategies of fish to better understand how these diverse food-webs work. The study provides pointers to future work such as on benthic-pelagic coupling, roles of sponges, contribution of SIA vs CSIA data and finer-detailed diet composition.

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Acronyms and Symbols

Abundance	N
Amino acid	AA
Bayesian standard ellipse area	SEA_B
Body mass (community level analysis)	B
Body mass (non-community level analyses)	M
Compound-specific isotope analysis	CSIA
Confidence interval	CI
Dissolved inorganic carbon	DIC
Dissolved inorganic nitrogen	DIN
Dissolved organic matter	DOM
Eccentricity	E
Epilithic algal matrix	EAM
Essential amino acid	EAA
Fatty acid	FA
Fork length	FL
High microbial abundance	HMA
Highest posterior median	HPM
Island mass effect	IME
Low microbial abundance	LMA
Particulated organic matter	POM
Predator-prey mass ratio	PPMR
Principal component analysis	PCA
Sample size corrected standard ellipse area	SEA_C
Size spectra	SS
Stable isotope analysis	SIA
Stable isotope Bayesian ellipse in R	SIBER
Standard deviation	SD
Standard ellipse area	SEA
Standard error	SE
Standard length	SL
Total length	L
Trophic enrichment factor	TEF
Trophic level	TL
Trophic position	TP
Underwater visual census	UVC

Chapter 1. Coral-reef fish community trophic structure and source partitioning

1.1 Introduction

Coral reefs are one of the most biodiverse ecosystems in the world (Spalding *et al.*, 2001) hosting diverse and abundant fish (Honda *et al.*, 2013) and providing important protein sources to humans (Watson and Pauly, 2001). However, a combination of local and global, and natural and anthropogenic stressors have threatened coral extinctions (Hughes *et al.*, 2003; Huang, 2012), significant reductions of reef fish stocks (Myers and Worm, 2003b; Shepherd and Myers, 2005; Pope *et al.*, 2006), collapse of fisheries (Jennings and Kaiser, 1998; Russ, 2002; Myers and Worm, 2003b) and alteration of subsequent fish production processes (Jennings and Lock, 1996; Jennings and Polunin, 1996). The stressors include fishing (Grigg, 1994; Jennings *et al.*, 1995; Hall, 1999; Fulton *et al.*, 2005; Fry *et al.*, 2006; McClanahan, 2011), pollution (Grigg, 1994), sea surface warming (Pauly, 1980; Mora and Ospina, 2001; Perry *et al.*, 2005; Hughes *et al.*, 2017) and habitat structural complexity reduction (Carpenter *et al.*, 1981; Martin-Smith, 1993; Grigg, 1994; Charbonnel *et al.*, 2002; Gratwicke and Speight, 2005a).

Community structure of coral-reef fish has been widely studied at species (McClanahan and McRoy, 1979; Jennings *et al.*, 1995; McClanahan, 2011) and trophic guild or functional group levels (Sano *et al.*, 1984; DeMartini *et al.*, 2008; Layman *et al.*, 2012; Richardson *et al.*, 2016). These studies provide important information on trophic interaction between species/groups and their responses towards certain stressors, and potential indications of their functional diversity to maintain the healthy states of coral reefs (e.g. ecosystem resilience, Bohnsack, 1983; Hughes *et al.*, 2003; Hughes *et al.*, 2007; Green and Bellwood, 2009; Heenan and Williams, 2013). More recently, community structures have been analysed solely on body size in many aquatic systems including coral reefs (Jennings *et al.*, 2002a; Jennings and Mackinson, 2003; Graham *et al.*, 2005; Arim *et al.*, 2010; Wilson *et al.*, 2010; Bell *et al.*, 2017; Woodson *et al.*, 2018). Size-based anslysis provides useful insights and allows quantification of community structure and large-scale comparisons among areas.

Many aforementioned studies applied gut contents-derived trophic categorisations to inform the trophic status of coral-reef fishes. Gut-contents data face limitations of temporal and spatial variation and provide only short-term dietary

information; in the setting of coral reefs, acquiring gut contents data can potentially be significantly impactful to the habitat or fish community. Stable isotope analysis has been advancing greatly and has a number of strengths in applying it to increase understanding of feeding behaviours and food-web structure and dynamics.

This review discusses some of the existing methods used to describe fish community structure and their applications with a particular focus on the potential of using stable isotope data to analyse the food webs of coral reefs. Scientific publications on detailed energy tracking of defined trophic categories are critically appraised as well as evidence confronting some supposed trophic strictness. Methodologies examining diet composition are compared focusing in particular on bio-tracer methods. Finally, current states of coral reefs and the objectives of this thesis are described.

1.2 Size structuring of coral-reef fish community

Body size is an important trait for animals, and can correlate with others such as productivity, fecundity and functional role (Peters, 1986; Boudreau *et al.*, 1991). Metabolic theory suggests that energy decreasing along a food chain can be one important factor causing the size structuring (Paul and Christensen, 1995; Brown and Gillooly, 2003). Thus, analysing community size structuring provides fundamental understanding of energy fluxes (Jennings *et al.*, 2002c; Rooney *et al.*, 2008), population dynamics, predator-prey interactions (Kingsford, 1992; Cohen *et al.*, 1993; Boyle and Pierce, 1994; Barnes *et al.*, 2010), food-web structure and function (Brose *et al.*, 2006), productivity (Banse and Mosher, 1980; Jennings *et al.*, 2002c) and ecosystem stability (Jennings and Warr, 2003). For example, in a single-source system (i.e. supported mainly by one production source type), abundance (N) scales with body mass (M) by $N \propto M^{-0.75}$ (Peters, 1983), whereas in a multiple-source system by $N \propto M^{-1.2}$ (Jennings and Mackinson, 2003); the latter suggests that production source types can also affect this relationship.

Quantifying size structuring helps address fish community changes in response to stressors such as fishing pressure (Dulvy *et al.*, 2004b; Graham *et al.*, 2005; DeMartini *et al.*, 2008; Robinson *et al.*, 2016), coral bleaching (Graham *et al.*, 2007) and habitat structural complexity degradation (Alvarez-Filip *et al.*, 2011). These stressors can affect size structuring directly through removing certain fish from the system such as through aggregation fishing (Sadovy and Domeier, 2005; Benoît and Swain, 2008), bycatch (Benoît and Swain, 2008) and fatality of juveniles (Pauly,

1980; Houde, 1989; Blaxter, 1991; Meekan *et al.*, 2003; Sponaugle and Grorud-Colvert, 2006) and indirectly such as by modifying habitat structural complexity (Alvarez-Filip *et al.*, 2009) and predator-prey relationships (e.g. top-down control, Benoît and Swain, 2008).

1.2.1 Size spectra

Size spectra indicate the relationship between $\log_{10}$ abundance and total length (for fish, toal length indicates the length from the tip of the snout to the tip of the longer lobe of the caudal fin) of all individuals in a community (Cohen *et al.*, 2003; Trebilco *et al.*, 2013). The relationship is normally negative linear in aquatic systems (Boudreau *et al.*, 1991; Rice and Gislason, 1996; Jennings *et al.*, 2002b; Jennings *et al.*, 2002c; Jennings and Mackinson, 2003; Graham *et al.*, 2005; Graham *et al.*, 2007; Al-Habsi *et al.*, 2008; Wilson *et al.*, 2010; Trebilco *et al.*, 2013; Robinson *et al.*, 2016), albeit with some exceptions (e.g. Rogers *et al.*, 2014). This relationship can respond to direct effects of stressors. For example, fishing tends to target fish above a certain size and the result is a decrease in the abundance of those sizes and steepening of the size spectrum (Figure 1.1). Global warming, on the other hand, can cause fatality of juvenile fish by disturbing their ontogenetic processes and metabolic rates (Pauly, 1980; Houde, 1989; Blaxter, 1991; Meekan *et al.*, 2003; Sponaugle and Grorud-Colvert, 2006), thus resulting in a milder slope of the size spectrum (Figure 1.1).

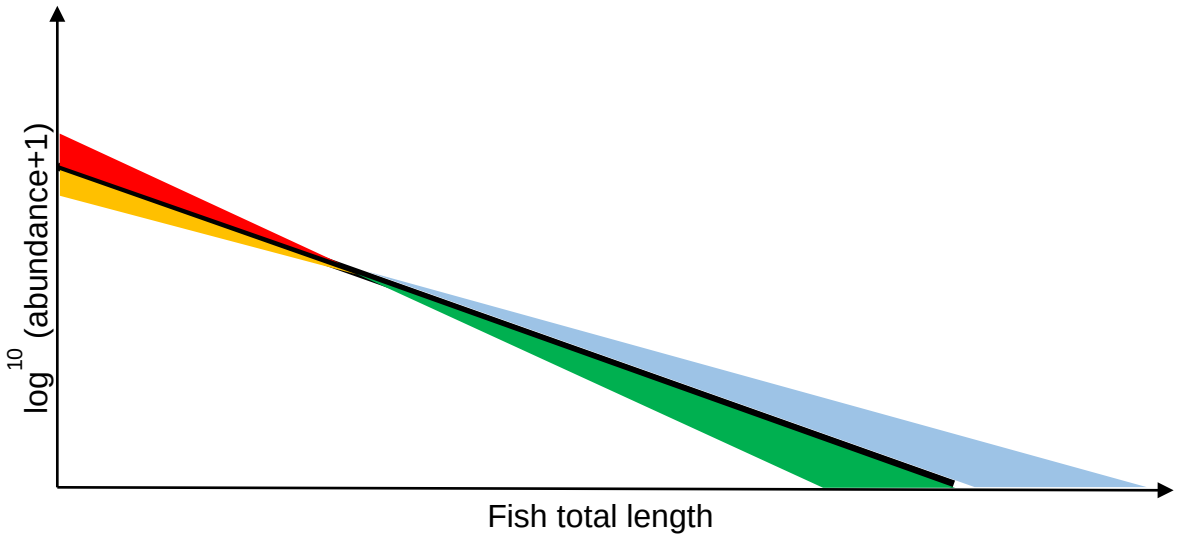


Figure 1.1 Size spectra and the responses towards certain stressors, indicated original size spectra (black line); removal of larger size fish through aggregation fishing (green area); increase of small individuals due to top-down effect of removing predators (red area); loss of small individuals due to complexity reduction and reduced survival rate from global warming (yellow area); and increase of larger individuals due to prey exposure as habitat degrades (blue area).

Changes in size spectra are evaluated by the linear regression coefficients slope, intercept and midpoint height (Trenkel and Rochet, 2003), and have been widely correlated with fishing pressure in temperate (Jennings *et al.*, 1995; Jennings *et al.*, 2001b; Jennings *et al.*, 2002a; Jennings *et al.*, 2002b) and tropical systems especially coral reefs (Dulvy *et al.*, 2004b; Graham *et al.*, 2005; Wilson *et al.*, 2010; Robinson *et al.*, 2016), including habitat structural complexity effects (Wilson *et al.*, 2008; Wilson *et al.*, 2010) at a large scale.

1.2.2 Trophic structure

In some cases, size spectra respond to indirect effects (e.g. benthic structural modification) only happen after a while such as through density dependent responses (Shin and Cury, 2004), community changes due to coral bleaching (Graham *et al.*, 2007), suspected evolution in response to harvesting (Law, 2000), top-down control (Dulvy *et al.*, 2004a) or bottom-up effects (Shin and Cury, 2004). For example, aggregation fisheries often target large predatory fish such as groupers (Sadovy and Domeier, 2005) thus resulting in fewer juveniles spawned, reducing the stock of such species (i.e. cause the total abundances of some large size classes to decrease) in the future and modifying predator-prey relationships (e.g. increase the survival rates of smaller individuals or prey fish). In addition, the large size classes may be replaced by low-TP species (e.g. parrotfish) causing the biomass to recover and size spectra to return to normal. Thus, in the short-term, the size spectrum will remain unchanged suggesting low sensitivity to such stressors.

Jennings *et al.* (2001a) developed another metric “size-based trophic structure” to quantify community size structuring which was potentially more sensitive to indirect effects. Such metric analyses the mean TP to body mass relationship in a community (i.e. size-based trophic structure or community level TP-body mass relationship) to allow further understanding of community trophic assemblages (e.g. trophic replacement, Graham *et al.*, 2017) and predator-prey relationships. Deriving the mean TP values for each body mass class requires species-level TP-body mass relationships and biomass composition data. Traditionally, estimating TP values for fishes requires gut-contents data whereas stable isotope ($\delta^{15}N$) provides a better approach (see 1.7.2).

In single-source food-webs, mean TP scales linearly and positively with body mass (Jennings *et al.*, 2001a; Jennings *et al.*, 2002a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008). This yields a predator-prey mass ratio by $PPMR = 2^{slope}$

(Jennings *et al.*, 2001a), where slope is calculated from the linear regression of mean TP against body mass. In aquatic systems, PPMR reflects constraints on community structure and can be used to evaluate general food-web properties such as food-chain length and stability (Jennings *et al.*, 2002c; Barnes *et al.*, 2010); smaller PPMR values indicate longer food chains (Jennings and Warr, 2003). The PPMR also provides a tool for food-web comparison among aquatic systems (Jennings *et al.*, 2001a; Jennings *et al.*, 2002c; Bode *et al.*, 2003; Bode *et al.*, 2006; Al-Habsi *et al.*, 2008; Morillo-Velarde *et al.*, 2018). However, no study has analysed the size-based trophic structure in coral reefs, thus the inferrable implications do not exist.

Compared with other aquatic systems from existing studies, coral reefs have many distinctive production sources and diverse feeding patterns exist, TP-body mass relationships among fishes are potentially more diverse (see 1.5) and at the community level such relationship is less likely to be linear positive as specific energy pathways potentially affect the relationship (Robinson and Baum, 2015). For example, small-sized but high-TP (Graham *et al.*, 2017) and large-sized but low-TP individuals (Hughes *et al.*, 2007) are considered abundant in coral reef systems. Thus, the linear relationship may not exist, and if that is the case then the analytical benefits that the relationship has facilitated elsewhere will not be possible for coral reefs.

1.3 Diverse production sources and feeding strategies

To better explain variations in community structure of coral-reef fish, understanding of major energy pathways is crucial. Such food webs are potentially supported by many sources including benthic algae, sponges, corals, detritus, plankton and efficient recycling pathways such as the sponge loop (de Goeij *et al.*, 2013). These production source types (Figure 1.2) can consist of multiple primary producers and mixotrophic species.

1.3.1 Benthic production sources

Much of the benthic primary production is thought to be from small benthic filamentous algae and microbial autotrophs (Goldberg, 2013). Their high rates of production support herbivore assemblages (Choat and Clements, 1998). In most cases, filamentous algae mixed with microbes and detritus, form the epilithic algal matrix (EAM) that is diverse and heterogeneous (Crossman *et al.*, 2001; Clements *et al.*, 2016; Adam *et al.*, 2018). Microbial autotrophic organisms such as euendolithic cyanobacteria and microalgae (known as microborers, Goldberg, 2013) occur within

the calcareous substrates. The epiphytic (attached to macroalgae such as *Sargassum* spp), epilithic and endolithic microbes are highly productive (Clements *et al.*, 2016), and can be accessed by some fish through direction ingestion, excavation and scraping.

There can also be a high biomass of macroalgae. Some of these are fed upon by several consumers (Paul *et al.*, 1990), while some others are not commonly palatable such as *Halimeda* spp due to its heavily calcified structure (Price *et al.*, 2011) and chemical defences (Paul and Van Alstyne, 1988; Cetrulo and Hay, 2000). These benthic algae are different in their bulk $\delta^{13}C$ values (Pinnegar and Polunin, 2000; Polunin and Pinnegar, 2002; Dromard *et al.*, 2013; Plass-Johnson *et al.*, 2013).

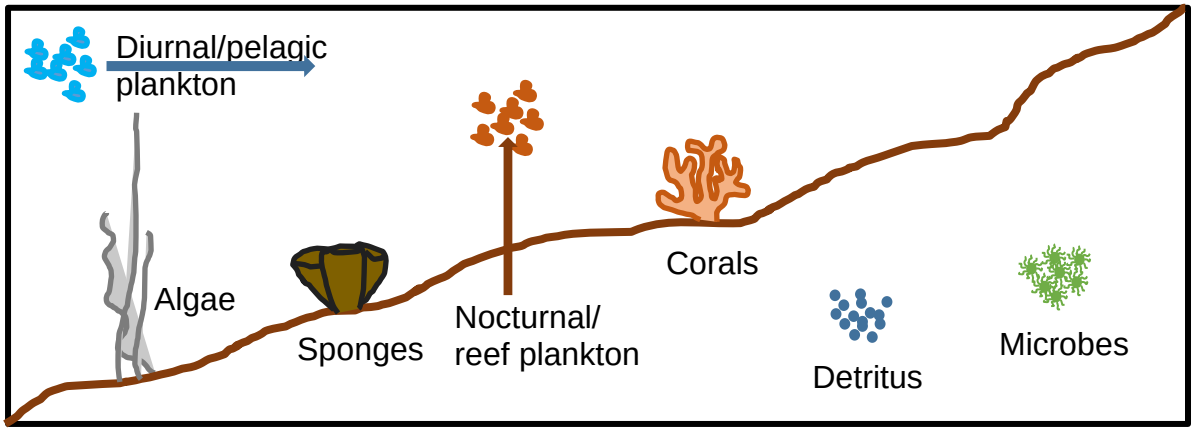


Figure 1.2 Illustration of likely major production sources in coral-reef food-webs including benthic sources: algae, sponges, corals, detritus and microbes; and pelagic sources: diurnal/pelagic and nocturnal/reef plankton. Some sources can mix with others (e.g. microbes and detritus).

Mixotrophic producers include symbiotic benthic animals such as sponges and hermatypic corals. They are capable of utilizing dissolved inorganic carbon and nitrogen (DIC and DIN), and dissolved organic matter (DOM) as well as feeding on other organisms (e.g. plankton, de Goeij *et al.*, 2017). Their associated microbial colonies can convert inorganic sources into organic form or organic sources (e.g. labile DOM) into other forms (Wild *et al.*, 2004a; Wild *et al.*, 2004b; de Goeij *et al.*, 2017), which are then used by the host (Baker, 2003; de Goeij *et al.*, 2017). Corals are well-studied in terms of symbionts and chemical cycles (Banaszak *et al.*, 1993; Trench, 1997; Baker, 2003; Williams and Grottoli, 2010; Radice *et al.*, 2019) but their stable isotope ecology in the food webs has not been well studied. Corals palatable to corallivorous fish are mainly in the genera *Acropora*, *Pocillopora* and *Porites* (Brooker *et al.*, 2013).

Sponges are also potentially important in coral-reef food-webs (de Goeij and Van Duyl, 2007; de Goeij *et al.*, 2008b; de Goeij *et al.*, 2013; de Goeij *et al.*, 2017), however, nutrient fluxes and ecological functions of reef sponges are complex and are even less studied than corals (de Goeij *et al.*, 2017). Sponges can be either sinks or sources for dissolved carbon, nitrogen, phosphate and silicate (de Goeij *et al.*, 2008b; Fiore *et al.*, 2013). DOM is potentially the largest source of organic matter on reefs (> 90% of total organic matter) and is the major form of carbon intake for sponges (e.g. 56-97% for a shallow reef species, de Goeij *et al.*, 2017), being processed by the sponge tissues (de Goeij *et al.*, 2008a) and associated microbes (Reiswig, 1971; de Goeij *et al.*, 2008a). As filter feeders, sponges also utilize particulate organic matter (POM) including plankton (Reiswig, 1971) which they prefer to DOM (de Goeij *et al.*, 2008a). Some sponges (e.g. cryptic species) are also capable of recycling nutrients from the water column back to the reef in the form of detritus through sponge loop by rapid turnover of filter cells (de Goeij *et al.*, 2013). Sponges intra- or extra-cellularly host a wide range of microorganisms such as archaea, heterotrophic bacteria, cyanobacteria, green algae, red algae, cryptophytes, dinoflagellates and diatoms (Lee *et al.*, 2001). The sponge-microbe symbiosis involves nutrient enrichment and safe habitat provision by sponges (Bultel-Poncé *et al.*, 1999) and includes intracellular digestion and translocation of metabolites (Wilkinson, 1987; Fiore *et al.*, 2013), nitrogen fixation (Wilkinson and Fay, 1979; Wilkinson *et al.*, 1999), stabilisation of the sponge skeleton (Wilkinson *et al.*, 1981) and participation in chemical defence (Paul, 1992) by the symbiotic microorganisms. Some of the sponge-microorganism relationships are species-specific (e.g. Lee *et al.*, 2001). Symbiotic microorganism mass in sponges varies among host species (Cleary *et al.*, 2013), thus sponges are normally categorised as high microbial abundance (HMA: containing 10^8–10^9 bacteria per gram of sponge tissue) and low microbial abundance species (LMA: 10^5–10^6) (Moitinho-Silva *et al.*, 2014; Morganti *et al.*, 2017).

Detritus is a mixture of dead organic matter, inorganic materials, associated fauna and microbes that include microalgae such as diatoms, dinoflagellates and cyanobacteria (Crossman *et al.*, 2001). These components may come from sources with distinctive baselines and their compositions can be highly variable, making detritus very heterogeneous. Its labile portions are important food sources with high nutritional value (i.e. high amino acid concentrations) for grazing fish (Crossman *et al.*, 2001). Detritus can be found in sediments or associated with algae, the detrital-

algal matrix being common on coral reefs (Adam *et al.*, 2018), yet the two types of food sources differ in amino acid and starch quantities and compositions (Crossman *et al.*, 2001). However, no study has investigated isotopic discriminability of individual detrital components on coral reefs, compared these with algal sources, or quantified diet compositions of reef-fish species in this way.

1.3.2 Pelagic production sources

Pelagic production sources include phytoplankton and zooplankton from the open ocean to the reefs dominating the plankton assemblage during the daytime (i.e. diurnal plankton). The former utilize DIC and DIN from the pelagic, upwelling or land runoff (Gove *et al.*, 2016) which can vary geographically and seasonally (Gove *et al.*, 2016; Garzon-Garcia *et al.*, 2018). Zooplankters feed on these phytoplankton, some herbivorous zooplankton and small zooplankton (Enright and Honegger, 1977; McClelland and Montoya, 2002; Sommer *et al.*, 2005; Kürten *et al.*, 2013). Functional and trophic roles of zooplankton can be seasonal (Kürten *et al.*, 2013), and zooplankton assemblages can be very variable due to their diel vertical migration (Zaret and Suffern, 1976; Enright and Honegger, 1977; Bollens and Frost, 1989). Plankton assemblage also includes nocturnal plankton that emerge after sunset. These include some zooplankton dwelling close to or in the reef substrate (e.g. holoplankton such as copepods and mysids) or inside crevices during the day (e.g. semipelagic organisms such as polychaetes, ostracods and crustacean larvae) and feeding on pelagic plankton in the water column after sunset (Zaret and Suffern, 1976; Enright and Honegger, 1977; Bollens and Frost, 1989; Hobson, 1991). Compared with diurnal zooplankton, nocturnal zooplankton are larger in size (Hobson, 1991), and occupy higher TPs as a result of size-based feeding patterns among pelagic zooplankton assemblages (McClelland and Montoya, 2002). Althought these zooplankters are not originated from the pelagic, they are considered as food sources derived from the pelagic plankton and thus as one type of pelagic production sources.

There is evidence of higher phytoplankton productivity around islands and atolls for a variety of reasons including upwelling and island mass effect (IME, Doty and Oguri, 1956; Hamner and Hauri, 1981; Gove *et al.*, 2016). These pelagic production sources can be expected to increase the abundance of planktivores. Planktivores potentially link pelagic production sources with coral-reef food-webs through predation (Randall, 1967), and provision of faeces which are eaten by reef

consumers (e.g. Robertson, 1982) and otherwise potentially distributing nutrients from pelagic sources to the reef (e.g. Francis and Côté, 2018).

1.4 Energy pathways

Most ecological research categorises coral-reef fish trophic guilds based on their principal diet (Hiatt and Strasburg, 1960; Jennings *et al.*, 1995; Polunin, 1996; McClanahan *et al.*, 1999; Hughes *et al.*, 2003; MacNeil *et al.*, 2015; D'Agata *et al.*, 2016; Graham *et al.*, 2017; Stamoulis *et al.*, 2017; Hadi *et al.*, 2018; Moustaka *et al.*, 2018). Such categorisations overlook the fact that the fish commonly rely on more than one source. Nine major trophic guilds are widely recognised in coral-reef fish, from low to high TP they are algivore, microphage, detritivore, corallivore, spongivore, diurnal planktivore, nocturnal planktivore, zoobenthivore and piscivore. Algivore and microphage, and sometimes also detritivore, species are normally grouped together as herbivores (e.g. Choat *et al.*, 2002; Burkepile and Hay, 2008; Green and Bellwood, 2009; Heenan and Williams, 2013; Dromard *et al.*, 2015), yet their food source types are potentially very different (Crossman *et al.*, 2001; Clements *et al.*, 2016). Nocturnal planktivores are typically categorised as zoobenthivores (e.g. fishbase.org), however, their food sources (i.e. nocturnal zooplankton) are different from zoobenthos due to their reliance on pelagic plankton (Hobson, 1991).

1.4.1 Algivory

Most algivorous fish (e.g. Acanthuridae, Siganidae, and Kyphosidae) specialize on different algae such as *Acanthurus leucosternon* being reported to feed mainly on filamentous rhodophytes (Robertson and Gaines, 1986) and *Naso elegans* feed mainly on phaeophytes (Ngugi *et al.*, 2017). Such specialisation is directly related with their symbiotic digestive microbes (Ngugi *et al.*, 2017).

1.4.2 Microphagy

Microphagous fish (e.g. parrotfish, Clements *et al.*, 2016) feed on microbial autotrophs. These fish mainly target endolithic, epilithic and epiphytic cyanobacteria by scraping them from hard surfaces (e.g. coral, rock) or exploiting endosymbiotic cyanobacteria (Clements *et al.*, 2016) through spongivory (Wulff, 1997; Dunlap and Pawlik, 1998).

1.4.3 Detritivory

Detritivorous fish (e.g. some Acanthuridae) forage on surfaces and sediments; *Acanthurus nigricauda* and *Ctenochaetus striatus* rely on epilithic and/or epiphytic dead organic matter (Robertson and Gaines, 1986) and associated heterotrophic bacteria and diatoms (Moriarty, 1976). Detritivore species tend to be considered less selective than consumers in other pathways (Crossman *et al.*, 2001), yet this issue has scarcely been studied. Some detritivores can utilize certain microbial autotrophs (e.g. diatoms and cyanobacteria) embedded in the detritus and some algivorous species (e.g. EAM feeders) can digest epiphytic detrital materials (Crossman *et al.*, 2001; Sanchez and Trexler, 2018).

1.4.4 Corallivory

Corallivorous fish can be either obligate (e.g. *Oxymonocanthus longirostris*) or facultative (e.g. *Balistapus undulatus*). Many specialize on certain coral component(s) (e.g. mucus [*Labrichthys unilineatus*], polyps [*Chaetodon* spp]), and skeletal material (e.g. tetraodontids). Some prefer *Acropora* and *Pocillopora* (Cole *et al.*, 2008). This specialization can potentially be a result of food availability and accessibility in relation to coral morphology (Cole *et al.*, 2008; Rotjan and Lewis, 2008; Brooker *et al.*, 2013). Most obligate corallivores belong to the family Chaetodontidae, with some from the Labridae (Cox, 1986). Their predation is generally assumed to have minimal influence on the coral community (Harmelin-Vivien and Bouchon-Navaro, 1983), and it may be beneficial in some respects (Cox, 1986).

1.4.5 Spongivory

Spongivorous fish (e.g. many pomacanthids) often target cryptic sponges. Some low-TP species (e.g. parrotfish) are also spongivorous (Wulff, 1997; Dunlap and Pawlik, 1998). Whether spongivores target particular sponge species is unknown.

1.4.6 Planktivory

The zooplanktivorous fish (e.g. Caesionidae, Atherinidae, and some Acanthuridae and Labridae) predate on zooplankton directly from the water column during the day time (Gliwicz, 1994). The Caesionidae feed mostly in large schools. There are two subfamilies (Caesioninae and Gymnocaesioninae), four genera (*Caesio*, *Pterocaesio*, *Gtmnocaesio* and *Dipterygonotus*) and six subgenera

(Odontonectes, Flavicaesio, Caesio, Pterocaesio, Pisinnicaesio and Squamosicaesio) based on several systematic analyses (Carpenter, 1990; Carpenter, 1993). Different zooplanktivorous species target different zooplankton based on predatory skills, prey preferences, prey behaviours and morphological characteristics (Zaret and Suffern, 1976). Some of them can feed on other food sources, and can have flexible diet (McMahon *et al.*, 2016). Some facultative planktivorours fish (e.g. some Acanthuridae) feed on both benthic production sources and plankton to some extent.

Nocturnal planktivorous fish such as most species of the genera *Myripristis* and *Apogon* forage mainly at night (Hobson, 1991). They feed on nocturnal zooplankton including calanoids from the open ocean, holoplankton residing close to the substrate during the day and migrating into the water column at night (e.g. copepods and mysids), and semi-pelagic organisms from the seafloor such as polychaetes, ostracods, mysids and amphipods (Bollens and Frost, 1989; Hobson, 1991). Some of these fish feed on their reefs, while some (e.g. *Myripristis murdjan*, *M. amaena* and *Priacanthus cruentatus*) migrate seaward to feed and return before sunrise (Hobson, 1991).

1.4.7 Zoobenthivory

Zoobenthivorous fish forage underneath rocks, inside holes in hard substrate, and in sediment for a wide range of benthic animal prey (e.g. polychaetes and amphipods). The zoobenthos is highly diverse; some of these fish feed relatively strictly on one type (e.g. *Orthopristis ruber*) while others do not (Zahorcsak *et al.*, 2000). Variation in zoobenthos preference can be related with ontogeny (e.g. *Umbrina coroides*, Zahorcsak *et al.*, 2000). Regardless of the high diversity of zoobenthos prey community (Garrison and Link, 2000), most literature has categorised fishes feeding on it as simply ‘zoobenthivorous’.

1.4.8 Piscivory

Piscivores (e.g. grouper) predate on other fish and are often considered to be generalist and opportunistic predators. Some also feed on zoobenthos (e.g. some lutjanids, Allen, 1985; Kulbicki *et al.*, 2005a; Layman and Allgeier, 2012). Pelagic piscivores (e.g. some *Caranx* spp) mostly feed on zooplanktivores, whereas reef piscivores can rely on both pelagic and benthic prey. Reef piscivores prefer smaller prey (e.g. juveniles) than large-sized individuals (Layman *et al.*, 2005), but their feeding strategies are potentially affected by the habitat complexity in the setting of

coral reefs (Beukers and Jones, 1998) and prey availability (Beukers-Stewart and Jones, 2004).

1.5 Feeding strategies of coral-reef fish

While some fish rely quite strictly on one energy pathway (e.g. obligate corallivores), many mainly feed on one source type but facultatively on others or frequently on more than one source type. Such trophic plasticity can sometimes be size-based. Large individuals generally feed at higher TPs (i.e. size-based feeding, Jennings *et al.*, 2001a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008; Romanuk *et al.*, 2011) as a result of ontogenetic dietary shifts (Shirota, 1970; Hunter, 1981; Winemiller, 1989; Gaughan and Potter, 1997; Labropoulou *et al.*, 1997; de la Morinière *et al.*, 2003; Meekan *et al.*, 2003; Uphoff *et al.*, 2019), morphometric changes including increasing gape size and post-maturity factors that influence foraging (Peters, 1986; Jennings *et al.*, 2001a; Jennings *et al.*, 2002b; Mumby *et al.*, 2006; Al-Habsi *et al.*, 2008; Robinson and Baum, 2015) and improved predation skills (Newman *et al.*, 2012). There are exceptions, for example, dietary shifts from high to low TP due to ontogeny (e.g. Chen, 2002; Layman *et al.*, 2005), seasonality in production sources (Bronk and Glibert, 1993; Rolff, 2000), and human disturbance (Pastorok and Bilyard, 1985; Graham *et al.*, 2017). Also, for some species (e.g. herbivores), TP can remain relatively unchanged with increasing body size (Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). These species-level relationships are also crucial to generate the community-level TP-body mass relationship and other important information (e.g. PPMR). Where this is not size-based, individuals can specialise in certain food source(s) to avoid competition or predation over time or in space as a result of variations in food availability (Matthews and Mazumder, 2004).

Mixed food-chain feeding allows high-TP predators to explore more productive food chains such as low-TP primary consumers (Layman *et al.*, 2005). Facultative feeding allows opportunists to feed on high quality food items, for example, some algivores are also coprophagous (Robertson, 1982). The broad trophic categorisations used tend to point to trophic redundancy (e.g. herbivores, Plass-Johnson *et al.*, 2013), whereas more detailed work is needed to understand the food-web dynamics and how different energy pathways affect community structures.

1.6 Diet analysis methods

It is clear that a more sophisticated typology with detailed dietary information is needed to gain greater understanding of coral-reef food-webs. Detecting mixed-

feeding patterns is sometimes difficult. For example, many microbial autotrophs are mixed with algae and detritus, and some diet data will underestimate contributions to an individual's nutrient requirements. There are many existing methods to analyse fish diet (Table 1.1). By observing feeding activities, diet can be broadly determined, for example by recording bites. Yet many production sources on the reef substrate are mixed with each other (e.g. EAM), and some fish might ingest a large chunk but only assimilate certain components which might not be visible (e.g. microbial autotrophs). Such methods therefore often miss important dietary details. Gut content analyses might include components ingested by accident, need huge sampling effort due for example to high spatial and temporal variability of diets (Jennings *et al.*, 2001a), and require identification of different items such as well digested prey and microbes. DNA barcoding can help; processing time is significantly reduced and accuracy of identification to species level is enhanced. Again, this method will not differentiate assimilated from unassimilated items or among mixed sources and may include environmental DNA (eDNA) which pervasively exists in the environment but is not fed by fishes. The procedures and type of primer used to extract and identify DNA can also affect the dietary interpretation; for example, a particular grinding strength can expose DNAs of some components but not others. Excessive grinding strength might release the DNAs of hard-shell components (e.g. diatoms) but also destroy the DNAs of some others. Through the PCR process, some DNA is amplified while some is not. DNA barcoding is also used to identify hindgut digestive symbionts to link back to the diet components (mainly for algivores) since many of the diet-digestive symbiont relationships are species-specific (Ngugi *et al.*, 2017) and relatively stable across individuals. However, this method might ignore food items which require no digestive symbionts. These gut contents methods require significant sampling efforts and sample sizes due to high spatial and temporal variation and the fact that only short-term dietary information can be obtained.

Techniques using bio-tracers such as fatty acids [FAs] and stable isotope ratios (the ratio between heavy and lighter isotopes) have several strengths for analysing diet. These methods derive a mean weighted and time-integrated signature of assimilated food items at a timescale depending on the turnover rate of the tissue analysed (Tieszen *et al.*, 1983). Fewer samples are needed than with gut contents data but both potential sources and consumers of interest must be collected. By comparing the values of bio-tracers, diet compositions are approximated. However, such methods are not as detailed as gut contents due to that the origins of these

biochemical compounds cannot be identified down to species level, thus, applying them to understand diet requires prior knowledge based on gut-contents.

Different bio-tracers trace differential compounds thus discriminability among production source types might differ depending on the bio-tracer used (Rooker *et al.*, 2006; Hanson *et al.*, 2010; De Troch *et al.*, 2012; Larsen *et al.*, 2013; McMahon *et al.*, 2016). The number of bio-tracers or determinants (FAs: total 37 [saturated, monounsaturated, polyunsaturated, highly unsaturated, branched and odds FAs], bulk stable isotopes: two [$\delta^{13}C$ and $\delta^{15}N$], $\delta^{13}C$ of FAs: varied among diet studies [can include both essential and non-essential], $\delta^{13}C$ of essential amino acids (EAAs): five principally) potentially affects the accuracy of the analytical results (Phillips and Gregg, 2001). Also, samles are preserved and treated differently. The FAs require samples to be frozen throughout the process and total lipid extraction. In the FA group analysis, FA composition data are used, whereas in FA stable isotope analysis, $\delta^{13}C$ data of selected FAs are used with trophic enrichment factors for every FA (Monson and Hayes, 1982; Uhle *et al.*, 1997b; Howland *et al.*, 2003; De Troch *et al.*, 2012). Stable isotope samples may be frozen or otherwise preserved (e.g. drying), and acidification, lipid extraction or urea removal may be needed.

Stable isotope analysis has two types: bulk and compound-specific stable isotope analyses. Bulk stable isotope analysis derives values of all organic components of a sample whereas compound-specific uses a special column in the gas chromatography and temperature settings to elude different compounds, and analyse their stable isotope values individually (e.g. $\delta^{13}C$-EAAs are only from the EAAs). Compared with bulk stable isotope data, the $\delta^{13}C$-EAA values of primary producers are more discriminable and more accurately recorded in the tissues of consumers with minimal $\delta^{13}C$ enrichment (Uhle *et al.*, 1997b; Howland *et al.*, 2003; McMahon *et al.*, 2010; Larsen *et al.*, 2013; Larsen *et al.*, 2015; McMahon *et al.*, 2016). Yet to isolate these compounds from the bulk material, extensive work (e.g. hydrolysis, elution and derivatisation) and costs are entailed. Nevertheless, both bulk $\delta^{13}C$ and $\delta^{15}N$ and $\delta^{13}C$-EAA data have potential strengths (e.g. moderate sample size, long-term dietary information) for elucidating trophic niches and pathways in the complex setting of coral-reef food-webs.

1.7 Bio-tracer diet analysis

1.7.1 Analysis approaches

The concept of isotopic niches has increasingly helped understanding of the trophic ecology of fish at species, genus or trophic guild levels and of their source partitioning. $\delta^{13}C$ and $\delta^{15}N$ values for example can indicate spatial and temporal patterns of resource utilization or width of trophic niches (Bearhop *et al.*, 2004) and potentially indicate feeding ecology, prey preferences and habitat choice (Leibold, 1995) within populations (Newsome *et al.*, 2007) and could be related with morphological traits such as gape or body size (Scharf *et al.*, 2000). However, uncertainty in these relationships is influenced by a number of factor including diet composition, isotopic variation among food sources (Matthews and Mazumder, 2004), and factors affecting assimilation (DeNiro and Epstein, 1981). The same apparent niche might result from different feeding pathways (Layman *et al.*, 2007a). Jackson *et al.* (2011) identified shortcomings including sensitivity to sample sizes (see also Podani, 2009) and lack of data on natural variability and further suggested Stable Isotope Bayesian Ellipses in R (SIBER) to define trophic niches where the width is based on the Standard Ellipse Area (SEA). This method has been applied in coral reefs to understand trophic interactions among certain groups such as species (Layman and Allgeier, 2012; O'Farrell *et al.*, 2014) and trophic guilds (Morillo-Velarde *et al.*, 2018), yet no study has analysed trophic interactions among trophic guilds in a single community which potentially addresses questions such as community source utilisation, trophic interaction among guilds and trophic redundancy (e.g. herbivores, Plass-Johnson *et al.*, 2015). Combining trophic guild- and species-level data can further help identify species crossing their trophic boundaries.

Finer dietary details are often needed to analyse trophic interactions and community structure. To quantify the diet, mixing models such as MixSIR (Moore and Semmens, 2008), SIAR (Parnell *et al.*, 2010) and MixSIAR (Stock and Semmens, 2016) are used which employ source and consumer stable isotope values and trophic enrichment factors (TEFs, which are the difference in stable isotope values between the diet and the consumer). For the model to work appropriately, all potential sources should be included and they should be isotopically discriminable. However, the diversity of food sources and low discriminability among these (especially in bulk stable isotope) limit the accuracy of diet analysis that the diet resolution is commonly at production source type level. Yet no study has analysed

the isotopic discriminability among production source types. The mixing model also requires appropriate TEFs (see below). In MixSIAR, prior information can be set with sufficient evidence, sufficient iterations are needed to ensure a model converges, and appropriate error structure (process and/or residual error) must be selected.

Using bulk data in the mixing model, TEFs are potentially variable depending on the system, location, energy pathway and species (DeNiro and Epstein, 1981; McCutchan *et al.*, 2003; Mill *et al.*, 2007; Strieder Philippsen and Benedito, 2013; Hussey *et al.*, 2014; McMahon *et al.*, 2015). Several studies applied pathway-specific TEFs from existing literature to analyse fish diet for herbivores (Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015), yet how accurate these values are to their specific systems remains unclear. Also, some reef production sources are similar in terms of their bulk stable isotope signatures (e.g. Pinnegar and Polunin, 2000), thus sensitivity analysis of the results to differential discriminability of sources and TEFs is needed.

Amino acids (AAs) are one of the most studied biochemical tracers in organic geochemistry (Larsen *et al.*, 2015), and contribute the more labile part of the bulk organic matter (Cowie and Hedges, 1994) in both plankton and sinking POM (Lee *et al.*, 2000; Hedges *et al.*, 2001). Due to the labile property, AA degradation such as through microbial processes can change AA compositions (Grutters *et al.*, 2002; Lomstein *et al.*, 2006). Early studies revealed the variation of stable isotope signatures of AAs was due to the kinetic isotope effect associated with both specific enzyme-catalysed biosynthetic pathways and metabolic branching ratios (Macko *et al.*, 1987; Uhle *et al.*, 1997a). These unique biochemical processes of EAA synthesis result in distinctive δ^{13}C-EAA values among different producers such as algae and bacteria (Larsen *et al.*, 2013) and potentially provide better source discriminability than bulk stable isotope data (Larsen *et al.*, 2015). Most animals (e.g. fish) cannot synthesize EAAs, thus, they must acquire EAAs directly from the source(s) with little modification in δ^{13}C-EAA values (McMahon *et al.*, 2010; Larsen *et al.*, 2013), resulting in δ^{13}C-EAA values being carried along food chains with minimal fractionation (McMahon *et al.*, 2015). Using δ^{13}C-EAA data in diet analysis potentially resolves the two limitations posed by bulk stable isotope data in dietary studies by improving discriminability among sources and eliminating uncertainties in trophic fractionation. By analysing δ^{13}C-EAA data and their patterns, pathways and food sources have became better resolved and more easily for example among C3 or C4 producers (Uhle *et al.*, 1997a; Hobbie and Werner, 2004), and among terrestrial,

Table 1.1 Comparison of existing diet analysis methods in the time span of diet, number of samples required for the analysis, time to process samples and data, variability, resolution (to which level diet components and size [+] can be identified) and other limitations.

Method	Variant	Diet time span	Sample number	Process time	Variability	Resolution	Limitations
Behavioural observation		Short	High	Short	High	Low	High sampling effort, individual tracking, diet composition may not be well characterised and quantified
Gut content		Short	High	Long	High	Medium+	High sampling effort, mortality of individual, highly digested/small items, by-ingestion
DNA barcoding	Gut contents	Short	High	Short	High	High	High sampling effort, high mortality, eDNA, process procedures, size of prey
	Hindgut microbes	Long	Medium	Short	Low	High	Some contents (that need digestive symbionts)
Biomarker (both source and consumer)	Fatty acids	Long	Low	Medium	Low	Medium	Sample preservation, only FAs, size of prey
	Bulk $\delta^{13}C$ and $\delta^{15}N$	Long	Low	Medium	Low	Medium+	Source resolution (only two determinents), trophic enrichment factors, all biochemical contents in the sample
	$\delta^{13}C$ of fatty acids	Long	Low	Long	Low	Medium	Sample preservation, trophic enrichment factors, costly, time consuming, only FAs, size of prey
	$\delta^{13}C$ of essential amino acids	Long	Low	Long	Low	Medium	Costly, time consuming, only EAAs, size of prey

aquatic and microbial sources (Chikaraishi and Naraoka, 2003; Larsen *et al.*, 2013; McMahon *et al.*, 2016). Specifically in coral-reef food-webs, McMahon *et al.* (2016) found that δ^{13}C-EAAs from sampled baseline sources (plankton, macroalgae, coral and detritus) were both isotopically diagnostic and accurately recorded in their direct consumer tissues and microbially reworked detritus provided important secondary carbon source for most species. Yet, there are other important producers in this food web such as sponges, turf algae and cyanobacteria which were not examined in their discriminability or diet contribution in McMahon *et al.* (2016). However, as the first application of δ^{13}C-EAA data in a coral-reef food-web, this study points to future directions of this high resolution trophic analysis in such diverse food webs.

Different indications using these bio-tracers (i.e. FAs, bulk δ^{13}C and δ^{15}N, δ^{13}C-FAs and δ^{13}C-EAAs) are expected as a result of the varied pathways of breaking down and synthesizing biochemical compounds. The differences might potentially address finer utilisation of specific nutrients. For example, through tracer experiements with bulk δ^{15}N, it has been shown that some sponges utilise DOM more for respiration and plankton for production (de Goeij *et al.*, 2017). These two source types differ in nutrient composition, yet little is known which components caused the different assimilation strategies. Tracing specific compounds might resolve this (paper in preparation).

1.7.2 Trophic position estimation

Stable isotope data are also used to estimate TP. In a single-source food web, an individual's TP can be calculated using the isotopic difference between it and the baseline of the food web, given a TEF ($TP_{fish} = TP_{base} + \frac{\delta^{15}N_{fish} - \delta^{15}N_{base}}{3.4}$). δ^{15}N is considered a better proxy for TP than δ^{13}C due to its significant range (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981), large TEF and less variation at the baseline (Hesslein *et al.*, 1991). However, TP estimation requires appropriate TEF values. Currently, constant, scaled and pathway-specific TEF values are being used. In the constant framework, the TEF value ($\Delta\delta^{15}$N = 3.4‰, DeNiro and Epstein, 1981) is the same for all trophic levels and energy pathways. In the scaled framework, the TEF varies to allow for assimilation differences at different TPs (Hussey *et al.*, 2014). In the pathway-specific framework, the TEF is set by production source types depended on. For example, herbivore $\Delta\delta^{15}$N may be higher than 3.4‰ (Mill *et al.*, 2007) or lower (Plass-Johnson *et al.*, 2013), and may be affected by location. However, these TEFs only work for strict feeders with known diet; for species with

highly mixed diets, TP values can be derived from the R package tRophicPosition (Quezada-Romegialli *et al.*, 2018) if there are only two distinctive source isotopic baselines.

TEFs are also one of the uncertainties in estimating mean PPMR especially in the constant framework (Jennings *et al.*, 2002c). From the scaled framework, mean PPMR value can be estimated more accurately (Hussey *et al.*, 2014; Reum *et al.*, 2015) and can provide a better basis for inter-systems comparison. The PPMR estimation accuracy might be further improved using pathway-specific TEFs, however, there is no published methodology for this at present.

1.8 Geography

Globally, most coral reefs are impacted by multiple stressors (Ban *et al.*, 2014) which have resulted in degradation of both the ecosystem and specifically fish communities (Wilson *et al.*, 2006; Graham *et al.*, 2017). The diverse Caribbean reef-forming corals and associated reef fish of the early 1980s have been structurally degraded by overfishing and global warming (Alvarez-Filip *et al.*, 2009), leaving simplified coral-reef fish communities (Acosta-González *et al.*, 2013; Alvarez-Filip *et al.*, 2015; Cruz *et al.*, 2015) and coral-algal phase shift (Hughes, 1994). Similar reef-fish community structure changes have occurred in the west Caribbean (Rogers *et al.*, 2014), the Pacific (Wilson *et al.*, 2008; Wilson *et al.*, 2010; Williams *et al.*, 2015; Robinson *et al.*, 2016) and the Indian Ocean (Graham *et al.*, 2007). With recent bleaching events in the Pacific (Sheppard, 2003; Perry and Morgan, 2017; Stuart-Smith *et al.*, 2018), adequate management strategies are needed, and these demand improved scientific understanding, which is the goal of this thesis.

1.9 Objectives of the thesis

In this thesis, I aim to better understand coral-reef fish community trophic structures and trophic plasticity in two locations (Bahamas [Chapter 2] and Maldives [Chapter 3-6]) and fine scale food source partitioning of coral-reef fishes using Maldivian stable isotope data (Figure 1.3).

Many coral-reef fishes feed outside their putative trophic guilds and very little attempt has been made to use stable isotope data to explore such trophic plasticity. In Chapter 2, I used bulk $\delta^{13}C$ and $\delta^{15}N$ data to explore the trophic plasticity of major coral-reef fishes and understand the interaction among trophic guilds in the Bahamas. In Chapter 3, this was futher applied in detail using the Maldivian data to demonstrate that many fishes were more omnivorous than previously thought and

fishes categorised as the same guild can rely on different production sources of the same type.

Much of the trophic plasticity is related with body size, I applied $\delta^{15}N$ and body mass data to explore TP-omnivory within fish species in both locations (Chapter 2 and 4) and to further prove that trophic pathways and some family-level traits can cause different $\delta^{15}N$-body mass relationships among species (Chapter 4).

My work in the Bahamas and Maldives showed that in many cases the trophic categorisations commonly applied to coral-reef fishes were often simplistic, if not incorrect. Therefore, I analysed non-size related trophic plasticity of several strict feeder fishes using bulk $\delta^{13}C$ and $\delta^{15}N$ data (Chapter 5) and ^{13}C-EAA data (Chapter 6) with improved analytical accuracy.

Because the positive community $\delta^{15}N$-$\log_{10}$ body mass relationship has had important implications for understanding other marine ecosystems, but has scarcely been studied in coral reefs, I used species-level $\delta^{15}N$-body mass and underwater visual census data to explore such relationship in the Bahamas (Chapter 2), and to test if the same linear positive relationship found in the Bahamas and other aquatic systems existed in a more intact and diverse coral reef system in the Maldives (Chapter 4).

In Chapter 7, I summerised the main findings of the thesis of individual chapters and related topics across chapters, addressed potential limitations of the studies and possible improvements, and shared opinions on where these researches can potentially lead to in a wider context.

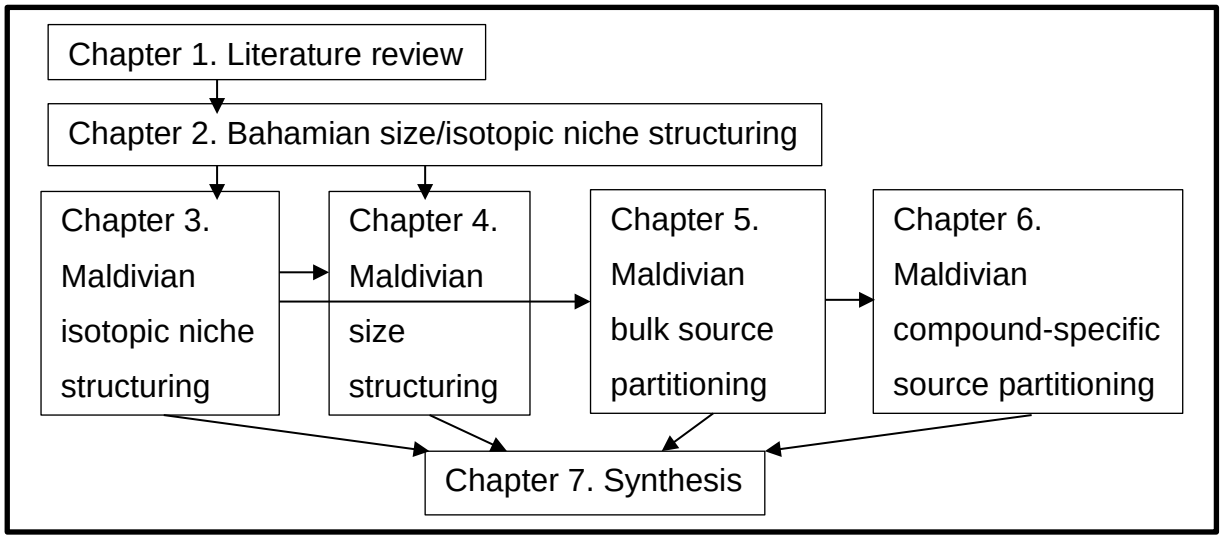


Figure 1.3 Illustration of the thesis structure

Chapter 2. Size structuring and trophodynamics of a Bahamian coral-reef fish community*

2.1 Introduction

In size-structured food-webs, numeric abundance (N) can scale negatively with body mass (M) due to energetic constraints (Paul and Christensen, 1995; Brown and Gillooly, 2003; Trebilco *et al.*, 2013) where $N \propto M^{-0.75}$ with a common energy source and $N \propto M^{-1.2}$ in a multi-energy source ecosystem (Brown and Gillooly, 2003; Jennings and Mackinson, 2003). Size structuring can provide understanding of energy fluxes (Jennings *et al.*, 2002c; Rooney *et al.*, 2008), population dynamics, predator-prey interaction (Kingsford, 1992; Cohen *et al.*, 1993; Boyle and Pierce, 1994; Barnes *et al.*, 2010), food-web structure and function (Brose *et al.*, 2006), secondary production (Banse and Mosher, 1980; Jennings *et al.*, 2002c) and ecosystem stability (Jennings *et al.*, 2001a; Post, 2002a). In aquatic food-webs, size-based feeding exists where large individuals generally feed at higher trophic positions (Jennings *et al.*, 2002b; Romanuk *et al.*, 2011). This is a result of ontogenetic dietary shifts, morphometric changes including increasing gape size and post-maturity factors that influence foraging (Jennings *et al.*, 2001a; Jennings *et al.*, 2002b; Mumby *et al.*, 2006; Al-Habsi *et al.*, 2008; Robinson and Baum, 2015). Body size alone does not constrain the trophic roles of fish (Jennings *et al.*, 2001a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008) because of multi-food chain feeding (Layman *et al.*, 2005), seasonal variation of pelagic production sources (Bronk and Glibert, 1993; Rolff, 2000), human disturbances (Graham *et al.*, 2017) and complex mixed-diet effects (e.g. ontogenetic change, migration; Bode *et al.*, 2006) and for some species their trophic positions remain relatively unchanged (e.g. strict herbivores).

In coral reef systems, size structuring of fish communities is understudied. Size spectra (SS) or abundance (N)-body size (total length, L) relationships have been used to describe fish community structure in relation to fishing pressure (Dulvy *et al.*, 2004b; Graham *et al.*, 2005; DeMartini *et al.*, 2008; Robinson *et al.*, 2016), coral bleaching (Graham *et al.*, 2007) and habitat structural complexity degradation where N normally scales negatively with L (Alvarez-Filip *et al.*, 2011; Zhu *et al.*, unpublished data). However, SS lack sensitivity in depicting community composition changes towards certain stressors (Graham *et al.*, 2017) and detecting species with ontogenetic dietary and functional role shifts (Plass-Johnson *et al.*, 2013; Dromard *et*

*Resubmited to Marine Biology

al., 2015). Coral reef-fish food-webs are supported by multiple production sources (both local and pelagic) and consumers of each production source may demonstrate specific abundance-body mass (M) relationships according to metabolic theory (Brown and Gillooly, 2003).

Investigating the functional roles of individuals within the community as well as the whole community by examining trophic position (TP)-body size relationships (size-based trophic structure) can improve understanding of size structuring, especially predator to prey relationships and energetic pathways (Romanuk *et al.*, 2011; Robinson and Baum, 2015). Stable isotopes can be used to estimate TP with less variability than gut content studies because stable isotopes provide a time-integrated signal of what has been assimilated from the diet (Jennings *et al.*, 2001a). The result is that complex trophic interactions can be captured (Post, 2002b) at both species and community levels (France *et al.*, 1998; Jennings *et al.*, 2001a; Jennings *et al.*, 2002a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008). The stable isotope ratio of nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$) is used as a proxy for TP because it has higher trophic enrichment factor (TEF) between predators and prey (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; McCutchan *et al.*, 2003; Strieder Philippsen and Benedito, 2013) and less variation at the baseline (Hesslein *et al.*, 1991) compared to carbon (^{13}C:^{12}C, expressed as $\delta^{13}C$) and sulphur stable isotope ratios (^{34}S:^{32}S, expressed as $\delta^{34}S$). Yet, there are few applications where stable isotope analyses have been used on coral reefs to look at the size structuring and trophodynamics of the fish community.

In aquatic systems, the predator-prey mass ratio (PPMR) can reflect constraints on community structure (Trebilco *et al.*, 2013) and can be used to evaluate general food-web properties such as food chain length and stability (Jennings *et al.*, 2002c; Barnes *et al.*, 2010). On an individual level, gut contents studies showed PPMR increased with predator body mass and TP, and decreased with transfer energy efficiency (Barnes *et al.*, 2010). On a community level, smaller mean PPMR values can indicate longer food chains (Jennings and Warr, 2003). PPMR may vary ten-fold among different aquatic systems (Jennings *et al.*, 2001a; Jennings *et al.*, 2002c; Bode *et al.*, 2003; Bode *et al.*, 2006; Al-Habsi *et al.*, 2008). Uncertainties in estimating the PPMR mainly come from the assumed nitrogen fractionation factor ($\Delta\delta^{15}N$) (Jennings *et al.*, 2002c; Reum *et al.*, 2015). Using $\Delta\delta^{15}N$ values that are scaled with TP affects PPMR estimation and a scaled fractionation

framework is thought to provide more accurate PPMR values and improve the basis for inter-systems comparison (Hussey *et al.*, 2014; Reum *et al.*, 2015).

Trophic structure can reveal the mean TPs of different size classes in local food-webs, however, species with similar mean TP values might have different trophic roles in their communities. Thus, combining $\delta^{15}N$ and $\delta^{13}C$, where $\delta^{13}C$ provides a signal for different food sources due to its high variability among food sources (Tieszen *et al.*, 1983), can delineate 'isotopic niches' (Newsome *et al.*, 2007) which, potentially describe resource utilization such as feeding ecology, prey preferences and habitat choice (Leibold, 1995) and could be determined by proxies such as mouth and body size (Scharf *et al.*, 2000). $\delta^{13}C$-$\delta^{15}N$ bi-plots can help to depict isotopic/trophic niches (Bearhop *et al.*, 2004) and feeding strategies within populations (Newsome *et al.*, 2007).

Here, underwater visual census and stable isotope data were used to explore the role of body size and trophic interactions in structuring a coral-reef fish community at Cape Eleuthera in the west Caribbean. Specifically, the study aims to: 1) examine whether the fish community shows size structuring through abundance-body size relationships (size spectra); 2) examine trophic niches at species andt rophic guild levels in order to understand energy pathways; 3) assess whether there are $\delta^{15}N$-body mass relationships at the species and community levels; and 4) examine predator-prey biomass ratios.

2.2 Materials and methods

2.2.1 Study site

Four accessible and conservation-protected reef sites (Figure 2.1, Table 2.1) on the Exuma side of Cape Eleuthera (the Bahamas) with relatively high structural complexity and diverse fish community were selected for visual surveys and fish sampling. These sites were close to each other and to shore, and they represented both bommies and patch reefs. Only scuba training and academic research are legally allowed at the sites which are monitored by Cape Eleuthera Marina and the Island School.

2.2.2 Survey and sample collection

2.2.2.1 Fish survey

Underwater visual census (UVC) was conducted by two divers (J. Atherton and Y. Zhu) using eight single-sweep 30 m x 5 m transects at all four sites to record

fish species, individual total lengths (L, to nearest cm) and numbers of individuals. Surveyors' estimation precision was repeatedly measured by conducting underwater fish-shaped object length estimation training (Bell *et al.*, 1985) to minimize error (± 5%). Transects ran parallel to each other to avoid intersection. Transects were carried out in the morning (0930-1130 hrs) or afternoon (1400-1600 hrs), while swimming at a steady speed for 30 minutes.

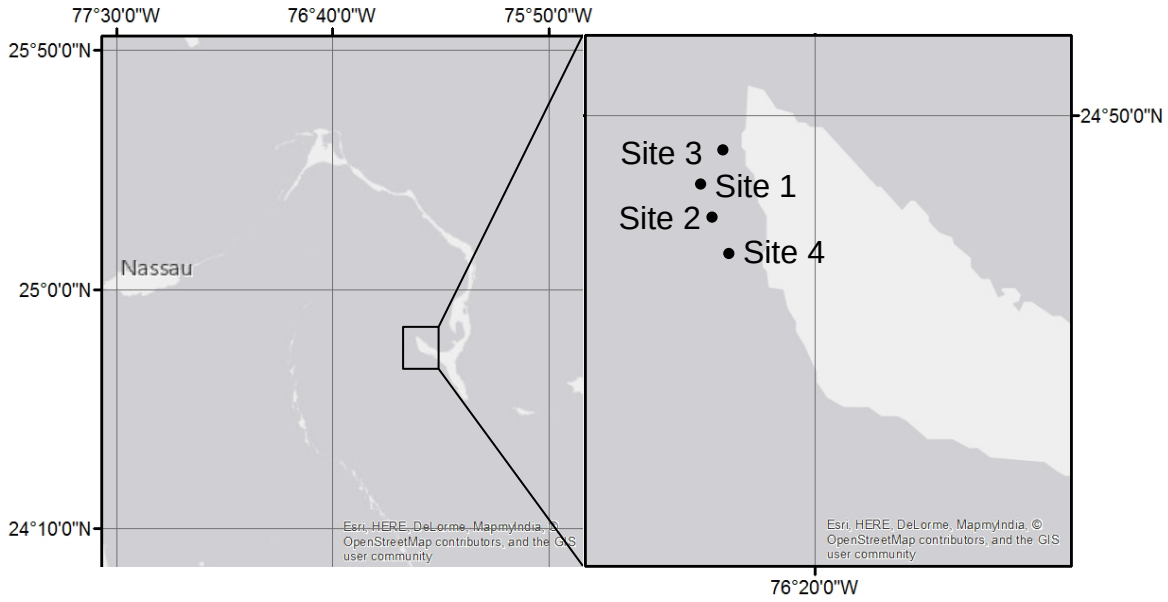


Figure 2.1 Map of survey sites at Cape Eleuthera (the Bahamas).

Table 2.1 Site information including location, reef type, survey method, and fishing method.

Site name	Location	Reef type	Survey method	Fishing method
Tunnel Rock (Site 1)	24°48'54.44"N 76°20'57.92"W	Large bommie; average depth of 12 m	UVC with 30 m x 5 m transects, 8 transects per site	Underwater fishing, gill net, and from fishermen
Cathedral (Site 2)	24°48'43.34"N 76°20'53.39"W	Large bommie; average depth of 11.6 m		
Some2C (Site 3)	24°49'4.72"N 76°20'53.03"W	Spread of coral patches; some small coral colonies, max. depth 6 m		Underwater fishing, gill net, BINCKE net, and from fishermen
Ike's Reef (Site 4)	24°48'25.54"N 76°20'38.01"W	Spread of coral patches; max. depth 5.5 m, drop to 2000 m		

Fish species were categorized as 'resident', 'wandering' (large home range, such as sharks and rays, often sighted gliding on top of reefs or resting underneath rocks or reefs), 'visiting' (feeding on reef only: pelagic fish such as Carangidae spp)

or forming large schools (such as Atherinidae spp). Those wandering and resting individuals were excluded since they were randomly spotted and not necessarily reef-associated, and also large schools of fishes since they were sighted only sporadically (Ferreira *et al.*, 2001).

2.2.2.2 Sampling for stable isotope analysis

Species composing 80% of the total biomass of each 5 cm L interval were selected for stable isotope analysis of the community trophic structure (Appendix 1). Body mass in g (M) was calculated from L using Equation i with published length to weight conversion factors *a* and *b* (Appendix 2; Froese *et al.*, 2014). For some species, conversion factors were linked with standard length/fork length rather than L, or L in units other than cm, thus L was converted into appropriate units or length types to comply with the conversion factors involved.

$$M = a \times L^b \qquad \text{Equation i}$$

Samples of selected species were collected through the size range recorded in UVC to adequately describe species δ^{15}N-log$_2$M relationships (Galván *et al.*, 2010). Size range cover ratio (r_L = $L_{SIA\ sample}$ range / L_{UVC} range) was used to check whether the sampling objective was met. Fish were collected using a variety of techniques depending on a species behaviour towards divers, feeding habits and swimming patterns. Hand net, 1 cm x 1 cm gill net, BINCKE net (Anderson and Carr, 1998), underwater fishing hook and line, static hook and line, spearfishing (local fishermen only) and hook and line surface trolling were all used in the sampling (Table 2.2). Fish were killed by spine dislocation in accordance with UK Home Office Scientific Procedures (Animals) Act and stored in an ice chest on board. After landing, approximately 2 g of dorsal white muscle tissue near the dorsal fin were dissected, rinsed with water and stored in individual whirlpack bags in a -20 °C freezer. All samples were dried in individual aluminium trays in an oven at 40 °C for ~12 h until fully dried, and then in individual sealed Eppendorf tubes in zip-lock bags.

At the four survey sites, benthic cover was dominated by the macroalga *Dictyosphaeria cavernosa* (> 70% of total living organism cover) which was fed on by most grazer species (e.g. Scarinae spp, fish observation data, D. Ruddock). Other producers were either constrained by time and equipment (e.g. plankton, detritus) or protected from sampling (e.g. hard corals), thus, only this alga was collected as a benthic food-web baseline. Samples were all collected within a one month period and from nearby sites to minimize large scale temporal isotopic variation which might

affect food-web baselines (Bronk and Glibert, 1993; Jennings *et al.*, 1997; Rolff, 2000; McCutchan *et al.*, 2003).

Table 2.2 List of fishing methods and targeting species. J: juvenile, A: adult, for codes see Table 2.3.

Fishing method	Targeting species	Examples of species
Hand net	Slow, approachable, small, disguising, territorial	Gobies, damselfish, RDL, ATT
Gill net	Fast, alert, non-thread shaped, schooling	J. Scarinae, A. labrids, J. haemulids, pomacentrids
BINCKE net	Fast swimming, swimming close to the ground, schooling	J. Scarinae, labrids
Underwater fishing	Large bodied, fast swimming, cryptic, smart, alert	A. haemulids, LSQ, some Scarinae, lutjanids, grouper
Line and hook	Pelagic, fast, alert	Carangids, lutjanids
Spearfishing	Fast, aggressive	A. Scarinae, acanthurids, RDL
Trolling	Pelagic, fast swimming	Carangids, GRB

2.2.2.3 Stable isotope analysis preparation

All dried samples were transported to Newcastle University under DEFRA permit TARP/2015/210, frozen and freeze dried, then ground with mortar and pestle, weighed to approximately 1.0 ± 0.1 mg in tin capsules with a Mettler MT5 microbalance, pelletized and stored in trays. The prepared samples were analysed by Iso-Analytical Ltd (Crewe, UK) by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS). The ^{15}N:^{14}N ratio ($\delta^{15}N$) was expressed relative to N_2 in air for nitrogen while that of ^{13}C:^{12}C ($\delta^{13}C$) was relative to Pee Dee Belemnite (PDB) of CO_2. Reference material used for this analysis was IA-R042 ($\delta^{13}C$ = -21.60 ± 0.05‰, $\delta^{15}N$ = 7.60 ± 0.06‰), with quality control check samples IA-R042, IA-R038 ($\delta^{13}C$ = -25.03 ± 0.09‰, $\delta^{15}N$ = -0.40 ± 0.13‰), a mixture of IA-R006 ($\delta^{13}C$ = -11.71 ± 0.03‰) and IA-R046 ($\delta^{15}N$ = 21.87 ± 0.21‰). IAR042 and IA-R038 were calibrated against and traceable to IAEA-CH-6 ($\delta^{13}C$ = -10.43‰) and IAEA-N-1 ($\delta^{15}N$ = 0.40‰), IA-R006 to IAEA-CH-6 and IA-R046 to IAEA-N-1. External standards (fish white muscle tissue, $\delta^{13}C$ = -18.87 ± 0.04‰, $\delta^{15}N$ = 12.94 ± 0.12‰) were also used for future reference. The precision of analysis for $\delta^{13}C$, $\delta^{15}N$, %C and %N was ±0.10‰, ±0.20‰, ±4% and ±1%, respectively. For individual samples, no lipid extraction was needed due to their total carbon to nitrogen ratios (C:N was determined from molar corrected elemental % data) were less than 3.7 (Fry *et al.*, 2003; Sweeting *et al.*, 2006).

2.2.3 Data analysis

All data were tested for normality and homogeneity of variance prior to analysis and analysed in R 3.24 (R Core Team, 2016) using linear regression (Wilkinson and Rogers, 1973; Bates *et al.*, 1992), the packages ggplot2 (Wickham and Chang, 2016) and siar (Parnell and Jackson, 2013). The assumptions of the ordinary least squares linear regression analyses were assessed using QQ plots, histograms of standardised residuals and plots of standardised residuals verses fitted values. Pearson's correlation coefficient was used to test correlations among variables. Significance was set at $p = 0.05$ in all cases. All errors are reported as ± 1SE unless otherwise stated.

2.2.3.1 Abundance-body size relationship (Size spectrum)

Fish counts of all eight transects of each site were summed (abundance, N) per 5cm total length (L) interval (Dulvy *et al.*, 2004b; Graham *et al.*, 2005). Linear regression was performed on individual sites' and the combined $\log_{10}(N+1)$ against L. Values for the intercept, slope and midpoint height were derived (Trenkel and Rochet, 2003).

2.2.3.2 Species and trophic guild stable isotope analyses

The bulk $\delta^{13}C$ and $\delta^{15}N$ data were used to interpret $\delta^{13}C$-$\delta^{15}N$ relationships in each species and trophic guilds. Isotopic niches of five trophic guilds, which included four benthic trophic guilds (benthivore, herbivore, omnivore and piscivore) and one pelagic trophic guild (planktivore), were investigated using SIBER from the package siar. This was achieved by calculating sample size corrected standard ellipse areas (SEA_C), standard ellipse parameters: eccentricity (E) and the angle in degrees (θ, 0° to 180 °) between semi-major axis and the x-axis, and Bayesian SEA (SEA_B) (Jackson *et al.*, 2011). θ and E values potentially to distinguish among isotopic niches where different species or trophic guilds have similar sized standard ellipses but different relationships between $\delta^{13}C$ and $\delta^{15}N$ (Reid *et al.*, 2016). θ values close to 0° or 90° suggest dispersion in only one axis: θ values close to 0° represent relative dispersion along the x-axis ($\delta^{13}C$), indicating multiple production sources, while θ values close to 90° highlight relative dispersion along the y-axis ($\delta^{15}N$), indicating

Table 2.3 List of sampled species of reef fish at Cape Eleuthera (the Bahamas), with scientific name, common name, species code, trohpic guild (Froese and Pauly, 2017), maximum total length (L_{max}) (Humann and DeLoach, 1989), UVC total length range, mean trophic position ± SE (Froese and Pauly, 2017), SIA sample size (*n*), SIA sample total length range, size range cover ratio r_L, mean $\delta^{15}N$ and $\delta^{13}C$ ± SE. *species sampled to represent other uncollected species on the list.

Scientific name	Common name	Code	Trophic guild	L_{max} (mm)	UVC L (mm)	Trophic position	*n*	SIA sample L (mm)	r_L	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Acanthurus chirurgus	Doctorfish	DCF	Herbivore	381	40–100	2.1±0.1	1	280	0%	5.60	-12.36
Acanthurus coeruleus	Blue tang	BLT	Herbivore	381	30–380	2.0±0.0	3	81–168	24.86%	4.71±0.25	-16.50±0.84
Acanthurus tractus	Ocean surgeonfish	OCF	Herbivore	381	90–210	2.0±0.0	9	84–280	100%	5.10±0.22	-13.64±0.50
Aulostomus maculatus	Atlantic trumpetfish	ATT	Piscivore	914	240–430	4.3±0.6	2	145–530	100%	5.92±0.63	-14.90±0.24
*Balistes vetula**	Queen triggerfish	QUT	Benthivore	610		3.8±0.1	4	320–419	N.A.	7.72±0.10	-12.36±0.30
Calamus pennatula	Pluma porgy	PLP	Benthivore	381		3.7±0.2	4	278–300	N.A.	8.20±0.28	-11.04±0.55
Caranx ruber	Bar jack	BAJ	Piscivore	610	190–450	4.3±0.1	5	273–392	45.77%	7.89±0.08	-13.16±0.52
Cephalopholis cruentata	Graysby	GSB	Piscivore	305	60–220	4.3±0.6	8	103–295	73.13%	8.35±0.24	-13.88±0.44
Chromis cyanea	Blue chromis	BLC	Planktivore	127	10–130	3.7±0.4	7	54–87	27.50%	5.23±0.10	-17.02±0.12
Clepticus parrae	Creole wrasse	CRW	Planktivore	305	10–250	3.4±0.2	8	71–113	17.50%	5.39±0.15	-17.22±0.09
Coryphopterus glaucofraenum	Bridled goby	BDG	Herbivore	64		2.7±0.4	1	34	N.A.	3.89	-10.92
Coryphopterus personatus	Masked/Glass goby	MGG	Herbivore	38	10–30	2.9±0.4	3	14–34	80.00%	3.99±0.08	-16.47±0.40
Elacatinus genie	Cleaning goby	CLG	Parasitivore	44	10–40	3.4±0.3	2	25–32	23.30%	8.92±0.78	-12.62±1.29
Epinephelus guttatus	Red hind	RDH	Benthivore	610	100–270	3.8±0.3	5	212–336	34.12%	7.89±0.18	-11.66±0.34
Epinephelus striatus	Nassau grouper	NSG	Piscivore	1219	250–600	4.1±0.0	6	361–423	17.71%	8.70±0.15	-11.94±0.21
Gramma loreto	Fairy basslet	FRB	Planktivore	76	10–80	3.3±0.4	4	22–35	18.57%	4.55±0.12	-16.99±0.17
Haemulon album	Margate	MGT	Benthivore	762	200–320	3.1±0.1	5	321–419	0%	7.45±0.18	-10.08±0.27
Haemulon flavolineatum	French grunt	FRG	Benthivore	305	50–180	3.5±0.1	9	96–233	64.62%	6.99±0.19	-10.85±0.26
Haemulon plumierii	White grunt	WTG	Benthivore	457	150–350	3.8±0.0	4	231–300	34.50%	8.10±0.17	-12.22±0.11
Haemulon sciurus	Bluestriped grunt	BSG	Benthivore	457	210–350	3.5±0.2	1	275	0%	7.45	-13.98

Halichoeres bivittatus	Slippery dick	SLD	Benthivore	229	30–100	3.8±0.1	2	120–133	0%	6.13±0.056	-10.64±0.22
Halichoeres garnoti	Yellowhead wrasse	YHW	Omnivore	203	10–190	3.7±0.2	5	36–135	55.00%	6.22±0.26	-13.66±0.33
Halichoeres maculipinna	Clown wrasse	CLW	Benthivore	165	50–140	3.3±0.2	1	117	0%	6.69	-13.72
Halichoeres pictus	Rainbow wrasse	RBW	Benthivore	76	10–100	3.5±0.3	4	16–30	15.56%	4.54±0.12	-17.43±0.15
Holacanthus ciliaris	Queen angelfish	QUA	Herbivore	457	140	3.0±0.0	5	195–325	0%	5.83±0.21	-14.76±0.36
*Holocentrus adscensionis**	Squirrel fish	SQF	Benthivore	406		3.5±0.4	2	273–308	N.A.	8.37±0.15	-12.04±0.86
Holocentrus rufus	Longspine squirrelfish	LSQ	Benthivore	318	70–270	3.6±0.4	6	135–275	67.50%	7.24±0.16	-13.59±0.18
Lutjanus apodus	Schoolmaster	SCM	Piscivore	610	120–460	4.3±0.4	3	202–246	12.94%	8.58±0.14	-11.96±0.62
Lutjanus griseus	Gray snapper	GRS	Piscivore	610	200–370	4.2±0.3	1	377	0%	8.73	-9.45
*Lutjanus synagris**	Lane snapper	LNS	Benthivore	381		3.8±0.2	5	200–300	N.A.	7.76±0.47	-10.57±0.88
Ocyurus chrysurus	Yellowtail snapper	YTS	Piscivore	762	160–550	4.0±0.3	9	260–378	30.26%	8.38±0.13	-11.93±0.40
Pomacanthus arcuatus	Gray angelfish	GRA	Herbivore	610	200–360	3.2±0.1	7	210–260	31.25%	6.29±0.16	-14.42±0.57
Pterois volitans	Red lionfish	RDL	Piscivore	381	60	4.4±0.4	11	155–400	N.A.	8.11±0.07	-12.24±0.39
Scarus iseri	Striped parrotfish	STP	Herbivore	254	10–380	2.0±0.0	10	62–130	18.38%	4.16±0.11	-13.92±0.23
Sparisoma aurofrenatum	Redband parrotfish	RBP	Herbivore	279	10–300	2.0±0.1	6	67–400	80.34%	3.88±0.18	-12.45±0.83
Sparisoma viride	Stoplight parrotfish	SLP	Herbivore	610	10–550	2.0±0.0	1	460	0%	4.59	-11.87
Sphyraena barracuda	Great barracuda	GRB	Piscivore	1829	440–1150	4.5±0.6	4	853–1100	34.79%	9.90±0.40	-11.78±1.52
Stegastes diencaeus	Longfin damselfish	LFD	Herbivore	152	40–150	2.0±0.0	7	57–85	25.45%	6.36±0.45	-14.03±0.65
Stegastes leucostictus	Beaugregory	BGG	Herbivore	102	10–80	3.1±0.2	1	26	0%	5.44	-14.47
Stegastes partitus	Bicolor damselfish	BID	Herbivore	102	20–70	2.0±0.0	2	67–70	6.00%	6.14±0.43	-14.08±0.25
Thalassoma bifasciatum	Bluehead wrasse	BHW	Omnivore	152	10–140	3.3±0.1	9	45–90	34.62%	5.35±0.12	-16.34±0.23

feeding across multiple trophic positions a uniform basal source. E explains the variance on the x- and y-axes: low E refers to similar variance on both axes with a more circular shape, while high E indicates that the ellipse is stretched along either x- or y-axis. In order to compare isotopic niche area among trophic guilds, a Bayesian approach was used that calculated 20,000 posterior estimates of SEA_B based on the data set. The mode and 95% credible intervals (CIs) were reported. A significant difference among SEA_B was interpreted graphically whereby if the 95% CI did not overlap then the SEA_B were deemed to be significantly different.

2.2.3.3 $\delta^{15}N$-body size relationship

All body mass data were $\log_2$ transformed to remove any effects of relationship between body size and phylogeny (Freckleton, 2000). Cross-species relationships between stable isotope data and M were analysed using linear regression between mean bulk $\delta^{13}C$ and $\delta^{15}N$ of each species and their maximum body mass (M_{max}) recorded by Humann and DeLoach (1989). Comparing fishes at a fixed proportion of maximum size (here ≥ 55% of L_{max}) reduced the risk of comparing different life stages (Charnov, 1993; Jennings *et al.*, 2001a; Galván *et al.*, 2010).

For each species, the $\delta^{15}N$-$\log_2 M$ relationship was generated using linear regression. The slope and intercept values (Appendix 4) from the linear relationships were used to calculate $\delta^{15}N$ values of other body mass individuals of the same species. To derive the community relationship between $\delta^{15}N$ and $\log_2 B$ (B was used instead of M to differentiate the community level analysis from the species level analysis), the mean weighted $\delta^{15}N$ value of each $\log_2 B$ class was derived by calculating 1) the mass ratio (*r*) of each individual (*i*) in each $\log_2 B$ class, using $r_i = M_i / \sum_{i=1}^{n} M_i$, where *n* is the total number of individuals in the $\log_2 B$ class; and 2) mean weighted $\delta^{15}N$ for each $\log_2 B$ class *j* of the whole community as $\delta^{15}N_j = \sum_{i=1}^{n} \delta^{15}N_i \times r_i$ (Al-Habsi *et al.*, 2008).

To obtain $\delta^{15}N$ values of species that were not sampled due to limitation of fishing techniques and natural abundances, several methods were used (Appendix 3): 1) using samples from similar species within the same genus if possible (order of priority: same genus, family, site, trophic position, diet and feeding habit from fishbase.org) by comparing 3 criteria: a) length to weight conversion factors, b) dietary similarities, and c) their feeding behaviours on site collected in this area; and 2) using existing data in the literature from nearby locations or elsewhere in the Caribbean with baseline adjustment (*D. cavernosa* to *D. cavernosa* if possible,

otherwise *D. cavernosa* to turf algae) to resolve temporal and/or spatial baseline variations (Cabana and Rasmussen, 1996).

2.2.3.4 Predator-prey mass ratio

The mean PPMR was calculated using the slope (*b*) of the regression line of the TP-log$_2$B relationship as $PPMR = 2^{1/b}$. However, the nitrogen TEFs ($\Delta\delta^{15}N$) between consumers and their diet vary (Post, 2002b); treating such TEFs as constant does not allow for variation in $\Delta\delta^{15}N$ based on trophic guilds (Mill *et al.*, 2007; Strieder Philippsen and Benedito, 2013) and varied trophic enrichment with increasing TP (Hussey *et al.*, 2014). Thus, two frameworks, additive (constant enrichment, Equation ii) and scaled (scaled enrichment, Equation iii) were used as follows:

$$TP_{additive} = TP_{base} + \frac{\delta^{15}N_{fish} - \delta^{15}N_{base}}{3.4} \quad \text{Equation ii}$$

where the $\Delta\delta^{15}N$ was assumed constant and equal to 3.4‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984), and the slope (*b*) of TP-log$_2$B relationship was obtained from the slope (*s*) of $\delta^{15}N$-log$_2$B relationship where $b_{additive} = s/3.4$. Thus, the PPMR was calculated as $PPMR = 2^{3.4/s}$.

$$TP_{scaled} = \frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{base}) - \log(\delta^{15}N_{lim} - \delta^{15}N_{TP})}{k} + TP_{base} \quad \text{Equation iii}$$

To calculate mean PPMR, the *b* values from both frameworks were calculated: b_{additive} was calculated as $b_{additive} = s/3.4$, while b_{scaled} was derived by calculating 1) TP_{scaled} with four coefficients: the saturating isotope limit as TP increases $\delta^{15}N_{lim}$ (Equation v), the isotope value for a known baseline $\delta^{15}N_{base}$, the consumer isotope value $\delta^{15}N_{TP}$, and the rate at which $\delta^{15}N_{TP}$ approaches $\delta^{15}N_{lim}$ per trophic level *k* (Equation iv), and 2) the b_{scaled}.

$$\mathrm{k} = -\log\left(\frac{\beta_0 - \delta^{15}N_{lim}}{-\delta^{15}N_{lim}}\right) \quad \text{Equation iv}$$

$$\delta^{15}N_{lim} = \frac{-\beta_0}{\beta_1} \quad \text{Equation v}$$

To calculate $\delta^{15}N_{lim}$ and *k*, β_0 and β_1 values were extracted from Hussey *et al.* (2014) where the 95% highest posterior median (HPM) uncertainty intervals for the β_0 and β_1 were [4.55, 7.33] and [-0.41, -0.14] respectively. A combination of $\beta_0 = 4.93$ and $\beta_1 = -0.37$ was chosen based on the $\delta^{15}N$ value of producer *D. cavernosa* and

the mean $\delta^{15}N$ value of the Scarinae *Scarus iserti*, *Sparisoma aurofrenatum* and *Sparisoma viride*, which gave $\Delta\delta^{15}N = 5.03 \pm 0.10‰$.

To calculate TP_{scaled}, the striped parrotfish (*S. iserti*) was used as the reference species ($\delta^{15}N_{base} = 14.30 \pm 0.30‰$; TP = 2.0) instead of the producer due to its low isotopic variation across sizes, time integration of seasonality from producers and adequate sample size (n = 10).

2.3 Results

2.3.1 Size spectra

In total 9055 individuals (L from 1 to 120 cm, M from 0.01 to 2742 g) were recorded in 32 UVCs over 4800 m^2 of reefs. Combined size spectra of these four sites showed a negative linear relationship between $\log_{10}$ fish abundance and total length (Figure 2.2): $\log_{10}$ (abundance + 1) = -0.052 ± 0.005 Total length +2.82 ± 0.17.

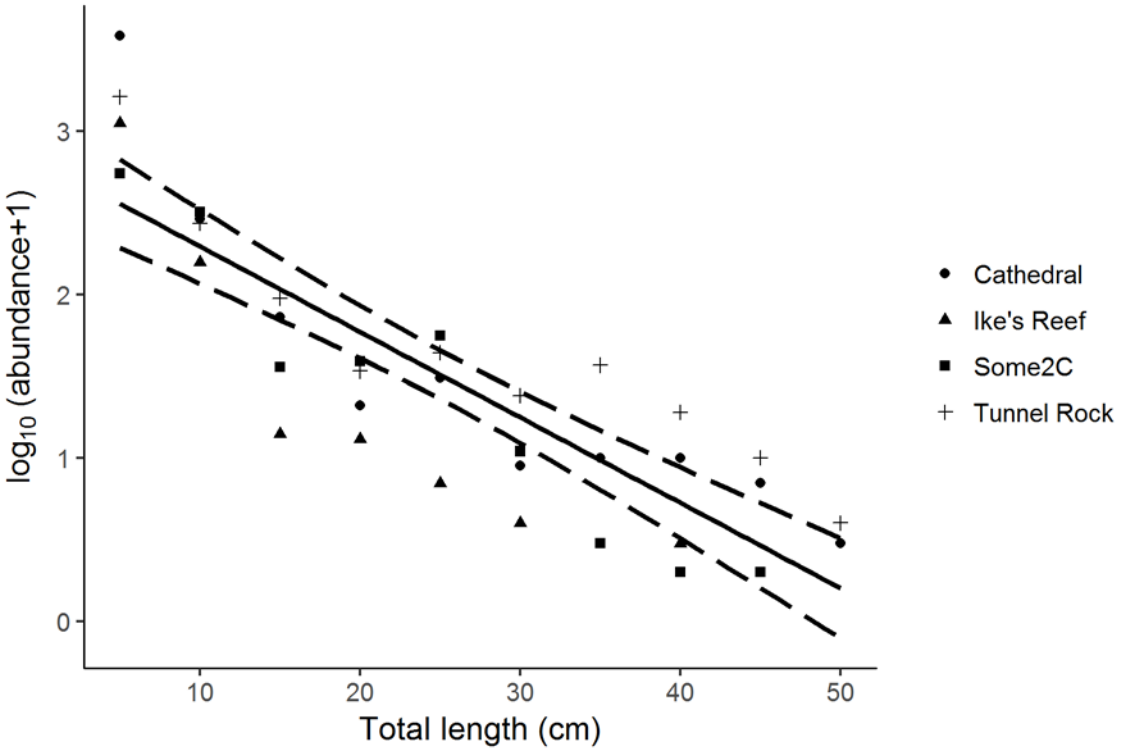


Figure 2.2 Combined relationship (linear regression) between $\log_{10}$ (abundance+1) and total length (cm) of 5cm interval of fish communities at Cape Eleuthera (the Bahamas). Solid line: linear regression line ($p < 0.05$, $r^2_{adjusted} = 0.73$), long dash line: 95% CI.

These sites demonstrated different slope and midpoint height values (Table 2.4) where Tunnel Rock had the highest slope and midpoint height values while Ike's Reef with lowest slope value and Some2C with lowest midpoint height value.

Table 2.4 Linear regression statistics of size spectra of fish communities at Cape Eleuthera (the Bahamas). Reef type: ^{B} = bommies, ^{P} = patch reefs.

Site	Slope	Intercept	Midpoint	Midpoint height	*p*	$r^2_{adjusted}$
Tunnel Rock^{B}	-0.045±0.005	2.89±0.18	30.0	1.555	< 0.05	0.87
Cathedral^{B}	-0.055±0.010	3.00±0.30	27.5	1.500	< 0.05	0.78
Some2C^{P}	-0.064±0.007	2.95±0.20	25.0	1.363	< 0.05	0.91
Ike's Reef^{P}	-0.093±0.018	3.12±0.36	17.5	1.492	< 0.05	0.83
Combined	-0.052±0.005	2.82±0.17			< 0.05	0.73

2.3.2 Species and guild stable isotope analysis

Of 41 fish species collected and analysed for $\delta^{15}N$, 11 had sample sizes under three, and three (*Coryphopterus glaucofraenum*, *Balistes vetula*, *Holocentrus adscensionis*) were sampled to represent certain uncollected species. The size range cover ratio (r_L) ranged from 0% to 100% (mean = 31.00 ± 5.03%). Mean species $\delta^{13}C$ ranged from -17.43 ± 0.15‰ (*Halichoeres pictus*) to -9.45 ± 0.00‰ (*Lutjanus griseus*) while mean $\delta^{15}N$ ranged from 3.88 ± 0.83‰ (*S. aurofrenatum*) to 9.90 ± 1.52‰ (*Sphyraena barracuda*). $\delta^{15}N$ values were significantly but weakly correlated with $\delta^{13}C$ ($p < 0.05$, $r^2_{adjusted} = 0.29$) at the species level (Figure 2.3). Some species had large SE values in $\delta^{13}C$ (≥ 1.00‰, e.g. *S. barracuda*) or $\delta^{15}N$ (≥ 0.50‰, e.g. *Aulostomus maculatus*), or both $\delta^{13}C$ and $\delta^{15}N$ (e.g. *Elacatinus genie*). Mean $\delta^{13}C$ and $\delta^{15}N$ values of strict pelagic planktivores (e.g. *Chromis cyanea*, TP = 3.7, $\delta^{13}C$ = -17.03 ± 0.12‰, $\delta^{15}N$ = 5.23 ± 0.10‰) and strict benthivores of similar TP (e.g. *B. vetula*, TP = 3.8, $\delta^{13}C$ = -12.36 ± 0.30‰, $\delta^{15}N$ = 7.72 ± 0.10‰) were significantly different.

The planktivore isotopic niche had a much lower $\delta^{13}C$ value than the others and was separated from the herbivore, benthivore and piscivore trophic guilds (Figure 2.3). The isotopic niche of the omnivores overlapped with those of the herbivores and planktivores as did those of the benthivore and piscivore. The isotopic niche of the herbivores was vertically separated from those of the benthivores and piscivores. E and θ values differed among trophic guilds (Table 2.5); the herbivore guild had the lowest E (0.77), while the omnivores had the highest (0.98). The benthivore (0.94) and planktivore (0.79) trophic guilds had very similar E values to the omnivore and herbivore trophic guilds, respectively. The θ values showed that the relationships between $\delta^{15}N$ and $\delta^{13}C$ within all the trophic guilds were positive; the herbivores had the lowest θ (6°) while the planktivores had the highest (76°).

Among the benthivore, omnivore, piscivore and herbivore trophic guilds, the isotopic niche was spread along the x-axis ($\theta < 45°$), while that of the planktivores was more vertically spread ($\theta > 45°$). Some species (the benthivore *H. pictus*, herbivores *Acanthurus coeruleus* and *Coryphopterus personatus* and omnivore *Thalassoma bifasciatum*) had isotopic coordinates close to the ellipse of the planktivore trophic guild, and one piscivore (*A. maculatus*) had an isotopic niche within the ellipse of the herbivore trophic guild. The SEA_B data (Figure 2.4, Table 2.4) suggested the herbivore and benthivore guilds had the largest isotopic niches, followed by the piscivore guild, then the omnivore guild. The planktivore guild had an isotopic niche significantly smaller than the others.

2.3.3 δ^{15}N-body mass relationships at species and community levels

There were significant but weak relationships across species between $\log_2 M_{max}$ (maximum body mass) and both $\delta^{15}N$ ($p < 0.05$, $r^2_{adjusted} = 0.12$; Figure 2.5a) and $\delta^{13}C$ data ($p < 0.05$, $r^2_{adjusted} = 0.17$; Figure 2.5b). The $\delta^{15}N$ values of several species did not scale positively with $\log_2 M_{max}$ (e.g. *E. genie*, *Sparisoma viride*, *Scarus iserti* and *S. aurofrenatum*). The SE values of $\delta^{13}C$ were generally higher than those of $\delta^{15}N$ regardless of M_{max}. $\delta^{15}N$ tended to scale positively with $\log_2 M$ for 24 species (Figure 2.6), significantly so e.g. *C. cyanea* (Appendix 4), negatively for five species, significantly so e.g. *Pomancanthus arcuatus* (Appendix 4). There was considerable variability around the regression line for nine species ($r^2_{adjusted} < 0.50$, e.g. *S. barracuda*), whereas this was not the case for others (e.g. *Clepticus parrae*, *Calamus pennatula*, *Halichoeres garnoti*). At the community level, the combined isotope data demonstrated a strong positive linear relationship between mean $\delta^{15}N$ and $\log_2 B$, the regression equation being $\delta^{15}N = 0.327 \pm 0.037\log_2 B + 4.03 \pm 0.21$ ($r^2_{adjusted} = 0.70$, $p < 0.05$, Figure 2.7).

2.3.4 Predator-prey mass ratio

TPs of all individuals were adjusted specific to this food web (Figure 2.8a). Low-TP individuals ($TP < 3$) demonstrated similar TPs in both frameworks, while high-TP individuals showed significant differences with their TPs higher in the scaled framework (e.g. *S. barracuda*: $TP_{additive} = 3.98$, $TP_{scaled} = 4.91$). Applying these two frameworks with different TEFs at the community level, the slope (b) of the relationship between mean TP and $\log_2 B$ were: $b_{additive} = 0.0990$ ($p < 0.05$) and $b_{scaled} = 0.1151$ ($p < 0.05$) (Figure 2.8b). Thus, the PPMRs were 1100 and 412 for the $PPMR_{additive}$ and $PPMR_{scaled}$, respectively.

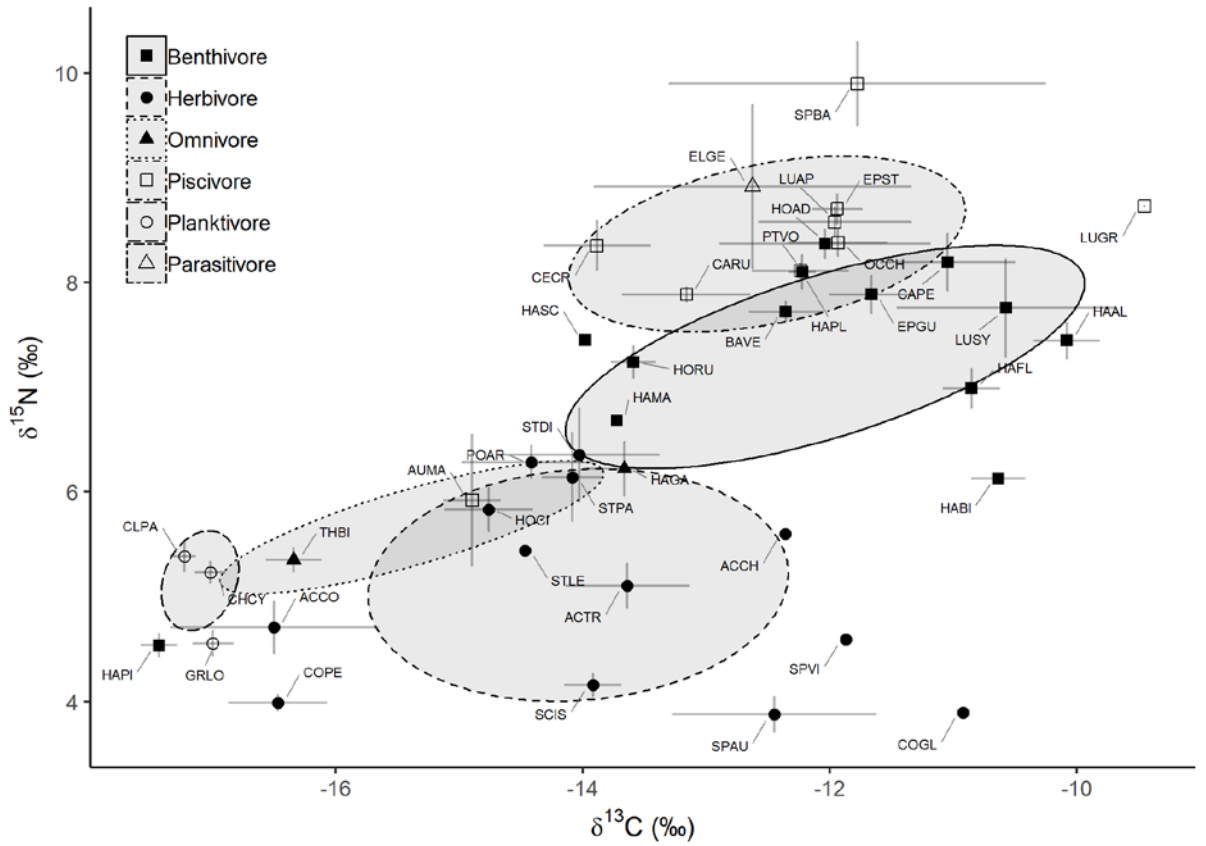


Figure 2.3 Plot of bulk $\delta^{15}N$ against $\delta^{13}C$ (mean±SE) relationship of all sampled fish species (for codes see Table 2.3) and small sample size-corrected standard ellipses (SEAc) (solid line-ellipses) for five trophic guilds (one species of parasitivore, CLG) of fish at Cape Eleuthera (the Bahamas).

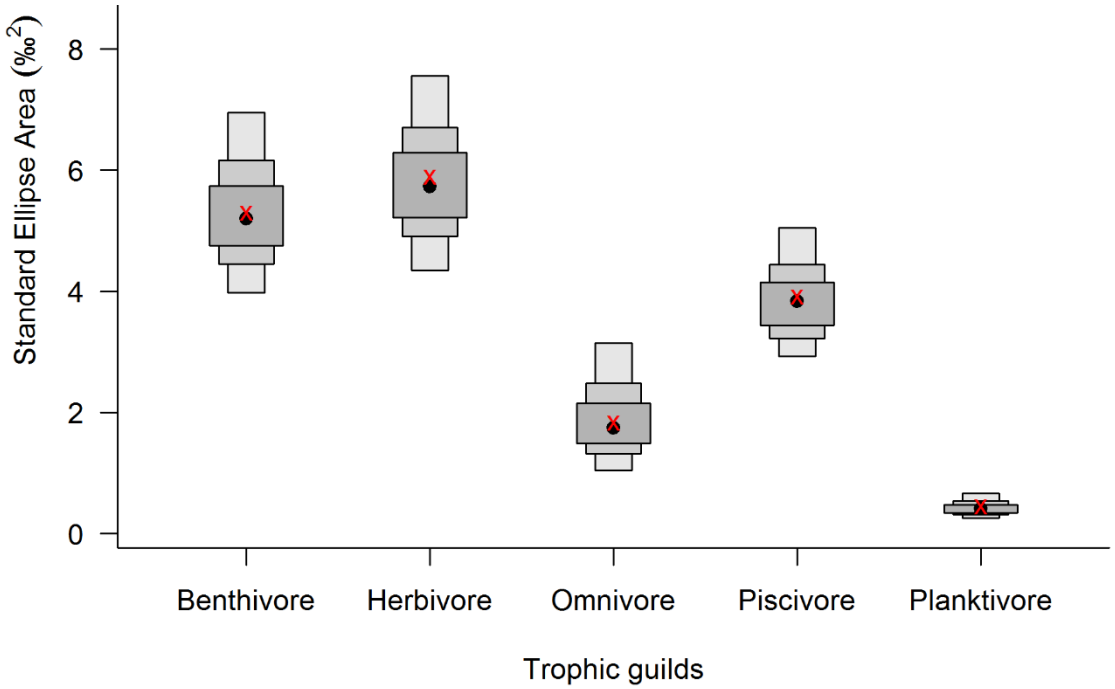


Figure 2.4 Posterior estimates of the standard ellipse areas (SEA_B) for the five fish trophic guilds at Cape Eleuthera (the Bahamas). The boxes represent the 95, 75 and 50% credible intervals in ascending order of size. Mode of the posterior Bayesian estimate area, SEA_B is indicated by a black circle. The maximum likelihood estimate and sample size-corrected ellipse area for the corresponding SEA_C is indicated by a red cross.

Table 2.5 Isotopic niche area (‰^{2}) estimates and parameters (eccentricity [E], the angle in degree between the semi-major axis of the isotopic niche and the x-axis [θ]) for five trophic guilds (benthivore, herbivore, omnivore, piscivore and planktivore) of coral reef-fish at Cape Eleuthera (the Bahamas). Estimates of isotopic niche areas are given as standard ellipse area (SEA), sample size-corrected standard ellipse area (SEA_C) and the mode of the Bayesian standard ellipse area (SEA_B) estimates. Upper and lower 95% credible intervals (CIs) indicate the uncertainty in the SEA_B estimates.

Trophic guild	SEA (‰^{2})	SEA_C (‰^{2})	E	θ (°)	SEA_B (‰^{2})	SEA_B 95% CIs
Benthivore	5.20	5.30	0.94	20.96	5.19	3.96 to 6.93
Herbivore	5.79	5.90	0.77	6.29	5.73	4.32 to 7.53
Omnivore	1.69	1.83	0.98	19.17	1.77	1.05 to 3.14
Piscivore	3.83	3.91	0.89	14.36	3.81	2.88 to 4.04
Planktivore	0.43	0.46	0.79	75.69	0.41	0.26 to 0.66

2.4 Discussion

2.4.1 Size spectra

Size spectra of the Cape Eleuthera coral reefs showed that the fish community was size-structured and was evidently subject to energetic constraints and possibly supported mainly by one production source type (Paul and Christensen, 1995; Brown and Gillooly, 2003; Jennings and Mackinson, 2003; Trebilco *et al.*, 2013). Previous studies showed that size spectra also existed among groups of individuals sharing the same production sources such as herbivore and carnivore (Brown and Gillooly, 2003; Robinson and Baum, 2015). However, in coral-reef food-webs where multiple production sources (e.g. benthic and pelagic), mixed-feeding and multi-food chain feeding patterns (especially from some large predator fish; Layman *et al.*, 2005) typically exist, it is rather complicated to look at size structuring for individual feeding groups because many fishes rely on multiple trophic pathways.

There were differences in size spectra (Table 2.4) between the two types of reefs which could be related to differences in physical habitat complexity (Harborne *et al.*, 2012; Zhu *et al.*, unpublished data), but other factors could also be involved, and to test this would require further data. Compared with other impacted coral-reef fish communities, the size spectrum slope (-0.052) from the Cape Eleuthera coral reef sites was steeper than lightly fished coral reefs in Fiji (-0.032 to -0.019, Dulvy *et al.*, 2004b), but smaller than Fijian coral reefs that were more heavily exploited (-0.235 to -0.208, Graham *et al.*, 2005) and post-bleached coral reefs in

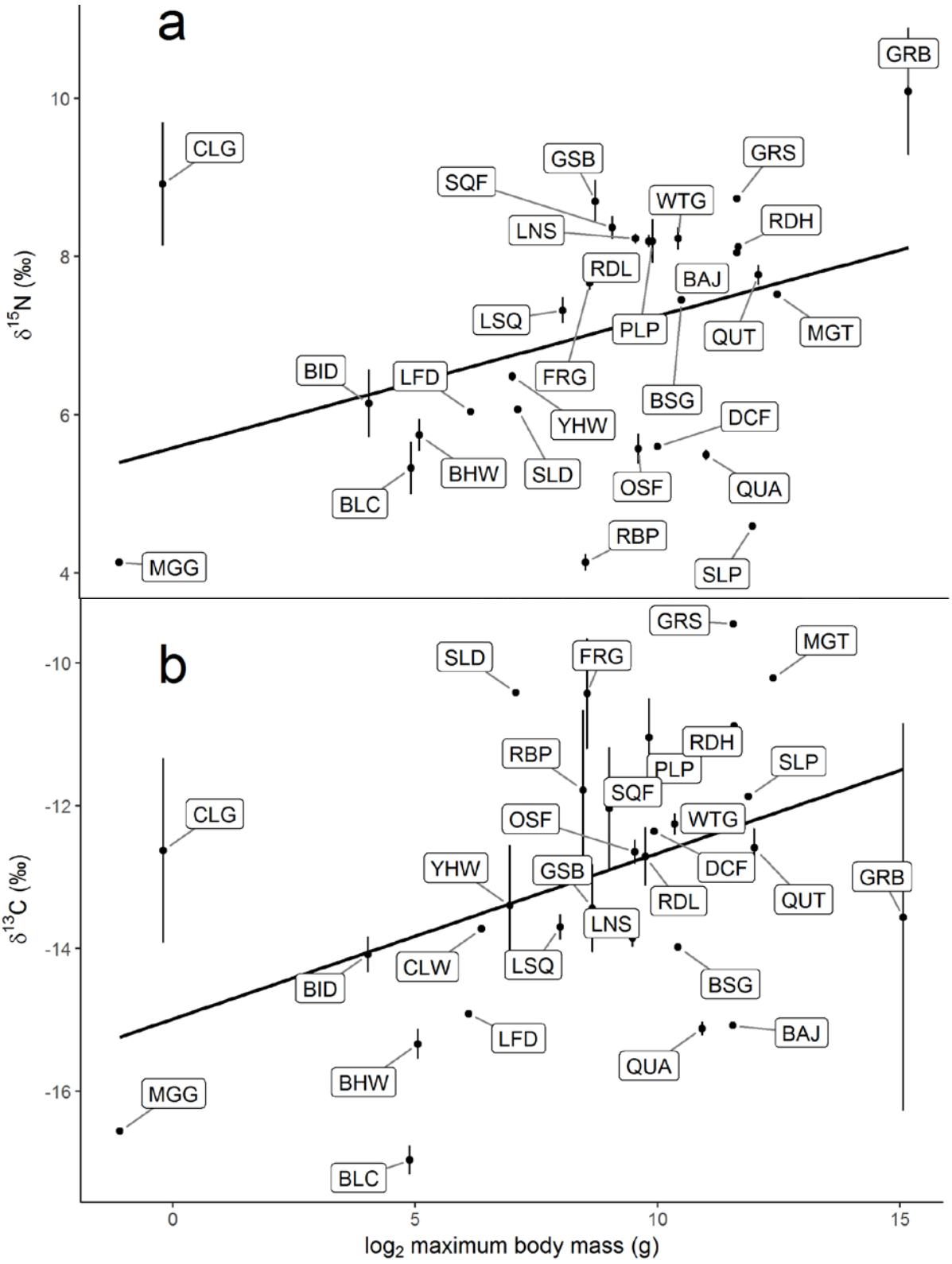


Figure 2.5 Plot of (a) bulk $\delta^{15}N$ and (b) bulk $\delta^{13}C$ (mean ± SE) vs. $\log_2$ maximum body mass for all collected fish species (for codes see Table 2.3) at Cape Eleuthera (the Bahamas) with mean isotope values of individuals bigger than 55% of their L_{max}. Solid line: linear regression line.

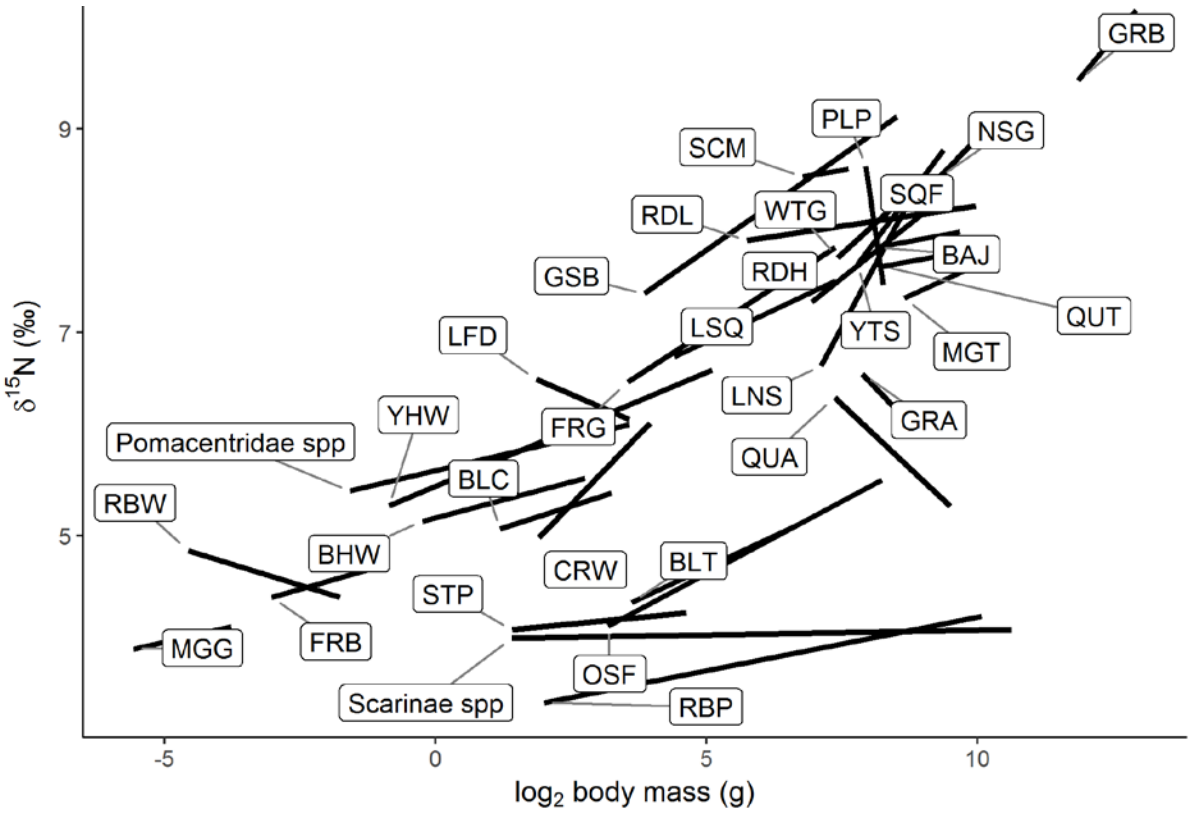


Figure 2.6 Plot of $\delta^{15}N$ against $\log_2$ body mass of all sampled species (slope ≠ 0) at Cape Eleuthera (the Bahamas). For codes see Table 2.3.

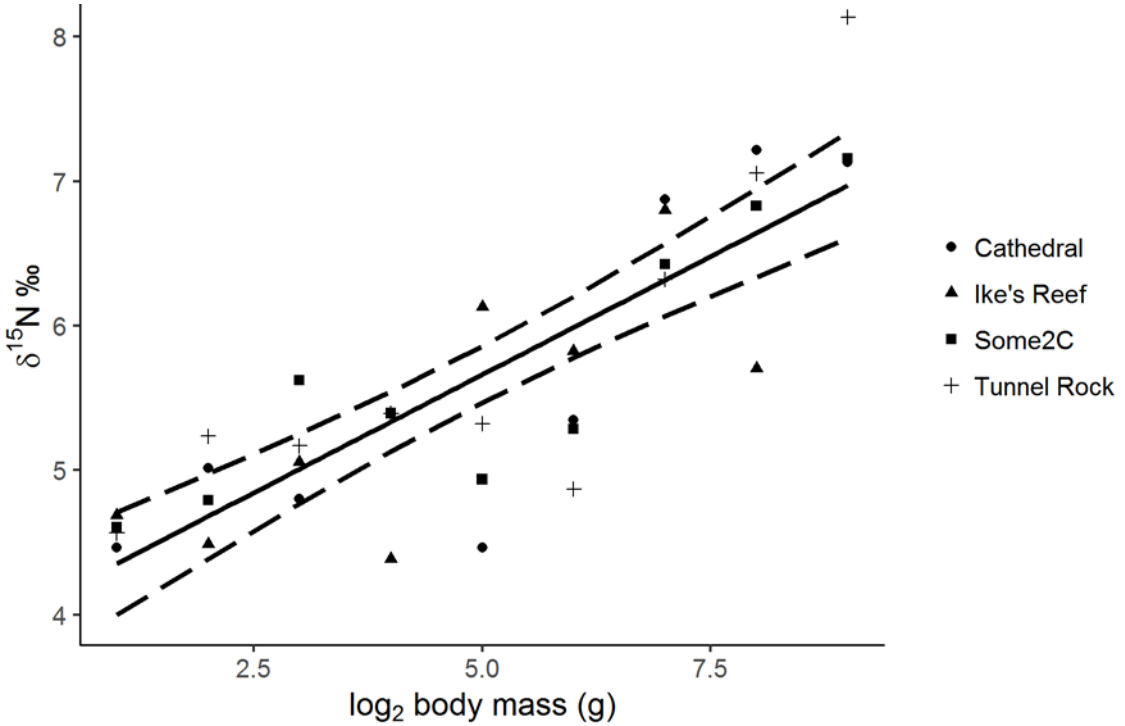


Figure 2.7 Combined relationship (linear regression) between $\delta^{15}N$ and $\log_2$ body mass for fish communities at Cape Eleuthera (the Bahamas). Solid line: linear regression line ($p < 0.05$), long dashed line: 95% CI.

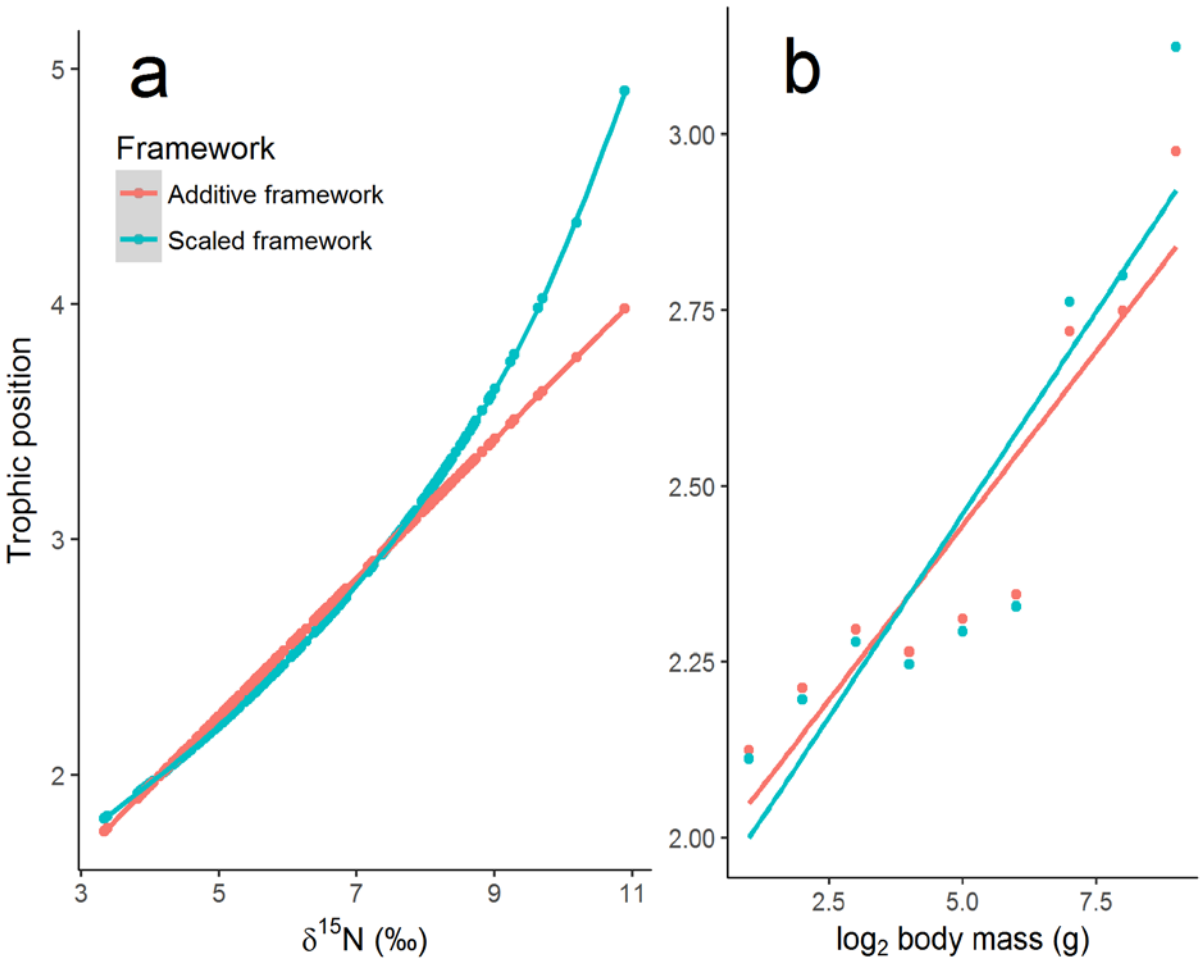


Figure 2.8 Plot of trophic position (TP) to (a) $\delta^{15}N$ relationship for all sampled individuals and (b) $\log_2$ body mass on community level, with additive and scaled fractionation frameworks at Cape Eleuthera (the Bahamas). Solid lines in (a) were smoothed with general additive model, in (b) were analysed with linear regression model.

Seychelles (-0.25 to -0.17, Graham *et al.*, 2007). Compared with other Caribbean reef sites, the Cape Eleuthera size spectrum slope was at the low end (-0.159 to -0.039, Zhu *et al.*, unpublished data). This suggested that there are potential drivers that lead to differences in the slopes of the size spectra among coral-reef fish communities. The Cape Eleuthera coral reefs are subject to conservation measures that protect large individuals from being fished, which may result in a shallower slope for the size spectrum. Alternatively, the low slope may be related to the degraded nature of the coral reef, which lacks the refuge space for small fish size classes (Alvarez-Filip *et al.*, 2009).

There are several limitations to applying size spectra on coral-reef fish communities. Since all existing studies were conducted in degraded reefs (e.g. heavily fished, structurally simplified), whether such method can be applied to more

intact or pristine reefs is unknown. Also, survey method is likely to influence reef-fish community size spectra. Rogers *et al.* (2014) found significantly higher abundances in smaller size classes with surveys covering all size classes, potentially due to fine spatial niche partitioning among these individuals (Alvarez-Filip *et al.*, 2011; Rogers *et al.*, 2014) and thus nonlinearity of size spectra. Other studies have found linear relationships where individuals with body mass < 20 g were excluded (Ackerman and Bellwood, 2000; Wilson *et al.*, 2010; Robinson *et al.*, 2016). It is important to note that the results in this study and most aforementioned studies relate only to non-cryptic diurnal fish due to limitations of reef visual survey at night, and some cryptic and nocturnal species (e.g. Holocentridae spp, Apogonidae spp) which might be highly abundant were scarcely included at all. Despite the limitations in this study, indications are that size spectra analysis can allow us to compare size structures among communities in impacted aquatic ecosystems, and potentially address drivers behind such as reef strcutural degradation.

2.4.2 Species and guild trophodynamics

Stable isotope analyses at both species and trophic guild levels indicated that at the Cape Eleuthera site mixed-feeding patterns existed and there were multiple energy pathways in the food web with different stable isotope baselines (e.g. benthic and pelagic production sources where species of similar TPs utilizing these two sources had different isotopic signatures).

High within-species variability in mean $\delta^{13}C$ and $\delta^{15}N$ values for some species suggested the existence of individual specialization (Matthews and Mazumder, 2004) in the food web where different individuals of the same species were consistently sampling different production sources. For example, similarly sized individual of the apex predator *S. barracuda* had similar $\delta^{15}N$ values but differed greatly in $\delta^{13}C$ values. The $\delta^{13}C$ values of approximately -16‰ were close to those within the planktivore trophic guild while the $\delta^{13}C$ values of ~10‰ were more consistent with the piscivore trophic guild. TP omnivory indicated by differences in $\delta^{15}N$ also occurs, for example in the parasitivore *E. genie* which feeds on parasites from fish at different trophic positions.

The SIBER analysis differentiated these two types of production sources, namely benthic (e.g. algae) and pelagic (e.g. plankton) based on the separated isotopic niches of strict feeders of each source type, and mixed-feeding patterns for some species that are typically regarded as relying solely on single types of source

materials (e.g. herbivore fish; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). In this study, isotopic niche areas (SEA_C and SEA_B) of the planktivores were significantly smaller than the other groups even though plankton can have highly variable isotopic signatures (McClelland and Montoya, 2002; Kürten *et al.*, 2013), which indicated a level of dietary consistency. High θ, low E and low SEA values of the planktivore trophic guild nevertheless suggested TP omnivory, with these fish feeding at TPs albeit from the same type of pelagic source (e.g. phytoplankton and zooplankton). The omnivore guild had high E, low θ and SEA values, suggesting source omnivory, these fish relying on distinctive sources (e.g. plankton and benthic algae) with similar $\delta^{15}N$ baselines. However, this might also be a result of the small number of species (n = 2). The benthivore, piscivore and herbivore trophic guilds, which share mostly benthic production sources, had similar SEA_C and SEA_B values which were much greater than those of the planktivores and omnivores, with their isotopic niche spread along the x-axis as indicated by E and θ values, suggesting source omnivory within the benthic producer category. Overlapping ellipse areas among the trophic guilds (e.g. piscivore and benthivore) suggested that they might share dietary resources to some extent; for example, some lutjanids are both piscivorous and feed on zoobenthos (Allen, 1985; Kulbicki *et al.*, 2005a; Layman and Allgeier, 2012). The vertical distribution in the SEA_C data for these four benthic trophic guilds reflected the herbivorous fish feeding at low trophic positions, while the omnivores, benthivores and piscivores utilized a wider range of energy sources from different TPs. There were species with stable isotope values outside the isotopic niche of their assumed trophic guilds, which suggested feeding on different food sources than previously known or those derived from snapshot diet studies. For example, the four benthic feeders (*H. pictus*, *A. coeruleus*, *C. personatus* and *T. bifasciatum*) were evidently relying on plankton sources, and the piscivore *A. maculatus* might be predating on smaller herbivores. Some herbivores came partly within the isotopic niche of other trophic guilds, indicating feeding on food sources in addition to algae such as invertebrates or planktivore faeces (Robertson, 1982; Wulff, 1997; Dunlap and Pawlik, 1998; Chen, 2002; Plass-Johnson *et al.*, 2013)**.**

2.4.3 $\delta^{15}N$-body mass relationship

The majority of species had positive relationships between $\delta^{15}N$ and $\log_2 M$ indicating they tend to feed at higher TPs as size increases. This could be a result of increasing gape size, predatory skill and fitness level allowing individuals to feed on

higher TP prey as they grow (Peters, 1986; Munday, 2001; Newman *et al.*, 2012). Those with negative or highly variable $\delta^{15}N$-$\log_2 M$ relationships potentially have dietary shifts from isotopically high value production sources to low or multi-pathway (e.g. exploitation of short food-chain) feeding patterns in their sampled size ranges, or otherwise assimilating significantly different isotopic baselines across the population (Jennings *et al.*, 2002a; Layman *et al.*, 2005). Unlike Robinson and Baum (2015) where $\delta^{15}N$-$\log_2 M$ relationships of all individuals in two separate trophic pathways (herbivore and carnivore) were investigated, species-level and whole-community level analyses were conducted in this study. The variation among species is attributable to differences in the trophic pathways supporting them, but to understand better how trophic pathways affect such relationships, more data are clearly needed. At community level, a positive linear relationship between $\delta^{15}N$ and $\log_2 B$ across the combined sites was found, indicating that TP tended to increase with body mass regardless of taxonomy and larger coral reef fish in this Cape Eleuthera community on average fed at higher TPs.

The weak cross-species relationship between isotopic signatures and $\log_2 M_{max}$ suggested that maximum body mass could scarcely constrain species' trophic capabilities in this food web in which there were small-bodied benthivores and planktivores and large-bodied herbivores. The body-size structuring is similar to those of North Sea and Western Arabian Sea community data (Jennings *et al.*, 2001a; Al-Habsi *et al.*, 2008). Here, the small-size class biomass data were dominated by herbivores rather than higher TP omnivores such as Labridae (Graham *et al.*, 2017), while the large-size classes were dominated by piscivores and omnivores rather than large-bodied herbivores such as Scarinae (Hughes *et al.*, 2007; Zhu *et al.*, unpublished data). Although the surveyed Cape Eleuthera sites are now legally protected, they were previously fished and are structurally degraded. The linearity of the $\delta^{15}N$-$\log_2 B$ relationship at Cape Eleuthera may not be generic; it could be influenced by the loss of habitat structural complexity and aspects of past overfishing (e.g. removal of large herbivores).

The present study also had limitations. All individuals were treated as if they had the same isotopic baseline, yet significant isotopic differences between benthic and pelagic sources are expected (McConnaughey and McRoy, 1979; Polunin and Pinnegar, 2002), whereas other baselines would have been taken into consideration when estimating the trophic positions of consumers with significantly mixed diets. Also, for some species, sample sizes failed to adequately fulfil requirements for

confidently describing stable isotope changes as a function of body mass (Galván *et al.*, 2010), yet linear regression was still applied to these species to explore their δ^{15}N-log$_2$M relationships. Low sample sizes (n < 3) and/or size ranges (r_L) meant that for some species, stable isotope data could not be derived across whole UVC size ranges; for these stable isotope data were assumed to be size-invariant. For missing species, the methods used to infer stable isotope values had limitations including species within the same genus or family having ontogenetic and/or dietary differences, some not meeting all three criteria and using published data from the same species could be subject to feeding strategies varying ontogenetically (Plass-Johnson *et al.*, 2013) or spatially (Jennings *et al.*, 1997; Matthews and Mazumder, 2004). Unlike studies using combined baselines (Mill *et al.*, 2007), here the benthic alga *D. cavernosa* was the sole baseline and this might not adequately represent the benthic producers those primary consumers fed on, which includes turf algae, cyanobacteria and other potential production sources.

2.4.4 Predator-prey mass ratio

Using the additive framework, the mean PPMR at Cape Eleuthera was estimated to be 1100:1, which indicates a relatively long food chain (Table 2.6). Estimated mean PPMR is sensitive to assumed level of fractionation. Reum *et al.* (2015) showed significant reduction from the additive to the scaled frameworks, and at Cape Eleuthera, a reduction in mean PPMR was also found (Table 2.6). However, the present study used different β coefficients from those in Reum *et al.* (2015), who applied the same scaled fractionation for all selected studies (Table 2.6) with β_0 and β_1 being the middle value of 95% HPM uncertainty intervals from Hussey *et al.* (2014). For the Cape Eleuthera analysis, β_0 and β_1 values were decided by fitting a similar fractionation value between *D. cavernosa* and the Scarinae ($\Delta\delta^{15}$N = 5.03‰). The revised relationship between δ^{15}N and TP using site-specific β coefficients delivered more realistic TP values for various species (e.g. Scarinae spp TP = 2, apex predator *S. barracuda* TP = 5). The indication is that β_0 and β_1 from the scaled fractionation framework could potentially depend on the specific ecosystem involved.

Table 2.6 Mean PPMR values of different communities from the literature using both additive and scaled frameworks. *calculated from Reum *et al.* (2015).

Community	$PPMR_{additive}$	$PPMR_{scaled}$	Reference
Central North Sea	109:1	49:1*	Jennings *et al.* (2002c)
North Sea	414:1	N.A.	Jennings and Warr
Cape Eleuthera	1100:1	412:1	Present study
Northern North Sea	1136:1	307:1*	Jennings *et al.* (2001a)
Puget Sound	4260:1	322:1*	Reum *et al.* (2015)
Galician upwelling	4500:1	N.A.	Bode *et al.* (2003)
Western Arabian	7792:1	90:1*	Al-Habsi *et al.* (2008)
Iberian Peninsula	1.8×10^5:1	N.A.	Bode *et al.* (2006)

The average estimated trophic fractionation value between the Scarinae and *D. cavernosa* ($\Delta\delta^{15}N = 5.03 \pm 0.10‰$) was higher than the 3.40‰ commonly proposed (DeNiro and Epstein, 1981) which was used in the additive framework, but similar to the values of Mill *et al.* (2007) and Lamb *et al.* (2012) who estimated $\Delta\delta^{15}N$ values between a mixed-algae baseline and wild of 4.78 ± 1.30‰ and 5.34 ± 0.18‰ respectively. However, some other studies have suggested much lower $\Delta\delta^{15}N$ values of 2.52 ± 2.50‰ (algae to herbivorous fish; Vander Zanden and Rasmussen, 2001), 1.70‰ (gut content to herbivorous fish; Wyatt *et al.*, 2010) and 2.30 ± 0.30‰ (Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). Herbivorous fish such as Scarinae may have a diet consisting of both high-protein (e.g. invertebrates) and low-protein foods such as algae (Wulff, 1997; Chen, 2002), however, only some benthic food sources (e.g. detritus, corals) were included in Dromard *et al.* (2015) and Plass-Johnson *et al.* (2013), while pelagic or planktivore-derived food items (e.g. faeces) can be important in the diet of some herbivorous fish (Robertson, 1982). In the literature $\Delta\delta^{15}N$ values range from 0.60‰ to 3.70‰ and $\Delta\delta^{13}C$ from 0.30‰ to 5.90‰; and TEFs could depend on type of aquatic ecosystem, research method (field or experimental), tissue and diet type or quality (McMahon *et al.*, 2010; Wyatt *et al.*, 2010; Strieder Philippsen and Benedito, 2013).

2.4.5 Conclusion

Stable isotope analysis suggested the existence of a wide range of production sources and mixed-feeding patterns of some coral-reef fish. Combining visual census and stable isotope data in size-based analysis evidently showed that coral reef-fish community at Cape Eleuthera was size structured. This is the first study finding a positive linear $\delta^{15}N$-$\log_2 B$ relationship in a coral reef system. It is unlikely that this is representative of the wider area; the sample size was low and conservation

measures the area subject to. Indications are that such trophic structure analysis can improve understanding of trophic interactions in coral-reef fish communities such as predator-prey relationships and trophic/energy pathways at community level, and potentially address drivers causing differences in trophic structures on a larger scale, trophic cascade and trophic replacement. Nevertheless, analysis here suggested the trophic ecology of coral-reef fish is understudied, for example little is known of the trophic versatility of some fish species.

Chapter 3. Resolving the trophic niches of Maldivian coral-reef fishes

3.1 Introduction

There is a tendency to braodly categorise trophic guilds of coral-reef fishes (Hiatt and Strasburg, 1960; Jennings *et al.*, 1995; Polunin, 1996; McClanahan *et al.*, 1999; Hughes *et al.*, 2003; MacNeil *et al.*, 2015; D'Agata *et al.*, 2016; Graham *et al.*, 2017; Stamoulis *et al.*, 2017; Hadi *et al.*, 2018; Moustaka *et al.*, 2018). This assumes that the categorisation is meaningful and that species scarcely cross trophic boundaries. The detail within these categories is suspected to be comparatively trivial, while important variation among and within species is assumed not to be masked by this categorisation. Yet many studies suggest the existence of mixed-feeding patterns such as trophic position (TP) and source omnivory: that some fish feed at higher TP as size increases (Jennings *et al.*, 2001a; Jennings *et al.*, 2001b; Chen, 2002; Jennings *et al.*, 2002a; Plass-Johnson *et al.*, 2013), or specialise on different sources within trophic categories (Matthews and Mazumder, 2004; Plass-Johnson *et al.*, 2013). For example, herbivores including the sub-family Scarinae selectively feed on microbial autotrophs while some species from the family Acanthuridae feed on filamentous benthic algae (Crossman *et al.*, 2001; Clements *et al.*, 2016). These two different primary production sources differ in protein content and some biomarkers (Brenner *et al.*, 1999; Clements *et al.*, 2016).Therefore the species feeding on only one primary producer should differ in some of their biomarkers. Some species also cross trophic boundaries as in the case of herbivores ingesting animal material (Randall, 1967; Robertson, 1982; Dunlap and Pawlik, 1998; Chen, 2002). A notion of dietary redundancy within simplistic categories needs to be replaced by one of higher trophic functional diversity (e.g. maintaining the healthy state of corals through herbivory, Thacker *et al.*, 2001; Burkepile and Hay, 2008; Green and Bellwood, 2009; Plass-Johnson *et al.*, 2015; Adam *et al.*, 2018) if the food-web structures of coral reef ecosystems are to be resolved in more detail.

To explore quantitatively the extent of trophic niche variation among coral-reef fish, bio-tracers are a tool that has certain advantages over gut-content analysis (Jennings *et al.*, 2002b) or DNA barcoding (Leal and Ferrier-Pagès, 2016) such as longer time integration of diets and exclusion of non-digested food items. However, such method does not provide diet informationas detailed as gut-contents (e.g. to species level of food items). Some biomarkers (e.g. stable isotopes, fatty acids)

represent time-integrated signatures of certain properties of individuals (Hobson and Welch, 1992; Jennings *et al.*, 2001a). Nitrogen stable isotope or $\delta^{15}N$ (the ratio between heavy and lighter isotopes of nitrogen, ^{15}N:^{14}N) is a good proxy for TP due to its steady enrichment from diet to consumer (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Jennings *et al.*, 2001a; McCutchan *et al.*, 2003; Strieder Philippsen and Benedito, 2013) and less variation in the baselines of food webs (Polunin and Pinnegar, 2002; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). In contrast, carbon stable isotope or $\delta^{13}C$ (^{13}C:^{12}C) data can be good proxies of food source types (Tieszen *et al.*, 1983; Polunin and Pinnegar, 2002; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015) because of the small ^{13}C enrichment from diet to consumer and differences among types of sources (DeNiro and Epstein, 1978; McCutchan *et al.*, 2003; Strieder Philippsen and Benedito, 2013). Pelagic production sources (e.g. phyto- and zooplankton) may be important for coral-reef fish food-webs (Robertson, 1982; de Goeij *et al.*, 2013; Gove *et al.*, 2016; Francis and Côté, 2018) but are expected to be isotopically different from those of reefs (McConnaughey and McRoy, 1979; Polunin and Pinnegar, 2002) due to the nutrient sources of phytoplankton (Gove *et al.*, 2016). $\delta^{13}C$ and $\delta^{15}N$ data can be used together to depict an isotopic niche (Newsome *et al.*, 2007), which relates to a trophic niche (Bearhop *et al.*, 2004) to some extent (Jackson *et al.*, 2011), and reflects dietary ecology (Elton, 1927), such as source partitioning (e.g. pelagic vs. benthic), prey preferences and habitat choice (Leibold, 1995). These data also reveal feeding strategies within species (Newsome *et al.*, 2007).

The package stable isotope ellipses in R (SIBER, Jackson *et al.*, 2011) was developed to reduce the uncertainty in estimating isotopic niches, and has been used to categorize generalist and specialist species (Layman and Allgeier, 2012). The model outputs standard ellipse area (SEA) indicating the isotopic niche of a group using 40% of the data and sample size corrected standard ellipse area (SEA_C) by correcting the SEA value based on the sample size. The SEA_C angle between semi-major axis and x-axis (θ) and eccentricity (E) can be used to compare feeding strategies among groups with similar SEA_C (Reid *et al.*, 2016). For example, a θ close to 90° suggests multiple TP feeding patterns while θ close to 0° suggests individuals within this group are feeding at the same TP. A value of E close to 1 suggests a relatively strict feeding pattern or a mixed-source and mixed-TP feeding (depending on the SEA value) while E close to 0 suggests mixed-source partitioning. The Bayesian SEA (SEA_B) can also be generated which estimates the probability

distribution of the SEA through iteration (Jackson *et al.*, 2011). Comparing SEA_B among groups allows a probabilistic interpretation of differences in isotopic niche size.

In coral reef systems, there are many types of primary production sources supporting the food web including turf algae and phytoplankton, and fish may be strict, facultative or generalist feeders and vary in these habits at different life stages (O'Brien, 1979; Robertson, 1982; Chen, 2002; Matthews and Mazumder, 2004; Plass-Johnson *et al.*, 2013). Categorizing among a small number of broad trophic guilds likely underestimates their trophodynamics and functional roles and may therefore misinform management. The aim here is to help resolve trophic niches of coral-reef fish using stable isotope to a much greater resolution than that of the broad trophic guilds commonly employed. This study, in North Malé Atoll (Maldives) sought to 1) use stable isotope data to compare and contrast putative trophic guilds and identify any overlap, and 2) analyse isotopic niches of individual species within the trophic guilds into which they are currently categorized.

3.2 Materials and methods

3.2.1 Study site and fish sampling

Twenty accessible inner-atoll reef sites (depth 4-7 m) of South West of North Malé Atoll (Maldives) were selected for fish tissue sampling (Figure 3.1). All reef sites maintained relatively high structural complexity one year after a bleaching event (Perry and Morgan, 2017).

Abundant species (Table 3.1) were determined through underwater visual census (for details, see Chapter 4). Individuals of these species were collected using a variety of techniques depending on species behaviour towards divers, feeding habits and swimming patterns. Hand net, BINCKE net (Anderson and Carr, 1998), clove oil, underwater fishing hook and line, static hook and line, Hawaiian sling and hook and line surface trolling were all used in the sampling (Table 3.1). Fish were killed by spine dislocation in accordance with the UK Home Office Scientific Procedures (Animals) Act and stored in an ice chest on board. After landing, approximately 2 g of dorsal white muscle tissue near the dorsal fin were dissected, rinsed with reverse osmosis treated water and stored in individual whirlpack bags in a -20 °C freezer. All samples were dried in individual tin trays in an oven at 50 °C for ~12 h until fully dried, and then stored in individual sealed Eppendorf tubes in zip-lock bags.

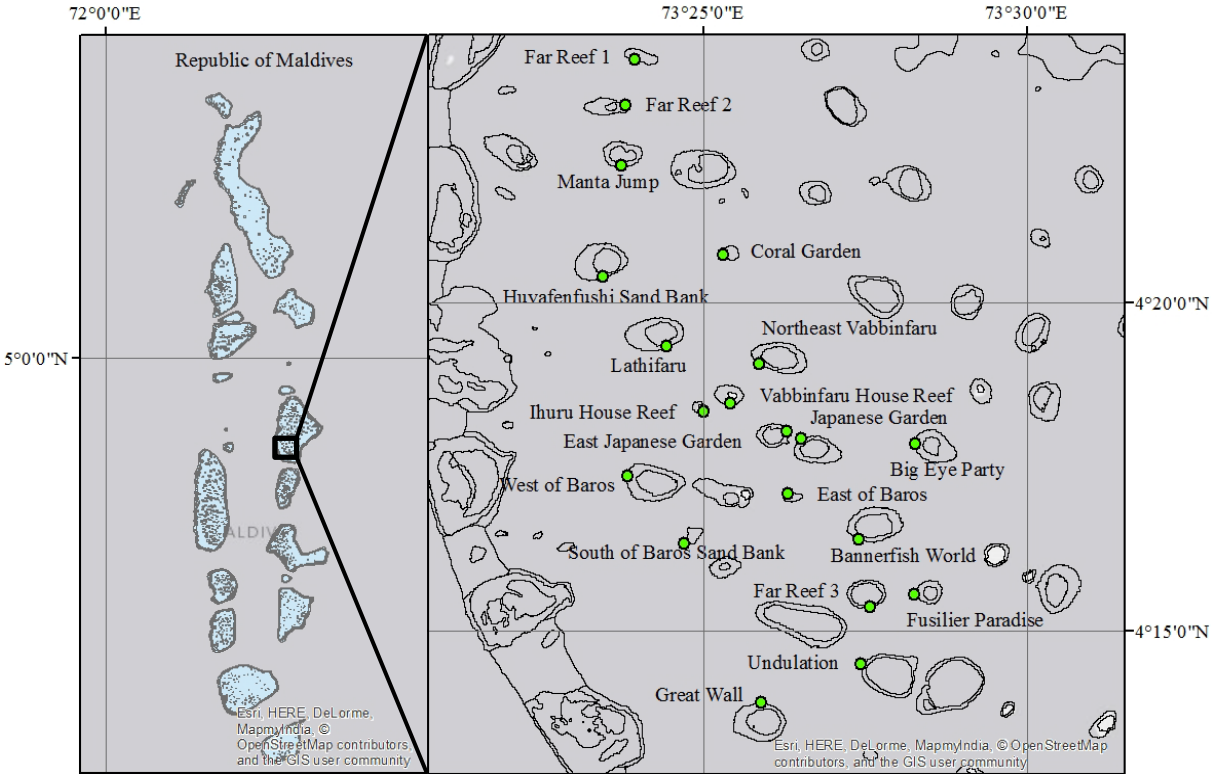


Figure 3.1 Map of survey sites (green dot) in North Malé Atoll (Maldives).

3.2.2 Baseline sampling

Benthic and pelagic production-source end-members for which strict primary consumers would be at a TP of 2.0 were characterised. The benthic end members were represented by strict algivorous, detritivorous and microphagous species. The algivorous species were *Acanthurus leucosternon* which feeds mainly on filamentous rhodophytes (Robertson and Gaines, 1986) and *Naso elegans* which feeds mainly on phaephytes (Ngugi *et al.*, 2017). Detritivorous species included *Acanthurus nigricauda* and *Ctenochaetus striatus* which rely on epilithic and/or epiphytic dead organic materials (Robertson and Gaines, 1986) and associated heterotrophic bacteria and diatoms (Moriarty, 1976). Microphagous species included the scraper Scarinae: *Scarus niger*, *Scarus scaber*, and *Scarus frenatus* and the excavator Scarinae: *Chlorurus sordidus* and *Chlorurus strongylocephalus*, which feed primarily on microbial autotrophs such as endolithic, epilithic, epiphytic or endosymbiotic cyanobacteria (Clements *et al.*, 2016). The pelagic end members included calanoid copepods separated from the plankton which was sampled with a 500 mm aperture plankton net (mesh size: 150 µm) surface towed at a steady speed of 3 knots for 20 min along the reef edge during the day (0900-1600 hrs). Copepoda samples were

stored in individual Eppendorf tubes in a -20 °C freezer, then transported back and freeze dried at Newcastle University.

Samples were all collected within a three month period (February - April 2017) and from nearby sites to minimize temporal and spatial isotopic variation which might affect food-web baselines (Bronk and Glibert, 1993; Jennings *et al.*, 1997; Rolff, 2000; McCutchan *et al.*, 2003).

3.2.3 Sample processing for stable isotope analysis

All samples were transported to Newcastle University under DEFRA permit ITIMP16.1258, frozen and freeze-dried (or refreeze dried for oven-dried samples). Fish samples were ground by hand with a mortar and a pestle. Ground samples were then weighed to 1.0 ± 0.1 mg in tin capsules with a Mettler MT5 microbalance, pelletized and stored in trays.

The prepared samples were analysed by Iso-Analytical Ltd (Crewe, UK) by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS). $\delta^{15}N$ was expressed relative to N_2 in air for nitrogen while $\delta^{13}C$ was relative to Pee Dee Belemnite (PDB) of CO_2. The reference material used for this analysis was IA-R068 ($\delta^{13}C$ = -25.22 ± 0.00‰, $\delta^{15}N$ = 1.00 ± 0.00‰), with quality control check samples IA-R068, IA-R038 ($\delta^{13}C$ = -25.11 ± 0.01‰, $\delta^{15}N$ = -0.53 ± 0.01‰) and IA-R069 ($\delta^{13}C$ = -18.87 ± 0.05‰, $\delta^{15}N$ = 11.76 ± 0.01‰), with quality control check samples IA-R068 and IA-R038, a mixture of IAEA-C7 ($\delta^{13}C$ = -14.46 ± 0.01‰) and IA-R046 ($\delta^{15}N$ = 21.88 ± 0.01‰). IA-R068, IA-R038 and IA-R069 were calibrated against and traceable to IAEA-CH-6 ($\delta^{13}C$ = -10.43‰) and IAEA-N-1 ($\delta^{15}N$ = 0.40‰), IA-R046 to IAEA-N-1. IAEA-C7, IAEA-CH-6 and IAEA-N-1 were inter-laboratory comparison standards. External standards (white muscle tissue of the coral reef serranid *Anyperodon leucogrammicus* with $\delta^{13}C$ = -13.53 ± 0.01‰, $\delta^{15}N$ = 12.64 ± 0.01‰) were also run. The precision of analysis for $\delta^{13}C$, $\delta^{15}N$, %C and %N was ±0.1‰, ±0.2‰, ±4% and ±1%, respectively. Where fish white muscle tissue samples had C:N ratios (determined from molar corrected elemental % data) higher than 3.7 (Fry *et al.*, 2003; Sweeting *et al.*, 2006), $\delta^{13}C$ mass balance arithmetic lipid correction was applied using Equation vi assuming lipid-protein $\delta^{13}C$ depletion was 7‰ (Sweeting *et al.*, 2006), and $C{:}N_{protein}$ was 3.7 (Fry *et al.*, 2003).

$$\delta_{protein} = \frac{\delta_{sample} \times C{:}N_{sample} + 7 \times (C{:}N_{sample} - C{:}N_{protein})}{\mathrm{C{:}\,N}_{sample}} \qquad \text{Equation vi}$$

Table 3.1 List of sampled species of reef fish at North Malé Atoll (Maldives), with scientific name, species code, trophic guild (fishbase.org, Froese and Pauly, 2017), mean trophic position or TP (fishbase.org, Froese and Pauly, 2017), SIA sample size (*n*), SIA sample total length range ($L_{SIA\ sample}$), mean $\delta^{15}N$ and $\delta^{13}C$ ± SE.

Scientific name	Code	Trophic guild	TP	*n*	$L_{SIA\ sample}$ (mm)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Acanthurus leucosternon	ACL	Herbivore	2.0	27	123-202	7.79±0.20	-15.26±0.19
Acanthurus nigricauda	ACN	Detritivore	2.2	7	201-277	8.86±0.14	-13.92±0.33
Acanthurus thompsoni	ACT	Zooplanktivore	3.6	8	125-181	10.05±0.10	-19.63±0.15
Aethaloperca rogaa	AER	Piscivore	4.2	12	164-318	12.97±0.08	-17.42±0.20
Amblyglyphidodon leucogaster	AML	Zoobenthivore	3.4	9	107-134	10.73±0.06	-19.23±0.08
Anyperodon leucogrammicus	ANL	Piscivore	3.9	12	238-414	13.05±0.07	-17.10±0.26
Balistapus undulates	BAU	Zoobenthivore	3.4	6	140-224	12.29±0.22	-16.45±0.30
Balistoides conspicillum	BAC	Zoobenthivore	3.3	3	253-303	11.08±0.18	-18.50±0.10
Caesio varilineata	CAV	Zooplanktivore	3.4	8	116-232	11.19±0.10	-19.54±0.13
Caesio xanthonota	CAX	Zooplanktivore	3.4	12	166-302	11.80±0.07	-18.75±0.11
Caranx melampygus	CAM	Piscivore	4.5	11	232-410	12.59±0.11	-17.66±0.12
Cephalopholis argus	CEA	Piscivore	4.5	13	186-342	12.92±0.05	-16.86±0.20
Cephalopholis leopardus	CEL	Zoobenthivore	4.0	2	112-118	11.90±0.02	-16.28±0.60
Cephalopholis miniata	CEMN	Piscivore	4.3	10	160-330	12.89±0.09	-18.05±0.08
Cetoscarus bicolor	CEB	Herbivore	2.0	1	350	8.37	-11.71
Chaetodon falcula	CHF	Zoobenthivore	3.5	4	141-149	11.49±0.11	-15.73±0.08
Chaetodon meyeri	CHM	Corallivore	3.3	16	118-148	10.42±0.15	-13.91±0.20
Chaetodon trifasciatus	CHTF	Corallivore	3.3	4	92-112	10.34±0.17	-12.66±0.26
Cheilinus fasciatus	CHEF	Zoobenthivore	3.4	3	213-289	11.06±0.19	-14.61±0.71
Cheilinus trilobatus	CHTR	Zoobenthivore	3.9	1	253	10.51	-12.90
Chlorurus sordidus	CHSD	Detritivore	2.6	8	134-235	7.99±0.13	-12.58±0.38
Chlorurus strongylocephalus	CHSC	Herbivore	2.0	7	172-442	7.88±0.10	-11.32±0.90
Chromis atripectoralis	CHA	Zooplanktivore	3.1	3	100-107	10.88±0.03	-19.46±0.07
Chromis ternatensis	CHTT	Zooplanktivore	3.4	2	98-112	10.20±0.02	-19.86±0.13
Ctenochaetus striatus	CTS	Detritivore	2.0	23	138-200	8.75±0.06	-14.03±0.15
Ctenochaetus truncatus	CTT	Herbivore	2.0	5	108-152	9.48±0.16	-14.69±0.33
Diodon liturosus	DIL	Zoobenthivore	3.5	4	265-404	11.06±0.16	-14.43±0.33
Epinephelus fuscoguttatus	EPF	Piscivore	4.1	1	420	12.58	-16.34
Epinephelus merra	EPM	Piscivore	3.8	1	138	11.50	-14.90
Fistularia commersonii	FIC	Piscivore	4.3	3	660-846	12.79±0.07	-17.76±0.26
Gnathodentex aureolineatus	GNA	Zoobenthivore	3.7	8	197-225	11.81±0.27	-13.75±0.57
Hemigymnus fasciatus	HEF	Zoobenthivore	3.5	2	197-229	12.40±0.11	-15.27±0.61
Hemitaurichthys zoster	HEZ	Zooplanktivore	3.3	7	129-154	11.93±0.11	-19.14±0.17
Lutjanus bohar	LUB	Zoobenthivore	4.3	4	243-316	12.92±0.15	-17.65±0.17
Lutjanus gibbus	LUG	Zoobenthivore	4.1	6	243-313	12.64±0.09	-17.26±0.22
Lutjanus kasmira	LUK	Zoobenthivore	3.9	2	219-240	13.04±0.24	-16.34±0.16
Melichthys indicus	MEI	Zoobenthivore	3.0	6	182-253	9.87±0.32	-16.75±0.33
Monotaxis grandoculis	MOG	Zoobenthivore	3.4	7	206-277	11.53±0.12	-14.19±0.15
Myripristis berndti	MYB	Zooplanktivore		3	174-224	12.12±0.10	-18.32±0.21
Myripristis murdjan	MYM	Zooplanktivore	3.4	6	164-182	11.75±0.09	-17.69±0.15
Myripristis pralinia	MYP	Zoobenthivore	3.5	2	167-174	11.75±0.07	-17.63±0.06
Myripristis violacea	MYVL	Zoobenthivore	3.5	20	154-187	11.64±0.06	-18.07±0.06
Myripristis vittata	MYVT	Zoobenthivore	3.8	3	156-173	12.05±0.14	-18.96±0.14
Naso brachycentron	NABC	Omnivore	2.7	1	271	11.25	-19.46
Naso brevirostris	NAB	Herbivore	2.2	3	210-242	10.38±0.59	-18.44±0.29

Naso elegans	NAE	Herbivore	2.0	5	270-410	7.92±0.30	-12.62±0.32
Naso fageni	NAF	Zooplanktivore	2.2	1	260	11.33	-19.52
Naso hexacanthus	NAH	Zooplanktivore	2.2	5	202-267	10.92±0.07	-19.63±0.05
Naso vlamingii	NAV	Zooplanktivore	2.2	2	255-340	9.61±0.43	-17.94±0.36
Odonus niger	ODN	Zoobenthivore	3.1	5	211-347	11.33±0.65	-17.87±1.02
Oxycheilinus digramma	OXD	Zoobenthivore	3.7	2	191-197	12.02±0.24	-15.56±0.12
Parupeneus macronema	PAM	Zoobenthivore	3.5	2	173-212	11.58±0.03	-14.87±0.41
Plectorhinchus vittatus	PLV	Zoobenthivore	3.9	6	321-441	12.07±0.17	-15.44±0.47
Plectropomus pessuliferus	PLP	Piscivore		2	334-370	12.69±0.16	-17.26±0.00
Pomacentrus indicus	POI	Omnivore	2.6	4	94-115	10.91±0.16	-19.08±0.22
Pomacentrus philippinus	POP	Omnivore	2.7	3	85-90	11.02±0.16	-19.46±0.03
Pterocaesio pisang	PTP	Zooplanktivore	3.4	6	114-150	11.24±0.05	-19.52±0.09
Pygoplites diacanthus	PYD	Spongivore	2.7	27	146-244	11.31±0.09	-17.88±0.05
Sargocentron caudimaculatum	SAC	Zoobenthivore	3.9	3	168-183	12.47±0.09	-16.49±0.27
Sargocentron diadema	SAD	Zoobenthivore	3.4	1	161	11.75	-15.29
Sargocentron spiniferum	SAS	Zoobenthivore	3.6	9	233-407	12.17±0.18	-16.13±0.27
Scarus frenatus	SCF	Herbivore	2.0	9	147-375	8.73±0.18	-13.10±0.52
Scarus niger	SCN	Herbivore	2.0	7	164-280	8.14±0.21	-13.68±0.58
Scarus rubroviolaceus	SCR	Herbivore	2.0	1	360	10.61	-17.68
Scarus scaber	SCS	Herbivore	2.0	4	182-270	8.54±0.05	-12.43±0.50
Scolopsis aurata	SCA	Zoobenthivore	3.6	1	264	12.22	-15.87
Variola louti	VAL	Piscivore	4.3	3	198-510	12.23±0.15	-16.49±1.34
Zanclus cornutus	ZAC	Zoobenthivore	2.5	6	144-155	10.97±0.11	-18.38±0.13
Zebrasoma scopas	ZES	Herbivore	2.0	3	118-124	9.72±0.70	-16.88±0.86

3.2.4 Data analysis

All data were analysed in R 3.24 (R Core Team, 2016) using the packages ggplot2 (Wickham and Chang, 2016) and SIBER (Jackson *et al.*, 2011). The data were tested for normality and homogeneity of variance prior to analysis. All errors are reported as ± 1SE unless otherwise stated.

3.2.4.1 Baseline correction

The pelagic source end members (n = 10, TP = 2.0, $\delta^{15}N$ = 8.13 ± 0.12‰) and benthic source endmembers/primary consumers (n = 88, TP = 2.0, $\delta^{15}N$ = 8.34 ± 0.08‰) were indistinguishable (p = 0.16), therefore no adjustment in baselines was needed between the benthic and pelagic pathways.

3.2.4.2 Trophic guild isotopic characterisation

Isotopic characterisation of the eight trophic guilds (Table 3.1), including six depending on reef production-sources (corallivore, detritivore, herbivore, piscivore, spongivore and zoobenthivore), one on mixed sources (omnivore) and one on pelagic production sources (zooplanktivore), were investigated by examining the dispersion of $\delta^{13}C$ and $\delta^{15}N$ values in iso-space. This was achieved by calculating

sample size corrected standard ellipse areas (SEA_C), including the isotopic niche parameters: semi-major axis (*a*), semi-minor axis (*b*), the angle in radians/degree (θ) between *a* and the x-axis, the eccentricity (E) and Bayesian SEA (SEA_B) (Jackson *et al.*, 2011). θ and E values have the potential to distinguish among isotopic niches where different species or trophic guilds have similar area values but there are differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ (Reid *et al.*, 2016). θ is generated as a value between 0 and π from the model and is reported here between 0° to 180°. θ values close to 0° or 90° suggest dispersion in only one axis: θ values close to 0° represent relative dispersion along the x-axis ($\delta^{13}C$) indicating individuals of the group are utilizing multiple production sources, while θ values close to 90° show relative dispersion along the y-axis ($\delta^{15}N$) and indicate multi-TP feeding patterns within a uniform basal source. θ values between 0° and 90° were categorised as positive inclination, whereas θ values between 90° and 180° were expressed as negative inclination. E explains the variance on the x- and y-axes: low E refers to similar variance on both axes with a more circular shape, while high E indicates that the ellipse is stretched along either x- or y-axis. In order to compare isotopic niche areas among trophic guilds, a Bayesian approach was used that calculated 20,000 posterior estimates of SEA_B based on the data set. The mode and 95% credible intervals (CIs) were reported. A significant difference among SEA_B was interpreted graphically whereby if the 95% CIs did not overlap then the SEA_B were deemed to be significantly different.

3.2.4.3 Species level isotopic niche analyses

Because main production source types in Maldivian coral reefs (e.g. plankton and benthic algae) are isotopically different (Chapter 5), fishes feeding strictly on these sources are deemed to be isotopically identical. Mean ± SE $\delta^{13}C$ and $\delta^{15}N$ values of individual species (i.e. isotopic niche) were plotted together with the trophic guild isotopic niche to examine their dietary strictness by the positions of the ellipses relative to their putative trophic guild based on Fishbase (fishbase.org). Species mean isotopic values within or slightly outside (with SE inside) of the isotopic niche were categorised as strict feeders according to their categorisation. Species well outside the isotopic niche and close to other isotopic niche were categorised as generalists of the two sources involved. Species within an isotopic niche other than that of which they had been assigned were categorised as strict feeders of that other source.

Extensive dietary studies have been conducted on herbivores (e.g. Acanthuridae and Scarinae) and diurnal and nocturnal planktivores (e.g. Caesionidae and *Myripristis*) including behaviour, anatomical and chemical (herbivore only) approaches (O'Brien, 1979; Carpenter *et al.*, 1981; Carpenter, 1990; Clements *et al.*, 2016), whereas diets of the other trophic guilds tend to be more simply categorised. Isotopic niches of three sub-guilds were created based on their distinctive diet compositions including algivorous Acanthuridae (*A. leucosternon* and *N. elegans*), microphagous Scarinae (*C. sordidus*, *C. strongylocephalus*, *C. bicolor*, *S. frenatus*, *S. niger* and *S. scaber*) and nocturnal planktivorous *Myripristis* (*M. berndti*, *M. murdjan*, *M. pralinia*, *M. violacea* and *M. vittata*). Isotopic niches of these groups were visualized and compared with other groups (after recategorisation of species) following the same method.

3.3 Results

3.3.1 Isotopic niches of trophic guilds

The isotopic niches of the eight trophic guilds were quite well spread, albeit with some overlap between particular pairs of trophic guilds (Figure 3.2). The detritivore isotopic niches was within that of the herbivore. This herbivore-detritivore cluster had a high mean $\delta^{13}C$ and a low mean $\delta^{15}N$ value and was distinct from other trophic guilds. The corallivore isotopic niche was approximately 3‰ higher in $\delta^{15}N$ than the herbivore-detritivore cluster (Figure 3.2). The omnivore isotopic niche was within that of the zooplanktivores. This zooplanktivore-omnivore cluster had on average an approximately 1‰ higher $\delta^{15}N$ but 5‰ lower $\delta^{13}C$ value than the corallivore isotopic niche. The spongivore isotopic niche was mostly within the zoobenthivore isotopic niche and was closer to the pelagic feeders than benthic feeders. This zoobenthivore-spongivore cluster was between the zooplanktivore-omnivore and corallivore clusters (Figure 3.2). The piscivore isotopic niche had approximately 1.5‰ greater $\delta^{15}N$ than the zoobenthivores and was close to the pelagic feeders.

SEA, SEA_C and mode of SEA_B values were similar to each other in each trophic guild (Table 3.2). Overall, the herbivore and zoobenthivore groups had large isotopic niche areas, the corallivore and zooplanktivore had medium-sized isotopic niche areas, whereas the others had small isotopic niche areas. The E and θ values differed among trophic guilds: the detritivore, herbivore, piscivore and spongivore groups had relatively round isotopic niches (E > 0.9) whereas others were

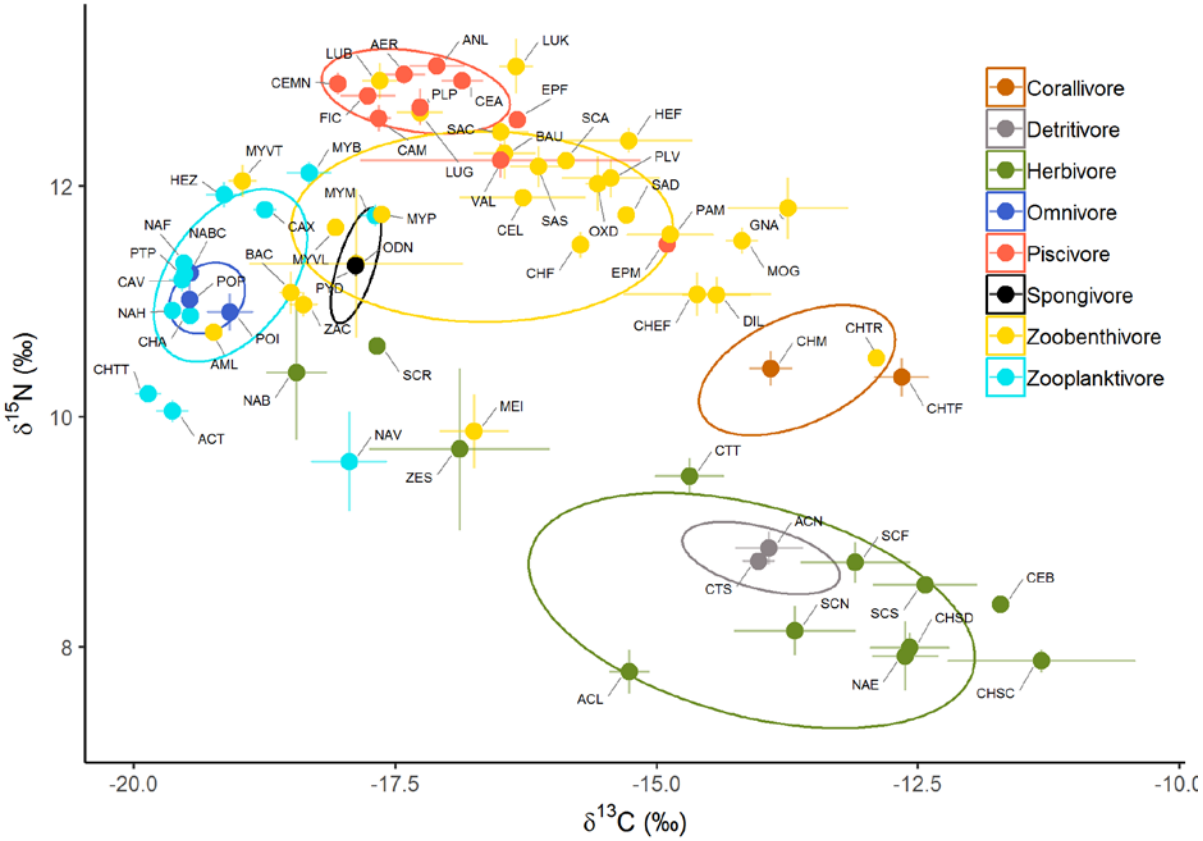


Figure 3.2 Plot of bulk $\delta^{15}N$ against $\delta^{13}C$ (mean ± SE) data of all sampled fish species (for codes see Table 3.) and small sample size-corrected standard ellipses/isotopic niches (solid line-ellipses from SIBER) for eight trophic guilds (corallivore, detritivore, herbivore, omnivore, piscivore, spongivore, zoobenthivore and zooplanktivore) of fish at North Malé Atoll (Maldives).

Table 3.2 Isotopic niche area (‰^{2}) estimates and parameters (eccentricity [E], the angle in degree between the semi-major axis of the isotopic niche and the x-axis [θ]) for eight trophic guilds of coral reef-fish at North Malé Atoll (Maldives). Estimates of isotopic niche areas are given as standard ellipse area (SEA), sample size-corrected standard ellipse area (SEA_C) and the mode of the Bayesian standard ellipse area (SEA_B) estimates. Upper and lower 95% credible intervals (CIs) indicate the uncertainty in the SEA_B estimates.

Trophic guild	SEA (‰^{2})	SEA_C (‰^{2})	E	θ (°)	SEA_B (‰^{2})	SEA_B 95% CIs
Corallivore	1.393	1.471	0.877	20.63	1.375	0.859-2.175
Detritivore	0.629	0.652	0.937	-12.95	0.610	0.433-0.896
Herbivore	6.208	6.287	0.907	-13.12	6.126	4.946-7.716
Omnivore	0.300	0.343	0.654	26.53	0.282	0.137-0.582
Piscivore	0.978	0.992	0.924	-8.19	0.960	0.757-1.234
Spongivore	0.279	0.290	0.944	70.82	0.283	0.190-0.411
Zoobenthivore	4.704	4.742	0.891	-0.34	4.753	3.931-5.589
Zooplanktivore	1.525	1.550	0.782	46.47	1.514	1.182-1.937

comparatively flat. The corallivore, omnivore, spongivore and zooplanktivore groups had isotopic niches with positively inclined θ values whereas those of the others were negatively inclined.

The SEA_B data (Figure 3.3, Table 3.2) indicated that the herbivore and zoobenthivore groups had similar SEA values, and these were significantly greaer than those of the other groups, followed by those of the corallivore and zooplanktivore groups that were significantly greater than those of the omnivore and spongivore groups. The SEA value of the piscivores was slightly lower than those of the corallivore and zooplanktivore groups but significantly higher than those of the omnivores and spongivores. The detritivore group had a SEA value slightly lower than the piscivores but slightly greater than those of the omnivores and spongivores.

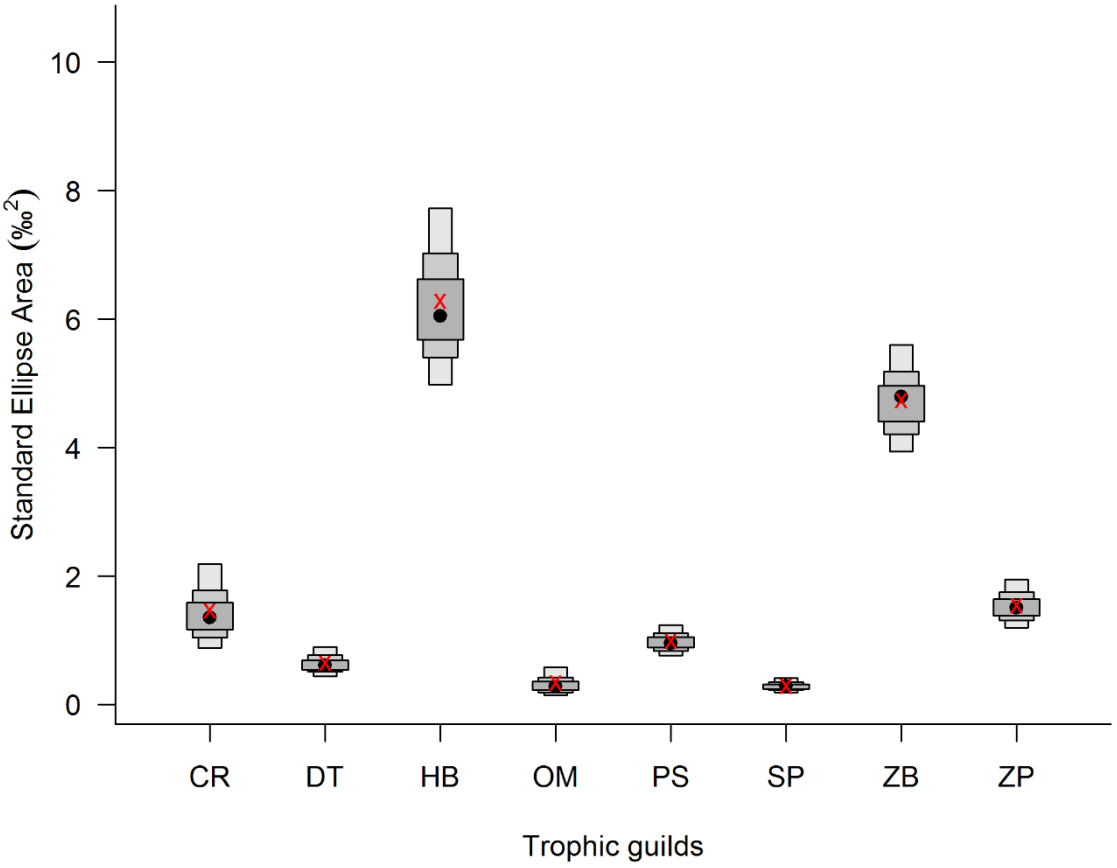


Figure 3.3 Posterior estimates of the standard ellipse areas (SEA_B) for the eight fish trophic guilds at North Malé Atoll (Maldives): corallivore (CR), detritivore (DT), herbivore (HB), omnivore (OM), piscivore (PS), spongivore (SP), zoobenthivore (ZB) and zooplanktivore (ZP). The boxes represent the 95, 75 and 50% credible intervals in ascending order of size. Mode of the posterior Bayesian estimate area, SEA_B is indicated by a black circle. The maximum likelihood estimate and sample size-corrected ellipse area for the corresponding SEA_C is indicated by a red cross.

3.3.2 Species isotopic niches

Most species had relatively small standard errors (SE < 0.5) in both stable isotope values (Table 3.1). There were 10 with SEs ≥ 0.5 in $\delta^{13}C$ (*S. frenatus*, *Gnathodentex aureolineatus*, *S. niger*, *Cephalopholis leopardus*, *Hemigymnus fasciatus*, *Cheilinus fasciatus*, *Z. scopas*, *C. strongylocephalus*, *Odonus niger* and *Variola louti*), three with SEs ≥ 0.5 in $\delta^{15}N$ (*N. brevirostris*, *O. niger* and *Z. scopas*), and two with high SEs in both $\delta^{13}C$ and $\delta^{15}N$ (*O. niger* and *Z. scopas*).

Corallivorous, detritivorous, omnivorous and spongivorous species were mostly within their ellipses (Figure 3.2). For the herbivore group, most species were within or close to the ellipse except for *Z. scopas*, *S. rubroviolaceus* and *N. brevirostris* which were close to the zooplanktivore-omnivore cluster. Most Scarinae species except *S. rubroviolaceus* (SCR) (*n* = 1) had isotopic niches with $\delta^{13}C$ less negative than the average herbivore, whereas herbivorous Acanthuridae species were more negative in $\delta^{13}C$ than other herbivores in the ellipse.

Of the zooplanktivorous species, *M. berndti* was close to, and *M. pralinia* was within, the zoobenthivore isotopic niche, *N. vlamingii* was close to the herbivore isotopic niche, and *Chromis ternatensis* and *Acanthurus thompsoni* were not close to any other isotopic niche and had lower $\delta^{15}N$ values than other zooplanktivores. Among zoobenthivorous species, *Amblyglyphidodon leucogaster*, *Balistoides conspicillum* and *Zanclus cornutus* were within, and *M. vitatta* and *M. violacea* were close to, the zoobenthivore-spongivore isotopic niche. *Cheilinus fasciatus*, *Diodon liturosus*, *Monotaxis grandoculis* and *Cheilinus trilobatus* were within, and *H. fasciatus* and *G. aureolineatus* were close to, the corallivore isotopic niche, while *Lutjanus bohar* was within, and *Lutjanus gibbus* and *Lutjanus kasmira* close to, the piscivore isotopic niche. Some piscivorous species such as *Variola louti* and *Epinephelus merra* were within and *Caranx melampygus*, *Plectropomus pessuliferus* and *Epinephelus fuscoguttatus* were close to the zoobenthivore isotopic niche.

3.3.3 Sub-trophic guild isotopic niches

When the herbivorous species were split into algivore and microphage sub-guilds and omnivorous species were separated out, the isotopic niches helped distinguish four groups within the herbivore-detritivore cluster with small overlaps (Figure 3.4), and the isotopic niches were reduced in size compared with that of the herbivores as a whole (Figure 3.5, Table 3.3). The omnivore isotopic niche was almost separated from the others, while there were still some overlaps among the

other three guilds. The detritivore isotopic niche was greater in $\delta^{15}N$ than the algivore and microphage groups. All species had isotopic niches (mean ± SE values) within the groups, except for *N. elegans* which was in the microphage isotopic niche rather than that of the algivores. The algivore isotopic niche was relatively flat compared with other groups' isotopic niches. The algivore SEA value tended to be greater than the other herbivore-detritivores, although not significantly so than the microphage and omnivore data (Figure 3.5, Table 3.3). Aside from the microphage group, the other three groups had isotopic niches with high negative inclination.

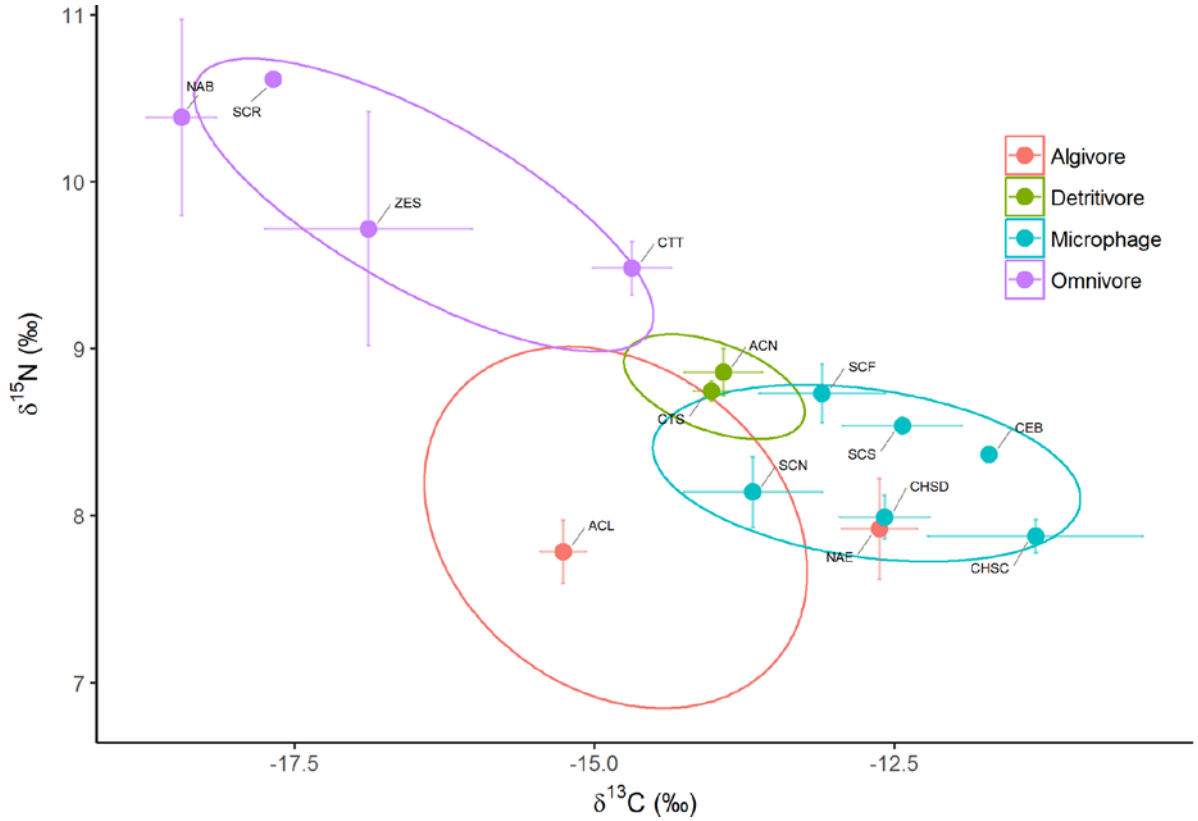


Figure 3.4 Plot of bulk $\delta^{15}N$ against $\delta^{13}C$ (mean ± SE) data of sampled fish species (for codes see Table 3.1) of two trophic guilds (detritivore and omnivore) and sub-guilds (algivore and microphage) and small sample size-corrected standard ellipses (solid line-ellipses from SIBER) of these four groups at North Malé Atoll (Maldives).

By categorising *Myripristis* spp as being in the nocturnal planktivore guild, when *N. vlamingii* and *M. indicus* were treated as omnivores and *N. brachycentron*, *P. indicus* and *P. philippinus* as zooplanktivores, the isotopic niches of zooplanktivore, nocturnal planktivore, zoobenthivore and omnivore groups became distinct from each other (Figure 3.6). This also discriminated the spongivore group from the zoobenthivore group, yet there was still overlap between the spongivores and nocturnal planktivores. The nocturnal planktivore and omnivore isotopic niches were relatively flat compared with those of the other three groups. The

zooplanktivore, omnivore and spongivore groups also had positively inclined ellipses whereas those of the nocturnal planktivores and zoobenthivores were negatively inclined (Table 3.4).

Table 3.3 Isotopic niche area (‰^{2}) estimates and parameters (eccentricity [E], the angle in degree between the semi-major axis of the isotopic niche and the x-axis [θ]) for four groups (two trophic guilds [detritivore and omnivore] and two sub-guilds [algivore and microphage]) of coral reef-fish at North Malé Atoll (Maldives). Estimates of isotopic niche areas are given as standard ellipse area (SEA), sample size-corrected standard ellipse area (SEA_C) and the mode of the Bayesian standard ellipse area (SEA_B) estimates. Upper and lower 95% credible intervals (CIs) indicate the uncertainty in the SEA_B estimates.

Trophic guild	SEA (‰^{2})	SEA_C (‰^{2})	E	θ (°)	SEA_B (‰^{2})	SEA_B 95% CIs
Algivore	5.019	5.258	0.778	-15.87	4.840	3.135-7.447
Detritivore	0.629	0.652	0.937	-12.95	0.623	0.428-0.896
Microphage	2.757	2.839	0.959	-5.33	2.659	1.958-3.767
Omnivore	3.147	3.461	0.964	-20.57	3.177	1.820-6.148

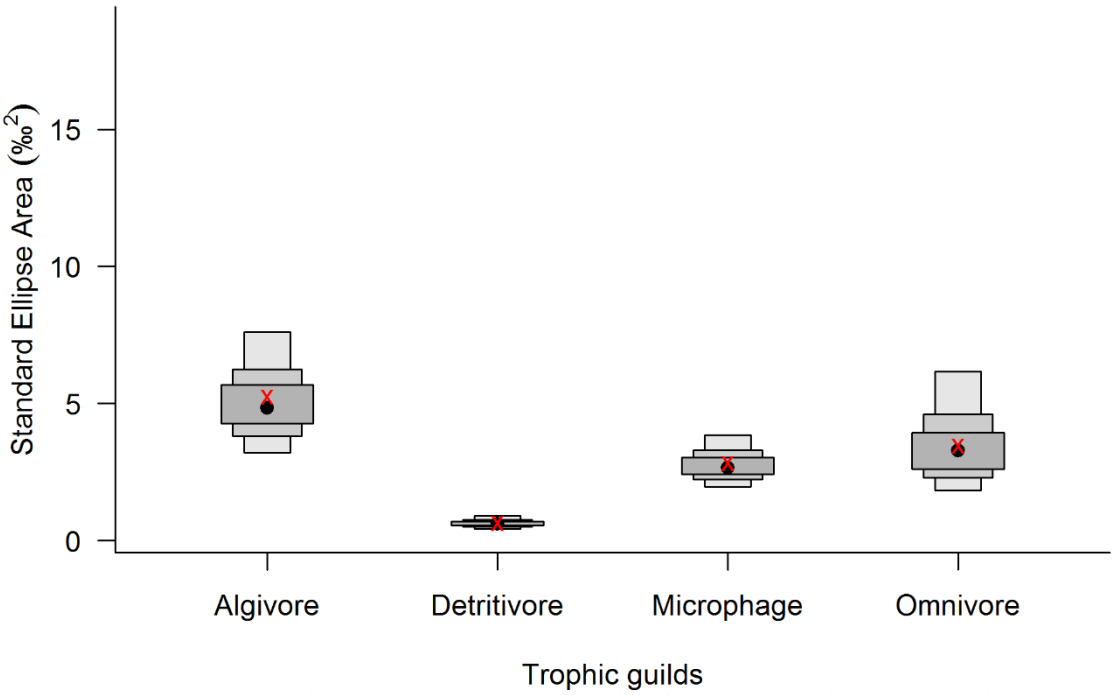


Figure 3.5 Posterior estimates of the standard ellipse areas (SEA_B) for the four fish groups (two trophic guilds [detritivore and omnivore] and two sub-guilds [algivore and microphage]) at North Malé Atoll (Maldives). The boxes represent the 95, 75 and 50% credible intervals in ascending order of size. Mode of the posterior Bayesian estimate area, SEA_B is indicated by a black circle. The maximum likelihood estimate and sample size-corrected ellipse area for the corresponding SEA_C is indicated by a red cross.

In the Bayesian analysis (Table 3.4), the omnivore (significantly higher than nocturnal planktivore and spongivore groups) and zoobenthivore groups (significantly higher than nocturnal planktivore, spongivore and zooplanktivore groups) had the highest SEA values.

3.4 Discussion

3.4.1 Isotopic characterisation of trophic guilds

The close SEA, SEA_C and mode SEA_B values for each of the eight trophic guilds indicated that they were not sensitive to sample size or data structure effects (Jackson *et al.*, 2011). The relative vertical position of SEA_C suggested that herbivores and detritivores were lower in $\delta^{15}N$ or TP (i.e. primary consumers); zooplanktivores, omnivores, spongivores, zoobenthivores and corallivores had a middling TP (i.e. secondary consumers); and piscivores occupied high TPs (i.e. tertiary consumers), as expected. The relative horizontal position suggested the existence of at least two types of primary production sources, namely benthic (higher in $\delta^{13}C$, e.g. algae) and pelagic (lower in $\delta^{13}C$, e.g. phytoplankton), and the reliance on either source differed among trophic guilds. The isotopic niches of these three clusters (herbivore-detritivore, zooplanktivore-omnivore and zoobenthivore-spongivore) and the other two trophic guilds (corallivore and piscivore) were discriminable to some extent.

The herbivore-detritivore cluster could result from 1) the herbivore and detritivore groups sharing similar production sources, or 2) bulk carbon and nitrogen stable isotopes did not discriminate them even though they were potentially supported by different pathways (Layman *et al.*, 2007a). Some detritivores can utilize certain microbial autotrophs (e.g. diatoms and cyanobacteria) embedded in the detritus and some algivorous species digest epiphytic detrital materials (Crossman *et al.*, 2001; Sanchez and Trexler, 2018). This detrital-algal matrix is common on coral reefs (Adam *et al.*, 2018). Yet these two types of food sources are different in masses of total extractable amino acids and starches, and amino acid compositions (Crossman *et al.*, 2001). However, no study has investigated isotopic discriminability of individual detrital components on coral reefs (e.g. dead animal material, faeces, microbes), compared these with algal sources (e.g. filamentous algae), or quantified diet compositions of reef-fish species in this way. Understanding the reasons behind the overlapping isotopic niches of these two trophic guilds requires further study.

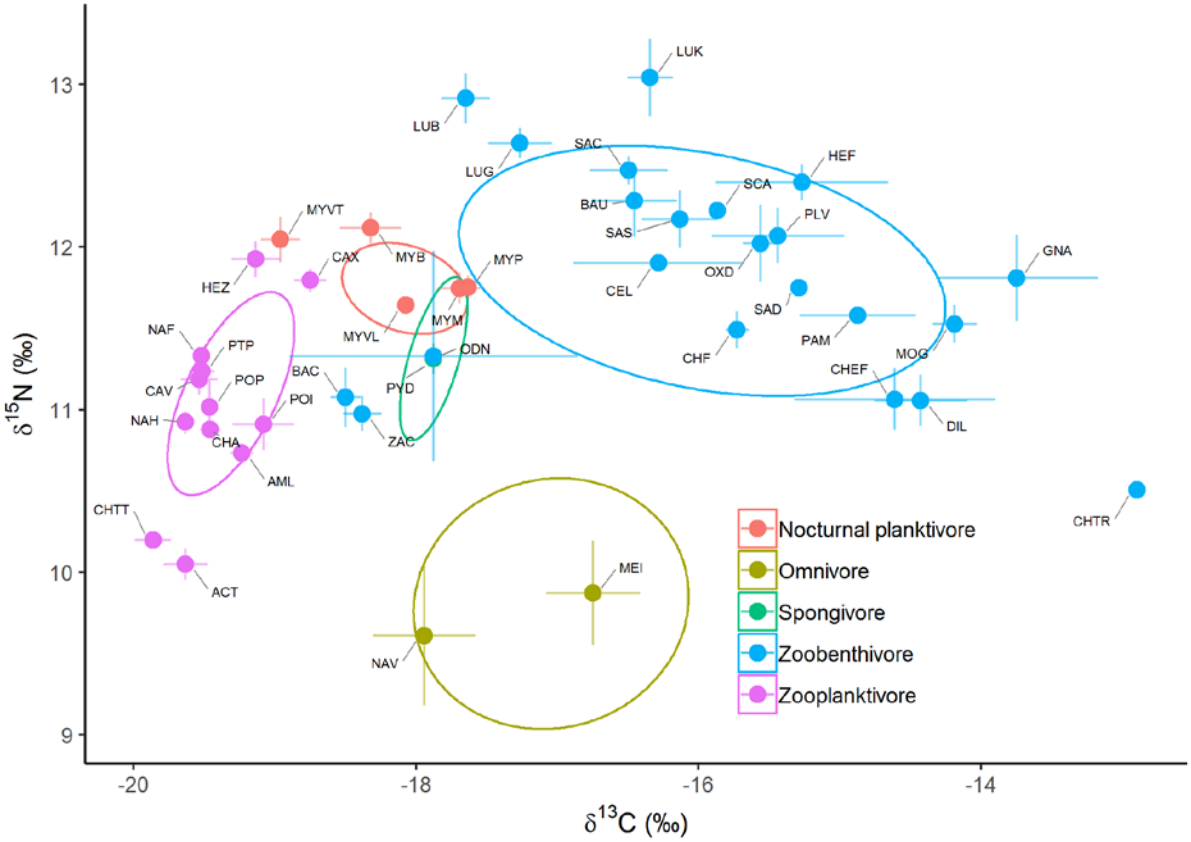


Figure 3.6 Plot of bulk $\delta^{15}N$ against $\delta^{13}C$ (mean ± SE) relationship of sampled fish species (for codes see Table 3.1) of four trophic guilds (omnivore, spongivore, zoobenthivore and zooplanktivore) and one sub-guild (nocturnal planktivore) and small sample size-corrected standard ellipses (solid line-ellipses) for these five groups at North Malé Atoll (the Maldives).

Table 3.4 Isotopic niche area (‰^{2}) estimates and parameters (eccentricity [E], the angle in degree between the semi-major axis of the isotopic niche and the x-axis [θ]) for five fish groups (four trophic guilds [omnivore, spongivore, zoobenthivore and zooplanktivore] and one sub-guild [nocturnal planktivore]) of coral reef-fish at North Malé Atoll (Maldives). Estimates of isotopic niche areas are given as standard ellipse area (SEA), sample size-corrected standard ellipse area (SEA_C) and the mode of the Bayesian standard ellipse area (SEA_B) estimates. Upper and lower 95% credible intervals (CIs) indicate the uncertainty in the SEA_B estimates.

Trophic guild	SEA (‰^{2})	SEA_C (‰^{2})	E	θ (°)	SEA_B (‰^{2})	SEA_B 95% CIs
Nocturnal planktivore	0.366	0.378	0.446	-14.15	0.363	0.256-0.505
Omnivore	2.018	2.355	0.618	7.39	1.907	0.755-4.109
Spongivore	0.279	0.290	0.944	70.82	0.274	0.190-0.411
Zoobenthivore	3.922	3.969	0.909	-9.34	3.904	3.127-4.813
Zooplanktivore	0.680	0.690	0.896	59.19	0.668	0.535-0.864

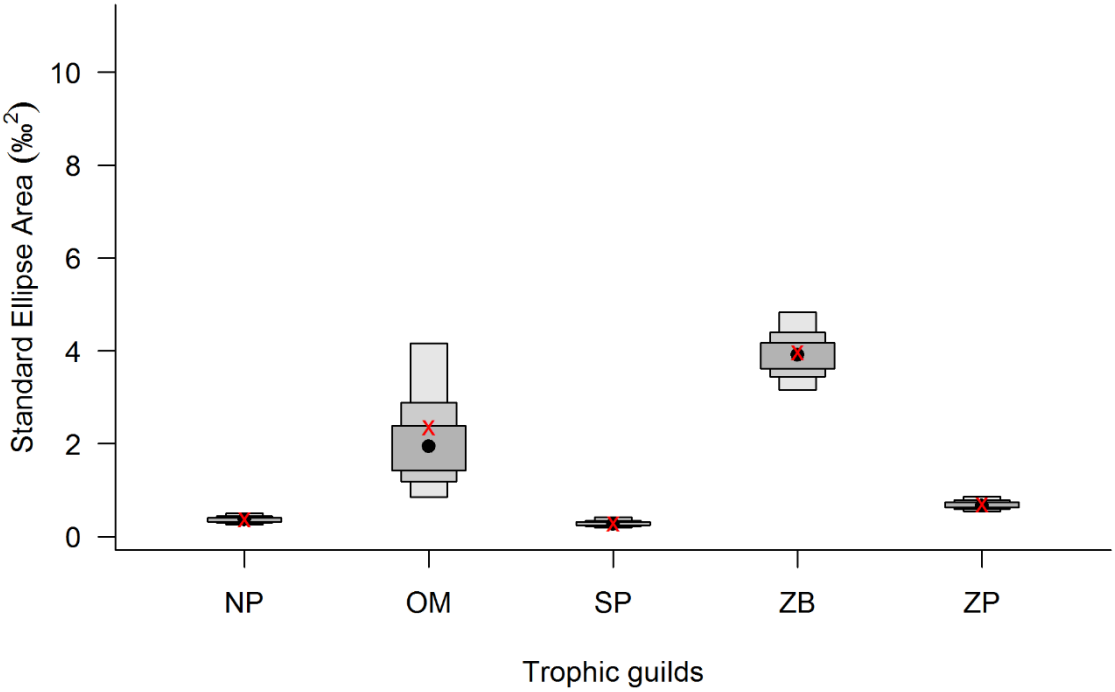


Figure 3.7 Posterior estimates of the standard ellipse areas (SEA_B) for the five fish groups (four trophic guilds [omnivore, spongivore, zoobenthivore and zooplanktivore] and one sub-guild [nocturnal planktivore]) at North Malé Atoll (Maldives): nocturnal planktivore (NP), omnivore (OM), spongivore (SP), zoobenthivore (ZB) and zooplanktivore (ZP). The boxes represent the 95, 75 and 50% credible intervals in ascending order of size. Mode of the posterior Bayesian estimate area, SEA_B is indicated by a black circle. The maximum likelihood estimate and sample size-corrected ellipse area for the corresponding SEA_C is indicated by a red cross.

The zooplanktivore-omnivore cluster suggested likely inaccurate categorisations of the sampled omnivores in the Maldives. Studies show *N. brachycentron*, *P. philippinus* and *P. indicus* feed on both zooplankton and benthic algae (Sommer *et al.*, 1996), and these two types of sources had very distinctive isotopic baselines, at least in the present study sites (see Chapter 5 and 6). Thus, these three species in the sampled size ranges at this location were evidently strict zooplanktivores based on their high affinity to the zooplanktivore group. Lastly, the zoobenthivore-spongivore cluster was potentially a result of overlapping diet, with some zoobenthivores other than strict spongivores also feeding on cryptic sponges (Randall, 1967).

3.4.2 Species isotopic niches

The isotopic niches indicated source partitioning of most species in each trophic guild (Jackson *et al.*, 2011). Species' isotopic niches outside the guild's isotopic niche could be a result of individual specialisation (Matthews and Mazumder, 2004) on sources, which were either isotopically similar to or totally different from the

diet of the majority depending on the relative position. Both situations were common at this study site, with some species likely to be inaccurately categorised.

3.4.2.1 Herbivores

The large, negatively inclined and relatively round isotopic niche (Figure 3.2) suggested these herbivores were likely to feed on many production sources of different isotopic baselines. Some potentially utilized sources from higher TP (e.g. zooplankton and zoobenthos) rather than just low-TP benthic algae. Thus, categorisation of these species as herbivores might underestimate their diverse source utilization that some animal-derived food items might be important to their diet.

At this study site, the herbivore trophic guild included microphagous Scarinae and algivorous Acanthuridae. These groups tend to feed on different benthic primary producers (microbial autotrophs and filamentous algae respectively, Crossman *et al.*, 2001; Clements *et al.*, 2016) and thus have potentially different functional roles in maintaining the health state of corals (Thacker *et al.*, 2001; Burkepile and Hay, 2008; Green and Bellwood, 2009; Plass-Johnson *et al.*, 2015; Adam *et al.*, 2018). By plotting the isotopic niches of these two sub-trophic guilds, such differences were reflected to some extent.

Comparing the isotopic niches between these two sub-guilds, some features remained in common; these included TP and source omnivory as indicated by the ellipse inclination (θ in Table 3.3). Previous studies have found herbivores feeding on other food sources than algae such as zooplankton, zooplanktivore faeces, invertebrates and cryptic sponges (Randall, 1967; Robertson, 1982; Wulff, 1997; Dunlap and Pawlik, 1998; Chen, 2002). For the microphagous group, digestive anatomy, gut content, gut bacteria and biomarker data indicate a strict diet in Scarinae consisting mainly of cyanobacteria (Clements *et al.*, 2016) through multiple approaches (e.g. bio-tracer and anatomy). High-TP feeding such as on invertebrates has only been observed in the initial phase of some Scarinae species (Chen, 2002), while spongivory (Wulff, 1997; Dunlap and Pawlik, 1998) is considered to be a mechanism to access symbiotic cyanobacteria within the sponges (Clements *et al.*, 2016). The mature species collected here were consistent in their TP. All Scarinae species except *S. rubroviolaceus* which was categorised as omnivore based on its affinity to the zooplanktivore isotopic niche, at this site and within their sampled size ranges evidently vary in the microbial autotroph sources which they rely on. This

source omnivory is also within species, for example, *C. strongylocephalus* has a large $\delta^{13}C$ SE and a low $\delta^{15}N$ SE attributable to feeding on multiple microbial autotrophs with a range of $\delta^{13}C$ baselines. More studies are required such as on microbial autotroph isotopic signatures to better understand this part of the food web.

In contrast, the negative inclination and affinity with the zooplanktivore group of algivorous Acanthuridae suggested source and TP omnivory. Existing literature suggests some algivorous Acanthuridae species are also coprophagous (Robertson, 1982), and here, the relative positions of *Z. scopas* and *N. brevirostris* in the iso-space (Figure 3.2) could be explicable in terms of their consuming faeces of zooplanktivores, while *C. truncatus* might feed on zoobenthos occasionally; these three species were thus categorised as omnivorous. After redefining trophic guilds (Figure 3.4), algivorous Acanthuridae species had a small yet negatively inclined isotopic niche and omnivorous acanthurids had a large yet very negatively inclined isotopic niche (Table 3.3) suggesting TP omnivory among them.

The SIBER plot of the microphage and algivore groups discriminated them rather well. However, the algivore *N. elegans* isotopic niche was within the microphage isotopic niche. *Naso elegans* has been categorised as a strict phaeophytivore based on gut content and DNA barcoding of digestive symbionts (Ngugi *et al.*, 2017). It may be that bulk carbon and nitrogen stable isotope data cannot discriminate between phaeophytes and some microbial autotrophs so that similar isotopic niches were perceived as species utilizing completely different pathways (Layman *et al.*, 2007a), or this species might utilize microbial autotrophs extensively at this Maldivian site. However, digesting some microbial autotrophs (e.g. cyanobacteria) does not require digestive symbionts; DNA barcoding of digestive symbionts confirmed phaephytes as one component of diet but not the entire diet composition. Bulk $\delta^{13}C$ values of benthic algae may vary very greatly (Pinnegar and Polunin, 2000; Polunin and Pinnegar, 2002; Dromard *et al.*, 2013; Plass-Johnson *et al.*, 2013), however, no study has looked at the isotopic signatures of both benthic algae and cyanobacteria in the same system except Pentecost and Spiro (1990) who studied carbon and oxygen stable isotopes of cyanobacteria and algae in a freshwater system. Thus, it remains unclear what the cause of the overlap between *N. elegans* and the microphage group is.

The herbivorous fish are trophically particularly diverse in this study, and the basis for this needs to be elucidated in future work. However, the algivore and microphage sub-guilds are isotopically characterised for the first time, categorising

this group as ‘herbivore’ has clearly masked a great deal of intriguing ecological detail.

3.4.2.2 Detritivores

The small SEA values and round yet negatively inclined isotopic niche of the detritivore group suggested their reliance on a relatively narrow production base. Detrital materials may include dead organic matter (e.g. algae, fish faeces and coral mucus), inorganic materials, microbial autotrophs (e.g. cyanobacteria), microalgae (e.g. diatoms and dinoflagellates) and associated meiofauna (Crossman *et al.*, 2001). These components may come from sources with distinctive baselines and the detritus can be highly varied spatially and temporarily in its composition. However, such heterogeneity was not reflected by the small detritivore SEA values here. This might be a result of 1) indistinguishable isotopic baselines among detrital components; 2) the detritus being a rather homogeneous mixing of the different components; or 3) these detritivores selectively focusing on certain component(s). Because these detrital sources have yet to be analysed isotopically, it is not possible to test these ideas. Using specific bio-tracer(s) from the detritus to the detritivores might resolve this because microbes tend to have diverse biosynthesis pathways for specific compounds such as essential amino acids or fatty acids (Larsen *et al.*, 2015).

The detritivore isotopic niche (Figure 3.4) sat between and slightly above those of the algivore and microphage, albeit with some overlap. The higher detrital $\delta^{15}N$ values than the other two groups suggested the influence of high-TP food sources; these might include faeces and meiofauna. However, the smaller detritivore SEA_C (Table 3.3) indicated either a more strict diet than the other groups or potentially a range of sources assimilated consistently by the species involved. Separating algivore and microphage groups (Figure 3.4) had the effect of better characterising the detritivores and further understanding their trophic functional roles. However, it is possible that if more detritivorous species had been analysed, the result might have been different.

3.4.2.3 Spongivores

Sponges are functionally important in coral-reef food-webs (de Goeij and Van Duyl, 2007; de Goeij *et al.*, 2008b; de Goeij *et al.*, 2013; de Goeij *et al.*, 2017) and provide a tool to monitor ecosystem state(s) (Orani *et al.*, 2018), whereas spongivory can be a beneficial function to reduce competition to maintain coral health (Hill, 1998;

Yahel *et al.*, 2003). The spongivore group had the smallest SEA values but a round yet vertical isotopic niche suggesting consistent source partitioning and TP omnivory (feeding on sponges of multiple TPs) rather than source omnivory of the single species (*P. diacanthus*) in this group found at other locations (e.g. algae, cnidarians and sponges, Alwany, 2009; Konow and Bellwood, 2011). However, only one species was included in this trophic guild, the result might have been different if more species had been analysed, also little is known regarding the exact sponges that spongivores feed on; to better understand the interaction between spongivores and sponges will require more data.

3.4.2.4 Corallivores

The middling SEA values and round and horizontal isotopic niche of the corallivores suggested their relative dietary strictness. The different isotope niches of the two species involved (*C. meyeri* and *C. trifasciatus*) could be a result of resource partitioning (Muscatine and Kaplan, 1994), specialisation on different genera or species of corals (Cole *et al.*, 2008; Brooker *et al.*, 2013) with different isotopic baselines (Appendix 5), morphologies (Brooker *et al.*, 2013), dietary components (e.g. mucus and polyps, Brooker *et al.*, 2013) and macroborer invertebrates (Cole *et al.*, 2008). Yet they both had very small SE in both isotope values (Table 3.1). The results here confirmed the strict and selective feeding pattern of these two corallivore species. Another Chaetodonidae species *C. falcula* was classified as corallivore (McClanahan *et al.*, 2005), however, from fishbase and this Maldives study, it is evidently zoobenthivorous.

3.4.2.5 Zooplanktivores

The zooplanktivore group also had medium SEA values, and a relatively round isotopic niche with an approximately 45° inclination, suggesting both source and TP omnivory. The trophic roles of zooplankton are often seasonal (Kürten *et al.*, 2013), the nutrient sources of phytoplankton can vary geographically and seasonally (Gove *et al.*, 2016; Garzon-Garcia *et al.*, 2018) and zooplankton assemblages can be very variable due to their diel vertical migration (Zaret and Suffern, 1976; Enright and Honegger, 1977; Bollens and Frost, 1989). Different zooplanktivorous species target different zooplankton based on predatory skills, prey preferences, prey behaviours and morphological characteristics (Zaret and Suffern, 1976). Thus, isotopic variation is expected among zooplankton prey (McClelland and Montoya, 2002; Kürten *et al.*, 2013) and their predators. Two species (*C. ternatensis* and *A. thompsoni*) had a

similar $\delta^{13}C$ but a lower $\delta^{15}N$ value than other zooplanktivores, suggesting they might be feeding on lower-TP zooplankton or even phytoplankton. One zooplanktivorous species, *N. vlamingii* had an isotopic niche very similar to the omnivores defined here (e.g. *Z. scopas*), suggesting its reliance on both zooplankton and benthic primary production sources, and thus was considered an omnivore. Replotting zooplanktivores with *Z. scopas* excluded (Figure 3.6), the SEA values were greatly reduced (from ~1.5 to ~0.68), as was their dietary variability. Yet, TP omnivory still existed based on the relatively flat and positively inclined isotopic niche.

3.4.2.6 Zoobenthivores

The zoobenthivore group had the second highest SEA values and a round and flat isotopic niche, suggesting that they can utilize a wide range of food sources from multiple TP. Its affinity to the zooplanktivore isotopic niche suggested that these species could utilize both benthic and pelagic sources to some extent. The overlap between the zoobenthivore and zooplanktivore groups was mainly attributed to *Myripristis* (nocturnal zooplanktivore), which could be a result of inaccurate categorization of this genus. Most *Myripristis* species feed at night on zooplankton (Hobson, 1991), which includes calanoids from the open ocean (larger than the diurnal), holoplankton residing close to substrate during the day and migrating into the water column at night (e.g. copepods and mysids), and semipelagic organisms from the seafloor (e.g. polychaetes, ostracods, copepods, mysids, isopods, amphipods and crustacean larvae). These zooplankton rely strictly or partially on pelagic phytoplankton such as those with diel migration patterns (Zaret and Suffern, 1976). Compared with diurnal zooplankton, isotopically the nocturnal ones tend to have 1) higher TP (a result of size-based feeding patterns among pelagic zooplankton, McClelland and Montoya, 2002) and 2) some inputs from benthic sources. In this study, *Myripristis* demonstrated both situation that *M. vittatta* and *M. berndti* had similar $\delta^{13}C$ values to the zooplanktivore isotopic niche but slightly higher $\delta^{15}N$; *M. violacea*, *M. murdjan* and *M. pralinia* were close to or inside the SEA_C of the zoobenthivores. Although some of these were categorised as zooplanktivores (*M. berndti* and *M. murdjan*), this genus should be considered as a special sub-guild (i.e. nocturnal planktivore), considering their distinctive diet composition. Other than mentioned pelagic/semipelagic organisms, sessile zoobenthos can also access pelagic production sources (e.g. phyto- and zooplankton, and associated DOMs) through multiple pathways (Zaret and Suffern, 1976; Robertson, 1982; de Goeij *et al.*,

2013; Francis and Côté, 2018). These pathways can potentially enhance benthic-pelagic coupling in such ecosystems and cause zoobenthivores to have lower $\delta^{13}C$ values closer to the pelagic baseline.

The zoobenthivore *A. leucogaster* had an isotopic niche similar to other zooplanktivores. They feed on prey such as amphipods, copepods, mysids and other invertebrates according to existing literature (Allen, 1991; Anderson and Hafiz, 1998), whereas in this particular site, this species might be specialising on pelagic zooplankton at the sampled size range. Two zoobenthivore species *Z. cornutus* and *B. conspicillum* had isotopic niches within the original zooplanktivore isotopic niche and close to that of the zoobenthivores (Figure 3.2), but their isotopic niches became isolated from the other isotopic niches (i.e. between the zooplanktivore and zoobenthivore-spongivore group, and below nocturnal planktivore) after replotting (Figure 3.6). Although these two species can feed on zoobenthos (Sano, 1984; Matsuura, 2001), this study suggested their high reliance on some pelagic sources; however, here they were still regarded as zoobenthivores.

The three lutjanids, *L. bohar*, *L. kasmira* and *L. gibbus* had isotopic niches with higher $\delta^{15}N$ values compared with other zoobenthivores, with *L. kasmira* located outside and to the right of the piscivore isotopic niche. These three species feed on zoobenthos and other fish (Allen, 1985; Kulbicki *et al.*, 2005a; Layman and Allgeier, 2012). However, in this study, these three species are evidently of higher TP than the other zoobenthivores; they might be feeding more on fish or higher-TP zoobenthos. To confirm this would require more replicates and data to analyse the diet of these species. Similar to *N. vlamingii*, *M. indicus* had an isotopic niche indicating its source and TP omnivory. One zoobenthivore species, *C. trilobatus* (n = 1) had an isotopic niche within the corallivore isotopic niche suggesting its reliance on corals as the main ultimate food source which contrasts with previous reports of its feeding on sea stars and sea urchins (McClanahan, 1995), and crustaceans, molluscs and fish (Myers, 1999), however, only one individual was collected for this species; more data are needed to analyse its trophic ecology.

The nocturnal planktivores occupied a specific space between the zooplanktivores and zoobenthivores, which corroborates the diet of *Myripristis* incorporating both benthic and pelagic sources. By doing so, the zoobenthivore isotopic niche was relocated to the right with similar $\delta^{13}C$ values to the algivore group. This result also suggested that there was a gap in $\delta^{13}C$ values between

benthic and pelagic sources at this location, which might be powerful in understanding benthic-pelagic coupling in these food webs.

3.4.2.7 Piscivores

Piscivorous species had relatively small SEA values, with a comparably round and slightly negatively inclined isotopic niche. This indicated their low level of source omnivory. Its affinity to the zooplanktivore isotopic niche suggested that these piscivorous species might prefer zooplanktivorous prey and thus be reliant on pelagic sources. Some piscivore species (*V. louti* and *E. merra*) had isotopic niches within the zoobenthivore isotopic niche suggested these species fed on zoobenthos more than fish; *V. louti* individuals especially, potentially and selectively fed on a wide range of zoobenthos within the sampled size range.

3.4.3 Conclusion

Bulk carbon and nitrogen stable isotope data can provided discriminable isotopic niches of major trophic guilds in coral-reef fish food-webs. Combining trophic guild isotopic niche with species isotopic niche data, omnivorous behaviours are evident in some groups, suggesting such strictly categorised consumers are relying on sources other than indicated by their categorisations, which are thus better understood (Appendix 6). Sub-trophic guild analyses of three established groups (algivore, microphage and nocturnal planktivore) further confirmed that stable isotope data help to disentangle dietary and trophic redundancy among groups sharing similar sources. However, categorising trophic guilds using this method might be subjective due to the definition of “dietary strictness”, the representability of the isotopic niche and the limitations of stable isotope data (e.g. temporal and spatial variation at the baseline, limitations of isotopic discriminability among sources), especially for understudied groups (e.g. zoobenthivore).

Chapter 4. Size-based trophic structuring of coral-reef fish communities at North Malé Atoll (the Maldives)*

4.1 Introduction

Body size is an important trait for animals (Peters, 1983), which correlates with many others including age, productivity, fecundity and functional role. In highly diverse ecosystems where many production sources and diverse trophic guilds exist, mixed feeding patterns are common, and individuals of the same species may vary in source partitioning (Wulff, 1997; Dunlap and Pawlik, 1998; Chen, 2002; Layman *et al.*, 2005; Layman *et al.*, 2007b; Lokrantz *et al.*, 2008; Layman *et al.*, 2012; Plass-Johnson *et al.*, 2013; Lobato *et al.*, 2014; Choat and Clements, 2018). Such variation can be size-related, with individuals feeding on different production sources as size increases due to energy constraints (Brown and Gillooly, 2003; Cohen *et al.*, 2003; Robinson and Baum, 2015), and ontogenetic and morphometric changes occur (Jennings *et al.*, 2001a; Chen, 2002; Jennings *et al.*, 2002b; de la Morinière *et al.*, 2003; Mumby *et al.*, 2006; Al-Habsi *et al.*, 2008; Green and Bellwood, 2009; Romanuk *et al.*, 2011; Plass-Johnson *et al.*, 2013; Robinson and Baum, 2015). The variation can also be a result of seasonality in pelagic production sources (Bronk and Glibert, 1993; Rolff, 2000), human disturbances (Pace *et al.*, 1999; Darimont *et al.*, 2015; Graham *et al.*, 2017), individual specialisation (Araújo *et al.*, 2011), feeding preferences (Wulff, 1997; Cocheret De La Morinière *et al.*, 2003; Layman *et al.*, 2005; Wyatt *et al.*, 2012; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015; McMahon *et al.*, 2016; Zhu *et al.*, unpublished data) and trophic functional roles (Lokrantz *et al.*, 2008; Plass-Johnson *et al.*, 2015).

Nitrogen stable isotope (^{15}N:^{14}N, expressed as $\delta^{15}N$) analysis has been widely used to examine trophic functional roles of fish (Layman *et al.*, 2005; Arim *et al.*, 2010). It can be used as a proxy for trophic position (TP) based on its trophic discrimination patterns between prey and predator and known isotopic baseline. Investigating size-based TP omnivory using $\delta^{15}N$ has found many fish species and individuals share similar production sources (Jennings *et al.*, 2002a; Jennings *et al.*, 2002b; de la Morinière *et al.*, 2003; Romanuk *et al.*, 2011; Plass-Johnson *et al.*, 2013; Robinson and Baum, 2015), but the strength of this relationship can differ among species (Galván *et al.*, 2010) as well as trophic guilds (de la Morinière *et al.*, 2003; Robinson and Baum, 2015). Some regional studies suggest that at the species level size does not necessarily constrain the TP of fish (Jennings *et al.*, 2001a; Al-

*Submitted to Coral Reefs

Habsi *et al.*, 2008; Robinson and Baum, 2015) while globally fish demonstrate a linear positive TP-body size relationship (Romanuk *et al.*, 2011). Variation in δ^{15}N-body size relationships among species is explicable in terms of differences among trophic pathways such as in source baseline and diet quality (McMahon *et al.*, 2010), morphological traits (Ríos *et al.*, 2019) and feeding ecology (Mill *et al.*, 2007; Plass-Johnson *et al.*, 2013; Clements *et al.*, 2016). Other factors may include digestibility (Polunin *et al.*, 1995) and growth or metabolic rate (Burkhardt *et al.*, 1999). These changes can be species-specific or source-specific. For example, herbivorous fishes mostly feed on benthic primary producers, mainly small filamentous algae and microbial autotrophs (Randall, 1967; Robertson, 1982; Wulff, 1997; Hill, 1998; Chen, 2002; Goldberg, 2013). These two types of production sources share similar carbon (i.e. dissolved inorganic carbon) but different nitrogen sources (nitrate and atmospheric nitrogen respectively). Therefore, they are different in terms of protein content and biomarker composition (e.g. stable isotopes and fatty acids), and are differentially utilized by various species of fish (Brenner *et al.*, 1999; Clements *et al.*, 2016). As a result, algivorous surgeonfish and microphage parrotfish can demonstrate distinctive δ^{15}N-body size relationships (Plass-Johnson *et al.*, 2013; Clements *et al.*, 2016). Carnivores have potentially more complex dietary patterns (Lazzaro, 1987; Layman *et al.*, 2007b; Layman *et al.*, 2012) because they can incorporate significant baseline shifts, which can link benthic and pelagic production. Examining δ^{15}N-body size relationships of specific source-consumer pairs can potentially explain the variation and improve understanding of source utilization and size-based feeding patterns in different trophic pathways.

At the community level, linear positive δ^{15}N-$\log_{10}$ body mass relationships exist in many aquatic systems (Jennings *et al.*, 2001a; Jennings *et al.*, 2002a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008) suggesting that as body size increases fish tend to feed at higher TP. These community-level studies were conducted in either temperate waters (e.g. Jennings *et al.*, 2001a) or a tropical demersal site (i.e. Al-Habsi *et al.*, 2008), but there is little known about coral reef systems. A positive linear δ^{15}N-$\log_{10}$ body mass relationship at the community level exists in a structurally degraded and overfished coral-reef fish food-web in Cape Eleuthera (Chapter 2). However, the fish assemblages do not resemble those of relatively intact coral reefs where small but high-TP species (e.g. Labridae spp) and large but low-TP species (e.g. Scarinae spp) are typically abundant (Hughes *et al.*, 2007; Graham *et al.*, 2017).

In healthy coral-reef food-webs, predator-prey relationships are likely to be more complex (Kingsford, 1992; Caley and Schluter, 2003; Dulvy *et al.*, 2004a; Duffy *et al.*, 2007; Feary *et al.*, 2007) than at Cape Eleuthera, and production sources are also likely to be more varied. In these healthy reefs, the presence of large algivores means that the TP of individuals may not be limited by their body size, and the mean TP may not scale linearly nor positively with body size at the community level; however this has yet to be tested. In the Maldives, although climate-driven bleaching events (Perry and Morgan, 2017) have influenced these reefs, reef-fishing and other anthropogenic pressures including pollution are considered to be small (Shepherd *et al.*, 1992; McClanahan, 2011). Thus, in this study, the objectives are to 1) identify which species undergo size-based shifts in TP, 2) establish whether different trophic pathways affect the δ^{15}N-$\log_{10}$ body mass relationship, 3) and analyse community compositional and functional changes at different body size classes of a site in the Maldives to test the existence of a positive linear δ^{15}N-body size relationship.

4.2 Materials and methods

4.2.1 Study site

Twenty accessible inner-atoll reef sites (depth 4-7 m) on South West North Malé Atoll (the Maldives) were selected for fish visual surveys (Figure 4.1). These sites and nearby sites within the atoll were selected for fish tissue sampling. All reef sites maintained relatively high habitat physical structural complexity one year after a major bleaching event (Perry and Morgan, 2017).

4.2.2 Survey and sample collection

4.2.2.1 Fish survey

Underwater visual census (UVC) was conducted at locations of continuous reef haphazardly selected at each of the 20 sites by Y. Zhu (buddied with research assistants from Banyan Tree Marine Labs Maldives) from January 23rd, 2017 to March 6th, 2017 during 0930-1700 hrs. The UVC involved one triple-sweep 30 m x 5 m transect (1st sweep: full transect of large and/or highly mobile individuals, 2nd and 3rd sweeps: half transect, left and right sides, of small and/or site attached/cryptic individuals) per site (mean duration: 45 min) to record non-cryptic diurnal fish with total length larger than 5 cm: species, individual total lengths (L, to nearest cm) and numbers of individuals. The surveyor's length estimation precision was repeatedly measured by conducting underwater fish-shaped object length estimation training

(Bell *et al.*, 1985) to minimize error (± 5%). All fish species in the water column from the reef substrate to the water surface were included in the survey except schools of Atherinidae spp (Ferreira *et al.*, 2001) which only appeared sporadically.

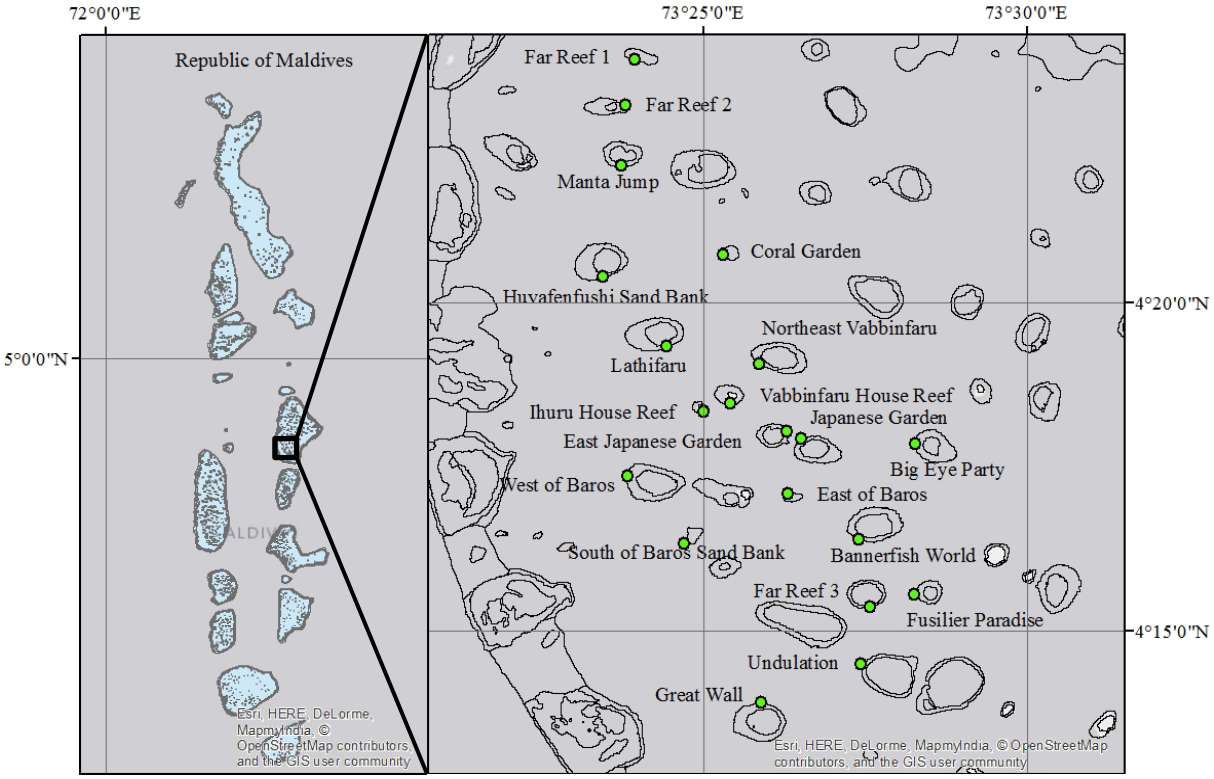


Figure 4.1 Map of survey sites (green dot) in North Malé Atoll (the Maldives). Green dots indicated the reef sites.

4.2.3 Fish sampling and preparation

Body mass or M (g) was calculated from L (cm) using Equation vii with published length to weight conversion factors *a* and *b* (Appendix 8) (fishbase.org, Froese *et al.*, 2014).

$$M = a \times L^b \qquad \text{Equation vii}$$

For some species, *a* and *b* were linked with standard length (SL) or fork length (FL) rather than total length (e.g. *Oxycheilinus diagramma* in SL), or L in units other than cm (e.g. *Aethaloperca rogaa* L in mm), thus, *a* and *b* were recalculated into L (cm) using nonlinear least square models (nls) (same as Equation vii) with precalculated starting values (using the original length format) (Table 4.1). Due to lack of available data, *a* and *b* of *Pygoplites diacanthus* and *Acanthurus thompsoni* were calculated by collecting individuals of varied sizes, recording their L (cm) and M (g), and using nls to fit L and M data into Equation vii, this gave: *P. diacanthus* (*n* = 12): *a* = 0.09051, *b* = 2.5686; *A. thompsoni* (*n* = 10): *a* = 0.01301, *b* = 3.0991. Species composing 80%

of the total biomass of each log_2B interval (B was used instead of M to differentiate the community level analysis from the species level analysis) were selected for stable isotope analysis of the community trophic structure (Table 4.2, Appendix 7).

Table 4.1 List of species with length to weight conversion factors from existing literature other than fishbase.org, different length units or types, or small sample L range. L: total length, SL: standard length.

Scientific name	Source	Note
Aethaloperca rogaa	Mapleston *et al.* (2009)	in mm, location: Great Barrier Reef
Cetoscarus bicolor	Kamikawa *et al.* (2015)	L: 18.7-48.5 cm, location: Guam
Chrysiptera glauca	Durville *et al.* (2003)	Location: Reunion Island, post-larva
Gomphosus caeruleus	*G. varius*, fishbase.org	
Hipposcarus harid	*H. longiceps*, fishbase.org	
Kyphosus cinerascens	Kamikawa *et al.* (2015)	L: 19.2-50.7 cm, location: Guam
Kyphosus vaigiensis	Kamikawa *et al.* (2015)	L: 18.5-49.7 cm, location: Guam
Oxycheilinus digramma	Fishbase.org	in SL, SL = 0.138 + 0.796L
Parupeneus pleurostigma	Fishbage.org	L: 24.6-26 cm
Siganus argenteus	Kamikawa *et al.* (2015)	L: 9.5-30.4 cm, location: Guam

Pempheris vanicolensis was not collected due to gear limitation. Samples of selected species were collected through the size range recorded in UVC to adequately describe species δ^{15}N-log_2M relationships (Galván *et al.*, 2010). Size range cover ratio (r_L = $L_{SIA\ sample}$ range / L_{UVC} range) was used to check whether the sampling objective was met. Fish were collected using a variety of technics depending on species behaviour towards divers, feeding habits and swimming patterns. Hand net, BINCKE net (Anderson and Carr, 1998), clove oil, underwater fishing hook and line, static hook and line, Hawaiian and hook and line surface trolling were all used in the sampling. Fish were killed by spine dislocation in accordance with the UK Home Office Scientific Procedures (Animals) Act and stored in an ice chest on board. After landing, approximately 2 g of dorsal white muscle tissue near the dorsal fin were dissected, rinsed with reverse osmosis treated water and stored in individual whirlpack bags in a -20 °C freezer. All samples were dried in individual tin trays in an oven at 50 °C for ~12 h until fully dried, and then in individual sealed Eppendorf tubes in zip-lock bags.

4.2.4 Baseline sampling

To apply δ^{15}N data to interpretation of coral-reef fish TPs, baselines are important, and because pelagic production sources may be significant (Robertson, 1982; de Goeij *et al.*, 2013; Gove *et al.*, 2016; Francis and Côté, 2018) and are likely

to be isotopically different (McConnaughey and McRoy, 1979; Polunin and Pinnegar, 2002), these and benthic sources (including macroalgae, microbes and detritus) were the two main pathways considered (see Chapter 3 for more details).

Both benthic (Dromard *et al.*, 2015) and pelagic (Wyatt *et al.*, 2012) production source endmembers (EMs) (strict primary consumers, TP = 2.0) were collected in order to correct for baseline differences. Benthic EMs were represented by species considered to be strictly algivorous, detritivorous or microphagous. Algivorous species included *Acanthurus leucosternon* which feeds on filamentous rhodophytes (Robertson and Gaines, 1986) and *Naso elegans* which feeds on phaeophytes (Ngugi *et al.*, 2017). Detritivorous species included *Acanthurus nigricauda* and *Ctenochaetus striatus* which feed on epilithic and/or epiphytic detritus (Robertson and Gaines, 1986), associated heterotrophic bacteria and diatoms (Moriarty, 1976). Microphagous species included the scraper Scarinae *Scarus niger*, *Scarus scaber* and *Scarus frenatus*, and the excavator Scarinae *Chlorurus sordidus* and *Chlorurus strongylocephalus*, which feed mainly on microbial autotrophs such as endolithic, epilithic and epiphytic or sessile endosymbiotic invertebrates (Clements *et al.*, 2016) cyanobacteria. Pelagic EMs included zooplanktonic calanoid copepods separated from the plankton sampled with a 500 mm aperture plankton net (mesh size: 150 µm) towed at a steady speed of 3 knots for 20 min along the reef edge during the day (0900-1600 hrs). Copepod samples were stored in individual Eppendorf tubes in a -20 °C freezer, and freeze dried at Newcastle University.

Samples were all collected within a three month period (February - April 2017) and from nearby sites to minimize temporal and spatial isotopic variation which might affect food-web baselines (Bronk and Glibert, 1993; Jennings *et al.*, 1997; Rolff, 2000; McCutchan *et al.*, 2003).

4.2.5 Stable isotope analysis preparation

All samples were transported to Newcastle under DEFRA permit ITIMP16.1258, frozen and freeze dried (or refreeze dried for oven dried samples). Fish samples were ground with mortar and pestle, then weighed to approximately 1.0 ± 0.1 mg in tin capsules with a Mettler MT5 microbalance, pelletized and stored in trays.

The prepared samples were analysed by Iso-Analytical Ltd (Crewe, UK) by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS). The ^{15}N:^{14}N ratio ($\delta^{15}N$) was expressed relative to N_2 in air for nitrogen while that of ^{13}C:^{12}C ($\delta^{13}C$) was

relative to Pee Dee Belemnite (PDB) of CO_2. Reference material used for this analysis was IA-R068 ($\delta^{13}C$ = -25.22 ± 0.00‰, $\delta^{15}N$ = 1.00 ± 0.00‰), with quality control check samples IA-R068, IA-R038 ($\delta^{13}C$ = -25.11 ± 0.01‰, $\delta^{15}N$ = -0.53 ± 0.01‰) and IA-R069 ($\delta^{13}C$ = -18.87 ± 0.05‰, $\delta^{15}N$ = 11.76 ± 0.01‰), with quality control check samples IA-R068 and IA-R038, a mixture of IAEA-C7 ($\delta^{13}C$ = -14.46 ± 0.01‰) and IA-R046 ($\delta^{15}N$ = 21.88 ± 0.01‰). IA-R068, IA-R038 and IA-R069 were calibrated against and traceable to IAEA-CH-6 ($\delta^{13}C$ = -10.43‰) and IAEA-N-1 ($\delta^{15}N$ = 0.40‰), IA-R046 to IAEA-N-1. IAEA-C7, IAEA-CH-6 and IAEA-N-1 were inter-laboratory comparison standards. External standards (*Anyperodon leucogrammicus* white muscle tissue, $\delta^{13}C$ = -13.53 ± 0.01‰, $\delta^{15}N$ = 12.64 ± 0.01‰) were also used for future reference. The precision of analysis for $\delta^{13}C$, $\delta^{15}N$, %C and %N was ±0.1‰, ±0.2‰, ±4% and ±1%, respectively. When fish white muscle tissue samples had carbon to nitrogen ratios (C:N determined from molar corrected elemental % data) higher than 3.7 (Fry *et al.*, 2003; Sweeting *et al.*, 2006), $\delta^{13}C$ mass balance arithmetic lipid correction (Equation viii) was applied to all fish white muscle tissue samples using the equation assuming 1) lipid-protein $\delta^{13}C$ depletion was 7‰ (Sweeting *et al.*, 2006), and 2) $C{:}N_{protein}$ was 3.7 (Fry *et al.*, 2003):

$$\delta_{protein} = \frac{\delta_{sample} \times C{:}N_{sample} + 7 \times (C{:}N_{sample} - C{:}N_{protein})}{\mathrm{C{:}N}_{sample}} \qquad \text{Equation viii}$$

4.2.6 Data analysis

All data were analysed in R 3.24 (R Core Team, 2016) using the packages: nls (Bates and Watts, 1988; Bates *et al.*, 1992) and ggplot2 (Wickham and Chang, 2016). All data were tested for normality and homogeneity of variance before analysis. Linear regressions were used to determine relationships between $\delta^{15}N$ and body mass. They were validated by assessing normality and homogeneity of variance using QQ plots, histograms of standardised residuals, and plots of standardised residuals verses fitted values. Significance was set at p = 0.05 in all cases. All errors are reported as ± 1SE unless otherwise stated.

Table 4.2 List of UVC sampled reef fish species at North Malé Atoll (the Maldives), with scientific name, species code, trophic guild (TG) (Froese and Pauly, 2017), mean trophic position (TP) (Froese and Pauly, 2017), underwater visual census (UVC) total length range (L_{UVC}), maximum total length (L_{max}) (Kuiter, 2014), SIA sample size (*n*), SIA sample total length range ($L_{SIA\ sample}$), size range cover ratio r_L, mean ± SE $\delta^{15}N$. *species sampled only for species and trophic pathway based analysis.

Scientific name	Code	TG	TP	L_{UVC} (cm)	L_{max} (cm)	*n*	$L_{SIA\ sample}$ (mm)	r_L (%)	$\delta^{15}N$ (‰)
Acanthurus nigricauda	ACN	Detritivore	2.2	26-30	45	7	201-277	42.5	8.86±0.14
Aethaloperca rogaa	AER	Piscivore	4.2	26-30	70	12	164-318	100	12.97±0.08
Amblyglyphidodon leucogaster	AML	Zoobenthivore	3.4	6-13	12	9	107-134	32.9	10.73±0.06
Anyperodon leucogrammicus	ANL	Piscivore	3.9	28-35	50	12	238-414	100	13.05±0.07
Balistapus undulates	BAU	Zoobenthivore	3.4	11-20	30	6	140-224	66.7	12.29±0.22
Balistoides conspicillum	BAC	Zoobenthivore	3.3	28-31	35	3	253-303	76.7	11.08±0.18
Caesio varilineata	CAV	Zooplanktivore	3.4	15-22	22	8	116-232	100	11.19±0.10
Caesio xanthonota	CAX	Zooplanktivore	3.4	14-20	20	12	166-302	56.7	11.80±0.07
Caranx ignobilis	CAI	Piscivore	4.2	66	100	0		0	
Caranx melampygus	CAM	Piscivore	4.5	35-40	100	11	232-410	100	12.59±0.11
Cephalopholis argus	CEA	Piscivore	4.5	26-37	45	13	186-342	74.5	12.92±0.05
Cephalopholis miniata	CEMN	Piscivore	4.3	39	40	10	160-330	0	12.89±0.09
Chaetodon falcula	CHF	Zoobenthivore	3.5	13-15	18	4	141-149	40	11.49±0.11
Chaetodon triangulum	CHTG	Corallivore	3.3	11-15	16	0		0	
Chaetodon trifasciatus	CHTF	Corallivore	3.3	11-14	15	4	92-112	6.7	10.34±0.17
Chlorurus sordidus	CHSD	Detritivore	2.6	6-28	40	8	134-235	45.9	7.99±0.13
Chlorurus strongylocephalus	CHSC	Herbivore	2.0	27-42	70	7	172-442	100	7.88±0.10
Chromis atripectoralis	CHA	Zooplanktivore	3.1	6-10	10	3	100-107	0	10.88±0.03
Chromis ternatensis	CHTT	Zooplanktivore	3.4	6-10	10	2	98-112	5	10.20±0.02
Chromis viridis	CHV	Zooplanktivore	2.9	6-10	10	0		0	
Chrysiptera glauca	CHG	Omnivore	2.4	6-10	10	0		0	
Ctenochaetus striatus	CTS	Detritivore	2.0	11-25	25	23	138-200	44.3	8.75±0.06
Ctenochaetus truncatus	CTT	Herbivore	2.0	11-15	18	5	108-152	100	9.48±0.16
Diodon liturosus	DIL	Zoobenthivore	3.5	35	45	4	265-404	100	11.06±0.16
Epinephelus fuscoguttatus	EPF	Piscivore	4.1	40	90	1	420	0	12.58
Fistularia commersonii	FIC	Piscivore	4.3	45	150	3	660-846	0	12.79±0.07
Gnathodentex aureolineatus	GNA	Zoobenthivore	3.7	16-20	30	8	197-225	7.5	11.81±0.27
Hemitaurichthys zoster	HEZ	Zooplanktivore	3.3	11-15	18	7	129-154	52.5	11.93±0.11
Lutjanus bohar	LUB	Zoobenthivore	4.3	50	80	4	243-316	0	12.92±0.15
Lutjanus gibbus	LUG	Zoobenthivore	4.1	26-29	50	6	243-313	100	12.64±0.09
Melichthys indicus	MEI	Zoobenthivore	3.0	26-28	24	6	182-253	0	9.87±0.32
Monotaxis grandoculis	MOG	Zoobenthivore	3.4	26-28	60	7	206-277	85	11.53±0.12
Myripristis murdjan	MYM	Zooplanktivore	3.4	16-20	25	6	164-182	45	11.75±0.09

Myripristis pralinia	MYP	Zoobenthivore	3.5	14-15	20	2	167-174	0	11.75±0.07
Myripristis violacea	MYVL	Zoobenthivore	3.5	13-20	25	20	154-187	47.1	11.64±0.06
Myripristis vittata	MYVT	Zoobenthivore	3.8	17	20	3	156-173	100	12.05±0.14
Naso brevirostris	NAB	Herbivore	2.2	18-30	50	3	210-242	26.7	10.38±0.59
Naso elegans	NAE	Herbivore	2.0	26-27	45	5	270-410	0	7.92±0.30
Naso fageni	NAF	Zooplanktivore	2.2	26	80	1	260	100	11.33
Naso hexacanthus	NAH	Zooplanktivore	2.2	27	50	5	202-267	0	10.92±0.07
Odonus niger	ODN	Zoobenthivore	3.1	16-24	40	5	211-347	36.3	11.33±0.65
Parupeneus macronema	PAM	Zoobenthivore	3.5	13-30	40	2	173-212	0	11.58±0.03
Pempheris vanicolensis	PEM	Zoobenthivore	3.5	14	15	0		0	
Plectorhinchus vittatus	PLV	Zoobenthivore	3.9	35-50	50	6	321-441	60.7	12.07±0.17
Pomacentrus caeruleus	POC	Omnivore	2.7	5-11	10	0		0	
Pomacentrus chrysurus	POCH	Omnivore	2.6	6-12	9	0		0	
Pomacentrus indicus	POI	Omnivore	2.6	6-11	11	4	94-115	32	10.91±0.16
Pomacentrus philippinus	POP	Omnivore	2.7	6-12	10	3	85-90	8.3	11.02±0.16
Pterocaesio pisang	PTP	Zooplanktivore	3.4	13-16	16	6	114-150	66.7	11.24±0.05
Pterocaesio trilineata	PTT	Zooplanktivore	3.4	15-17	16	0		0	
Pygoplites diacanthus	PYD	Spongivore	2.7	26-29	25	27	146-244	0	11.31±0.09
Sargocentron spiniferum	SAS	Zoobenthivore	3.6	26-30	45	9	233-407	100	12.17±0.18
Scarus frenatus	SCF	Herbivore	2.0	16-34	47	9	147-375	64.4	8.73±0.18
Scarus niger	SCN	Herbivore	2.0	11-30	40	7	164-280	0	8.14±0.21
Scarus rubroviolaceus	SCR	Herbivore	2.0	35	70	1	360	0	10.61
Scarus scaber	SCS	Herbivore	2.0	28-30	35	4	182-270	100	8.54±0.05
Variola louti	VAL	Piscivore	4.3	39-50	80	3	198-510	0	12.23±0.15
Zanclus cornutus	ZAC	Zoobenthivore	2.5	12-15	22	6	144-155	13.3	10.97±0.11
Zebrasoma scopas	ZES	Herbivore	2.0	11-15	20	3	118-124	0	9.72±0.70
*Acanthurus leucosternon**	ACL	Herbivore	2.0	N.A.	20	27	123-202	N.A.	7.79±0.20
*Acanthurus thompsoni**	ACT	Zooplanktivore	3.6	N.A.	25	8	125-181	N.A.	10.05±0.10
*Chaetodon meyeri**	CHM	Corallivore	3.3	N.A.	20	16	118-148	N.A.	10.42±0.15
*Cheilinus fasciatus**	CHEF	Zoobenthivore	3.4	N.A.	35	3	213-289	N.A.	11.06±0.19
*Myripristis berndti**	MYB	Zooplanktivore	N.A.	N.A.	70	3	174-224	N.A.	12.12±0.10
*Sargocentron caudimaculatum**	SAC	Zoobenthivore	3.9	N.A.	23	3	168-183	N.A.	12.47±0.09

$\delta^{15}N$-$\log_2 M$ relationships of 1) all individuals, 2) individuals of selected pathways including a) individuals of two TP (primary consumer and carnivore) groups and b) family-source pairs (strict feeding individuals of the same family sharing the same source [to the finest level based on dietary studies]), and 3) species level (individuals of the same species, $n \geq 3$) were analysed using linear regression to understand the variation of such relationships at different levels and potential causes (e.g. family, source). Primary consumers included algivore, detritivore and

microphage individuals (Table 4.3). These individuals were analysed at both TP group and source level (Hobson, 1974; Masuda and Allen, 1993; Randall, 2001; Chen, 2002; Cole *et al.*, 2008; Clements *et al.*, 2016; Ngugi *et al.*, 2017). Other individuals were grouped as carnivores in the TP group level analysis: strict corallivores, spongivores and zooplanktivores were analysed at family-source level (Table 4.3).

Table 4.3 List of individuals selected for family-source pair $\delta^{15}N$-$\log_2 M$ relationships analysis: trophic guild, known source, family and species.

Trophic guild	Source	Family	Species
Primary consumer	Detritus	Acanthuridae	*Acanthurus nigricauda, Ctenocheatus striatus*
	Cyanobacteria	Scarinae	*Chlorurus sordidus, Chlorurus strongylocephalus, Scarus frenatus, Scarus niger, Scarus scaber*
	Phaephyte	Acanthuridae	*Naso elegans*
	Rhodophyte	Acanthuridae	*Acanthurus leucosternon*
Corallivore	Corals	Chaetodontidae	*Chaetodon falcula, Chaetodon meyeri, Chaetodon trifasciatus*
Spongivore	Sponges	Pomacanthidae	*Pygoplites diacanthus*
Zooplanktivore	Zooplankton	Acanthuridae	*Acanthurus thompsoni, Naso brachycentron, Naso fageni, Naso hexacanthus*
		Caesionidae	*Caesio varilineata, Caesio xanthonota, Pterocaesio pisang*
		Chaetodontidae	*Hemitaurichthys zoster*
		Pomacentridae	*Chromis atripectoralis, Pomacentrus indicus, Pomacentrus philippinus*

The relationship between $\delta^{15}N$ and $\log_2 B$ at community level was analysed using quadratic linear regression with $\log_2 B$ range from 3 to 13 that excluded undersampled cryptic species (e.g. gobies and blennies) and undersampled/oversampled large home range and highly mobile individuals (e.g. sharks). Linear regression coefficients (slope and intercept) from species $\delta^{15}N$-$\log_2 M$ relationships (Appendix 9) were used to calculate $\delta^{15}N$ values of other-body-mass individuals. Mean values or single values were used for species with $n < 3$. Due to the small collected size range ($n = 3$, L = 11.8-12.4 cm, $r_L = 0\%$) and highly variable $\delta^{15}N$ (9.72 ± 0.70‰), mean $\delta^{15}N$ value was used for *Z. scopas* regardless of differences in M. Values for uncollected species which contributed much to biomass

classes were derived from values of species from the same genus or family with similar TPs and length to weight relationships (*Pomacentrus caeruleus* from *Pomacentrus philipinus*, *Pomacentrus chrysurus* and *Chrysiptera glauca* from *Pomacentrus indicus*, *Chromis viridis* from *Chromis atripectoralis*, and *Pterocaesio trilineata* from *Pterocaesio pisang*). The mass ratio (*r*) of each individual (*i*) at each $\log_2$B class as the weighting factor was then calculated by

$$r_i = M_i / \sum_{i=1}^{n} M_i$$

where *n* is the total number of individuals in the $\log_2$B class. Combining δ^{15}N and *r* for each individual based on Al-Habsi *et al.* (2008), the mean weighted δ^{15}N at each $\log_2$B class (*j*) of the whole community was calculated as

$$\delta^{15}N_j = \sum_{i=1}^{n} \delta^{15}N_i \times r_i$$

where *j* is the $\log_2$B class. Mean weighted δ^{15}N per $\log_2$B class ($\delta^{15}N_{original,j}$) were standardized to 80% biomass composition from the collected biomass percentage of each $\log_2$B class ($Biomass\%_j$) using:

$$\delta^{15}N_{standardized,j} = {}^{\delta^{15}N_{original,j}}\Big/_{Biomass\%_j} \times 80\%.$$

Because isotopic baselines may differ between benthic and pelagic feeders, the $TP_{adjusted}$ of each individual sample was calculated using tRophicPosition (Quezada-Romegialli *et al.*, 2018), the baselines of pelagic source endmembers/primary consumers (*n* = 10, TP = 2.0, δ^{15}N = 8.13 ± 0.12‰) and benthic source endmembers/primary consumers (*n* = 88, TP = 2.0, δ^{15}N = 8.34 ± 0.08‰) were however indistinguishable (*p* = 0.16), therefore no adjustment was needed.

4.3 Results

4.3.1 Species stable isotope data

Fifty-one species with sample sizes ≥ 3 were included in the species-level analysis. The size range cover ratio (r_L) ranged from 0% to 100% (mean = 40.3%). Mean species δ^{15}N ranged from 7.88 ± 0.10 ‰ (*C. strongylocephalus* [CHS]) to 13.05 ± 0.07 ‰ (*A. leucogrammicus* [ANL]). Body mass ranged from 4 g (*C. ternatensis* [CHT]) to 6974 g (*S. niger* [SCN]). The largest individuals were algivores (e.g. *S. niger*) whereas the smallest ones fed at higher TPs (e.g. *P. philippinus*) (Figure 4.2).

4.3.2 δ^{15}N-body mass relationships at individual and TP group levels

When all individual fish were pooled, there was a significant positive δ^{15}N-$\log_2$M but this was weak (Table 4.4, Figure 4.2). Primary consumers had a non-significant δ^{15}N-$\log_2$M relationship (Table 4.4, Figure 4.2), whereas carnivores had a significant relationship (Table 4.4, Figure 4.2). The pooled primary consumers had lower slope and intercept values compared to the carnivores.

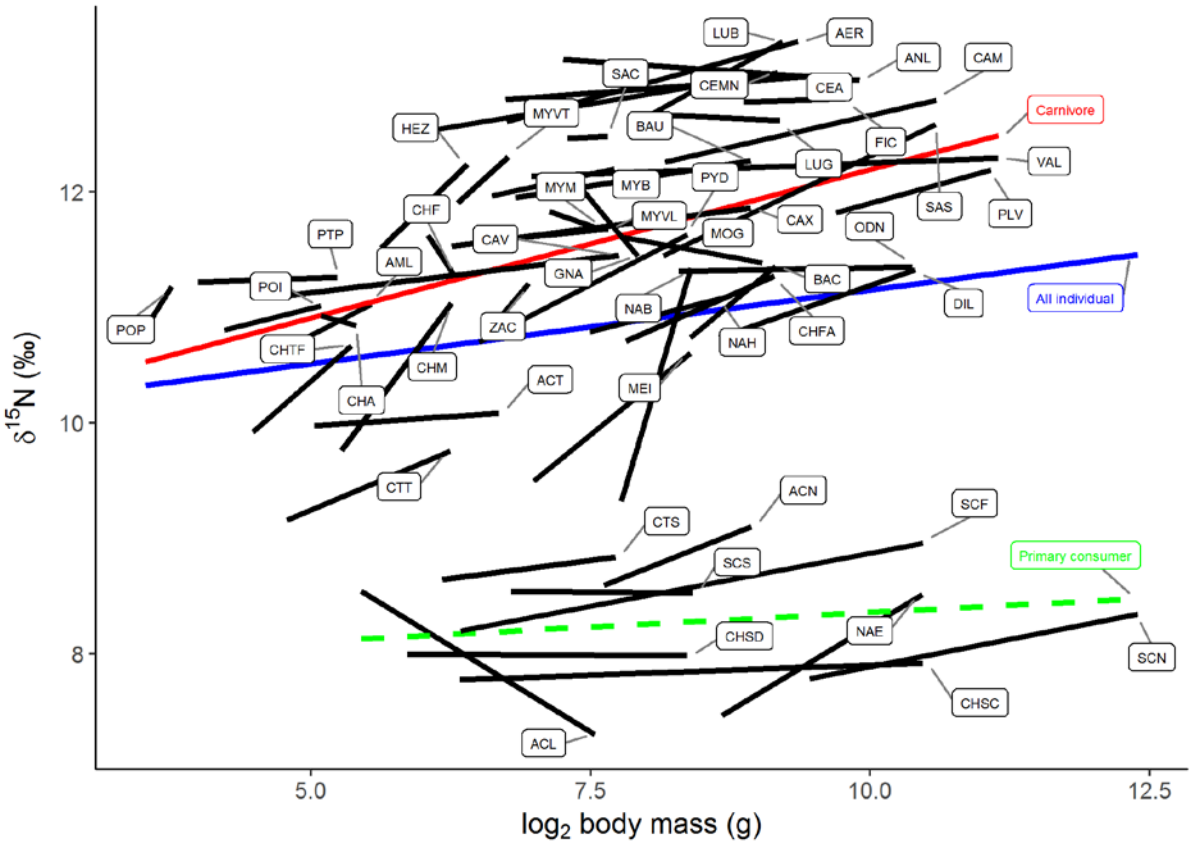


Figure 4.2 Plot of δ^{15}N-$\log_2$ body mass relationships of all individuals (blue), carnivorous individuals (red), primary consumer individuals (green) and individual species with slope ≠ 0 (black) at North Malé Atoll (the Maldives). δ^{15}N values of species with n < 3 and *Zebrasoma scopas* were the mean values in Table 4.2. For codes see Table 4.2.

4.3.3 δ^{15}N-body mass relationships at family-source level

Of ten family-source pairs (Figure 4.3), six had significant δ^{15}N-$\log_2$M relationships (p < 0.05, Table 4.5), among which three had strong linear relationships ($r^2_{adjusted}$ > 0.50). The four non-significant relationships were for detritivorous Acanthuridae, phaephytivorous Acanthuridae, zooplanktivorous Pomacentridae and microphage Scarinae. Individuals of the same family feeding on different sources demonstrated different slope and intercept values (e.g. Acanthuridae), and this also applied to the finer scale of source (different classes of sources from the same type,

e.g. Phaephyceae and Rhodophyta). Individuals of different families feeding on the same source also demonstrated different slope and intercept values (e.g. zooplanktivore).

Table 4.4 Linear regression parameter estimates for $\delta^{15}N$-$\log_2$ body mass relationships in individual and TP based analyses.

Model	Coefficient	Estimate	Standard error	*p* value	$r^2_{adjusted}$
All Individual	Intercept	9.880	0.403	< 0.05	0.01
	$\log_2$ body mass	0.127	0.052	< 0.05	
Carnivore	Intercept	9.625	0.245	< 0.05	0.13
	$\log_2$ body mass	0.257	0.036	< 0.05	
Primary consumer	Intercept	7.856	0.365	< 0.05	0.00
	$\log_2$ body mass	0.050	0.045	0.27	

Table 4.5 Linear regression parameter estimates for $\delta^{15}N$-$\log_2$ body mass relationships in family-source based analyses, species included in each source-family pair were from Table 4.2.

Model	Coefficient	Estimate	Standard error	*p* value	$r^2_{adjusted}$
Acanthuridae (detritivore)	Intercept	7.823	0.622	< 0.05	0.05
	$\log_2$ body mass	0.130	0.083	0.13	
Acanthuridae (phaephytivore)	Intercept	2.394	2.343	< 0.05	0.54
	$\log_2$ body mass	0.286	0.247	0.10	
Acanthuridae (zooplanktivore)	Intercept	7.601	2.343	< 0.05	0.76
	$\log_2$ body mass	0.410	0.247	< 0.05	
Acanthuridae (rhodophytivore)	Intercept	11.778	1.294	< 0.05	0.26
	$\log_2$ body mass	-0.594	0.187	< 0.05	
Caesionidae (zooplanktivore)	Intercept	10.433	0.169	< 0.05	0.62
	$\log_2$ body mass	0.158	0.025	< 0.05	
Chaetodontidae (corallivore)	Intercept	5.683	1.427	< 0.05	0.32
	$\log_2$ body mass	0.860	0.249	< 0.05	
Chaetodontidae (zooplanktivore)	Intercept	6.245	1.064	< 0.05	0.82
	$\log_2$ body mass	0.937	0.175	< 0.05	
Pomacanthidae (spongivore)	Intercept	7.459	1.245	< 0.05	0.25
	$\log_2$ body mass	0.499	0.161	< 0.05	
Pomacentridae (zooplanktivore)	Intercept	11.781	0.645	< 0.05	0.11
	$\log_2$ body mass	-0.207	0.136	0.16	
Scarinae (microphage)	Intercept	7.846	0.476	< 0.05	-0.01
	$\log_2$ body mass	0.045	0.052	0.39	

4.3.4 δ^{15}N-body mass at species level

Of 50 species with $n \geq 3$ (Figure 4.2, Appendix 9), 40 showed δ^{15}N-$\log_2$M relationships that were positive (e.g. *Odonus niger* [ODN]) and 10 that were negative (e.g. *Chaetodon falcula* [CHF]) with slope values ranging from -1.498 (*C. falcula*) to 3.2486 (*Naso brevirostris* [NAB]). Eight of the fifty species showed significant linear relationships ($p < 0.05$) between δ^{15}N and $\log_2$M (n = 3-27), that were positive except that of *A. leucosternon* (ACL). Among all species, 13 showed δ^{15}N values invariant with size (|slope| < 0.1, e.g. *C. sordidus* [CHS]). Other species had less than 0.5 trophic level-equivalent δ^{15}N value shifts (± 1.70‰) within the sampled size range except *Naso brachycentron* (δ^{15}N change = 2.02‰). Linear regression fitted well for 11 species ($r^2_{adjusted} > 0.50$, e.g. *Balistoides conspicillum* [BAC]) whereas there were considerable variabilities around the regression line for 28 species ($r^2_{adjusted} < 0.20$, e.g. *O. niger*).

4.3.5 δ^{15}N-body mass relationship at community level

Across $\log_2$B class (i.e. 3-13), composition of species with different TP (e.g. herbivore, carnivore and others undefined) varied (Appendix 7, Figure 4.4). At lower body mass classes, biomass was mainly contributed by high-TP species. In the $\log_2$B = 3 size class (4-32 g), biomass was dominated by Pomacentridae (e.g. *Chromis ternatensis*, *P. indicus*, *P. philippinus*, *C. glauca*, *Amblyglyphidodon leucogaster* and *C. atripectoralis*); in 32 - 64g, by Pomacentridae (e.g. *C. ternatensis* [31.74%] and *C. atripectoralis* [6.78%]) and Caesionidae (e.g. *P. trilineata* [22.36%], *Caesio xanthonota* [10.15], and *P. pisang* [7.18%]); 64-128 g, by Caesionidae (e.g. *P. trilineata* [27.30%] and *Caesio varilineata* [16.35%]) and *Myripristis* spp (e.g. *M. pralinia* [6.83%], *M. vittata* [6.51%] and *M. violacea* [5.88%]); 128-256 g, by *C. varilineata* (50.61%) and *O. niger* (38.82%); 256-512 g, by *O. niger* (66.57%), *C. striatus* (5.35%) and *S. niger* (3.03%). Above $\log_2$B = 9 (512 g), the biomass contribution shifted from high-TP to low-TP species (e.g. *S. niger*, *S. frenatus* and *C. strongylocephalus*).

Mean weighted δ^{15}N ranged from 7.21 to 8.95‰, a range of 1.73‰ equivalent to ~0.5 trophic level. At the community level, there was a significant quadratic relationship between standardized δ^{15}N and $\log_2$B given by the equation $\delta^{15}N = -0.076 (\log_2 B)^2 + 1.206 \log_2 B + 5.685$, $p < 0.05$, $r^2_{adjusted} = 0.86$ (Figure 4.5).

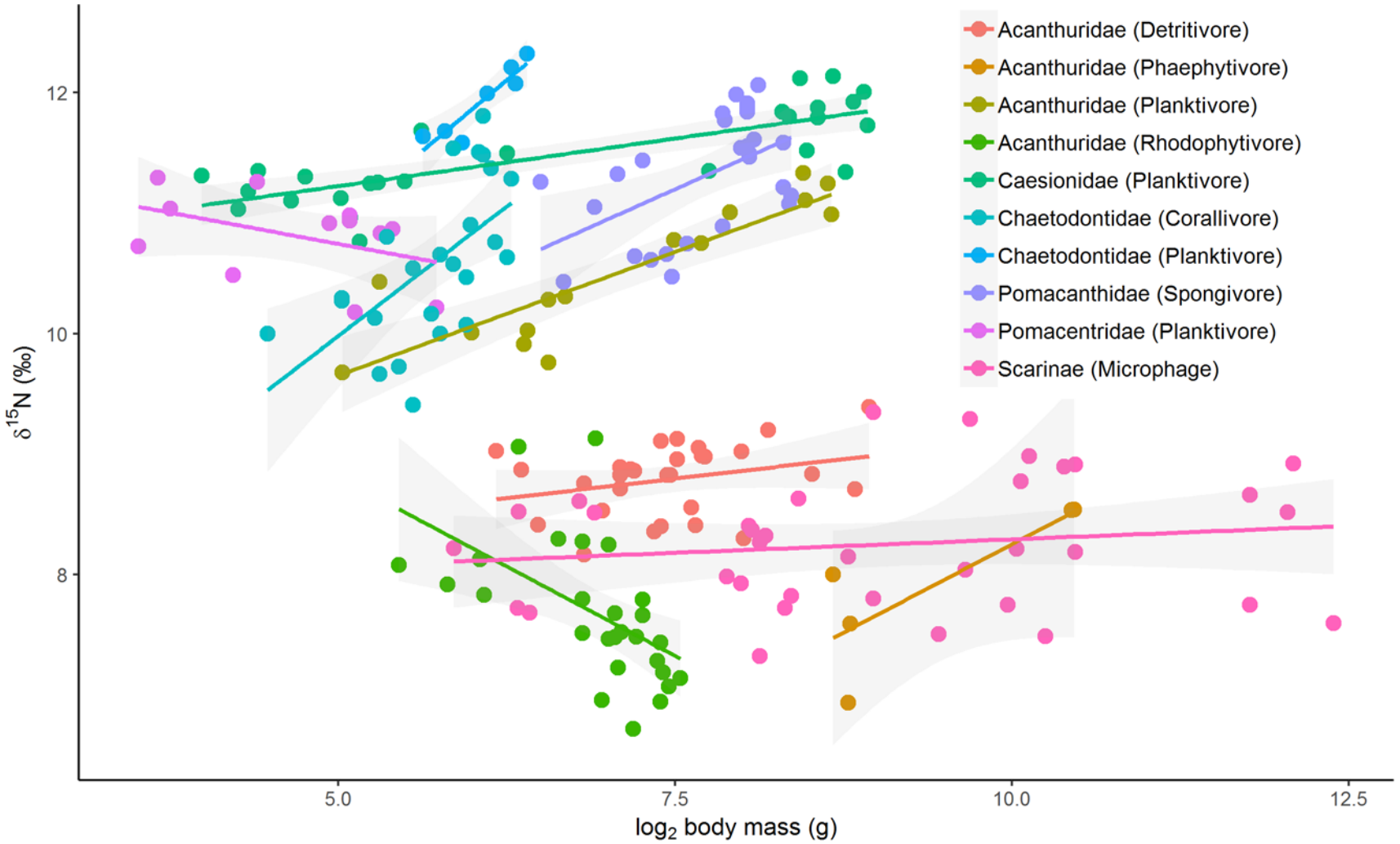


Figure 4.3 Relationships between $\delta^{15}N$ and $\log_2$ body mass of individuals of the same family feeding strictly on certain production sources. Shaded area: 95% CIs

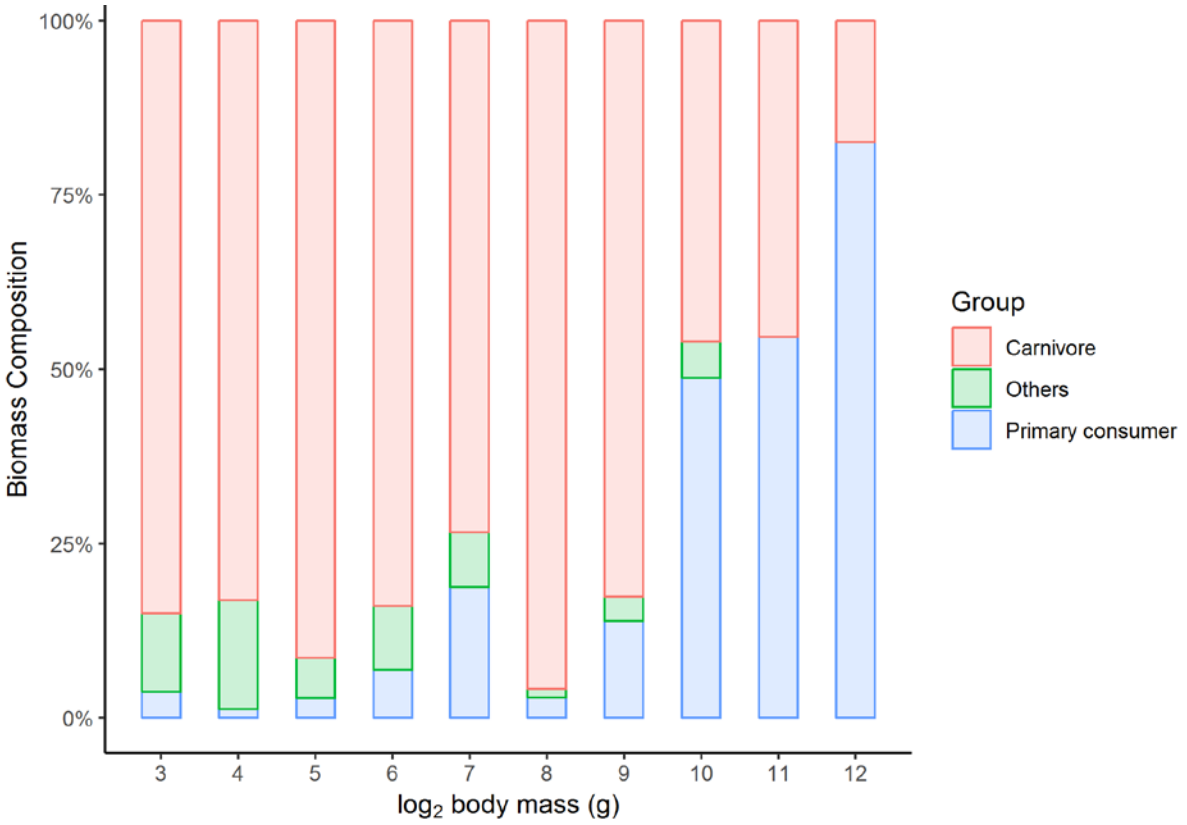


Figure 4.4 Biomass composition of sampled fish including carnivores, primary consumers and others (undefined) per log_2 body mass class.

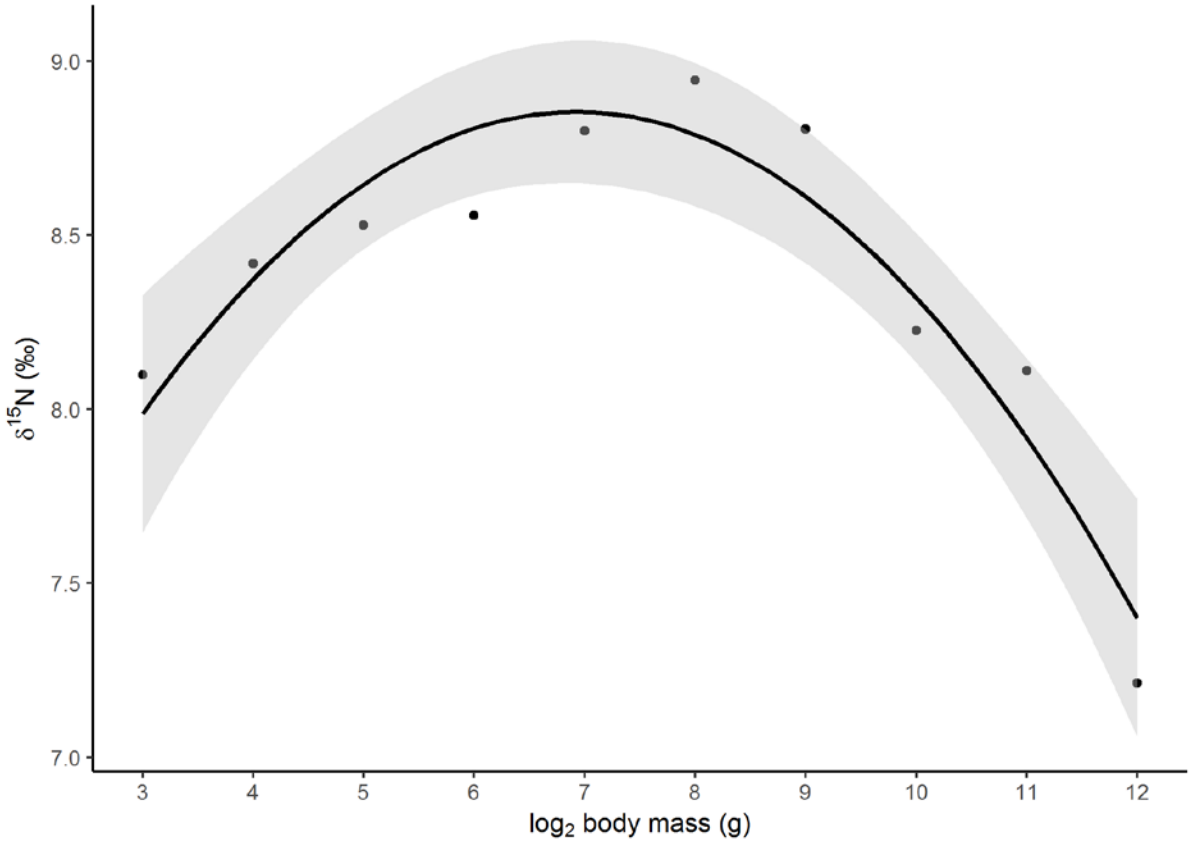


Figure 4.5 Combined quadratic relationships between mean $\delta^{15}N_{standardized}$ (80% biomass) and log_2 body mass for fish communities at North Malé Atoll (the Maldives). Shaded area: 95% CIs.

4.4 Discussion

4.4.1 Individual and species δ^{15}N-body mass relationships

The all-individual $\delta^{15}N$-$\log_2 M$ relationship was significant but weak in these reefs, which was similar to the findings in the global data (with existence of large top predators such as sharks) (Romanuk *et al.*, 2011), Bahamian coral-reefs ($\delta^{15}N_{individual} = 0.2793\log_2 M + 5.0408$, $p < 0.05$, $r^2_{adjusted} = 0.40$; Chapter 2) and Republic of Kiribati coral reefs (δ15Nindividual = 0.067log2M + 2.761, p < 0.00, r2m = 0.17; Robinson and Baum, 2015). Comparing among these three coral reef-based studies, different intercept values could be a result of different baselines, whereas different slope values might be due to 1) methodology, 2) sample size, 3) state of the system or 4) community composition. In terms of methodologies, benthic-pelagic baselines were onlyexamined in the Maldivian data, but in the other two studies, likely different $\delta^{15}N$ benthic-pelagic baselines were not corrected for. Specifically, Robinson and Baum (2015) used data without baseline correction and found that corallivores (TP = ~3.7) had a mean $\delta^{15}N$ value approximately 5.00‰ higher than that of zooplanktivores (TP = ~3.4), whereas here benthic and pelagic baselines had similar $\delta^{15}N$ values for the two types of indicator species involved (corallivore ~11.40‰; zooplanktivore ~10.40‰). All three studies focused only on abundant species determined by UVC. However, numbers of species and sample sizes differed (N species = 69, 41 and 23, sample size = 424, 193 and 344, in the Maldives, the Bahamas and Kiribati respectively). Number of species within the same trophic guilds also differed, for example in herbivorous species (N = 6, 13 and 4), as did the UVC sampled maximum body mass of herbivorous species ($M_{max,\ herbivorous\ species}$ = 5366, 1590 and 794 g). These three sites had different fish assemblages attributable to geographic, natural and anthropogenic disturbance (e.g. overfishing of parrotfish) differences. The Maldivian site potentially resembled less impacted coral reefs where large herbivores are abundant (Lewis, 1986; Choat *et al.*, 2002; Hughes *et al.*, 2007; Burkepile and Hay, 2008; Heenan and Williams, 2013; Dromard *et al.*, 2015; Plass-Johnson *et al.*, 2015) even though it was surveyed one year after the mass bleaching event (Perry and Morgan, 2017). Thus, the 3rd assumption (different states) was more appropriate in explaining the result in the Maldives. The indication is that in more dynamic and less impacted food-webs with multiple sources, individual $\delta^{15}N$ values might not correlate linearly with body size.

Linear $\delta^{15}N$-$\log_2M$ relationships indicated the existence of size-based feeding patterns in the sampled size ranges of individual species, which is in line with other marine habitats (Jennings *et al.*, 2002a; Romanuk *et al.*, 2011; Plass-Johnson *et al.*, 2013). Positive slope values suggested those species fed at higher TP as size increased. This could potentially be due to increasing gape width or predatory skills, causing them to feed on larger individuals of the same prey species (Layman *et al.*, 2005) or different prey of higher TP (Jennings *et al.*, 2002b). The former may be applicable to most species with positive slope values due to their small $\delta^{15}N$ increase with size, whereas the latter could apply to *N. brevirostris* (NAB) which increased by ~0.5 trophic level with size. Its initial $\delta^{15}N$ value was very close to those of primary consumers while its ultimate $\delta^{15}N$ value was within the range of carnivores. This suggested that this species could be shifting diet from benthic algal sources to salps or other invertebrates (Choat *et al.*, 2002), and it is typically categorised as an omnivore (Randall, 1985).

The species with negative slope values in contrast were potentially shifting diet from high to low TP sources at larger sizes (e.g. from animals to algae). As one example, *A. leucosternon* (slope = -0.594, $p < 0.05$, $r^2_{adjusted} = 0.26$) feeds on the epilithic algal matrix (EAM) and has a diet composed of rhodophytes (e.g. *Polysyphonia* sp., *Laurencia* sp., *Champia* sp. and Gelidiaceae sp.) and cyanobacteria (*Lyngbya* sp.) (Robertson and Gaines, 1986) with the last possessing a lower $\delta^{15}N$ value (Brenner *et al.*, 1999). This species potentially shifted diet from rhodophytes to cyanobacteria at a bigger size, although this could only be confirmed through diet analysis (e.g. size-based diet analysis; Chen, 2002; Plass-Johnson *et al.*, 2013).

High variation around some $\delta^{15}N$-$\log_2M$ relationships suggested potential individual specialisation (Araújo *et al.*, 2011) in the sampled size ranges, or their diet compositions were highly variable relative to tissue turnover rate. *Odonus niger* (slope = 0.020, $p = 0.99$, $r^2_{adjusted} = -0.33$) feeds mostly on zoobenthos and zooplankton (Matsuura, 2001), and in the Maldives was observed schooling while feeding on zooplankton. Weak correlation between size and $\delta^{15}N$ potentially suggested that this species was utilizing both sources regardless of size. *Chlorurus sordidus* (slope = -0.005, $p = 0.98$, $r^2_{adjusted} = -0.16$) feeds on detritus or other animal contents at a smaller size (Chen, 2002) and gradually switches to mainly cyanobacteria through excavating carbonate substrate in the adult phase (Chen, 2002; Clements *et al.*, 2016). The sampled size range of this species did not include

the size (3 cm) where this species ceased feeding on invertebrates in the post-settlement dietary shift study (Chen, 2002). Thus, it should have a rather flat δ^{15}N-$\log_2$M relationship. However, detrital materials include dead organic matter (e.g. algae, fish faeces and coral mucus), inorganic material, microbes, microalgae (diatoms, dinoflagellates and cyanobacteria) and associated meiofauna (Crossman *et al.*, 2001). The composition of detritus could vary significantly across sites. This might help explain the variation observed in the δ^{15}N data and the highly varied δ^{15}N-$\log_2$M relationship, features which were present in *C. striatus* and *A. nigricauda*.

4.4.2 Pathway-specific δ^{15}N-body mass relationships

Primary consumer and carnivore groups showed different δ^{15}N-$\log_2$M relationships from each other, and this is supported by the metabolic theory of energy constraints (Brown and Gillooly, 2003). Compared to Robinson and Baum (2015) (coral reef only) who found similar slope values, and de la Morinière *et al.* (2003) (included mangrove, seagrass and reef; no zooplanktivore included; analysed ontogenetic change in δ^{15}N-FL relationships) who reported different slope values among these two groups, the Maldivian primary consumer group had a weaker δ^{15}N-$\log_2$M relationship with a lower slope value (slope = 0.0503, p = 0.27, n = 91) compared to the carnivore group (slope = 0.2567, $p < 0.05$, n = 333). The variation among studies in the relationships might be due to sample size, baseline correction, and/or human impact. The indications are that size-based feeding patterns are common for carnivorous species, but not for primary consumers. This is unsurprising given that algivores and detritivores are reliant on the same type of production source regardless of size.

Individuals of different trophic pathways demonstrated differential δ^{15}N-$\log_2$M relationships. These relationships showed improved significance level and stronger fitting than the two TP group analyses, suggesting that pathway can potentially explain the variation in these to some extent. Individuals of the same family share some ontogeny and phylogeny traits which constrain morphology and growth rate (Choat *et al.*, 1996; Choat *et al.*, 2002), digestibility (Polunin *et al.*, 1995) and maximum body mass to some extent. Different sources can have different diet quality (e.g. protein content) which potentially influences δ^{15}N trophic fractionation (McMahon *et al.*, 2010), feeding rates and thus δ^{15}N values in the white muscle tissue. Higher slope value and $\delta^{15}N_{max}$ of the zooplanktivorous chaetodontid *Hemitaurichthys zoster* compared with other large-bodied zooplanktivorous families

suggested the certain traits (e.g. morphological or fitness) along growth make such species adapt faster in high TP feeding pattern, i.e. changing diet towards higher-TP larger zooplankton (Hobson, 1974). This could also be the case of the higher slope value of the phaephytivorous acanthurid *N. elegans* (i.e. rapid adaptation of feeding from low- to high δ15N-value algae due to growth-related traits; Ngugi *et al.*, 2017). Similar to species-based analysis, those with negative slope values evidently changed their diet from high to low $\delta^{15}N$ food items. The three planktivorous Pomacentridae species (*C. atripectoralis*, *P. indicus* and *P. philippinus*) feed on both zooplankton and benthic algae (Masuda and Allen, 1993), but this may be size-based. This may also be the case for the rhodophytivorous acanthurid *A. leucosternon*. However, those two groups with negative linear $\delta^{15}N$-$\log_2 M$ relationships potentially showed two distinctive feeding strategies considering the trophic pathways they used, with the former from high to low quality sources and the latter the opposite (Clements *et al.*, 2016). This might relate to the protein content. The strategy of zooplanktivorous Pomacentridae species could be to exploit a short and productive food chain to avoid competition and predation, whereas that of *A. leucosternon* could result from improved digestibility of cyanobacteria imbedded in EAM to supplement their protein requirement at bigger size. Detailed studies need to be conducted to understand the mechanisms behind whether this is species (e.g. diet preference, digestibility, anti-competition) or source related (e.g. protein content, availability). Pathways with non-significant linear relationships could be due to sample size (e.g. phaeophytivorous Acanthuridae), highly variable composition of detrital material, omnivorous behaviour (zooplanktivorous Pomacentridae) or facultative feeding patterns (e.g. microphage Scarinae; Robertson, 1982; Wulff, 1997; Plass-Johnson *et al.*, 2013).

4.4.3 Community $\delta^{15}N$-body mass relationship

The significant quadratic relationship between $\delta^{15}N$ and $\log_2 B$ at community level suggested dramatic compositional and functional changes in the trophic structure as body size increases. The majority of fish biomass was from higher TP species as size increased up to 512 g body mass, but at greater sizes, primary consumers gradually dominated the biomass of each body mass class while number of species decreased. This could be explained by M_{max} of fish, human exploitation (extracting large carnivorous fish from the system) and growth rate (e.g. rapid growth of Scarinae, Choat *et al.*, 1996). Up to 512 g body mass, most biomass-important

species (e.g. Caesionidae, Pomacentridae) were high-TP but small in M_{max}. There are exceptions of large-bodied Labridae (e.g. *Cheilinus undulatus*) and some Serranidae (e.g. *Plectropomus laevis*) in the area, but they were not encountered in UVCs, which might be a result of their large home range. It is important to note that this study was confined to non-cryptic diurnal fish due to limitations of reef visual survey at night, and some cryptic and nocturnal species (e.g. Holocentridae spp, Apogonidae spp) which might be highly abundant but were underestimated.

This is the first time that such a quadratic $\delta^{15}N$-$\log_2 B$ relationship had been found in a coral reef system. It indicates a significant cross-community trophic shift with body size in relatively intact coral reefs. Previous studies observed linear positive $\delta^{15}N$-$\log_2 B$ relationships between predators and prey at community level (Jennings *et al.*, 2001a; Al-Habsi *et al.*, 2008). However, this only applies to communities sharing a single source or type of sources with similar baselines, which does not apply here.

Despite the variation caused by baselines and geographic features, fish community and habitat structural degradation (Alvarez-Filip *et al.*, 2009; Rogers *et al.*, 2014; Plass-Johnson *et al.*, 2015) and human impacts (Pace *et al.*, 1999; Darimont *et al.*, 2015; Graham *et al.*, 2017) could potentially explain different relationship found at Cape Eleuthera (linear positive) and the Maldives (quadratic) sites. Large herbivores were fished out while large Serranidae (e.g. *Mycteroperca tigris*) aggregated in protected reefs in the Bahamas case, whereas large herbivores were common and large predators seemed relatively scarce in the Maldives. Herbivory is an important ecological function to maintain the healthy state of coral reefs by cleaning out competitive organisms such as algae (Lewis, 1986; Hughes *et al.*, 2007; Burkepile and Hay, 2008; Green and Bellwood, 2009; Heenan and Williams, 2013; Plass-Johnson *et al.*, 2015); large herbivores are thought to provide such function better than smaller individuals (Chen, 2002; Burkepile and Hay, 2008; D'agata *et al.*, 2016). In the Maldives, Scarinae accounted for the greatest biomass at the largest size classes. Although Scarinae do not digest much benthic algae, they behaviourally remove such algae to access their target food source extensively (Clements *et al.*, 2016). The $\delta^{15}N$-$\log_2 B$ relationship can provide a powerful tool to understand trophic and functional compositions of marine communities, but the quadratic form of this in the Maldives precludes the derivation of community attributes such as PPMR and food-chain transfer efficiencies made possible

elsewhere (Jennings *et al.*, 2001a; Jennings *et al.*, 2001b; Jennings *et al.*, 2002b; Jennings *et al.*, 2002c; Al-Habsi *et al.*, 2008).

4.4.4 Conclusion

This study confirmed the existence of size-based TP-omnivory among Maldivian coral-reef fish species using $\delta^{15}N$ data. Although trophic pathway can affect individual $\delta^{15}N$-$\log_2 M$ relationships, at community level the quadratic $\delta^{15}N$-$\log_2 B$ relationship for the first time indicates the more complex community compositional and functional variations in a dynamic and less degraded coral reef system compared with others.

Chapter 5. Stable isotope data belie simplistic trophic categorisations of coral-reef fishes

5.1 Introduction

Production sources in coral-reef food-webs are very diverse. These include primary producers such as benthic algae (Hallock and Schlager, 1986; Cowen, 1988), plankton (Doty and Oguri, 1956; Gove *et al.*, 2016), and products of what are considered to be highly efficient recycling pathways (Alongi, 1988; Gast *et al.*, 1998; de Goeij *et al.*, 2013). Based on the major trophic energy pathway used, reef-fish species are typically assigned to a single trophic guild, yet this assumes feeding is strictly confined to single food source types and crossing trophic boundaries is unlikely. Evidence suggests the opposite. While there are fish occupying narrow trophic niches (e.g. some Chaetodontidae feeding exclusivelyon Scleractinia corals), many fishes may rely on multiple food sources. Some change diet (Chen, 2002) and trophic position as they grow (Mumby, 2006; Romanuk *et al.*, 2011; Plass-Johnson *et al.*, 2013; Zhu *et al.*, unpublished data). Also, some herbivores feed facultatively on zooplankton (O'Brien, 1979), on faeces (Robertson, 1982), microbial autotrophs (Paul and Valerie, 1999; Choat and Clements, 2018) and cryptic sponges (Dunlap and Pawlik, 1996; Thacker *et al.*, 1997; Wulff, 1997; Pawlik, 1998). There are differences also within species and maturity levels in the production sources on which they rely, due to predation, competition (Matthews and Mazumder, 2004) and availability of food sources, which vary with depth (Malcolm *et al.*, 2011), geographic features (Gove *et al.*, 2016), benthic cover (Floeter *et al.*, 2007) and seasonality (Rolff, 2000).

These diverse dietary patterns help define the trophic interactions among fish, by defining trophic functional roles and predator-prey relationships. Different methods have been used to assign species into trophic guilds and in turn analyse fish food-webs. Gut contents data provide a snapshot of the most recent meal of an individual, and can be highly variable due to seasonality, quickly/highly digested food items and temporal and spatial effects, and thus require massive sampling effort (Jennings *et al.*, 2001a). DNA barcoding to identify ingested food items can address predator-prey interactions and disentangle complex marine food-webs; however, it has only limited ability to quantify diet composition due to methodological issues (e.g. grinding methods, primer), differential DNA degradation rates, amplification biases and interference from existing environmental DNA (eDNA, exists in the environment but is not part of the diet) (Leal and Ferrier-Pagès, 2016) and provides only a snapshot of

recent diet. Fatty acid (FA) analysis offers a time-integrated method to trace the flow of fatty acids, but samples readily decompose without adequate freezing. Stable isotope analysis (SIA) also provides a time integrated method to understand the flow of carbon from production sources to consumers and elucidate trophic positions (DeNiro and Epstein, 1978; Hesslein *et al.*, 1991; Jennings *et al.*, 2002a; Jennings *et al.*, 2002c; McCutchan *et al.*, 2003; Hannides *et al.*, 2009; Hussey *et al.*, 2014; Reum *et al.*, 2015) and trophic niches (DeNiro and Epstein, 1978; Tieszen *et al.*, 1983; Cocheret de la Morinière *et al.*, 2002; Bearhop *et al.*, 2004; Jackson *et al.*, 2011; Carreón-Palau *et al.*, 2013; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015; Reid *et al.*, 2016), while correctly dried samples are unlikely to degrade in storage.

Stable isotope mixing models help resolve likely diet compositions, trophic niches and production sources in food-webs (Bond and Diamond, 2011). MixSIAR (Semmens *et al.*, 2013) was developed to improve existing frameworks by introducing a more process-based formulation of uncertainty in mixing models especially for systems with narrow consumer stable isotope data.

Here data were collected to explore trophic pathways of some coral reef-fish using bulk stable isotope analysis. Samples of fish white muscle and production sources were collected during January - April 2017 in North Malé Atoll (Maldives) to 1) test the isotopic discriminability of likely production sources, 2) assess the extent to which fish species considered to be reliant on single production sources might be dependent on other sources and 3) how this dependence might vary between two types of reef habitat.

5.2 Materials and methods

5.2.1 Study site

Twenty accessible inner-atoll sites (depth 4-7 m) and six outer-atoll reefs (depth 10-20 m) in the South West of North Malé Atoll (Maldives) were randomly selected for fish and production source sampling (Figure 5.1).

5.2.2 Sample collection

Six major trophic energy pathways were identified according to the biomass composition data of different trophic guilds (Chapter 4): corallivory, detritivory, herbivory, diurnal and nocturnal planktivory, and spongivory. A primary consumer and diet/source end member (EM) were collected for each energy pathway (Table 5.1).

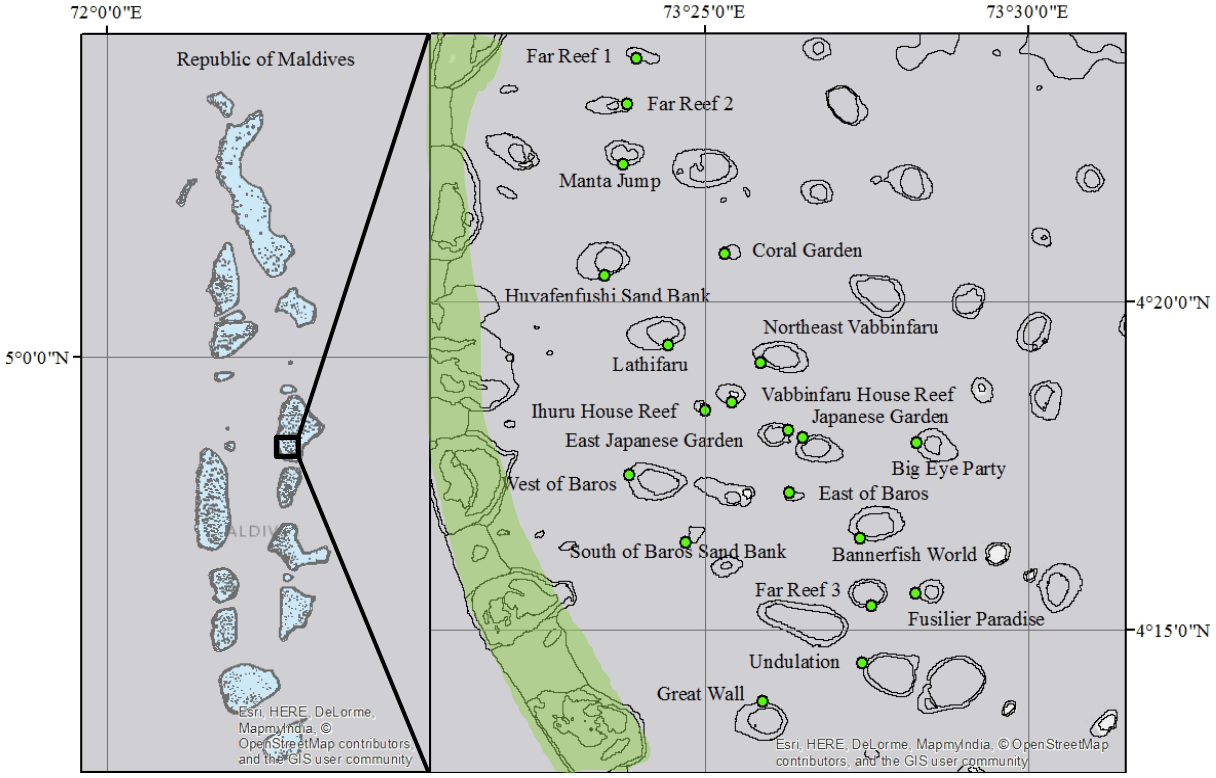


Figure 5.1 Diagramatic map of reef sites in North Malé Atoll, the Maldives. Green dots: inner-atoll sites; green-shaded area: six outer-atoll reefs.

Corals were isotopically represented by common species palatable to corallivorous Chaetodontidae: *Acropora austera*, *Acropora divaricate* and *Pocillopora verrucosa* (Brooker *et al.*, 2013), and the strict corallivore was *Chaetodon meyeri* (Sano, 1984). Detritus was represented by an EM, a non-burrowing holothurian *Pearsonothuria graeffei* (Semper, 1868) that feeds strictly on reef detritus (Purcell *et al.*, 2012) and the strictly detritivorous acanthurid fish *Ctenochaetus striatus* (Crossman *et al.*, 2001). Benthic algae were represented by two common pervasive macroalgae *Halimeda opuntia* and *Tydemania expeditionis*, and the benthic algivore here was *Acanthurus leucosternon* (Robertson and Gaines, 1986). Diurnal and nocturnal plankton included a whole range of pelagic organisms such as phyto- and zooplankton, larvae and particulate organic matter. There were two strict diurnal planktivores: *Caesio varilineata* and *Caesio xanthonota*, and three strict nocturnal planktivores: *Myripristis violacea*, *Myripristis berndti* and *Myripristis murdjan* (Hobson, 1991). Sponges were represented by a high microbial abundance (HMA) species, *Hyrtios erecta* (Kennedy *et al.*, 2014; Cleary *et al.*, 2015), an unarmoured thorectid with heavily cored primary and secondary fibres (Custódio and Symposium, 2007), yet palatable to known predators including *Thalassoma klunzingeri* and *Diadema*

setosum (Burns et al., 2003). The paired strict spongivore was the angelfish *Pygoplites diacanthus*.

5.2.2.1 Fish sampling and preparation

Most fish were collected with a Hawaiian sling. Caesionidae spp from the outer-atoll were collected by Maldivian fishermen. After being captured, fish were kept in a holding bag until brought on board. Fish were killed by spine dislocation in accordance with the UK Home Office Scientific Procedures (Animals) Act and stored in an ice chest on board. After landing, approximately 2 g of white muscle tissue near the dorsal fin were dissected from each fish, rinsed with reverse osmosis water and stored in an individual whirlpack bag in a -20 °C freezer. All samples were later dried in individual tin trays in a fanned oven at 50 °C for ~12 h until fully dried, then stored in individual sealed Eppendorf tubes in zip-lock bags.

Table 5.1 List of selected major energy pathways, primary consumers and their paired production source type and production source or end member (EM).

Energy pathway	Consumer	Production source type	Production source or end member (EM)
Corallivory	*Chaetodon meyeri*	Coral	*Acropora austera, A. divaricate, Pocillopora verrucosa*
Detritivory	*Ctenocheatus striatus*	Detritus	*Pearsonothuria graeffei* (EM)
Herbivory	*Acanthurus leucosternon*	Benthic algae	*Halimeda opuntia, Tydemania expeditionis*
Diurnal planktivory	*Ceasio varilineata, C. xanthonota*	Pelagic plankton	Day time planktonic assemblage ≥ 150 µm
Nocturnal planktivory	*Myripristis violacea, M. berndti, M. murdjan*	Reef plankton	Night time planktonic assemblage ≥ 150 µm
Spongivory	*Pygoplites diacanthus*	Sponge	*Hyrtios erecta*

5.2.2.2 Production source sampling

Corals (5 cm newly grown branch), the sponge and benthic algae (20 g of fronds) were collected with a dive knife. *Pearsonothuria graeffei* individuals were collected on corals, transported to the boat with a mesh net, then 2 cm x 2 cm dermal tissue dissected in a tray with seawater. After landing, these samples were rinsed

with reverse osmosis water and stored in individual whirlpack bags in a -20 °C freezer.

Pelagic production sources (diurnal and nocturnal plankton) were collected with a plankton net (500 mm aperture, 150 µm mesh) towed at the sea surface at a steady speed of 3 knots for 20 min along the reef edge. Plankton samples were kept on the net filter for each tow in an ice chest. After landing, the filters were rinsed with reverse osmosis water, and the plankton materials were removed and stored in individual Eppendorf tubes in a -20 °C freezer. The diurnal plankton was sampled at most sites in the inner- and outer-atoll, whereas the nocturnal plankton sampling was confined to two adjacent reefs and one channel between them in the outer-atoll, and two reef sites (Vabbinfaru and Ihuru) in the inner-atoll due to limited access.

Benthic macroalgae *H. opuntia* and *T. expeditionis* samples were dried in individual tin trays in an oven at 50 °C for ~12 h until fully dried, and stored in individual whirlpak bags, while other production source samples were kept frozen before transportation back to the UK, and then freeze dried (or refreeze dried for oven dried samples) and stored in a fridge. All samples were transported in a Thermos insulated bag with frozen ice pads under DEFRA permit ITIMP16.1258.

5.2.3 Stable isotope analysis sample preparation

All samples were frozen and then freeze-dried. Dermal tissues of *P. graeffei* were ground with a Cryomill while the other samples were ground by hand with mortar and pestle. Benthic algae and plankton were ground and acidified with 1N HCl, rinsed (dropwise untill bubbling ceased), oven dried, rinsed with Milli-Q water four times, frozen and freeze-dried. Ground samples 1.0 ± 0.1 mg (fish white muscle tissues) or 3.0 ± 0.3 mg (production sources or EMs) were then weighed in individual tin capsules with a Mettler MT5 microbalance, pelletized and stored inside plate-well sample trays.

The prepared samples were analysed by Iso-Analytical Ltd (Crewe, UK) by Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS). The ^{15}N:^{14}N ratio was expressed relative to N_2 in air for nitrogen, while that of ^{13}C:^{12}C was relative to Pee Dee Belemnite (PDB). Reference material used for this analysis was IA-R068 ($\delta^{13}C$ = -25.22 ± 0.00‰, $\delta^{15}N$ = 1.00 ± 0.00‰), with quality control check samples IA-R068, IA-R038 ($\delta^{13}C$ = -25.11 ± 0.01‰, $\delta^{15}N$ = -0.53 ± 0.01‰) and IA-R069 ($\delta^{13}C$ = -18.87 ± 0.05‰, $\delta^{15}N$ = 11.76 ± 0.01‰), with quality control check samples IA-R068 and IA-R038, a mixture of IAEA-C7 ($\delta^{13}C$ = -14.46 ± 0.01‰) and IA-R046 ($\delta^{15}N$ =

21.88 ± 0.01‰). IA-R068, IA-R038 and IA-R069 were calibrated against and traceable to IAEA-CH-6 ($\delta^{13}C$ = -10.43‰) and IAEA-N-1 ($\delta^{15}N$ = 0.40‰), IA-R046 to IAEA-N-1. IAEA-C7, IAEA-CH-6 and IAEA-N-1 were inter-laboratory comparison standards. External standards (*Anyperodon leucogrammicus* white muscle tissue, $\delta^{13}C$ = -13.53 ± 0.01‰, $\delta^{15}N$ = 12.64 ± 0.01‰) were also used for future reference.

5.2.4 Data analysis

All data were analysed in R 3.24 (R Core Team, 2016) using multiple packages: SIBER (Jackson *et al.*, 2011), MixSIAR (Semmens *et al.*, 2013) and ggplot2 (Wickham and Chang, 2016). Differences in $\delta^{13}C$ and $\delta^{15}N$ values among the six production source types or EMs at both inner- and outer-atoll reefs were tested using one-way ANOVA for each stable isotope ratio. Tukey tests were used for multiple pairwise comparisons of significant effects identified by the ANOVAs (Appendix 10).

5.2.4.1 Source discrimination

$\delta^{13}C$ and $\delta^{15}N$ values of six production source types or their EMs at both inner- and outer-atoll reefs were visualized using SIBER by examining the dispersion of $\delta^{13}C$ and $\delta^{15}N$ values in iso-space. This was achieved by calculating sample size corrected standard ellipse areas (SEA_C) using the majority of the data per group (40%), and the isotopic niche parameters semi-major axis (*a*), semi-minor axis (*b*), angle in degrees (θ) between *a* and the x-axis, eccentricity (E) and Bayesian SEA (SEA_B) (Jackson *et al.*, 2011). θ and E values have the potential to distinguish among isotopic niches where different species or trophic guilds have similar sized isotopic niches but there are differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ (Reid *et al.*, 2016). The θ is a value between 0 and π, and reported here in degrees between 0° and 180°; θ values close to 0° represent relative dispersion along the x-axis ($\delta^{13}C$), indicating multiple production sources, while θ values close to 90° show relative dispersion along the y-axis ($\delta^{15}N$), indicating multiple trophic positions within a uniform basal source. The E value explains the variance on the x- and y-axes: low E refers to similar variance on both axes with a more circular shape, while high E indicates that the ellipse is stretched along either the x- or y-axis. In order to compare isotopic niche areas among trophic guilds, the Bayesian approach calculated 20,000 posterior estimates of SEA_B based on the data set; the mode and 95% credible intervals (CIs) were reported. A significant difference among SEA_B was interpreted

graphically whereby if the 95% CI did not overlap then the SEA_B values were deemed to be significantly different.

Table 5.2 Bulk carbon and nitrogen stable isotope trophic enrichment factors ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) for specific trophic pathway of six major trophic pathways in coral-reef fish food-webs at North Malé Atoll (the Maldives).

Energy pathway	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	Reference
Corallivory	1.70 ± 1.00	3.10 ± 1.20	Sweeting *et al.* (2006)
Detritivory	0.00	0.00	N.A.
Herbivory	0.50	1.90	Wyatt *et al.* (2010)
Diurnal planktivory	0.60	2.40	Wyatt *et al.* (2010)
Nocturnal planktivory	0.60	2.40	Wyatt *et al.* (2010)
Spongivory	1.70 ± 1.00	3.10 ± 1.20	Sweeting *et al.* (2006)

5.2.4.2 Source mixing models in consumers

$\delta^{13}C$ and $\delta^{15}N$ values of source types or EMs relative to the six fish trophic guilds were calculated using MixSIAR (Stock and Semmens, 2016). Mean ± SD $\delta^{13}C$ and $\delta^{15}N$ values and sample sizes (*n*) of production sources/EMs of each location and raw $\delta^{13}C$ and $\delta^{15}N$ values of fish were used in the model. Each trophic group was analysed separately. The model was run with sufficient iteration until MCMC chain length reached convergence where all variables in the Gelman-Rubin diagnostic were < 1.05 and ≤ 5% of variables were outside ± 1.96 in the Geweke diagnostic (Semmens *et al.*, 2013). Pathway specific trophic enrichment factor (TEF) values were chosen where possible (Table 5.2). A single TEF value was applied across energy pathways ($\Delta\delta^{13}C$ = 1.7 ± 1.0‰, $\Delta\delta^{15}N$ = 3.1 ± 1.2‰, Sweeting *et al.*, 2006) except that between detritus EM *P. graeffei* and the detritivore ($\Delta\delta^{13}C$ = 0.00‰, $\Delta\delta^{15}N$ = 0.00‰) to test the sensitivity of diet composition towards different TEFs (Appendix 12). Both process and residual errors were included in the model to reduce variations from within-population trophodynamics and individual physical differences such as digestability (Stock and Semmens, 2016). The output of MixSIAR was visualized using box and whisker plots with the mode (as black bars), 25 and 75% quantiles (as boxes) and 0 and 100% quantiles (as lines). Diet composition was considered to be significantly different (non-overlapping boxes), different (mode not inside the box of the other) or similar (mode inside the box of the other).

5.3 Results

5.3.1 Discriminability of production sources/end members

The isotopic niches of the six production sources or EMs (regardless of location) were distinguishable to some extent (Figure 5.2). However, the SEA_C of nocturnal plankton was within that of the diurnal plankton, while those of the benthic algae and *H. erecta* overlapped significantly. The relative vertical positions of the six groups suggested the plankton production sources/EMs (diurnal and nocturnal plankton) and *P. graeffei* had higher $\delta^{15}N$ values or trophic positions than benthic primary production sources (benthic algae and *H. erecta*), and the corals had an intermediate $\delta^{15}N$ value. The plankton production sources/EMs had similar $\delta^{13}C$ values to benthic algae and *H. erecta*, while the corals had higher $\delta^{13}C$ values and *P. graeffei* had the highest $\delta^{13}C$ value (Figure 5.2).

SEA, SEA_C and mode of SEA_B values were similar to each other in each production source type/EM (Table 5.3). The E values indicated algae, coral, diurnal plankton and nocturnal plankton had relatively round isotopic niches ($E > 0.9$), whereas those of *H. erecta* and *P. graeffei* were relatively flat. The algae, coral, diurnal plankton, *P. graeffei* had rather horizontal isotopic niches ($|\theta| < 5°$) whereas *H. erecta* and nocturnal plankton with negative θ values were more vertical. *Hyrtios erecta* had the highest modal SEA, followed by algae, diurnal plankton, coral, *P. graeffei* and nocturnal plankton (Figure 5.3, Table 5.3). The SEA probability distribution suggested algae and *H. erecta* were similar with 95% probability of having a greater SEA than coral, nocturnal plankton and *P. graeffei*. Corals, nocturnal plankton and *P. graeffei* had similar SEAs, and 95% probability of these being greater than nocturnal plankton (Figure 5.3, Table 5.3).

5.3.2 Diet compositions

The corallivorous *C. meyeri* appeared to feed almost entirely on the hard corals with small but non-significant differences in diet proportion between inner- and outer-atoll (Figure 5.4a). Some reliance on detritus was evident, but this was relatively small. Using the constant TEF value in the mixing model provided similar results (Appendix 12).

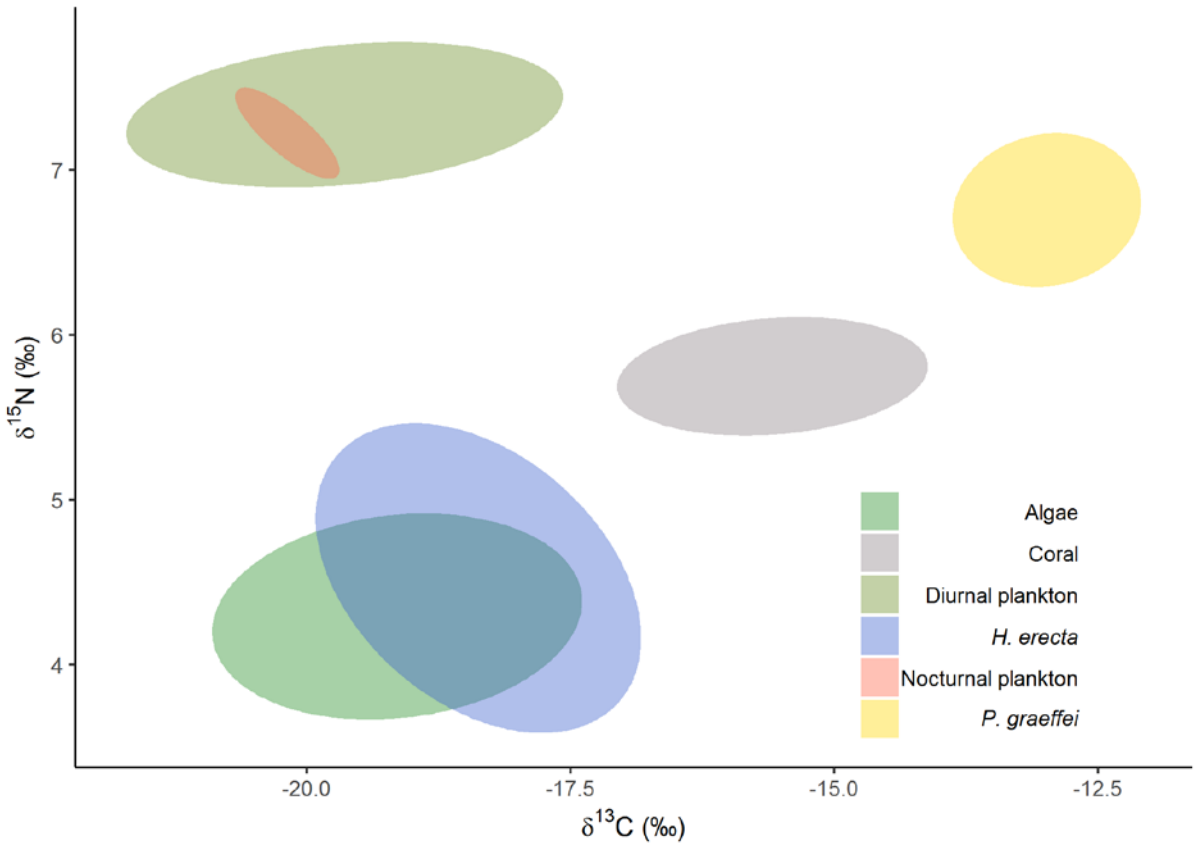


Figure 5.2 Plot of bulk $\delta^{15}N$ against $\delta^{13}C$ showing the small sample size-corrected standard ellipses/isotopic niche (solid line) of six production source types or end members at North Malé Atoll (Maldives). D. plankton = diurnal plankton, *H. erecta* = *Hyrtios erecta*, N. plankton = nocturnal plankton, and *P. graeffei* = *Pearsonothuria graeffei*.

Table 5.3 Standard ellipse areas (‰^{2}) and eccentricity [E], the angle in degree between the semi-major axis of the isotopic niche and the x-axis [θ] parameters for six production sources types or end members at North Malé Atoll (Maldives). Estimates of isotopic niche areas are given as standard ellipse area (SEA), sample size-corrected standard ellipse area (SEA_C) and mode of the Bayesian standard ellipse area (SEA_B) estimates. Upper and lower 95% credible intervals (CIs) indicate the uncertainty in the SEA_B estimates.

Production source/End member	SEA (‰^{2})	SEA_C (‰^{2})	E	θ (°)	SEA_B (‰^{2})	SEA_B 95% CIs
Algae	3.329	3.405	0.936	3.44	3.282	2.422-4.423
Corals	1.598	1.637	0.971	2.58	1.569	1.155-2.122
Diurnal plankton	2.572	2.770	0.979	3.27	2.494	1.397-4.282
Hyrtios erecta	3.983	4.193	0.855	-18.45	3.860	2.539-6.065
Nocturnal plankton	0.202	0.252	0.963	-26.30	0.227	0.092-0.582
Pearsonothuria graeffei	1.229	1.301	0.856	4.07	1.145	0.756-1.915

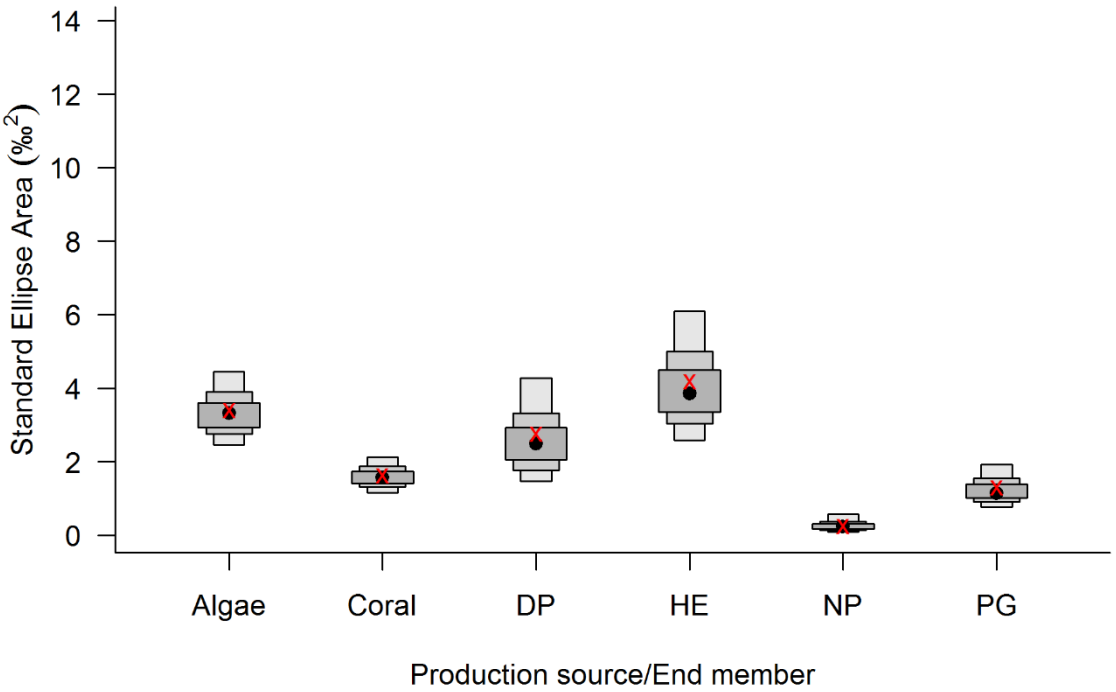


Figure 5.3 Posterior estimates of the standard ellipse areas (SEA_B) of the six production source types/end members (DP: diurnal plankton, HE: *Hyrtios erecta*, NP: nocturnal plankton, PG: *Pearsonothuria graeffei*). The boxes represent the 95, 75 and 50% credible intervals in ascending order of size, with the mode indicated by the black circles. The maximum likelihood estimate for the corresponding SEA_C is indicated by the red squares.

For the detritivore (*C. striatus*), the inner- and outer-atoll data were similar (Figure 5.4b). However, the isotopic data pointed to this species relying on corals rather than on the detritus represented by *P. graeffei*. This tended to be to a greater extent in the outer-atoll than the inner-atoll, the latter tending to be less detritus driven. This result was similar to that using a constant TEF value in the mixing model (Appendix 12).

The stable isotope data of the diurnal planktivore Caesionidae spp suggested that individuals in the inner-atoll relied on diurnal and nocturnal plankton and *H. erecta* while those in the outer-atoll relied mostly on diurnal plankton (Figure 5.4c). The constant TEF analysis showed that these fish at the inner- and outer-atoll sites relied almost exclusively on diurnal and nocturnal plankton, especially the latter (Appendix 12).

For the herbivorous *A. leucosternon*, the principal source was identified as detritus in both locations (Figure 5.4d). Diet proportions of other sources were mostly very low, the greatest being coral. The analysis using a single TEF showed similar results but no difference in diurnal plankton between the locations (Appendix 12).

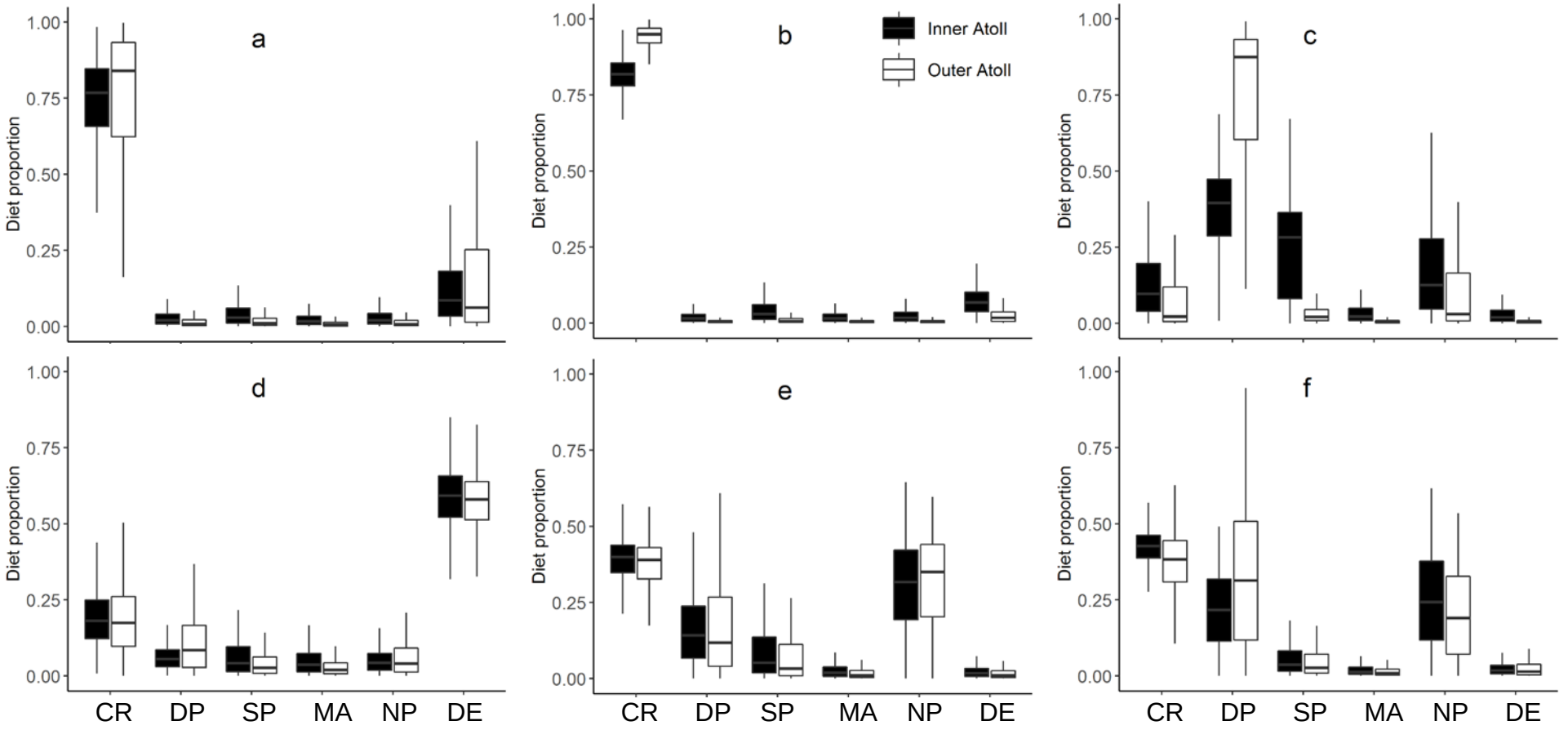


Figure 5.4 Modelled proportions of different proportion source types (CR = coral, DP = diurnal plankton, SP = sponge, MA = macroalgae, NP = nocturnal plankton and DE = detritus) to the a) corallivore *Chaetodon meyeri*, b) detritivore *Ctenochaetus striatus*, c) diurnal planktivores *Caesio varilineata* and *C. xanthonota*, d) herbivore *Acanthurus leucosternon*, e) nocturnal planktivores *Myripristis violacea*, *M. berndti* and *M. murdjan*, and f) spongivore *Pygoplites diacanthus* on inner- (black box) and outer-atoll reefs (white box) at North Malé Atoll (Maldives).

The nocturnal planktivore utilized both nocturnal plankton and coral sources (mode ~ 40%) with some reliance on diurnal plankton (mode ~15%, Figure 5.4e). The analysis using a single TEF value indicated that these fish were mostly reliant on nocturnal plankton at both locations (Appendix 12).

The spongivore *P. diacanthus* showed high reliance on corals and diurnal plankton (mode ~ 20-30%) in addition to nocturnal plankton (mode ~ 20-25%) with no difference between the locations (Figure 5.4f). The constant TEF analysis highlighted the dietary importance of nocturnal plankton which was greater in the inner-atoll (mode ~ 55%) than the outer-atoll (mode ~ 40%), with some dependence on diurnal plankton and corals that was greater in the outer-atoll than in the inner (Appendix 12). Neither TEF assumption highlighted substantial reliance on the sponge *H. erecta* (Figure 5.4f, Appendix 12).

5.4 Discussion

Six production source types were discriminated to some extent from each other using the stable isotope data, but of the six consumers, only the corallivore complied with its single putative trophic category. In contrast, the others relied either on multiple production source types or on a type of source other than that to which it is assigned. Some of these consumers demonstrated location-dependent source-partitioning. It is evident that the putative trophic categorisations of fish are often inaccurate or simplistic.

5.4.1 Source discrimination

The isotopic niche plot indicated that the major production source types represented by the selected organisms were discriminable to some extent. One exception was the overlap between diurnal and nocturnal plankton. This was most likely a result of their sharing the same carbon and nitrogen sources. Both include phytoplankton, pelagic and reef zooplankton with some reef zooplankton being from within or on the reef substrate (e.g. holoplankton such as copepods and mysids) or inside crevices during the day (e.g. semipelagic organisms such as polychaetes, ostracods and crustacean larvae) and feeding in the water column after sunset (Zaret and Suffern, 1976; Enright and Honegger, 1977; Bollens and Frost, 1989; Hobson, 1991). Compared with the diurnal zooplankton, nocturnal zooplankton may be larger in size (Hobson, 1991) and might be greater in TP (McClelland and Montoya, 2002). The nocturnal planktivore isotopic niche was contained within that of the diurnal planktivores. In this study, all plankton were sampled at the sea surface (0-50 cm),

and this may only incompletely indicate the whole zooplankton assemblage ultimately supporting the consumers whereas depth could be an important factor in determining the abundance and composition of zooplankton (Bollens and Frost, 1989; Hobson, 1991). The SEA values of the nocturnal plankton were much smaller than that of diurnal plankton, potentially attributable to less varied composition, lower sample size ($n_{diurnal}$ = 15, $n_{nocturnal}$ = 6) and smaller spatial coverage (See Methods).

Reasons for the overlapping ellipses of *H. erecta* and macroalgae are unclear. The macroalgae isotopic data pertained only to frond tissues, while the *H. erecta* data will have derived from sponge tissues and microbes (10^8-10^9 bacteria per gram of sponge tissue). *Hyrtios erecta* is non-cryptic, and expected to have photosynthetic microbes. If it is a photoautotrophic sponge, this might explain its isotopic niche overlap with the selected benthic macroalgae. However, production to respiration [P/R] ratio and microbe assemblages of this species are unknown.

Pearsonothuria graeffei the detritus end-member was separated from other production sources. Most aquatic TEFs apply to particular food sources and their consumers (Strieder Philippsen and Benedito, 2013) and are sensitive to tissue type and compound effects (e.g. protein and fatty acids, Tieszen *et al.*, 1983; Hobson and Clark, 1992a), however the detritus *P. graeffei* fed on and its isotopic signature are unknown, and it is likely to have been influenced by a number of ultimate sources. Compositions of amino acids (Crossman *et al.*, 2001) and fatty acids (Sanchez and Trexler, 2018) have been used as bio-tracers to categorise detritus and track its energy flow in aquatic systems. Yet, stable isotope characterisations of detritus in the tropics often use whole sediment samples to represent it isotopically (e.g. Chong *et al.*, 2001) which masks the heterogeneity of detritus, differential isotopic fractionation of the different components involved (e.g. bacteria, diatoms) and potentially biomarkers of interest (e.g. bacterial fatty acids, Monson and Hayes, 1982). These unknowns make it hard to further characterise diet sources and designate appropriate TEF values. Here, the EM used was expected to isotopically represent the assimilatable components of the detritus, and the TEF between the dermal tissue of this EM and white muscle tissues of fish was suspected to be much less than from food source to white muscle tissues. Thus, it is not sure whether detritus can be distinguished from other primary production source types and whether this EM is a good indicator of those detrital components preferred by the detritivore. Further studies are needed to understand the composition of the detritus matrix, palatable

components and their respective biomarker and TEF values, and potential consumer(s).

The isotopic niche parameters indicated benthic algae, corals, diurnal plankton and detritus were more variable in $\delta^{13}C$ than $\delta^{15}N$, while the converse was the *H. erecta* and nocturnal plankton. This suggests that *H. erecta* could be a mixed-trophic sponge species relying on photosynthetic and plankton feeding sources and thus a high level of TP omnivory. Compared with the diurnal plankton which potentially included individuals with different TPs and $\delta^{13}C$ baselines, the nocturnal plankton mainly included zooplankton of multiple trophic positions sharing a single $\delta^{13}C$ baseline (Hobson, 1991). Although the numbers of species used for each production source type varied, the SEA, SEA_C and SEA_B values were not necessarily related to this; benthic algae (two species) and sponge (one species) had greater isotopic niche areas than corals (three species), suggesting natural isotopic variation within and between species.

5.4.2 Diet composition of the consumers

Diet compositions of the corallivore, and diurnal and nocturnal planktivores were mostly in accordance with their assigned trophic guilds. For the corallivore, the TEF was the same throughout due to there being no pathway-specific data available, and *C. meyeri* was linked to hard corals on inner and outer reefs. Previous dietary studies have shown that many chaetodontid species including *C. meyeri* are obligate corallivores (Sano, 1984). The coral species selected were evidently representative of its food source type, and the assumed TEF seems to have been appropriate.

However, in the dietary analyses of the diurnal and nocturnal planktivores, significant differences arose. For the diurnal planktivore, both TEF assumptions pointed to the importance of diurnal and nocturnal plankton as important food sources. The overlapping isotopic niches of diurnal and nocturnal plankton must have reduced their discriminability (Semmens *et al.*, 2013). Both results showed a higher diet proportion of diurnal plankton at outer-atoll than inner-atoll locations. Regardless of the overlap between diurnal and nocturnal plankton and the uncertainties of the TEFs, it may be that these Caesionidae preferred diurnal plankton where it was more available. For the nocturnal planktivore, both TEF assumptions showed the importance of nocturnal plankton and some influence of diurnal plankton, although the pathway-specific TEFs pointed to corals as also important. The corallivore TEF value remained the same, thus, the differences were mainly caused by changes in

the TEFs of nocturnal planktivory. *Myripristis* spp feed on nocturnal zooplankton including larger calanoids, polychaetes, ostracods, copepods, mysids, isopods, amphipods and crustacean larvae (Hobson, 1991). These in turn likely rely on multiple food sources including detritus, zoobenthos and zooplankton. It is worth nothing that the nocturnal plankton overlapped isotopically with diurnal plankton, but not with corals. However, the nocturnal plankton was collected from the surface, and potentially included a wide spectrum of organisms compared to the diurnal plankton (Hobson, 1991). To tease apart the different components, identify which are relied on by consumers, it is necessary to understand their biomarkers and TEF values better. Here, it is suspected the unexpectedly high diet proportion of corals was a result of some of the *Myripristis* prey feeding on corals or coral detritus (e.g. mucus) which were absent from the nocturnal plankton samples, and the lower TEF of nocturnal planktivory might be incorrect at this location. The effect was to position the nocturnal planktivores closer to corals and away from the nocturnal/diurnal plankton cluster.

Other cases where the diet proportion derived from the isotopic data was not in accordance with existing categorisation were those of the detritivore, herbivore and spongivore. The detritivore data indicated it was feeding substantially on hard corals albeit with small contributions from other sources such as detritus. The detritivorous species *C. striatus* is reported to feed mostly on detritus and sediment (Robertson and Gaines, 1986; Choat *et al.*, 2002) and diatoms (Purcell and Bellwood, 1993). Its fatty acid composition is close to that of Scarinae but very different from Acanthuridae in other trophic guilds such as the planktivores and algivores (Clements *et al.*, 2016). Its gut microbial assemblage is distinct from that of most other trophic guilds (Miyake *et al.*, 2015). It is very likely that *C. striatus* at North Malé atoll was selectively feeding on detritus (e.g. coral mucus or debris) and microbial autotrophs (e.g. cyanobacteria) in the detritus matrix. However, microbial autotrophs which are isotopically different from algal sources (Pentecost and Spiro, 1990) were not sampled. It is evident that *P. graeffei* was not an appropriate bulk $\delta^{13}C$ and $\delta^{15}N$ end member for whatever *C. striatus* was feeding on or the TEF (0.00‰) used for both isotopes were not appropriate. In contrast, the herbivore *A. leucosternon* isotopic data pointed to high reliance on detritus. This species evidently feeds on turf algae (Robertson and Gaines, 1986) but these algae were not sampled. The epilithic algal matrix involved includes a wide range of potential production sources (Crossman *et al.*, 2001; Adam *et al.*, 2018) and can be isotopically different from other macroalgae (Pinnegar and Polunin, 2000; Plass-Johnson *et al.*, 2015). In this study, benthic algae

were represented by *Halimeda* spp because they were single species and assumed to be isotopically similar to other benthic algae although they are not commonly palatable due to their heavily calcified structures (Price *et al.*, 2011) and chemical defences (Paul and Van Alstyne, 1988; Cetrulo and Hay, 2000). The result here may be because of that turf algae were more isotopically similar to detritus than to *Halimeda* spp. Yet this herbivorous species will only be better defined isotopically when the components of the food matrix, their isotope values and TEFs are clear.

The spongivore *P. diacanthus* was linked to hard corals, and diurnal and nocturnal plankton sources which suggests greater pelagic influence than previous studies (Alwany, 2009; Konow and Bellwood, 2011). The isotopic niche of *P. diacanthus* is similar to that of the nocturnal planktivore *Myripristis* (Appendix 11). The data suggest that *P. diacanthus* relied more on diurnal plankton and less on nocturnal plankton at outer-atoll sites than inner-atoll sites. This may reflect food availability for the sponge that *P. diacanthus* feeds on. However, the sponges preferred by *P. diacanthus* are unknown. Although the sponge *H. erecta* used in the mixing model suggests a small contribution to the diet of this angel fish, other sponge species collected had lower $\delta^{13}C$ but higher $\delta^{15}N$ values resembling the pelagic sources (Appendix 11) similar to a study in Papua New Guinea (Weisz, 2006). The replication in this study was insufficient to use these in the mixing models. Such high-TP sponges may be preferred by this spongivore. With more information, the diet could be better parameterised in the future.

Location (inner- and outer-atoll) generated significantly different dietary proportions for fish relying on pelagic sources (e.g. Caesionidae, *P. diacanthus*) and the detritivore *C. striatus*. It is possible that for the caesionids plankton availability was greater at outer-atoll sites and this also affects the *P. diacanthus* feeding on planktivorous sponges; *C. striatus* might be a similar case of food availability in that more corals survived a recent bleaching event (Perry and Morgan, 2017) at outer-atoll sites.

The TEF values used here only indicated single trophic level consumer-diet relationships, yet several other trophic pathways including faeces feeding (Robertson, 1982) and the sponge loop (de Goeij *et al.*, 2013) might not be exactly one trophic level of enrichment from the food source. DNA barcoding of the faeces of fish can reflect the diet composition to some extent (Stamoulis *et al.*, 2017), but digestive loss of the DNA of certain food items, type of primer used, presence of eDNA and insufficient grinding of samples may all affect results. Also, the stable

isotope signatures of some element(s) are suspected to change through biochemical reactions with digestive enzymes and intestinal bacteria (Macko *et al.*, 1986; Morasch *et al.*, 2002; Casciotti *et al.*, 2003; Morasch *et al.*, 2004). Thus, such pathways might not be accurately captured in the mixing model. The sponge loop detritus comes from the rapidly replaced filter cells (de Goeij *et al.*, 2013). Its isotopic signatures are considered different to the sponge as stable isotope values are sensitivity to the tissue turnover rate (Tieszen *et al.*, 1983). Moreover, the detritus was only looked at as diet for zoobenthos in the tracer experiment of de Goeij *et al.* (2013) but how it becomes integrated into fish (e.g. direct detritivory, indirect carnivory) remains unclear.

5.4.3 Limitations

This is the most detailed study of its kind to date, but there were several limitations. The TEFs used here were pathway-specific values from existing studies which might not be appropriate for this location or the selected consumers (Post, 2002b; McCutchan *et al.*, 2003; Mill *et al.*, 2007; Dromard *et al.*, 2013; Plass-Johnson *et al.*, 2013; Strieder Philippsen and Benedito, 2013). Modelled dietary proportions differed significantly between constant and variable TEF analyses of some consumers. It is likely that more pathway-specific TEF data would help increase the confidence in the modelled production source mixes, especially for this complex ecosystem, and while six sources were explored here, it might be that additional sources would have better explained the present results. These sources might include benthic autotrophs (e.g. turf algae and microbes), cryptic autotrophic sponges and semi-pelagic zooplankton that migrate down after sun set. However some of these sources might not be isotopically discriminable (Polunin and Pinnegar, 2002; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). Although several production sources or end members were used to represent one particular production source type, these might not be precise indicators of the types of sources targeted. The identification of production sources, their isotopic characterisation and the mixing models used to resolve them in these complex food webs is a rapidly developing field, and uncertainties over the TEF will hopefully be reduced in future. For now this is the most detailed such study of coral reefs.

5.4.4 Conclusion

The bulk stable isotope data have helped to distinguish between major production source types to some extent. By using MixSIAR with both production

source type and consumer fish stable isotope data, some strictly categorised fish are evidently feeding cross their trophic boundaries. This indicates that many trophic categorisations belie a more complex trophodynamic foundation of these coral-reef food-webs. Some fish also demonstrated flexible diet composition in the two types of reef habitats suggesting certain level of dietary versatility.

Chapter 6. Fish trophic specialism vs generalism on coral reefs: an amino-acid compound-specific stable isotope comparison

6.1 Introduction

Trophic specialist (e.g. Brooker *et al.*, 2013) and generalist fishes (O'Brien, 1979; Robertson, 1982; Mumby *et al.*, 2006; Romanuk *et al.*, 2011; Plass-Johnson *et al.*, 2013; Zhu *et al.*, unpublished data) occur in coral-reef food-webs, yet, in many large scale studies (e.g. MacNeil *et al.*, 2015; D'Agata *et al.*, 2016; Graham *et al.*, 2017; Stamoulis *et al.*, 2017; Hadi *et al.*, 2018), fish species have very commonly been assigned to single trophic guilds which mask dynamic source partitioning and potential flexibility in functional roles at species level or below within the food web. Understanding energy flow of such diverse food webs in finer detail requires better resolution of the production sources involved. Reef-fish generalism has been analysed through behavioural studies (O'Brien, 1979; Robertson, 1982; Fernando, 1994; McEdward, 1997; Wulff, 1997) and bio-tracers such as bulk stable isotope data, but these either do not incorporate long-term diet or have analytical limitations. For example, bulk stable isotope data provide time-integrated signatures of assimilated food items into tissues but lack resolution among production sources and are sensitive to the variation of isotopic enrichment factors (Post, 2002b; McCutchan *et al.*, 2003; Mill *et al.*, 2007; Dromard *et al.*, 2013; Plass-Johnson *et al.*, 2013; Strieder Philippsen and Benedito, 2013).

Recent studies have improved carbon flow tracing in ocean environments by analysing the carbon stable isotope values ($\delta^{13}C$) of specific compounds such as amino acids. Amino acids are one of the most studied biochemical tracers in organic geochemistry (Larsen *et al.*, 2015), and are a more labile part of the bulk organic matter (Cowie and Hedges, 1994) in both plankton and sinking particular organic matter (Lee *et al.*, 2000; Hedges *et al.*, 2001). Unique biochemical processes of synthesizing or degrading essential amino acids (EAAs) result in distinctive $\delta^{13}C$-EAA values among primary production sources such as algae and bacteria (Larsen *et al.*, 2013) providing greater power to distinguish among sources than bulk stable isotope data (Larsen *et al.*, 2015). Most animals cannot synthesize EAAs, and must acquire them directly from the source(s) with little modification in $\delta^{13}C$-EAA values (McMahon *et al.*, 2010; Larsen *et al.*, 2013), values which are thus carried along food chains with minimal fractionation (McMahon *et al.*, 2015). This method can potentially resolve two limitations of bulk stable isotope data in dietary studies, namely by

improved discrimination among sources and reduced uncertainties in trophic fractionation from the use of separate isotopic baseline materials.

Here to understand better the trophic ecology of coral-reef fish, carbon compound-specific stable isotope data of essential amino acids are used to 1) test the discriminability of production sources, 2) compare isotope ecologies of several fish categorised as trophic specialists in six trophic pathways, and 3) compare these ecologies between two major reef locations.

6.2 Materials and methods

6.2.1 Study site

Twenty accessible inner-atoll sites (depth 4-7 m) and six outer-atoll reefs (depth 10-20 m) at South West of North Malé Atoll (the Maldives) were selected for fish and production source sampling (Figure 6.1).

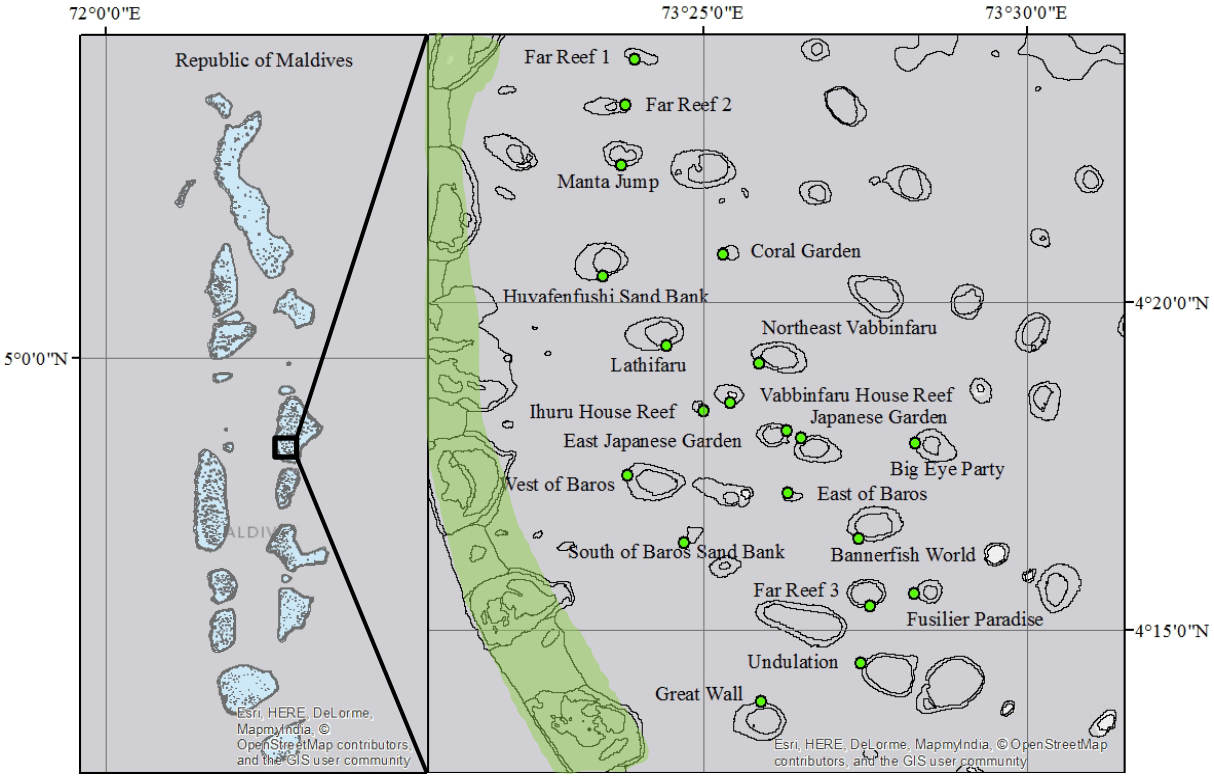


Figure 6.1 Map of reef sites in North Malé Atoll, the Maldives. Green dots: inner-atoll sites; green-shaded area: six outer-atoll reefs.

6.2.2 Sample collection

6.2.2.1 Production source type selection

Corallivory, detritivory, herbivory, diurnal and nocturnal planktivory, and spongivory were studied as major potential energy pathways on the coral reef. For

each of these a primary consumer and its known main diet source or source end member (EM) (Table 6.1) were also studied.

The corals were the common palatable species *Acropora austere* and *A. divaricate* (Brooker *et al.*, 2013), and the strict corallivores were *Chaetodon meyeri*, *C. falcula* and *C. trifasciatus* (Sano, 1984; Cole *et al.*, 2008). Detritus was represented by an EM, a non-burrowing holothurian *Pearsonothuria graeffei* (Semper, 1868; Purcell *et al.*, 2012) that feeds strictly on reef detritus (Purcell *et al.*, 2012), and the strict detritivore was the acanthurid *Ctenochaetus striatus* (Crossman *et al.*, 2001). The benthic alga was the macroalga *Halimeda opuntia*. The benthic algivore was *Acanthurus leucosternon* (Robertson and Gaines, 1986).

Table 6.1 List of major energy pathways, primary consumer(s) and their paired production source type (as main diet), and source(s) or endmember.

Energy pathway	Consumer	Production source type	Source or endmember (EM)
Corallivory	*Chaetodon meyeri, C. falcula, C. trifasciatus*	Coral	*Acropora austera, A. divaricata*
Detritivory	*Ctenocheatus striatus*	Detritus	*Pearsonothuria graeffei*
Herbivory	*Acanthurus leucosternon*	Macroalgae	*Halimeda opuntia*
Diurnal planktivory	*Ceasio varilineata, C. xanthonota*	Pelagic plankton	Day time planktonic assemblage ≥ 150 µm
Nocturnal planktivory	*Myripristis violacea, M. berndti, M. murdjan*	Reef plankton	Night time planktonic assemblage ≥ 150 µm
Spongivory	*Pygoplites diacanthus*	Sponge	*Hyrtios erecta*

Diurnal and nocturnal plankton were represented by the whole planktonic assemblages from plankton net tows, which included phyto- and zooplankton, larvae and particulate organic matter (POM). For the diurnal plankton, calanoid copepods were separated out to compare with the whole homogenate. The strict diurnal planktivores were the fusilier *Caesio varilineata* and *C. xanthonota*, and the strict nocturnal planktivores were the soldierfish *Myripristis violacea*, *M. berndti* and *M. murdjan* (Hobson, 1991).

Sponges were represented by a high microbial abundance (HMA) species, *Hyrtios erecta* (Abdelmohsen *et al.*, 2010; Radwan *et al.*, 2010; Kennedy *et al.*, 2014; Cleary *et al.*, 2015) which is palatable (Burns et al., 2003). The selected strict spongivore was the pomacanthid fish *Pygoplites diacanthus*.

6.2.2.2 Fish sampling and preparation

Most fish were collected using a Hawaiian sling. Caesionidae spp from the outer-atoll were collected by Maldivian fishermen. After being captured, fish were kept in a holding bag until brought on board. Fish were killed by spine dislocation in accordance with the UK Home Office Scientific Procedures (Animals) Act and stored in an ice chest on board. After landing, approximately 2 g of white muscle tissue near the dorsal fin was dissected, rinsed with reverse osmosis water and stored in an individual whirlpack bag in a -20 °C freezer. All samples were dried in individual tin trays in a fanned oven at 50 °C for ~12 h until fully dried, then stored in individual sealed Eppendorf tubes in zip-lock bags.

6.2.2.3 Production source sampling

Benthic production sources (5 cm newly grown branch for coral, 5 cm newly grown branch or 20 g encrusting tissue for sponge, and 20 g of frond for macroalgae) were collected with a dive knife. *Pearsonothuria graeffei* individuals were collected from the reef, transported to the boat with a mesh bag, 2 cm x 2 cm dermal tissue was dissected in a tray with sufficient seawater and the sea cucumbers were then released to the sea. Diurnal and nocturnal plankton were collected with a plankton net (500 mm aperture, 150 µm mesh) towed at a steady speed of 3 knots for 20 min along the reef edge during the day and after sunset. All samples were kept in individual whirlpak bags or filter caps (plankton only) in an ice chest on board. For diurnal plankton, half of the material was used to separate out calanoid copepods by naked eye. After landing, samples were rinsed with reverse osmosis water and stored in individual whirlpak bags or Eppendorf tubes (depending on the size) in a -20 °C freezer.

Halimeda opuntia samples were dried in individual tin trays in an oven at 50 °C for ~12 h until fully dried, and stored in individual whirlpak bags, while other production source samples were kept frozen during transportation back to Newcastle and then freeze dried and stored in a fridge at Newcastle University (NU). All samples were transported in a THERMOS insulated bag with frozen ice pads to NU under DEFRA permit ITIMP16.1258.

6.2.3 Amino acids compound-specific stable isotope analysis preparation

Dermal tissue of *P. graeffei* was ground with a Cryomill. Other samples were ground manually with mortar and pestle. All ground samples were derivatised.

Samples were weighed (fish: ~2 mg, *H. opuntia*: ~10 mg, others: 6-8 mg) into individual Pyrex® culture tubes (16-150 mm), with enough 6M hydrochloric acid (HCl_{aq}) to submerge samples, and 25 µL of 800 µg/mL DL-norleucine internal standard dissolved in 0.1M HCl_{aq} was added. The tubes were then filled with N_2 gas to displace any oxygen present and capped with Corning® phenolic cap with PTFE liner and tightly sealed with PTFE tape. Culture tubes were heated at 100°C for 24 hours with a heating block to hydrolyse the proteinaceous matter liberating hydrolysable amino acids (AAs). The 6M HCl_{aq} was then evaporated under N_2 gas at 70 °C and each sample was stored in approximately 1 mL 0.1M HCl_{aq}. Dowex® 50WX8 hydrogen form resin (200-400 mesh) was prepared by soaking overnight in 3M $NaOH_{aq}$, washing five times with Milli-Q® water then soaking overnight in 6M HCl_{aq}. Ion exchange chromatography was employed to separate and collect the AA fraction: first marking level of 1mL Milli-Q® water in a flash column, pipetting prepared resin to the marked level, washing resin with 2 mL Milli-Q® water three times; slowly adding the sample with a pipette on top of the resin and eluting salts with 2 mL Milli-Q® water (three times) into a waste bottle; finally eluting the AA fractions with 2 mL of 2M NH_4OH_{aq} (three times if necessary) into a new culture tube. The 2M NH_4OH_{aq} was evaporated under N_2 gas at 60 °C, ready for the first stage of the derivatisation process.

The AAs were esterified by adding 0.25 mL of a 4:1 (volumetric) isopropanol (IP, ≥ 99.5%) and acetyl chloride (AC, 98%) mixture, capped and sealed then heated to 100 °C for 1 hour. The reaction was then quenched in a freezer at -5 °C. Excess reagents were then evaporated using a gentle stream of N_2 gas whilst heating the tubes on a heating block at 40 °C. 0.25 mL aliquots of dichloromethane (DCM, anhydrous, ≥ 99.8%) were added to each tube and again evaporated under a gentle stream of N_2 gas at 40 °C to remove residual reagents. Dried esterified AA fractions were then acylated by adding 2 mL of a 5:2:1 (volumetric) acetone (HPLC grade), trimethylamine (≥ 99%) and acetic anhydride (≥ 99%) mixture, capped and sealed, and heated to 60°C for 10 minutes. Excessive reagents were then evaporated using a gentle stream of N_2 gas at room temperature. Liquid-liquid separation was conducted three times per sample to isolate the derivitised AA fraction by dissolving into 2 mL of ethyl acetate (EA, anhydrous, 99.8%) and 1 mL of saturated $NaCl_{aq}$, vortexing, drawing off the organic layer into a new culture tube, and evaporating the residual solvent using a gentle stream of N_2 gas at room temperature. Residual

solvent was further removed by adding 1 mL of DCM and evaporating under a gentle stream of N_2 gas in an ice bath.

6.2.4 Carbon stable isotope analysis of amino acids (δ^{13}C-AAs)

Dried derivatised AA fractions were examined by GC/FID to assess concentration and to ensure that derivatisation had been successful. The concentration of each sample were adjusted where necessary after screening and analysed by GC/C/IRMS, conditions as in Table 6.2:

Table 6.2 GC conditions for GC/FID and GC/C/IRMS.

GC	GC/FID	GC/C/IRMS
Instrument	Agilent 7890	Thermo Fisher Scientific Delta V GC/C/IRMS (GC IsoLink, Conflow IV interface)
Ionisation mode	N.A.	Electron ionisation (EI)
Injection mode	Cold on Column (COC)	PTV
Injection volume	1 µL	
Carrier gas	He	
Flow rate	2 mL/min	1 mL/min
Column	DB-35 30m x 0.32mm id x 0.5µm	
Initial oven	70 °C	40 °C
Hold time	2 min	5 min
Ramp 1	15 °C/min up to 150 °C	15 °C/min up to 120 °C
Ramp 2	2 °C/min up to 210 °C	3 °C/min up to 180 °C
Ramp 3	8 °C/min up to 150 °C	1.5 °C/min up to 210 °C
Ramp 4	N.A.	4 °C/min up to 270 °C
Hold time	10 min	7 min

Pulses of reference gas (CO_2) were introduced into the IRMS instrument during the analysis giving rise to peaks with known δ^{13}C value (^{13}C:^{12}C ratio relative to Pee Dee Belemnite [PDB]); these reference pulses were used to calculate the analyte peaks in each chromatogram. Identification of the derivatised AAs was achieved by matching the peak elution times with those from a mixed AA standard (derivatised) containing: Alanine (Ala), Glycine (Gly), Valine (Val), Leucine (Leu), Norleucine (Nle), Threonine (Thr), Serine (Ser), Proline (Pro), Aspartic Acid (Asp), Glutamic Acid (Glu), Hydroxyproline (Hyd), Phenylalanine (Phe), Lysine (Lys) and Tyrosine (Tyr). δ^{13}C data of five EAAs (Val, Leu, Thr, Phe and Lys) were used in the analysis.

6.2.5 Data analysis

δ^{13}C-EAA values were analysed in R 3.24 (R Core Team, 2016) using MixSIAR (Semmens *et al.*, 2013) and ggplot2 (Wickham and Chang, 2016).

6.2.5.1 δ^{13}C-EAA data correction

Prior to the analysis, automatically generated peaks in the chromatography were screened and corrected by redefining background and/or peak. Those values from peaks with peak height above or below the designated range (200-20000 mv) were remeasured by either diluting or concentrating (50-200 mv; lower than 50 mv, no further concentration) the solution. The values from either dilution or concentration were examined again at their peak heights. Only values with peak heights within the range were used. For peaks with interfering background, the background value was redefined by finding the closest flat line before the peaks. For coeluting peaks, the peaks were redefined by selecting the start and end points of the peaks by comparing the shape with the reference pulse peaks (and then adjusting background if needed). Peaks with substantial fronting or tailing were also remeasured.

Data were then corrected for the carbon added during esterification and acylation processes in the derivatisation. Standards (composed of Ala, Gly, Val, Leu, Thr, Ser, Pro, Asp, Glu, Phe, Lys and Tyr) with known δ^{13}C-AA values were derivatised using the same reagents as the samples and their derivatised δ^{13}C-AA values were used to calculate correction factors (Corr, Table 6.3) for each AA by the mass balance equation (Docherty *et al.*, 2001):

$$Corr = \frac{n_{cd}\delta^{13}C_{cd.std} - n_c\delta^{13}C_{c.std}}{n_d}$$

where *n* is the number of carbons per molecule, subscript *c* denotes the compound of interest, *d* refers to the derivative groups, *cd* is the derivatised compound and *std* is the standard. The *Corr* value takes into account the derivative groups and any kinetic effects during derivatisation. By applying the correction factor, the original δ^{13}C values were calculated as:

$$\delta^{13}C_c = \frac{n_{cd}\delta^{13}C_{cd} - n_d Corr}{n_c}$$

6.2.5.2 δ^{13}C-EAA data analysis

Individual δ^{13}C-EAAs of the six production source types and end members at both inner- and outer-atoll reefs were compared using ANOVAs with production source or source end member and location as fixed factors. Tukey's honest

significant difference tests were used for multiple pairwise comparisons of significant effects identified by the ANOVAs (Appendix 14). Multivariate signatures of δ^{13}C-EAAs of production sources, source end members and fish trophic guilds were visualized by principal component analysis (PCA) using the covariance matrix to plot ellipses of each group with 95% confidence intervals (Larsen *et al.*, 2013; McMahon *et al.*, 2016). Unlike bulk stable isotope data where the relative positions in the δ^{15}N-δ^{13}C biplot can separate pelagic or benthic sources (i.e. pelagic sources have lower δ^{13}C values than benthic sources), it is unclear where these two source types position in the PCA plot. Thus, the calanoid copepods collected here were used as an indicator of pelagic source. Benthic sources were indicated by *H. opuntia*. Other sources were examined and categorised as benthic or pelagic based on their relative position to these two indicators. Location was not included in the PCA because δ^{13}C-EAA values of the same production source type or end member did not differ between inner and outer atoll areas (Appendix 13). However, δ^{13}C-EAA values per location were included in the mixing model. For both the sources and the consumers, the strength of each δ^{13}C-EAA affecting variation among δ^{13}C-EAA values was examined by the length of arrow, and the correlation between δ^{13}C-EAAs was indicated by the relative position of the arrows.

Table 6.3 List of amino acids for carbon compound-specific isotope analysis (δ^{13}C-AA), numbers of carbon atoms per AA, added in derivative group, total of derivative group, δ^{13}C-AAs of standard, correction factors for samples derivatised in University of Bristol (UoB) and Newcastle University (NU).

AA	No. of C atoms (C*c*)	No. of C atoms added in derivative group (C*d*)	Total C atoms in derivative group (C*cd*)	Underivateised δ^{13}C values (‰) of standard (*std*)	Correction factor (UoB)	Correction factor (NU)
Ala	3	5	8	-26.11	-37.8406	-39.7417
Gly	2	5	7	-40.99	-36.9675	-39.6570
Val	5	5	10	-26.17	-42.7660	-43.7503
Leu	6	5	11	-22.53	-40.2410	-44.5537
Thr	4	5	9	-30.56	-47.3923	-49.5706
Ser	3	5	8	-36.50	-45.3112	-44.7961
Pro	5	5	10	-10.64	-43.4055	-40.1033
Asp	4	8	12	-7.69	-42.1848	-44.0720
Glu	5	8	13	-13.30	-33.5178	-36.6337
Phe	9	5	14	-30.27	-51.5252	-50.5851
Lys	6	5	11	-22.24	-45.7066	-47.2030
Tyr	9	5	14	-16.94	-51.7315	-58.6845

Relative δ^{13}C-EAA data of the sources for the six fish trophic guilds at both locations were calculated using MixSIAR (Stock and Semmens, 2016). Mean ± SD δ^{13}C-EAA values and sample sizes (*n*) of sources at each location and raw δ^{13}C-EAA values of fish at each location with a minimal trophic enrichment factor (0.1 ± 0.1‰) (McMahon *et al.*, 2010) were used in the model in each trophic group one at a time with iteration or MCMC chain length to reach convergence where all variables in the Gelman-Rubin diagnostic were < 1.05, with ≤ 5% of variables outside ± 1.96 in the Geweke diagnostic (Semmens *et al.*, 2013). Both process and residual errors were included in the model to reduce effects of within-population trophodynamics and individual physical differences such as digest ability (Stock and Semmens, 2016). The output of MixSIAR was visualized using box and whisker plots with the mode (as black bars), 25 and 75% quantiles (as boxes) and 0 and 100% quantiles (as lines). Diet composition was considered to be significantly different (non-overlapping boxes), different (mode not inside the box of the other) or similar (mode inside the box of the other).

6.3 Results

6.3.1 Isotopic discrimination among sources or EMs

The PCA biplot explained 75.3% of the total variation with the PC1 axis explaining 53.4% and PC2 21.9% (Table 6.4). The two benthic production sources *H. opuntia*, *Acropora* spp and single end member (*P. graeffei*) were separated out (Figure 6.2) whereas the others (diurnal and nocturnal plankton and *H. erecta*) overlapped with the calanoid copepod (i.e. pelagic cluster). Within the pelagic cluster, the copepod ellipse encompassed that of *H. erecta*, most diurnal plankton and half of the nocturnal plankton. The ellipse of benthic algae was on the right and below that of the copepod, the ellipse of *P. graeffei* was to the left of nocturnal plankton, and the ellipse of *Acropora* spp was comparably far away from the pelagic cluster.

The arrows of the pyruvate-derived aliphatic AAs (Leu and Val) δ^{13}C were close to each other and had greater strengths whereas the other three (two oxaloacetate-derived AAs [Thr and Lys] and one aromatic AA [Phe]) were close and pointing in the opposite direction along the PC2 axis with lower strengths.

6.3.2 Isotopic discrimination of consumers

PC1 explained 64.2% of the variance while PC2 explained 17.0% (total 81.2%; Table 6.5). The benthic algivore, corallivore and detritivore trophic guilds

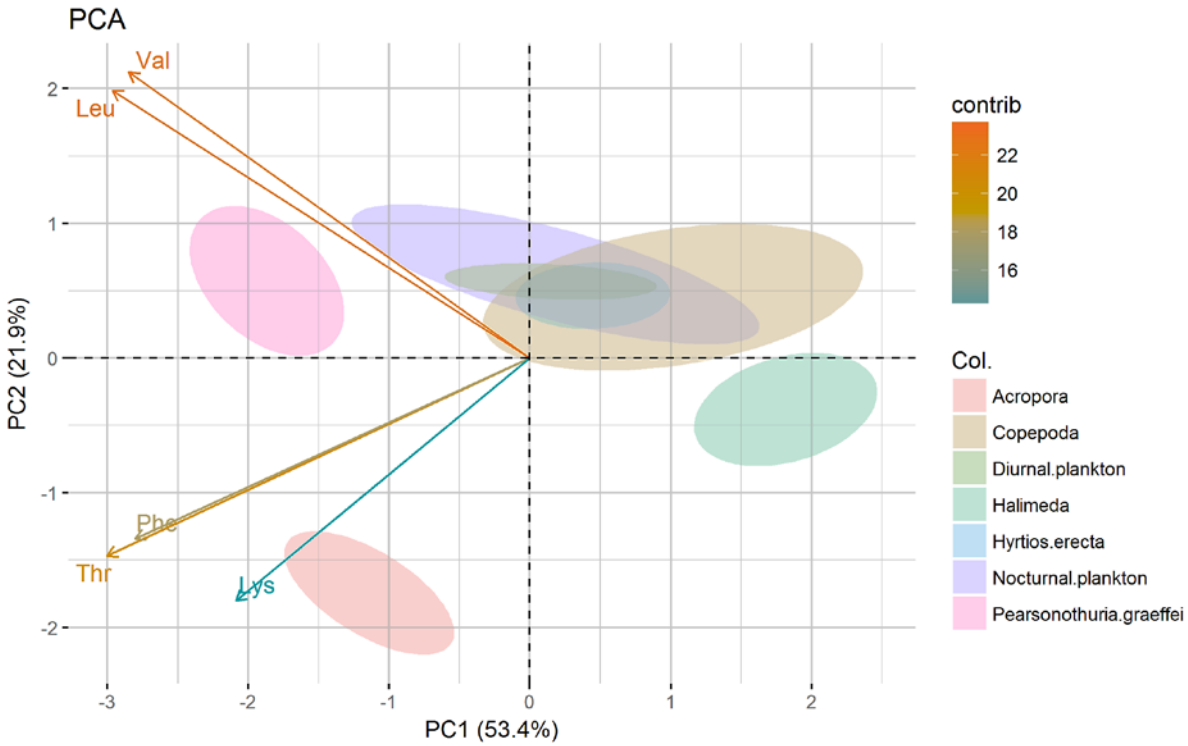


Figure 6.2 Multivariate separation of the seven primary production sources and end members in North Malé Atoll (Maldives) using the principal components (PCs) 1 and 2 from the principal component analysis of $\delta^{13}C$ values of the essential amino acids ($\delta^{13}C$-EAAs) valine (Val), leucine (Leu), threonine (Thr), phenylalanine (Phe) and lysine (Lys). Ellipses indicate 95% confidence limits of each primary production source or end member (*Acropora* spp, Copepoda spp, diurnal plankton, *Halimeda opuntia*, *Hyrtios erecta*, nocturnal plankton and *Pearsonothuria graeffei*). The strength of arrow of each $\delta^{13}C$-EAA in determining the variation in PC1 and 2 was indicated by the color of "contrib".

Table 6.4 Eigenvectors and variance explained (%) for the five principal components (PCs) in the principal component analysis (PCA) of $\delta^{13}C$ values of the essential amino acids valine, leucine, threonine, phenylalanine and lysine from the seven primary production sources and end members (*Acropora* spp, Copepoda spp, diurnal plankton, *Halimeda opuntia*, *Hyrtios erecta*, nocturnal plankton and *Pearsonothuria graeffei*) in North Malé Atoll (Maldives).

$\delta^{13}C$-EAA	PC1	PC2	PC3	PC4	PC5
Valine	-0.46	-0.54	-0.15	0.08	-0.69
Leucine	-0.48	0.50	-0.03	-0.04	0.72
Threonine	-0.49	-0.37	0.24	-0.75	-0.10
Phenylalanine	-0.45	-0.34	0.53	0.63	-0.00
Lysine	-0.34	-0.46	-0.8	0.18	0.07
Variance (%)	53.4	21.9	14.2	6.3	4.2

were close to each other with some overlaps, and located almost 100% on the positive side of the PC1 axis (Figure 6.3). Among these, the algivore ellipse was closest to the pelagic feeding groups while the corallivore was the furthest away. The other three (distinct spongivore guild and the overlapping diurnal and nocturnal planktivore guilds) were located on the negative side of the PC2 axis. The arrows of $\delta^{13}C_{Val}$ and $\delta^{13}C_{Leu}$ were positively associated with PC1 and PC2, whereas $\delta^{13}C_{Thr}$, $\delta^{13}C_{Phe}$ and $\delta^{13}C_{Lys}$ were negatively associated with PC2.

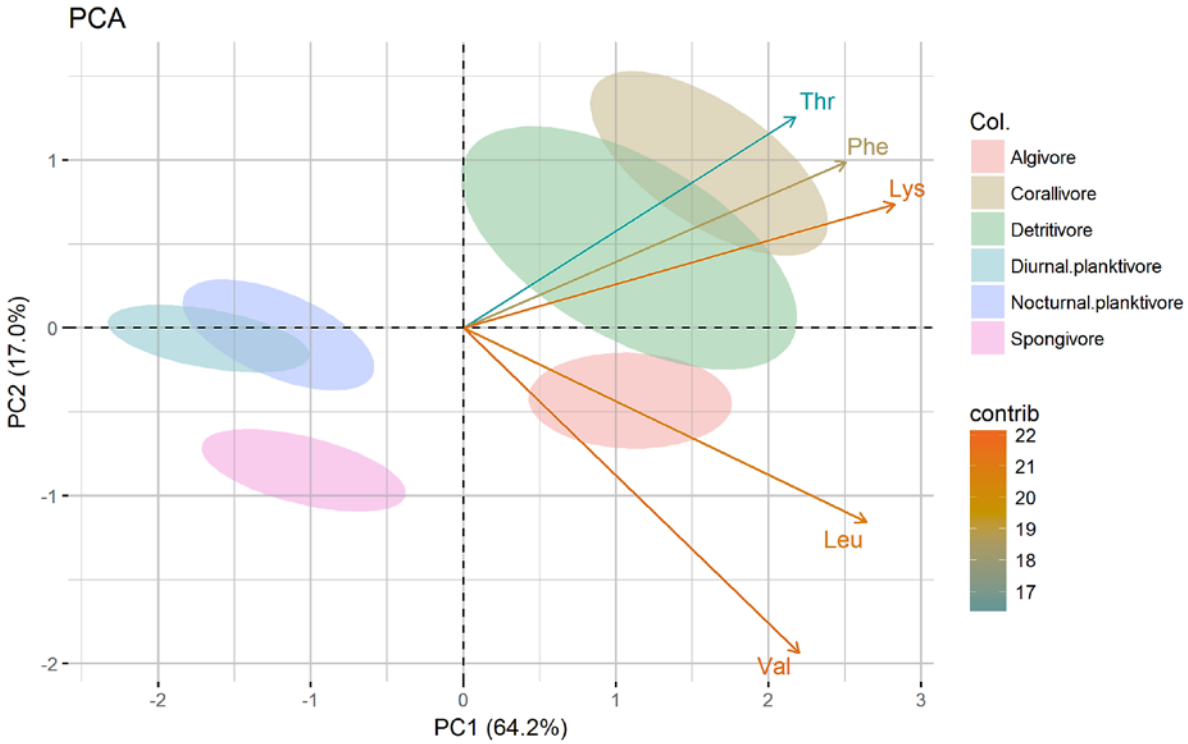


Figure 6.3 PCA plot of the six trophic guilds in North Malé Atoll (Maldives) using PC1 and PC2 of $\delta^{13}C$ data of the five essential amino acids valine (Val), leucine (Leu), threonine (Thr), phenylalanine (Phe) and lysine (Lys). Ellipses indicate 95% confidence of each trophic guild. The strength of arrow of each $\delta^{13}C$-EAA in determining the variation in PC 1 and 2 was indicated by the color of "contrib". The fish species in each trophic guild were: algivore (*Acanthurus leucosternon*), corallivore (*Chaetodon meyeri*), detritivore (*Ctenochaetus striatus*), diurnal planktivore (*Caesio varilineata* and *C. xanthonota*), nocturnal planktivore (*Myripristis violacea*, *M. berndti* and *M. murdjan*) and spongivore (*Pygoplites diacanthus*).

6.3.3 Diet composition

The data indicated the consumer was either 1) a specialist feeding strictly on the type of source suggested by the putative trophic guild, or 2) a specialist on another source type, or 3) a generalist feeding on more than one source type. Location (inner- and outer-atoll) was a significant factor in diet composition of some consumer groups.

Table 6.5 Eigenvectors and variance explained (%) for the five principal components (PCs) in the principal component analysis (PCA) of $\delta^{13}C$ values of the essential amino acids valine, leucine, threonine, phenylalanine and lysine from the six trophic guilds in North Malé Atoll (the Maldives). The fish species in each trophic guild were: algivore (*Acanthurus leucosternon*), corallivore (*Chaetodon meyeri*), detritivore (*Ctenochaetus striatus*), diurnal planktivore (*Caesio varilineata* and *C. xanthonota*), nocturnal planktivore (*Myripristis violacea*, *M. berndti* and *M. murdjan*) and spongivore (*Pygoplites diacanthus*).

$\delta^{13}C$-EAA	PC1	PC2	PC3	PC4	PC5
Valine	0.40	-0.68	0.31	-0.36	-0.40
Leucine	0.48	-0.40	-0.26	0.62	0.40
Threonine	0.39	0.44	0.72	0.36	-0.11
Phenylalanine	0.45	0.35	-0.56	0.03	-0.60
Lysine	0.51	0.26	-0.05	-0.60	0.56
Variance (%)	64.2	17.0	12.3	3.8	2.7

6.3.3.1 Corallivore

The corallivores had a diet derived mainly from corals and detritus (Figure 6.4a). For those from the inner-atoll, detritus contributed significantly more to diet (mode = 65%) than corals (mode = 25%), whereas in the outer-atoll, the detritus input was similar to that of corals. These results indicate the corallivores at outer-atoll sites fed on both corals and detritus whereas in the inner atoll they fed more on detritus than corals.

6.3.3.2 Detritivore

Regardless of the location, the detritivores relied substantially on detritus (mode = 65%, Figure 6.4b). Inputs from other sources were small.

6.3.3.3 Planktivore

Both diurnal and nocturnal plankton (mode = 25%) were important food sources for the diurnal planktivores (Figure 6.4c), with some small inputs from detritus (mode = 10%). Those from the outer-atoll (mode = 58%) tended to feed slightly more on diurnal plankton than those from the inner-atoll (mode = 50%).

The diet composition of the nocturnal planktivores was somewhat generalist regardless of location (Figure 6.4e), including diurnal ($mode_{inner}$ = 42%, $mode_{outer}$ = 38%) and nocturnal plankton ($mode_{inner}$ = 20%, $mode_{outer}$ = 12%), detritus ($mode_{inner}$ = 22%, $mode_{outer}$ = 27%) and corals ($mode_{inner}$ = 10%, $mode_{outer}$ = 15%), regardless of the location.

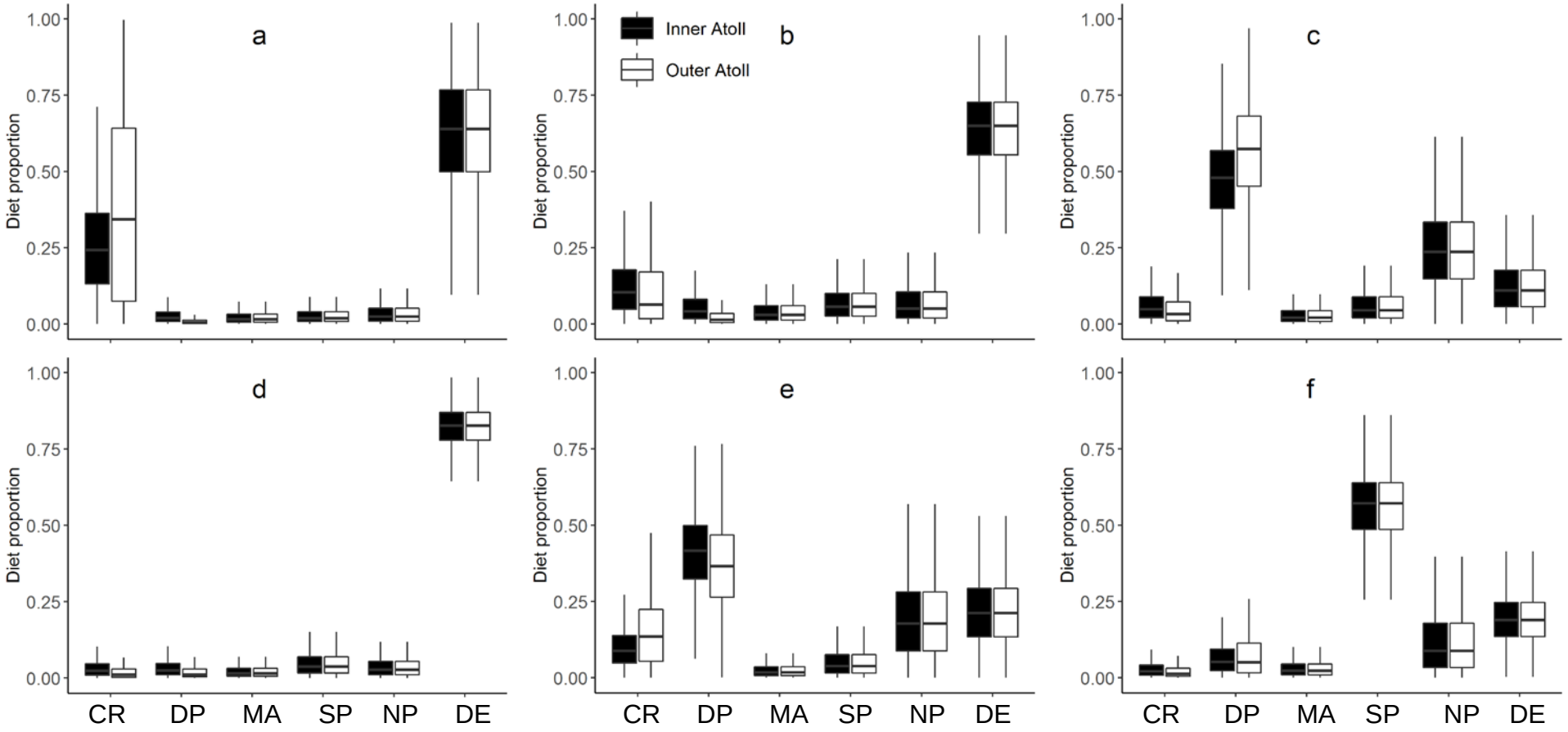


Figure 6.4 Diet composition of the a) corallivores *Chaetodon meyeri*, *C. falcula* and *C. trifasciatus*, b) detritivore *Ctenochaetus striatus*, c) diurnal planktivores *Caesio varilineata* and *C. xanthonota*, d) herbivore *Acanthurus leucosternon*, e) nocturnal planktivores *Myripristis violacea*, *M. berndti* and *M. murdjan*, f) spongivore *Pygoplites diacanthus* in inner- (black box) and outer-atoll sites (white box) of North Malé Atoll (Maldives). Diet components were CR = *Acropora* spp, DP = diurnal plankton, MA = *Halimeda opuntia*, SP = *Hyrtios erecta*, NP = nocturnal plankton and DE = *Pearsonothuria graeffei*.

6.3.3.4 Herbivore

Unlike its putative categorization, the herbivores showed a very strict preference for detritus (mode = 85%) regardless of location (Figure 6.4d).

6.3.3.5 Spongivore

The spongivores diet was dominated by sponge (mode = 56%, Figure 6.4f), but with some small inputs from detritus (mode = 20%), and diurnal (mode = 8%) and nocturnal plankton (mode = 23%).

6.4 Discussion

The finding for these fish which are typically categorised as trophic specialists relying on one production source help to question some of the trophic categorisations commonly applied to coral-reef fishes, increase understanding of the extent of source omnivory and highlight examples of location-dependent individual specialisation.

6.4.1 Source and consumer discrimination

In the PCA, the pyruvate-derived AAs Val and Leu contributed to the variation differently from the oxaloacetate-derived and aromatic groups. This is similar to comparisons of different production sources among systems (terrestrial and aquatic, Larsen *et al.*, 2013) and also within coral reefs (McMahon *et al.*, 2016). The oxaloacetate-derived AAs (Thr and Lys) and aromatic AA (Phe) had similar PCA arrow trends, which resembles McMahon *et al.* (2016) but differs from Larsen *et al.* (2013). It might be that within the same system (here coral reefs), the synthesis pathways of Thr, Lys and Phe are similar, whereas between aquatic and terrestrial systems, these two groups of AAs are synthesized differently. It is possible that some groups of AA might not be significantly different within the same systems but can potentially provide a powerful tool in cross-system comparisons.

The mean ± SD δ^{13}C-EAA values (Appendix 13) suggested significant differences among the types of sources (Appendix 14). The slight differences in δ^{13}C-EAA values of the same production source types between inner and outer atoll locations (Appendix 14) are likely related to different nutrient baselines, but overall they were indistinguishable, unlike in McMahon *et al.* (2016). This may be a result of the high oceanographic porosity of this atoll in the Maldives allowing nutrients from the open ocean to flow through the whole atoll. However, the spatial and temporal scale of this study was too small to test the homogeneity of δ^{13}C-EAA values more widely acroos the atoll. A larger survey would be needed for this.

Benthic algae and corals had distinct ellipses that are partly explicable in terms of the underpinning sources. Benthic algae photosynthesise organic carbon using dissolved inorganic carbon (DIC such as CO_3^-), while corals are mixotrophic, relying on photosynthesis of their symbionts and also planktonic food items. Detritus is likely derived from a variety of ultimate sources including dead organic matter (e.g. algae, fish faeces and coral mucus), inorganic materials, microbial autotrophs (e.g. cyanobacteria), microalgae (e.g. diatoms and dinoflagellates) and associated meiofauna (Crossman *et al.*, 2001). Although these or some components in the case of corals and detritus all possess some kind of photosynthetic mechanisms which possibly share the same inorganic carbon origin (i.e. CO_2), their distinct ellipses suggest several possibilities: 1) differences in EAA synthesis pathways among different autotrophic sources/components; 2) isotopically distinguishable non-autotrophic components; and 3) the influence of heterotrophy in corals was small and the EAA synthesis pathways are so different from pelagic sources (arrows of Phe, Thr and Lys were in the opposite direction) resulting in its significantly separation from the pelagic PCA cluster. However, since there has been no study of individual components of these composite sources, it is not possible to distinguish between these. A limitation here is that one benthic macroalga was sampled and the representativeness of this with respect to other benthic algae is unclear. Larsen *et al.* (2013) found overlapping ellipses among Phaeophyceae, Rhodophyta and microalgae together with other broadly categorised food sources in aquatic systems using a similar visualisation method (linear discriminant function analysis). However, it is yet to be tested whether algal sources are discriminable among themselves using δ^{13}C-EAA values in the Maldives. The affinity between the pelagic cluster and the benthic algae found in this study is similar to McMahon *et al.* (2016); this suggested that at least *H. opuntia* and pelagic microalgae had similar δ^{13}C-EAA values due to possibly similar synthetic pathways. Based on the relative positions of the macroalgae, corals and detritus sources, the benthic sources are much more diverse in origin than the pelagic sources.

For the detritus, *P. graeffei* is reported to feed only on reef detritus (Purcell *et al.*, 2012), yet the exact diet is unknown. The affinity to the pelagic source cluster suggested that it might be relying more on detritus derived from the pelagic than that from the reefs. Pelagic detritus on the reef substrate may come directly from the pelagic organisms (e.g. dead materials of pelagic bacteria such as pico- or nanoplankton, pelagic consumers, and their faeces) and indirectly through recycling

pathways such as the sponge loop (de Goeij *et al.*, 2013). However, for any indirectly processed pelagic detritus, the δ^{13}C-EAA values may be modified by bacterial reworking (Grutters *et al.*, 2002; Lomstein *et al.*, 2006). Understanding the diet of *P. graeffei* needs future work.

Bulk stable isotope data could not clearly differentiate between corals and detritus (Chapter 5), but here, they were distinct. Although the temporal variation can affect discriminability, the CSIA data provide a more robust means of discriminating sources providing no fractionation factor is needed.

6.4.2 Diet compositions of consumers

The similar PCA results of the δ^{13}C-EAAs between production source and consumer data suggested these six production source types were adequate to capture the main diet of these fishes or there was no dramatically different source missing, since introducing such sources might generate different PC/arrow patterns (Larsen *et al.*, 2013). From the results, I found there were fish that relied solely on the source expected for their trophic group, relied on a source other than this, or depended on more than one source.

6.4.2.1 Corallivore

In contrast to their putative categorisation, the corallivores here were more aligned with detritus as a source than with the *Acropora* spp. While the detrital material indicated by the *P. graeffei* δ^{13}C-EAA data remain unclear, there are three possibilities: one is that the corallivores fed on these *Acropora* species but also other corals, which had δ^{13}C-EAA values closer to the *P. graeffei* and the *P. graeffei* may specialise on the detritus from those other corals. Another possibility is that the corallivores specialise on certain components of the corals which had δ^{13}C-EAA values closer to the coral detritus assimilated by *P. graeffei* rather than the whole coral tissue. A third possibility is that although corals and detritus were distinct production source types (Figure 6.2), the major difference was along PC2 axis which only explained 21.9% of the variance, thus, they might be very similar (especially if the *P. graeffei* fed only on coral detritus), and the samples collected were from a very short period of time (3 months); the stable isotope 6-12 months turnover period thus might have reduced the difference at consumer level. The first two imply ignored sources or components of these, whereas the third suggests lack of temporal variability and insufficient discriminability. These limitations are common in CSIA

studies and more research to understand source discriminability and diet compositions is needed.

6.4.2.2 Detritivore, herbivore and spongivore

These three groups had strict diets which varied little with location. The detritivores and spongivores were feeding in accordance with their putative food sources, whereas the herbivores appeared to be detritivorous.

The results confirmed that *P. graeffei* is an end member of the selected detritivore species (which remained unknown in Chapter 5), or at least EAA-wise providing that the source it represents was distinct from others in δ^{13}C-EAA value pattern. Yet the exact component(s) of detritus fed by *P. graeffei* and *C. striatus* remain to be studied.

Refuting the putative categorization of *A. leucostern* may be premature due to missing sources (see MixSIAR criteria 1 in Chapter 5) such as algal turf (Robertson and Gaines, 1986). The benthic alga *H. opuntia* differed in both PC axes from the detritus. Yet, the adjacent herbivore and detritivore ellipses suggested the isotopic similarity of their diets; the benthic alga used here was clearly not a good indicator of the main diet of this herbivore species.

The spongivore data indicated its diet was strictly in accordance with its trophic categorisation , unlike other studies which suggested *P. diacanthus* is an omnivore (Alwany, 2009; Konow and Bellwood, 2011). Despite of the high affinity of the bulk isotopic ellipses of the sponge *H. erecta* and *Halimeda* spp, here the ellipse of the sponge was within the calanoid copepod ellipse. As a group, sponges rely on a mixture of trophic sources including microorganisms (Wilkinson and Fay, 1979; Wilkinson, 1987; Wilkinson *et al.*, 1999; Fiore *et al.*, 2013), DOM (de Goeij *et al.*, 2008b; Fiore *et al.*, 2013) and pico- or nanoplankton (Reiswig, 1971). Yet sponges utilize these nutrient sources very differently. In a tracer experiment, one sponge shows preference for planktonic sources over DOM, the former used more for production and the latter for respiration (de Goeij *et al.*, 2017). Thus, the isotopic values of sponges can be expected to differ between bulk and compound-specific analyses; the bulk data may represent the whole body including filtered DOM, whereas the δ^{13}C-EAA values indicate AA fractions from filtered planktonic sources used to build sponge body tissues. Most sponges are mixotrophic, and if such nutritional utilization preferences are common, their δ^{13}C-EAA patterns are expected to be similar to those of plankton. Alternative food-web tracers might be useful here.

It is also the case that spongivores might prefer specific parts of the sponges; for example, they might digest the symbionts rather than the sponge tissues from the HMA sponges. To understand these complications, more studies are needed.

6.4.2.3 Planktivores

The results suggested these caesionids fed on both diurnal and nocturnal plankton in spite of being diurnal feeders; however there was no isotopic discrimination between diurnal and nocturnal plankton. Compared with diurnal zooplankton, nocturnal zooplankton include larger calanoids, polychaetes, ostracods, copepods, mysids, isopods, amphipods and crustacean larvae (Hobson, 1991). These animals rely on multiple food sources including detritus, zoobenthos and zooplankton. The characterisation may be a result of inadequate sampling. The nocturnal plankton collected in the study was from the surface (0-50 cm), and not examined and identified in terms of its components. Thus, it might not include those nocturnal zooplankton staying close to the reef substrate (McClelland and Montoya, 2002). These diurnal planktivores may have been feeding on zooplankton mainly from the pelagic or utilizing pelagic sources (as a secondary food source themselves). The nocturnal planktivores indicated some source omnivory, preferring zooplankton but also utilizing other zoobentho sources.

6.4.3 Effects of location

Mean δ^{13}C-EAA values of some production sources tended to differ between inner- and outer-atoll sites (e.g. $\delta^{13}C_{Val}$ of corals), however, due to the small sample sizes the significance of these could not be estimated. Different δ^{13}C-EAA values of production sources were used for these two locations in MixSIAR assuming they were different according to the findings of McMahon *et al.* (2016), yet it might be that such differences do not pertain to this atoll in the Maldives.

Greater reliance of diurnal planktivores on diurnal plankton at outer-atoll sites is potentially attributable to higher abundances of zooplankton at atoll edges (Gove *et al.*, 2016). McMahon *et al.* (2016) indicated that some diurnal planktivores can alter source partitioning based on food availability. The finding that the corallivores were feeding slightly more corals at outer-atoll sites is also notable. It is possible that preferred corals were more available at outer-atoll sites following the recent coral bleaching event (Perry and Morgan, 2017). Both these examples further support the likelihood of spatial differences as a result of spatial variability in food source availability (Matthews and Mazumder, 2004).

6.4.4 Conclusion

Using δ^{13}C-EAA data, production sources were discriminated to some extent, especially the benthic sources. There might be drawbacks comparing the δ^{13}C-EAA with those of the bulk analyses and limitations in source discrimination, but this method suggested a new way of tracing carbon flows in this system, and potentially revealed different carbon utilization mechanisms by the same organism together with the bulk data.

Applying production source and consumer data in the mixing model, diet compositions suggested some putative trophic categorizations are inaccurate. From the source affinity analysis and diet compositions of fish, it was suspected the pelagic sources to be more important in supporting coral-reef food-webs than expected; some fish might not feed directly from the pelagic sources but may utilize forms of production sources that derived from the pelagic (e.g. spongivores and detritivores). Finer-scale data on more food sources such as algal turf and cyanobacteria, and on individual components of some highly mixed sources (e.g. detritus, sponges) are required.

Chapter 7. Isotope ecology of coral-reef fish community: synthesis and recommendations

7.1 Summary

This is the first community-level size-based trophic structure analysis of coral-reef systems. In the Bahamas and Maldives, the mean TP to body mass relationship differs greatly. Isotopic niches of species and trophic guild data suggest some species relied mainly on either pelagic or benthic (reef) production sources while some potentially relied on both. Also, many species demonstrated TP omnivory where the variance of prey TP increases as their body mass increases. Specifically, at the Maldivian site, stable isotope data revealed that the fish typically categorised as relying on a single food source type may be incorrectly characterised, and instead their trophic plasticity should be acknowledged. Further, pelagic production sources are potentially important to coral-reef fish food-webs as indicated by the bulk and compound-specific stable isotope data.

7.2 Coral-reef fish community structure

7.2.1 Size structuring

At community level, the Bahamian site demonstrated a positive linear TP to body mass relationship, indicating that mean TP increases with body mass and small mass classes were dominated by low-TP species (e.g. parrotfish) whereas in large mass classes high-TP species (e.g. groupers) predominated. This pattern resembles findings of several studies in temperate systems (Jennings *et al.*, 2001a; Jennings *et al.*, 2002a; Jennings *et al.*, 2002b; Al-Habsi *et al.*, 2008) suggesting one dominant production source type was supporting this food web. In contrast, at the Maldivian site where reefs were more intact, the relationship was parabolic, suggesting low-TP species dominated at both of the body mass range ends and high-TP species were most important in the middle. Size structuring among systems potentially depends on the number of dominant types of production source (Jennings and Mackinson, 2003). In the Maldives case, the existence of large-bodied carnivores and parrotfish in one system suggests both benthic and pelagic producers were important. These two source types differed in isotopic baseline value (McConnaughey and McRoy, 1979; Polunin and Pinnegar, 2002) and might affect the mean $\delta^{15}N$-body mass relationships. Thus, it is crucial to examine and correct the isotopic baseline. In the Bahamian study, these two baselines differed while in the Maldivian study, they did

not. However, baselines were not corrected in the Bahamian study because no pelagic producer was sampled.

Size-based trophic structure can indicate community-wide predator to prey relationships (e.g. predator-prey mass ratio or PPMR) and certain food-chain properties (Jennings *et al.*, 2001a). Yet, it only applies to linear mean TP to $\log_2$ body mass relationships (Jennings *et al.*, 2001a; Al-Habsi *et al.*, 2008; Hussey *et al.*, 2014). In the Maldivian study, the large herbivores with considerably high biomass distorted the linearity at large body mass classes. However, they are suspected not to support the higher trophic levels as prey due to their size (C. Skinner pers. comm), yet this raises a question as to what happens ultimately to that biomass. A cut-off body size of these herbivore species, above which data would not be included, might generate a linear relationship that could be used to calculate a pelagic and benthic-based PPMR. This cut-off value was not estimated here, thus, exclusion of these individuals was not possible for the analyses, although it would rely on these being a linear predator-prey relationship below the cut-off. The present data on particular species are insufficient to assign pelagic and benthic sources reliability across all body mass intervals. Yet, the distinctive size-based trophic structures might ultimately be explicable, for example in relation to overfishing and severity of reef degradation (Richardson *et al.*, 2016; Graham *et al.*, 2017; Chapter 2 and 4). However, it is too early to begin to adequately explain the different relationships found. These are the only existing such studies and more spatial and temporal data are needed to address the causes. The size-based trophic structure might ultimately prove useful for comparing system states.

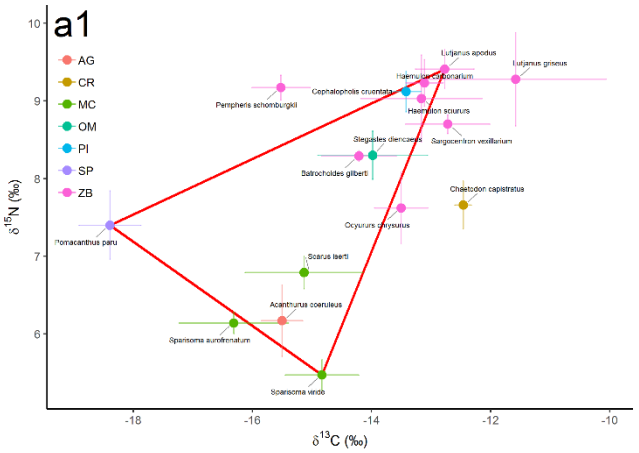
7.2.2 Isotopic niche structuring

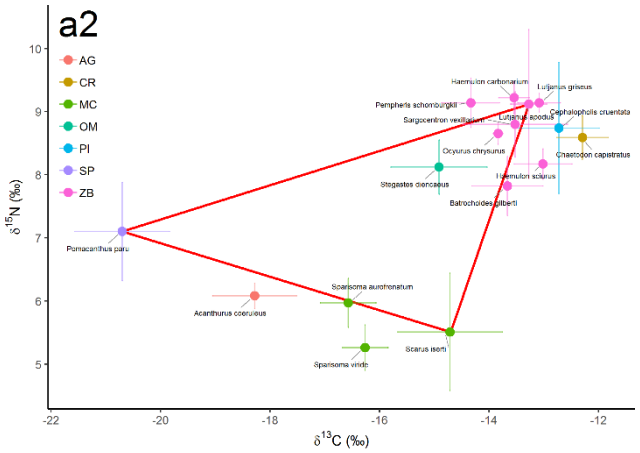
The relationships of isotopic niches of species and trophic guilds to each other provide some insights into how such food-webs are structured in terms of source partitioning and allow to detect species feeding across the trophic boundary. The importance of both benthic and pelagic sources was further supported by the distinctive isotopic niches of strictly pelagic and strictly benthic trophic guilds and those positioned between these two. However, this is the first study analyzing both species and trophic guild data in one food web, and there is no clear definition on the level of omnivory of fishes in relation to distance between their isotopic niche and the trophic guild isotopic niche, more data are needed to quantify this relationship. Combined with species-level stable isotope data (isotopic niche and size-based TP

omnivory analyses), source partitioning patterns among fishes seem to differ between the seas and this community-level difference needs to be better understood.

Existing trophic niche/diet analysis methods address univariate property(-ies) at a single TP, species or community level. For example, diet analyses often focus on few species in a single community and location; or the size-based trophic structure does not point to source reliance. High-TP predatory fishes are considered to play key functional roles in community structure (e.g. Jennings and Polunin, 1997; Myers and Worm, 2003a; Almany, 2004; Dulvy *et al.*, 2004a; Layman *et al.*, 2007b; Myers *et al.*, 2007; Rotjan and Lewis, 2008). Although isotopic niches of these fishes might be similar, they may nevertheless be supported by different trophic pathways (Layman *et al.*, 2007a); while inadequate sample sizes can limit detection of differences. The time-integration effect inherent in stable isotope data especially in long-lived slow-growing apex predators means that the range of $\delta^{13}C$ (from both pelagic and benthic sources) decreases as TP increases. Such a TP-based pattern in $\delta^{13}C$ values can be visualized and can potentially elucidate the overall source reliance at community level and food-chain length.

To understand the overall source reliance and food-chain length of a fish community, I propose a new metric which I refer to as the “iso-niche triangle”. This requires three vertices in the $\delta^{15}N$-$\delta^{13}C$ biplot of species or trophic guild level isotope data: mean piscivore data or the highest (apex), mean zooplankton data or the zooplanktivore species with the lowest mean $\delta^{13}C$ value (pelagic vertex), and mean benthic primary consumer data preferably Scarinae or other algivores or algivore/microphage species with the highest mean $\delta^{13}C$ value (benthic vertex). The angle at the pelagic point might indicate the extent of reliance on the pelagic sources of the whole system; i.e. the greater the angle the higher the reliance. If pelagic and benthic vertices have similar $\delta^{15}N$ and TP values, the vertical distance from the top vertex (or apex) to the bottom edge may indicate food-chain length (i.e. the greater the distance the longer the food-chain). Yet baseline adjustment and/or selecting other species with similar TP might be needed. I applied this to existing aquatic studies that included at least these three vertices groups with $\delta^{15}N$-$\delta^{13}C$ biplots (Figure 7.1) and plotted iso-niche triangles for each system with selected vertices (Table 7.1).


a1
AG
CR
MC
OM
PI
SP
ZB
Lutjanus apodus
Lutjanus griseus
Haemulon carbonarium
Pempheris schomburgkii
Cephalopholis cruentata
Haemulon sciurus
Sargocentron vexillarium
Stegastes diencaeus
Batrochoides gilberti
Chaetodon capistratus
Ocyurus chrysurus
Pomacanthus paru
Scarus iserti
Acanthurus coeruleus
Sparisoma aurofrenatum
Sparisoma viride
δ15N (‰)
δ13C (‰)


a2
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SP
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Lutjanus apodus
Cephalopholis cruentata
Ocyurus chrysurus
Chaetodon capistratus
Haemulon sciurus
Stegastes diencaeus
Batrochoides gilberti
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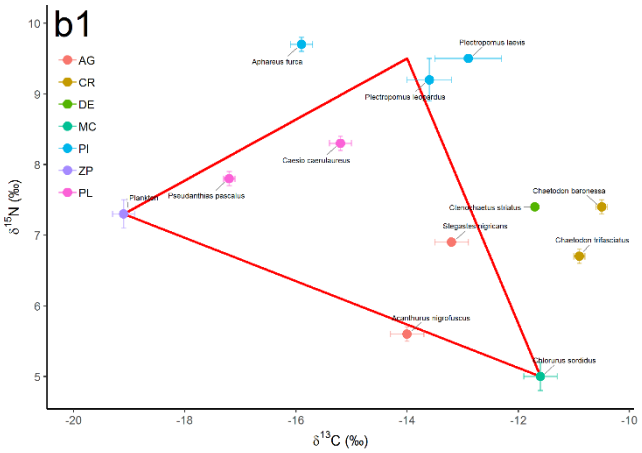
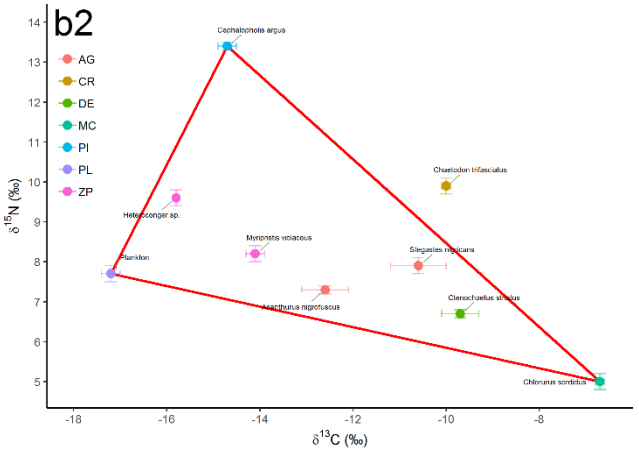
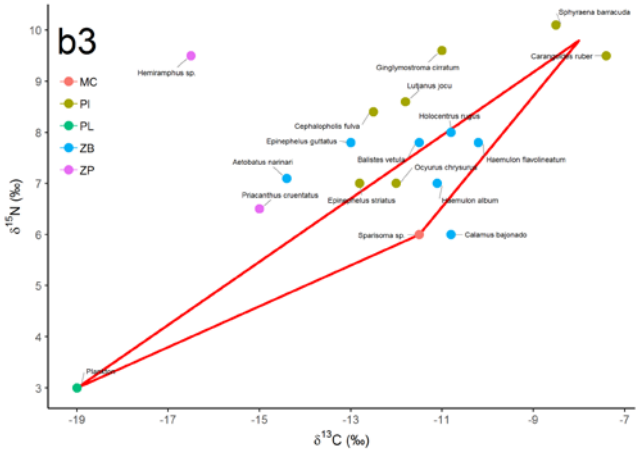
Scarus iserti
Sparisoma viride
δ15N (‰)
δ13C (‰)


b1
AG
CR
DE
MC
PI
ZP
PL
Plectropomus laevis
Acharaus furca
Plectropomus leopardus
Caesio caerulaureus
Plankton
Pseudanthias pascalus
Chaetodon baronessa
Clenochaetus striatus
Stegastes nigricans
Chaetodon trifasciatus
Acanthurus nigrofuscus
Chlorurus sordidus
δ15N (‰)
δ13C (‰)


b2
AG
CR
DE
MC
PI
PL
ZP
Cephalopholis argus
Chaetodon trifasciatus
Heteroconger sp.
Myripristis violacea
Plankton
Stegastes nigricans
Acanthurus nigrofuscus
Clenochaetus striatus
Chlorurus sordidus
δ15N (‰)
δ13C (‰)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MC
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ZP
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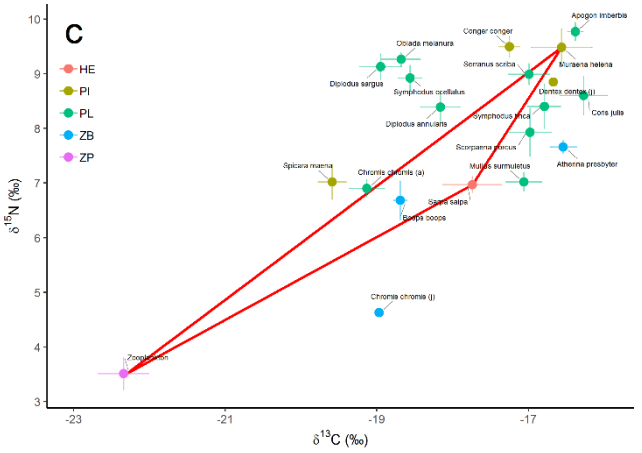
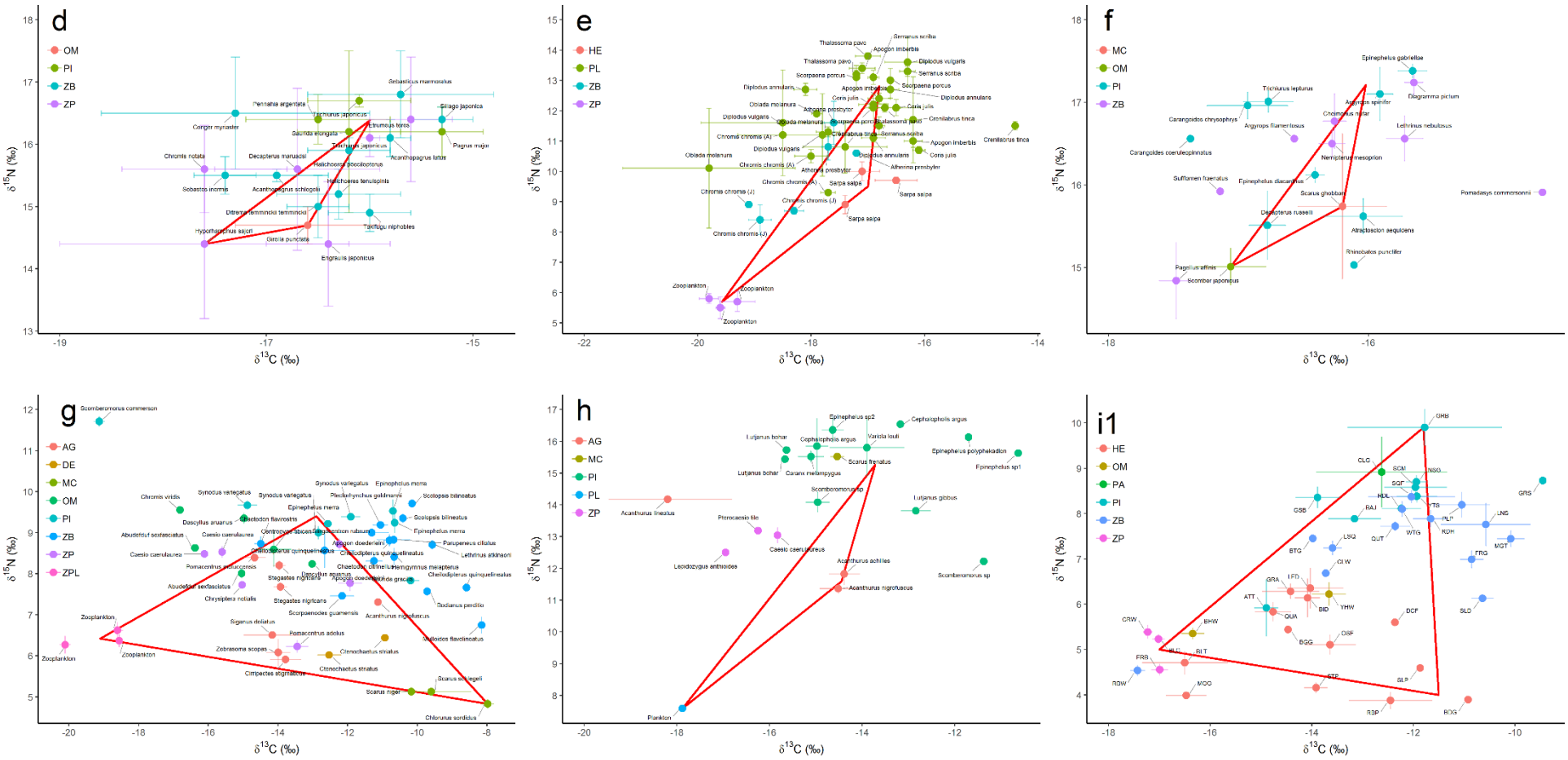
Hemiramphus sp.
Ginglymostoma cirratum
Caranx latus ruber
Lutjanus jocu
Holocentrus rufus
Cephalopholis fulva
Epinephelus guttatus
Haemulon flavolineatum
Aetobatus narinari
Balistes vetula
Ocyurus chrysurus
Priacanthus cruentatus
Epinephelus striatus
Haemulon album
Sparisoma sp.
Calamus bajonado
Plankton
δ15N (‰)
δ13C (‰)


c
HE
PI
PL
ZB
ZP
Apogon imberbis
Conger conger
Oblada melanura
Serranus scriba
Mycteroperca rubra
Diplodus sargus
Symphodus ocellatus
Diplodus annularis
Symphodus tinca
Coris julis
Scorpaena porcus
Spicara maena
Chromis chromis (a)
Mullus surmuletus
Sarpa salpa
Boops boops
Atherina presbyter
Chromis chromis (j)
Zooplankton
δ15N (‰)
δ13C (‰)


d
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δ15N (‰)
δ13C (‰)

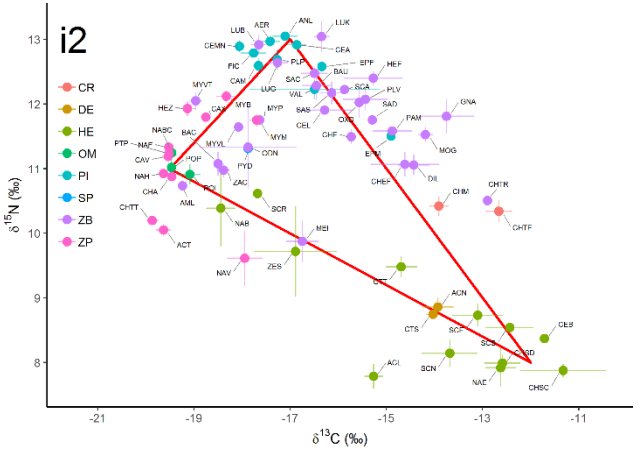

Figure 7.1 Application of iso-niche triangle concept to existing studies: a. Morillo-Velarde *et al.* (2018) (1. Limones; 2. Bonanza), b. Polunin and Pinnegar (2002) (1. Great Astrolabe Reef, Fiji; 2. Tiahura Moorea, French Polynesia; 3. Caribbean), c. Pinnegar and Polunin (2000), d. Ahmad-Syazni *et al.* (2013), e. Jennings *et al.* (1997), f. Al-Habsi *et al.* (2008), g. Carassou *et al.* (2008), h. Polunin *et al*. (unpublished data), i1. Chapter 2 (for species codes see Table 2.3), i2. Chapter 3 (for species codes see Table 3.1). Error bars indicating SD (a1, a2, c, d, e, f, h) or SE (b1, b2, g, i1, i2) or were not available in the original data (b3). Each plot used one data set, except e, g and h used several data set from multiple nearby sites in each study. Note: axis range varies among plots. Trophic guild codes are AG = algivore, CR = corallivore, DE = detritivore, HE = herbivore, MC = microphage, OM = omnivore, PA = parasitivore, PI = piscivore, PL = plankton, SP = spongivore, ZB = zoobenthivore, ZP = zooplanktivore and ZPL = zooplankton.

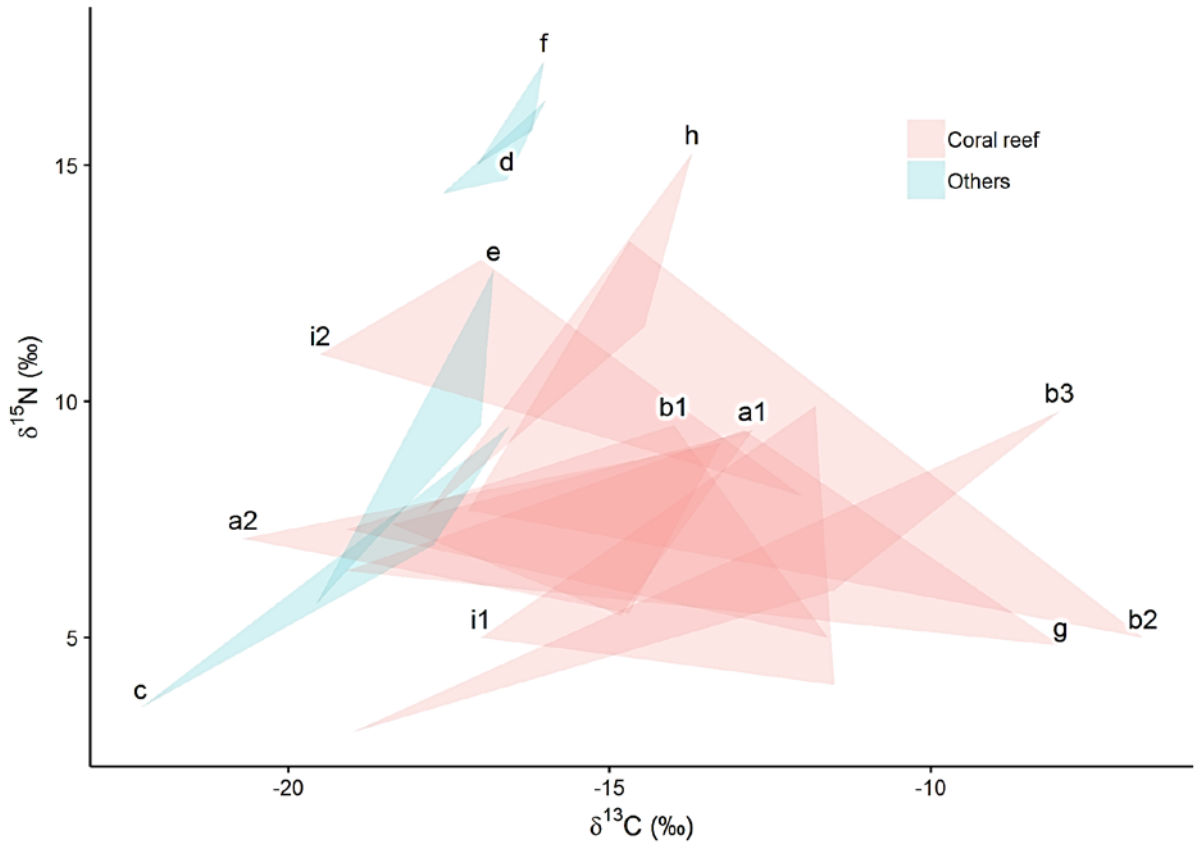

Figure 7.2 Plot of all iso-niche triangles derived from existing studies. Type of aquatic system includes coral reefs and others. Study sources in Figure 7.1.

Six possible outcomes follow from this characterization which potentially explain the proportion of benthic-pelagic coupling in the food webs: the community has a 1) pelagic-dependent long food-chain; 2) pelagic-dependent short food-chain; 3) benthic-dependent long food-chain, 4) benthic-dependent short food-chain, 5) benthic and pelagic dependent long food-chain, and 6) benthic and pelagic dependent short food-chain. Although only study i2 (my Maldivian study) examined benthic-pelagic baselines, here the food-chain length was simply determined by the TP of the apex. Type 1 includes Maldives (i2) and French Polynesia studies (b2); there appear to be no published studies of Types 2 and 6; Type 3 includes the Caribbean (b3), Corsica (c), Japan (d), Spain (e), Western Arabian Sea (f), Christmas Island (h) and Bahamian studies (i1); Type 4 includes both studies in Mexico (a1 and a2); and Type 5 includes the Fiji (b1) and New Caledonia studies (g). Type 5 iso-niche triangle particularly suggests the importance of benthic-pelagic coupling in these two reef sites. There is no clear pattern among oceans, latitudes or levels of severity of impact except seemingly higher reliance on benthic sources among Caribbean reefs (a1, a2, b3 and i1) and wider $\delta^{13}C$ range among benthic and pelagic production sources (Figure 7.2). The indication is that there are variations among

systems in the source partitioning and food-chain length using this metric even at small spatial scales (sites in Mexico, or sites with similar latitude in the same ocean [e.g. Fiji and French Polynesia]), and replicating this in other systems could potentially help reveal community properties at different scales.

Table 7.1 Example of iso-niche triangles and their vertices (pelagic, benthic and apex) from existing literature. Study sources in Figure 7.1. *TP values were not indicated in the study, and are derived from fishbase.org. ^TP values were assumed.

Study	Location	Pelagic	Benthic	Top
a1	Limones, Mexico	*Pomacanthus paru* (TP = 2.7)	*Sparisoma viride* (TP = 2.1)	*Lutjanus apodus* (TP = 3.2)
a2	Bonanza, Mexico	*P. paru* (TP = 2.4)	*Scarus iserti* (TP = 2.2)	*L. apodus* (TP = 3.1)
b1	Fiji	*Pseudanthias pascalus*	*Chlorurus sordidus*	mean predator
b2	French Polynesia	*P. pascalus* (TP* = 3.3)	*C. sordidus* (TP* = 2)	*Cephalopholis argus* (TP* = 4.5)
b3	Caribbean	Plankton (TP* = 2)	*Sparisoma* spp (TP* = 2)	mean predator (TP* = 4.4)
c	Corsica	Zooplankton (TP* = 2)	*Sarpa salpa* (TP* = 2)	*Muraena Helena* (TP* = 4.2)
d	Hiroshima, Japan	*Hyporhamphus sajori* (TP* = 3.4)	*Girella punctata* (TP* = 2.9)	mean predator (TP* = 4.2)
e	Spain	Zooplankton	*S. salpa*	mean predator (TP* = 3.9)
f	Western Arabian Sea	*Scomber japonicus* (TP^ = 3.4)	*Scarus ghobban* (TP* = 2)	mean predator (TP* = 4.4)
g	New Caledonia	Zooplankton (TP^ = 2)	*C. sordidus*	mean predator (TP* = 4)
h	Christmas Island	Plankton (TP^ = 2)	mean algivore (TP^ = 2)	mean predator (TP* = 4.4)
i1	Bahamas	mean zooplanktiovre (TP^ = 2)	mean Scarinae (TP^ = 2)	*Sphyraena barracuda* (TP* = 4.5)
i2	Maldives	mean zooplanktivore	mean Scarinae	mean predator (TP* = 4.5)

To provide a good basis for inter-system comparison, this approach requires detailed fish survey (species and biomass), TP (existing data or new measurement) data and adequate fish samples (e.g. $n \geq 6$ per species) for a complete community

isotopic niche plot. There are also limitations: 1) the estimated food-chain length depends on the assumed $\Delta\delta^{15}N$ of that specific system (see Chapter 2); 2) in systems where production of top predators is based on either pelagic or benthic source, a single triangle might not be appropriate.

7.3 Community mean PPMR and food-chain length

Mean PPMR indicates food-chain length (Jennings and Warr, 2003) and through a scaled fractionation framework the estimation can be more accurate (Reum *et al.*, 2015). However, the analysis at the Bahamian site suggests there are potential location-specific scaling factors which could only be resolved with more data. The predator-prey relationship from the mean PPMR relates to individuals sharing the same source (e.g. plankton in the North Sea). As mentioned, the diversity of production sources in these coral reefs and feeding patterns among fishes might substantially affect mean PPMR, for example for individuals switching food-chains (e.g. resilient top predator, Layman *et al.*, 2007b). This might generate shorter food-chain length due to the uncertain weighting of $\delta^{15}N$ values of predators feeding on both long (high-TP) and short (low-TP) food-chains.

Mean PPMR values allow for large scale food-chain length comparison. Compared with other methods (e.g. "iso-niche triangle" or maximum food-chain length in Morillo-Velarde *et al.*, 2018), this method provides a better basis because maximum food-chain length might depend on survey methods and not be representative of the community.

7.4 Coral reef-fish source partitioning

7.4.1 Trophodynamics

Body size affects TP-omnivory for many coral reef-fishes either positively or negatively. In addition to generic pathways (e.g. herbivores and carnivores, Robinson and Baum, 2015), fine-scale pathway (e.g. planktivore and algivore) and family can modify the TP-body size relationship. This is potentially attributable to size-related morphological variation among different species (Ríos *et al.*, 2019), but size-based TP-omnivory does not seem to apply to strict microphages (e.g. Scarinae spp).

Fish trophodynamics can also be size-independent, for example individuals of the same species and size may feed on different sources from each other (i.e. source-omnivory). Specifically in the Maldives, several strictly categorised primary (e.g. some Acanthuridae) and secondary consumers (e.g. *M. violacea*) were feeding

on more than one production source type. Also some species were mainly feeding on a source type other than their putative one. However, this might potentially be affected by the study design or the methods used (see below).

Using single TP values or trophic guilds to describe coral reef-fish is likely to mask significant details in the functional ecology of these fish. Many models (e.g. EcoPath) and studies (e.g. Hiatt and Strasburg, 1960; Jennings *et al.*, 1995; Polunin, 1996; McClanahan *et al.*, 1999; Hughes *et al.*, 2003; MacNeil *et al.*, 2015; D'Agata *et al.*, 2016; Graham *et al.*, 2017; Stamoulis *et al.*, 2017; Hadi *et al.*, 2018; Moustaka *et al.*, 2018) have used simple trophic categorisations to help understanding of coral-reef food-web structure and function (e.g. food-chain length estimation, Post, 2002a). Using the main or sometimes wrong trophic and functional roles of some fish to infer predator-prey relationships, ecosystem services and community structure is likely to lead to significant misunderstanding. For example, some Acanthuridae and most Scarinae have typically been classified as 'herbivores', however, these groups are isotopically different; the former feeds on benthic filamentous algae and the latter on microbial autotrophs (Clements *et al.*, 2016). Studies have often attempted to correlate benthic algal cover with herbivorous parrotfish abundance (e.g. McClanahan *et al.*, 1999; Green and Bellwood, 2009), however, some of these fishes graze only to exploit the epiphytic/epilithic cyanobacteria. Thus, interpretation of such correlation might be spurious. From a conservational point of view, to avoid exacerbating a phase shift from coral- to algal-dominated state, it seems more reasonable to protect species feeding significantly on blooming algal species (e.g. some Acanthuridae) than on all herbivores. Also, many of these fish feed partially on pelagic sources (e.g. faeces, Robertson, 1982), thus, fishing reef-pelagic species (e.g. Caesionidae) should also be regulated.

7.4.2 Dietary strictness

It is also the case that some species' trophic categorisations were appropriately classified for this study site. For example, in the isotopic niche study (Chapter 3), the detritivore *C. striatus* and *A. nigricauda* had very small isotopic niches with low SE values of $\delta^{15}N$ and $\delta^{13}C$. For *C. striatus*, both bulk $\delta^{15}N$ and $\delta^{13}C$ and $\delta^{13}C$-EAA values suggest its high dietary strictness, albeit with some discrepancies potentially because of the two different methods employed. These suggest that *C. striatus* is a strict detritivore. However given the potential heterogeneity of detritus, this species might potentially feed on specific component(s)

within the detritus matrix or be effective at foraging on different detrital components from different sources.

Some Acanthuridae were potentially feeding on both benthic algae and pelagic sources based on the isotopic niche analysis, but *A. leucosternon* was not one of them and evidently had a strict diet. Both bulk and CSIA data indicated this species' heavy reliance on detritus. It may be the case that this fish also selects and assimilates detritus while grazing filamentous algae. However, since I did not collect algal turf, this cannot be confirmed (turf algae collected by C. Skinner during January 2019, are currently being analysed).

For the spongivore *P. diacanthus*, results differed between the bulk and compound-specific stable isotope data. In the bulk data, this species relies on both benthic and pelagic sources whereas in the compound-specific data, it relies significantly on the sponge *H. erecta*. This is potentially caused by 1) differential discriminability of the source types (see 7.5.1), 2) the two methods tracing different types of biochemical compounds (see 7.5.2), and 3) the complex trophic roles of sponges (see 7.6).

7.5 Bulk vs compound-specific stable isotope data

These two techniques gave similar results in source discrimination and some different results in diet analyses of fishes. The analytical accuracy was improved using compound-specific stable isotope data, yet the greater cost of this techqniue are to be aware of (Table 7.2).

Table 7.2 Time and cost for each sample in bulk and compound specific stable isotope analysis. Preparation time: from dried sample to being ready to go through GC; analytical time: from entering the GC to generating data; preparation cost: chemicals and apparatus; analytical cost: GC cost.

Stable isotope technique	Preparation time	Analytical time	Preparation cost	Analytical cost
Bulk stable isotope analysis	20 min	96 samples per day; 2-4 weeks waiting (done by Iso-Analytical)	£1	£6
Compound-specific stable isotope analysis	1 week	12 samples per day; 4 months waiting and screening data (done by oneself)	£10	£200

7.5.1 Source type discriminability

The production source types studied were discriminated to some extent by both bulk $\delta^{15}N$ and $\delta^{13}C$ and $\delta^{13}C$-EAA data, but it was not analysed that which data had a better discriminability because PCA only explains variation partially and there was no function to calculate the overlap areas among PCA ellipses. In both analyses, there were significant limitations. Selecting particular species to represent each production source type assumed their isotopic representability was appropriate, yet individual species within the same type can be isotopically different (e.g. Pinnegar and Polunin, 2000; Rolff, 2000; Plass-Johnson *et al.*, 2013; Dromard *et al.*, 2015). Also a few production sources which can be potentially important in fish diet, such as algal turf (as discussed in 7.4.2), cyanobacteria and cryptic sponges, were not sampled. In addition some of the mixed-sources collected (e.g. detritus, plankton) might be analysed down to into their individual components to better understand their discriminability and trace them through the food web.

Both data showed separation of benthic algae, corals, detritus and the diurnal-nocturnal plankton cluster. However, sponges (represented by the single species *H. erecta*) overlapped with benthic algae in the bulk data and within the plankton cluster in the compound-specific data, suggesting different bio-tracers might not always end up telling the same story.

This is the most detailed study on isotopic discriminability of production source types in a coral reef to date, and has begun to resolve some complex energy flow questions in coral-reef food-webs such as benthic-pelagic coupling (e.g. contribution of pelagic sources to the diet of reef fishes), ontogenetic dietary shift (e.g. TP-omnivory) and food-web resilience (e.g. flexible diet composition of some reef fishes), but there are clearly many points for future development.

7.5.2 Comparing bulk and compound-specific stable isotope data

The two stable isotope methods track energy flow through different biochemical components. In the bulk stable isotope method, all components of the samples are analysed which may include inorganic materials, carbohydrates, amino acids, fatty acids and DNAs, all of which vary in their isotopic turnover rate (Tieszen *et al.*, 1983) potentially causing variability in the stable isotope data. In addition, bulk stable isotope values are enriched with each trophic step (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981). In contrast, the compound-specific stable isotope method used here ($\delta^{13}C$-EAA here) tracks only the carbon of essential amino acids. The

δ^{13}C-EAA value pattern differs greatly among primary producers and minimal enrichment is involved (McMahon *et al.*, 2010; Larsen *et al.*, 2013; Larsen *et al.*, 2015; McMahon *et al.*, 2016). Differences in dietary results are thus not unexpected with these two tracking methods (See 7.5.3 and 7.6, as an example), but these differences might improve understanding in compound-specific source utilization (e.g. the sponge's mixed trophic roles).

Both bulk and compound-specific isotope data had within variations (SE or SD), and the variations differed among isotopes or compounds with no clear pattern between these two methods. Such variations are normally considered related with dietary strictness (i.e. the smaller SD the more strict the diet of the species), however, this might not be the case for individuals. For example, *S. barracuda* in the Bahamian study and *V. louti* in the Maldivian study had a greater SE in δ^{13}C than most other local species, which was unexpected for top predators. This might be due to individual specialisation (Matthews and Mazumder, 2004), the individuals sampled selectively and consistently feeding on different sources. In contrast, the detritivores in the Maldives had very small SEs in both bulk δ^{15}N and δ^{13}C, despite being thought to be feeding on a very heterogeneously mixed source. It might be that they were specialising on certain components within, or all individuals were utilizing many components consistently across the population. Currently, individual-based diet data mainly come from gut contents examination, yet there is potential for using stable isotope mixing models for individuals. However, this would be costly in terms of analytical running time. The indication here is that mixed-source partitioning might be the case for species with both great and small isotopic variations, and to understand what these data mean requires further study.

7.5.3 Mixing model results

I found discrepancies in diet proportions of some consumers using the two sets of data. Despite the inherited limitations of each method (bulk: only two determinants, variability in enrichment factors; compound-specific: only tracking the amino acids), these results might answer my questions from different perspectives. For example, the corallivore in the Maldives had strict reliance on corals using the bulk data but both detritus (~ 62%) and corals (~ 25-35%) were indicated as important in the compound-specific data. Detritus and corals were isotopically discriminable in both data sets although it is conceivable much of the detritus on these reefs is from corals (e.g. mucus or dead components), yet being reworked by

microbes is presumably different from the coral tissues. The bulk isotopic signatures of corals (whole tissue) and detritus (dermal tissue of *P. graeffei*) might include greater amounts of chemical components that are nutritionally important other than amino acids; the bulk data might suggest the main non-AA carbon sources were from the corals, while compound-specific data likely indicate the AA carbon sources were mainly from the detritus fed on by *P. graeffei*.

From a statistical point of view, source discrimination was not significantly improved using compound-specific stable isotope data, but the calculation was due to more determinants included. This heavily relies on the laboratory-based studies in non-$\delta^{13}C$ fractionating EAAs. However, my PCA plot of these five EAAs showed correlation among some (e.g. Lys and Leu) which might reduce the power of the analysis to some extent. Like bulk stable isotopes, $\delta^{13}C$ values of non-EAAs fractionate along food chain (McMahon *et al.*, 2010), yet these TEFs were never studied in vivo. I believe there belies the opportunity of introducing these non-EAAs into the mixing model provided known TEFs to further improve source discriminaibilty and analytical accuracy.

7.5.4 Other indications

From CSIA data, the detritus *P. graeffei* fed on was identical to that fed on by *C. striatus* because detritus represented by this end member was distinct from other production source types and had $\delta^{13}C$-EAA values similar to the fish. It appears that the isotopic enrichment factors of bulk $\delta^{15}N$ and $\delta^{13}C$ of this trophic pathway or between such end members ($\delta^{15}N$ = 4.76 ± 0.45‰, $\delta^{13}C$ = -12.99 ± 0.87‰) and consumers ($\delta^{15}N$ = 8.75 ± 0.29‰, $\delta^{13}C$ = -12.79 ± 0.73‰) are $\Delta\delta^{15}N$ = 2.00 ± 0.10‰ and $\Delta\delta^{13}C$ = 0.20 ± 0.30‰.

7.6 Trophic roles of sponges

The trophic ecology of sponges is complex (Reiswig, 1971; Wilkinson and Fay, 1979; Wilkinson and Garrone, 1980; Wilkinson *et al.*, 1981; Wilkinson *et al.*, 1999; de Goeij and Van Duyl, 2007; de Goeij *et al.*, 2008a; de Goeij *et al.*, 2008b; Fiore *et al.*, 2013; de Goeij *et al.*, 2017). They acquire nutrients from both benthic and pelagic environments in different ways. Studies have variously pointed to sponges mainly utilizing DOM which is processed by both sponge tissues (de Goeij *et al.*, 2008a) and associated microbes (Reiswig, 1971; de Goeij *et al.*, 2008a) and preferring plankton (de Goeij *et al.*, 2008a). As a HMA sponge species, bulk stable isotope data of *H. erecta* suggested its microbes were likely photoautotrophic (i.e. using ambient DIC)

and the host might acquire nutrients from its associated microbes or DOM for metabolism and at the same time use plankton for tissue building (J. de Goeij pers. comm). Although many microbes can synthesize EAAs with isotopic value patterns different from those of benthic algae (Larsen *et al.*, 2013), the microbes associated with sponges are scarcely studied at all and it is unclear how close their isotopic value patterns are to pelagic sources. It is possible that: 1) sponge microbes assimilate a portion of the plankton intake from the sponge and do not synthesize much of the EAAs unless critical; 2) the microbes synthesize EAAs from their DIC and DOM intake through pathways similar to those of phytoplankton (i.e. similar δ^{13}C-EAA pattern), and transport them largely to the host; 3) the microbes synthesize EAAs, but there is no or little transportation of these to the host, thus, the host has to acquire EAAs from plankton feeding; and 4) this sponge is not photoautotrophic and both its tissue and associated microbes have to acquire EAAs from the plankton and nutrients for metabolism from the DOM.

7.7 Future directions

My stable isotope analyses give insight into understanding of how coral-reef fish communities are trophically structured, but it is clear that much remains unresolved. More data are needed, for example to better address the causes of variations in community structure. Ideally this would include large scale data, and involve benthic and environmental variables and covariates. As some of these links between community structure responses and predictors can be correlated potentially with lag effects (e.g. coral bleaching, Graham *et al.*, 2007), tools such as structural equation models will likely enhance understanding of the interactions. My dietary studies suggested some strictly categorised reef fishes are feeding on more than one source based on available stable isotope data and assumptions. More work focusing on production source isotopic discriminability and consumer trophic categorisation by including more producers is essential, as is applying these data in mixing models to analyse diet along the food chain. A limitation in the present thesis was the sampling of primary producers (algae) and sponges. In particular, to better understand benthic-pelagic coupling in reef systems, greater understanding of the trophic ecology of sponges has become crucial. It may be that the sponge *H. erecta* utilises DOM and plankton based on unique bio-tracer analyses, but there are clearly many species of sponge which remain completely unstudied in this regard. Hopefully, pathways of other chemical compounds in other sponge species can be investigated in the future.

Stable isotope analysis has been rapidly developed over the past few decades and stable isotope data show great strengths in food-web studies. I have demonstrated several novel strategies in applying such data to understand the community structure, energy flow and trophic interaction in coral-reef food-webs without significantly impacting the ecosystem itself. With many stressors as well as the uncertainties in the future coral reefs are facing, I believe applying this is the way to empower us to understand coral reefs better especially in small-scale studies. However, when one is considering to use either bulk or compound-specific stable isotope data to understand any food-web, they are advised to start with the bulk data as a pilot study and then adopt compound-specific data if it is feasible time and funding-wise. With advancing technologies in GC or LC (liquid chromatography) or introducing new bio-tracer(s), compound-specific stable isotope analysis might become much cheaper and faster. On a larger scale, with the development of online archives (e.g. IsoBank), meta-analysis is a very promising means to address changes globally and compare across systems spatially and temporarily.

Appendices

Appendix 1

Table A1. Species list with biomass composition (%) per 5 cm total length interval with scientific name, code and biomass composition (%) and sum (%). *Species were unable to collect during fieldwork period.

Scientific name	Code	5	10	15	20	25	30	35	40	45	50	55	60	80	120
Aulostomus maculatus	ATT					0.1				1.2					
Caranx ruber	BAJ				7.4	16.0	15.3	8.4	6.5	6.5					
Stegastes leucostictus	BGG	0.9	1.4												
*Kyphosus sectatrix**	BGC								13.4	27.2	18.5				
Stegastes partitus	BID	0.6	0.9												
*Mycteroperca bonaci**	BLG						1.9							100.0	
*Caranx lugubris**	BLJ								10.4						
Chromis cyanea	BLC	8.4	23.4	0.9											
Acanthurus coeruleus	BLT	0.8	3.1	19.4	16.0	1.2		3.3	5.1						
Thalassoma bifasciatum	BHW	8.4	13.7	4.0											
Haemulon sciurus	BSG				8.5	15.7	13.7	4.4							
Coryphopterus glaucofraenum	BDG	0.4	0.7												
*Chromis multilineata**	BRC	0.2	2.4	1.8											
Elacatinus genie	CLG	0.7													
Halichoeres maculipinna	CLW		0.3	1.6											
*Stegastes variabilis**	CCD	1.8	1.7												
Clepticus Parrae	CRW	1.7	2.3	2.3	1.4	1.2									
Acanthurus chirugus	DCF	0.1	0.3												
Gramma loreto	FRB	7.2	1.3												

*Chaetodon capistratus**	FEB	0.1	1.2									
Haemulon flavolineatum	FRG		1.6	6.0	2.6							
*Gnatholepis thompsoni**	GSG	0.2	0.8									
Pomacanthus arcuatus	GRA				1.7		3.9		5.7			
Lutjanus griseus	GRS				0.9	2.2	4.3	8.2	6.4			
Cephalopholis cruentata	GSB		0.1	2.1	3.6	1.5						
Sphyraena barracuda	GRB									3.0		100.0
*Sparisoma atomarium**	GBP	0.2	0.8									
*Serranus tigrinus**	HLB	0.1	1.0									
*Caranx latus**	HEJ										23.6	
Stegastes diencaeus	LFD	0.7	13.8	5.9								
Holocentrus rufus	LSQ		0.4	1.6	3.3	0.9	1.3					
Haemulon album	MGT				0.5	1.3		1.9				
Coryphopterus personatus	MGG	52.3										
Epinephelus striatus	NSG					0.7	2.1	4.0	6.3	12.8		100.0
Acanthurus tractus	OSF		1.1	2.3	3.7	1.5						
Calamus pennatula	PLP		0.1		2.2	0.9						
*Scarus taeniopterus**	PCP	3.2	2.4	3.3		0.7						
*Halichoeres radiates**	PWW			0.4	2.8						19.9	
Holacanthus ciliaris	QUA			2.1		1.2		6.4				
*Scarus vetula**	QUP			0.5	2.0	4.2	4.9		3.7	7.6	20.8	
Halichoeres pictus	RBW	1.5	0.7									
Epinephelus guttatus	RDH		0.1	0.4								
Pterois volitans	RDL		0.1	0.4								
Sparisoma aurofrenatum	RBP	2.3	2.6	8.0	4.0	4.2	7.6					
*Sparisoma chrysopterum**	RTP				0.9		2.3					

*Holacanthus tricolor**	ROB		0.2		1.7										
Lutjanus apodus	SCM			1.2	6.9	10.0	14.6	42.2	15.5	6.3	17.2				
*Abudefduf saxatilis**	SGM		2.8	9.3	2.5										
*Canthigaster rostrate**	SNP	0.6	1.6												
Halichoeres bivittatus	SLD	0.1	0.6												
*Bodianus rufus**	SPH	0.1	0.3			0.9	2.7	2.6							
*Chaetodon ocellatus**	SFB			3.3											
*Pseudupeneus maculatus**	SPG		0.1	2.5	1.8										
*Scorpaena plumieri**	SPS					0.8			3.5						
Sparisoma Viride	SLP	1.8	2.4	2.3	3.0	3.4	5.0	2.5	15.3	23.5		53.6			
Scarus iserti	STP	1.5	8.2	14.4	2.2				2.7						
*Mycteroperca tigris**	TGG			0.4				2.1	3.3			46.4			
Haemulon plumierii	WTG			0.4	8.2	21.7	8.7	6.3							
Halichoeres garnoti	YHW	3.1	4.2	1.7	2.8										
*Sparisoma rubripinne**	YTP								2.4						
Ocyurus chrysurus	YTS				8.3	9.6	11.8	7.6		11.8					
Sum		99.4	98.6	98.7	99.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Appendix 2

Table A2. Length to weight conversion factors *a* and *b*. Updated in September 2016 from fishbase.org (Froese and Pauly, 2017).

Scientific name	*a*	*b*
Abudefduf saxatilis	0.01905	3.00
Acanthemblemaria maria	0.00457	3.08
Acanthurus chirurgus	0.02042	2.96
Acanthurus coerules	0.02512	2.96
Acanthurus tractus	0.01862	2.91
Atherinidae spp	0.00537	3.11
Aulostomus maculatus	0.00396	2.87
Bodianus rufus	0.01440	3.05
Calamus calamus	0.02455	2.93
Calamus penna	0.03020	2.86
Canthigaster rostrata	0.02239	2.96
Caranx latus	0.02188	2.95
Caranx lugubris	0.01820	2.94
Caranx ruber	0.01698	2.94
Cephalopholis cruentata	0.01122	3.07
Cephalopholis fulva	0.01000	3.02
Chaetodon capistratus	0.02512	3.09
Chaetodon ocellatus	0.02570	3.02
Chaetodon sedentatius	0.02291	3.03
Chromis cyanea	0.01479	2.99
Chromis multilineata	0.01479	2.99
Clepticus parrae	0.00955	3.05
Coryphopterus personatus/hyalinus	0.00740	3.10
Coryphopterus spp	0.00683	3.10
Echeneis naucrates	0.00275	3.15
Elacatinus evelynae	0.00589	3.13
Epinephelus adscensionis	0.01349	3.09
Epinephelus guttatus	0.01148	3.04
Epinephelus striatus	0.01148	3.04
Ginglymostoma cirratum	0.00417	3.08
Gnatholepis cauerensis	0.00933	3.20
Gramma loreto	0.01122	3.04
Gramma melacara	0.00389	3.12
Gymnothorax miliaris	0.00182	3.07
Haemulon album	0.01259	2.99
Haemulon flavolineatum	0.01318	3.00
Haemulon plumierii	0.01479	2.98

Haemulon sciurus	0.01549	2.98
Halichoeres bivittatus	0.00933	3.06
Halichoeres garnoti	0.01000	3.13
Halichoeres maculipinna	0.01047	3.20
Halichoeres poeyi	0.01000	3.08
Halichoeres radiatus	0.01310	3.04
Hamlet Juvenile	0.01778	3.03
Holacanthus ciliaris	0.03090	2.89
Holacanthus tricolor	0.03388	2.91
Holocentrus rufus	0.01122	2.90
Hypoplectrus puella	0.00900	3.04
Kyphosus sectatrix/biggibus	0.01413	3.00
Lucayablennius zingaro	0.00457	3.08
Lutjanus apodus	0.01413	2.98
Lutjanus griseus	0.01445	2.98
Malacoctenus boehlkei	0.00933	3.03
Malacoctenus triangulatus	0.00891	3.00
Monacanthus tuckeri	0.02754	3.07
Mulloidichthys martinicus	0.00977	3.14
Mycteroperca bonaci	0.01000	3.05
Mycteroperca tigris	0.01122	3.06
Ocyurus chrysurus	0.01479	2.95
Opistognathus aurifrons	0.00389	3.12
Pomacanthus arcuatus	0.03236	2.92
Pseudupeneus maculatus	0.01000	3.12
Pterois volitans	0.01122	3.09
Scarus iserti	0.01096	3.01
Scarus taeniopterus	0.01350	3.00
Scarus vetula	0.01413	3.03
Scorpaena plumieri	0.01514	2.99
Serranus tabacarius	0.01072	3.06
Serranus tigrinus	0.01000	3.05
Sparisoma atomarium	0.01210	3.03
Sparisoma aurofrenatum	0.01047	3.13
Sparisoma chrysopterum	0.01047	3.10
Sparisoma rubripinne	0.00891	3.04
Sparisoma viride	0.01349	3.05
Sphyraena barracuda	0.00851	2.92
Stegastes adustus	0.01995	2.99
Stegastes diencaeus	0.01995	2.99
Stegastes leucostictus	0.01995	2.95
Stegastes partitus	0.01479	3.02

Stegastes planifrons	0.02138	2.96
Stegastes variabilis	0.01820	2.97
Thalassoma bifasciatum	0.00912	3.01

Appendix 3

Methods to retrieve stable isotope values of missing species from the species list

i. Using data from similar species

Scarus taeniopterus and *S. iserti* were observed swimming and foraging in mixed groups as juveniles in all surveyed sites. They have similar length to weight conversion factors *a* and *b* (Nagelkerken and Van Der Velde, 2004; Froese *et al.*, 2014): 0.01350 and 3.00 (*S. taeniopterus*), 0.01096 and 3.01 (*S. iserti*). The $\delta^{15}N_{S. taeniopterus}$ was reported to be 4.30 ± 0.10‰ (Dromard *et al.*, 2015) with the baseline $\delta^{15}N_{\text{turf algae}}$ = 1.70‰ between September and November 2010 in Guadeloupe. Taking natural seasonal variations of isotopic signatures of algae into consideration, $\delta^{15}N$ of *S. taeniopterus* was represented by that of local *S. iserti*. Scarinae spp in the Bahamas were all recorded with the same trophic position (2.0) in fishbase.org. Thus the $\delta^{15}N$ of all other missing Scarinae spp were represented by the combined $\delta^{15}N$ of collected Scarinae spp.

Chromis multilineata and *C. cyanea* were also observed feeding plankton in mixed groups, and their length to weight ratio coefficient *a* and *b* are the same. Although Aguilar *et al.* (2008) suggested a much higher $\delta^{15}N_{C. multilineata}$ = 7.21‰ with a high combined baseline of $\delta^{15}N$ = 5.54‰, the high input of anthropogenic pollution in northern coast of Cuba, west of Havana City did not resemble the situation in Cape Eleuthera, thus the $\delta^{15}N$ of *C. multilineata* was represented by that of local *C. cyanea*.

Cephalopholis fulva which only appeared in the most degraded site (Ike's Reef) had similar trophic position and length to weight ratio conversation factors to *Cephalopholis cruentata* and *Epinephelus striatus*. However, Keegan and DeNiro (1988) recorded $\delta^{15}N_{C. fulva}$ = 2.00‰ at Nassau with same baseline which did not match the reported trophic position (4.1). Thus, the $\delta^{15}N$ of *C. fulva* was replaced by the combined $\delta^{15}N$ of *C. cruentata* and *E. striatus*. *Epinephelus adscensionis* and *Epinephelus guttatus* had similar trophic positions and length to weight conversion factors, thus their $\delta^{15}N$ were treated equivalent. Both *Mycteroperca bonaci* and *Mycteroperaca tigris* have high trophic positions and biomass contribution, but could not be sampled. Their $\delta^{15}N$ values were replaced by combined $\delta^{15}N$ of other groupers.

Holacanthus tricolor was spotted traveling and foraging solely which was different from *P. arcuatus* and *Holacanthus ciliaris*. However, these three species

share similar trophic positions, length to weight conversion factors and potentially similar food items (e.g. sponges), thus the $\delta^{15}N$ of *H. tricolor* was replaced by the combined $\delta^{15}N$ of *P. arcuatus* and *H. ciliaris*.

ii. Using published data

Abudefduf saxatilis was not sampled due to no available gears, and it does not share similar trophic position, length to weight conversion factors and foraging strategies with other pomacentrids in the surveyed area. We used a constant baseline-adjusted value ($\delta^{15}N$ = 3.552‰) from the Gulf of Mexico (Rooker *et al.*, 2006).

Same method was applied to *Bodianus rufus*, *Canthigaster rostrata*, *Chaetodon capistratus*, *Kyphosus sectatrix*, *Mulloidichthys martinicus*, *Stegastes planifrons* and *Stegastes variabilis*.

iii. Species with no available data

$\delta^{15}N$ of the rest of species were not obtained due to 1) sole species in its family was surveyed thus no alternative species to refer to, 2) morphologically and trophically different from other species surveyed in its genus/family, 3) no research on its stable isotope values, 4) existing data was not applicable for this location, and 5) contributed little biomass to its 5 cm L class.

Appendix 4

Table A3. Statistical terms of linear regression analysis on the relationship between $\delta^{15}N$ and $\log_2$ body mass (M) of collected fish species and two families: Pomacentridae and Scarinae at Cape Eleuthera (the Bahamas): slope and intercept values of linear regression, minimum and maximum of collected M ($\log_2$ transformed), *p* and r^2 values. Constant $\delta^{15}N$ values (‰) of species: ATT* (5.92), BDG* (3.89), BID* (6.14), BGG* (5.4), CLG* (8.92), CLW* (6.68), BSG* (7.45), DCF* (5.60), GRS* (8.73), GSG* (3.89), SLD* (6.13), SLP* (4.59), SGM^ (3.55), SPH^ (3.90), TSD^ (4.45), SNP^ (6.40), BGC^ (6.85), CCD^ (7.33), FEB^ (7.94). * collected in this study, ^ from existing literature. For codes see Table 2.3.

Code	Slope	Intercept	$\log_2 M_{min}$	$\log_2 M_{max}$	*p*	r^2
BAJ	0.097	7.05	8.12	9.68	0.58	0.12
BHW	0.140	5.18	-0.25	2.76	0.28	0.17
BLC	0.170	4.87	1.20	3.25	0.28	0.23
BLT	0.249	3.44	3.62	6.73	0.84	0.06
CRW	0.551	3.93	1.91	3.96	0.00	0.88
FRB	0.150	4.85	-3.02	-0.98	0.45	0.30
FRG	0.345	5.29	3.54	7.38	0.00	0.90
GRA	-0.561	11.01	7.88	8.78	0.34	0.19
GRB	0.647	1.81	11.85	12.92	0.62	0.15
GSB	0.373	5.94	3.85	8.51	0.00	0.82
LFD	-0.229	6.97	1.86	3.58	0.82	0.01
LNS	0.995	-0.41	7.12	8.94	0.16	0.54
LSQ	0.251	5.65	4.41	7.39	0.10	0.54
MGG	0.121	4.56	-5.57	-3.76	0.35	0.73
MGT	0.241	5.25	8.65	9.80	0.66	0.08
NSG	0.549	3.42	9.28	9.98	0.33	0.23
OSF	0.284	3.21	3.19	8.24	0.02	0.54
PLP	-3.598	37.21	7.94	8.26	0.04	0.92
Pomacentridae	0.126	5.64	-1.58	3.58	0.09	0.36
QUA	-0.502	10.06	7.37	9.50	0.10	0.65
QUT	0.096	6.86	8.25	9.41	0.76	0.06
RBP	0.105	3.15	2.01	10.08	0.06	0.63
RBW	-0.165	4.10	-4.56	-1.76	0.07	0.87
RDH	0.426	4.34	6.95	8.97	0.08	0.69
RDL	0.081	7.44	5.74	9.97	0.11	0.26
Scarinae	0.032	3.93	1.41	10.63	0.33	0.06
SCM	0.087	7.94	6.78	7.62	0.90	0.03
STP	0.051	4.01	1.41	4.63	0.61	0.03
WTG	0.487	4.12	7.42	8.54	0.25	0.57
YHW	0.224	5.49	-0.86	5.11	0.01	0.92
YTS	0.707	2.16	7.79	9.38	0.76	0.01

BAJ	0.097	7.04	8.15	9.68	0.58	0.12
BHW	0.140	5.18	-0.25	2.76	0.28	0.17
BLC	0.170	4.87	1.20	3.25	0.28	0.23
BLT	0.249	3.44	3.62	6.73	0.84	0.06
CRW	0.551	3.93	1.91	3.96	0.00	0.88

Appendix 5

Table A4. Bulk $\delta^{13}C$ and $\delta^{15}N$ data (mean ± SD) of eight coral genus cultured at the Swire Institute of Marine Science, the University of Hong Kong during Junle 2013 (Baker *et al*., unpublished data).

Genus	$\delta^{13}C$	$\delta^{15}N$	*n*
Acropora	-20.75 ± 0.76	7.13 ± 0.52	10
Favites	-16.60 ± 0.87	8.96 ± 0.38	5
Goniopora	-19.70 ± 0.80	8.42 ± 0.56	10
Pavona	-21.46 ± 0.73	8.76 ± 0.28	10
Platygyra	-17.78 ± 0.67	8.66 ± 2.06	4
Porites	-17.63 ± 0.08	7.70 ± 0.07	3
Turbinaria	-18.62 ± 0.55	8.94 ± 0.55	5

Appendix 6

Table A5. Updated trophic guilds (TGs) of fish (for codes see Table 3.1) based on their relative position to the isotopic niches of major trophic guilds of coral-reef fish in North Malé Atoll (the Maldives).

Code	Original TG	Amended TG
ACL	Herbivore	Algivore
AML	Zoobenthivore	Zooplanktivore
CEB	Herbivore	Microphage
CHSC	Herbivore	Microphage
CHSD	Herbivore	Microphage
CTT	Herbivore	Omnivore
MEI	Zoobenthivore	Omnivore
MYB	Zooplanktiovre	Nocturnal planktivore
MYM	Zooplanktiovre	Nocturnal planktivore
MYP	Zoobenthivore	Nocturnal planktivore
MYVT	Zoobenthivore	Nocturnal planktivore
NAB	Herbivore	Omnivore
NABC	Omnivore	Zooplanktivore
NAE	Herbivore	Algivore
NAV	Zooplanktiovre	Omnivore
POI	Omnivore	Zooplanktivore
POP	Omnivore	Zooplanktivore
SCF	Herbivore	Microphage
SCN	Herbivore	Microphage
SCR	Herbivore	Omnivore
SCS	Herbivore	Microphage
ZES	Herbivore	Omnivore

Appendix 7

Table A6. Biomass contribution (%) of top five species contributing 80% of the total biomass of each log_2 body mass class (in the order of biomass contribution). For codes see Table 4.1.

3		4		5		6		7		8	
CHT	69.75	POP	29.02	CHT	39.07	CHT	31.74	PTT	27.30	CAV	50.61
POI	6.88	CJT	25.85	AML	14.71	PTT	22.36	CAV	16.35	ODN	38.82
POP	4.27	CHG	11.22	POP	14.04	CAX	10.15	MYP	6.83		
		POI	6.63	CHA	10.78	PTP	7.18	MYVT	6.51		
		POC	3.79	POI	9.51	CHA	6.78	MYVL	5.88		
9		10		11		12		13			
ODN	66.57	SCN	34.53	CHS	26.44	SCN	77.00	SCN	79.32		
CTS	5.35	NAB	9.50	PLV	20.58	PLV	7.13	CAI	12.67		
SCN	3.03	SCF	6.78	CAM	19.80						
PYD	2.08	CEA	5.74	SCN	18.23						
SCF	1.81	CAM	5.58								

Appendix 8

Table A7. List of length-weight ratio conversion factors *a* and *b* of UVC species from North Malé Atoll (the Maldives). References: 1. (Letourneur *et al.*, 1998), 2. (Gumanao *et al.*, 2016), 3. (Murty, 2002), 4. (Kamikawa *et al.*, 2015), 5. (Mapleston *et al.*, 2009), 6. (Froese, 1998), 7. (Kulbicki *et al.*, 2005b), 8. (Smith *et al.*, 1993), 9. (Longenecker and Langston, 2016), 10. (Schroeder, 1982), 11. (Letourneur, 1998), 12. (Fry *et al.*, 2006), 13. (Choat *et al.*, 2002), 14. (Choat *et al.*, 1996), 15. (Craig and Axe, 1997), 16. (Edwards and Shaher, 1991), 17. (González-Sansón *et al.*, 2014), 18. (Hussain *et al.*, 2010), 19. (Harmelin-Vivien and Bouchon-Navaro, 1983), 20. (Peyton *et al.*, 2016), 21. (Roldan and Muñoz, 2004), 22. (Dalzell, 1988), 23. (Mohamad Kasim and Ameer Hamsa, 1994), 24. (Seki, 1986), 25. (Sudekum *et al.*, 1991), 26. (Van der Elst, 1993), 27. (Pauly *et al.*, 1996), 28. (Torres Jr, 1991), 29. (Uchida and Uchiyama, 1986), 30. (Kochzius, 1997), 31. (Pauly, 1980), 32. (Erguden *et al.*, 2009), 33. (Brouard and Grandperrin, 1984), 34. (Ralston, 1988), 35. (Blanco *et al.*, 2003), 36. (Holloway *et al.*, 2015), 37. (Jehangeer, 2003), 38. (Taskavak and Bilecenoglu, 2001), 39. (Abdurahiman *et al.*, 2004), 40. (Thomas *et al.*, 2003), 41. (Cabanban, 1984), 42. (Gajeelee, 1980), 43. (Kimani *et al.*, 2008), 44. (Zhu *et al.*, unpublished data), 45. (Durville *et al.*, 2003). Others are from fishbase.org (Froese and Pauly, 2017). Several species' *a* and *b* values were from species indicated as alternatives.

Scientific name	*a*	*b*	Reference	Alternative
Acanthurus auranticavus	0.02344	2.96		
Acanthurus leucosternon	0.02860	2.92	3	
Acanthurus lineatus	0.02290	3.00	3,4,8,15	
Acanthurus nigricauda	0.03800	2.85	1,3,7,8,9,21	
Acanthurus thompsoni	0.01301	3.10	44	
Aethaloperca rogaa	0.05252	2.73	5	
Allocoris cuvieri	N.A			
Allocoris formosa	N.A.			
Amblyglyphidodon leucogaster	0.02284	2.94	7	
Amphiprion clarkii	0.02291	2.99		

Amphiprion nigripes	0.01122	3.04		
Anampses meleagrides	0.01000	3.06		
Anyperodon leucogrammicus	0.00410	3.32	1,2,7	
Apogon apogonides	0.00646	3.19		
Apolemichthys trimaculatus	0.03020	2.89		
Arothron nigropunctatus	0.00000	3.00		
Aulostomus chinensis	0.00400	3.34	6,7	
Balistapus undulatus	0.03090	3.11	2,12,21	
Balistoides conspicillum	0.02512	2.94		
Bodianus axillaris	0.01202	3.05		
Bodianus diana	0.01202	3.05		
Caesio lunaris	0.019290	2.97	2	
Caesio varilineata	0.01259	3.10		
Caesio xanthonota	0.01259	3.10		
Cantherhines dumerilii	0.02507	2.79		
Canthigaster valentini	0.05130	2.72	7	
Caranx ignobilis	0.02510	2.98	1,7,10,16,20,23,24,25,26,27	
Caranx melampygus	0.02690	2.95	1,2,4,7,10,20,28,29	
Caranx sexfasciatus	0.02570	2.94	2,4,17,18,20	
Centropyge multispinis	0.03020	2.89		
Cephalopholis argus	0.01170	3.12	1,3,4,7,11	
Cephalopholis leopardus	0.01660	2.99		*C. miniata*
Cephalopholis miniata	0.01660	2.99	1,7	
Cephalopholis nigripinnis	0.01259	3.05		
Cephalopholis sexmaculata	0.01660	2.99		*C. miniata*
Cetoscarus bicolor	0.02760	2.92	4	

Chaetodon andamanensis	0.02291	3.01	
Chaetodon auriga	0.03240	2.92	17,21
Chaetodon citrinellus	0.03800	2.81	17
Chaetodon collare	0.02291	3.01	
Chaetodon falcula	0.02291	3.01	
Chaetodon guttatissimus	0.02291	3.01	
Chaetodon kleinii	0.04470	2.96	2,21
Chaetodon lineolatus	0.02291	2.95	
Chaetodon madagaskariensis	0.02291	3.01	
Chaetodon meyeri	0.02291	3.01	
Chaetodon oxycephalus	0.02291	3.01	
Chaetodon triangulum	0.02291	3.01	
Chaetodon trifascialis	0.03470	2.86	1,7
Chaetodon trifasciatus	0.02240	3.11	1,3,7,11
Chaetodon xanthocephalus	0.02291	3.01	
Cheilinus chlorourus	0.02880	2.93	1,2,7
Cheilinus fasciatus	0.02190	3.02	1,2
Cheilinus trilobatus	0.02190	3.02	1,2,7,11
Cheilodipterus quinquelineatus	0.01380	3.04	1,7,30
Chlorurus capistratoides	0.01413	3.04	
Chlorurus sordidus	0.01910	3.09	3,4,7,8,14
Chlorurus strongylocephalus	0.01413	3.04	
Chromis atripectoralis	0.01910	3.25	1,7
Chromis dimidiata	0.01820	3.00	
Chromis flavipectoralis	0.01820	3.00	
Chromis ternatensis	0.02630	3.15	1,7

Chromis viridis	0.03800	2.73	1,7	
Chromis xutha	0.01820	3.00		
Chrysiptera glauca	0.09000	2.41	4,6	
Cirrhilabrus exquisitus	0.01660	2.95		
Ctenochaetus striatus	0.02344	3.06	1,7,8,11,13	
Ctenochaetus truncatus	0.02344	2.97		
Dascyllus aruanus	0.04470	2.74	1,3,7,11,21	
Dascyllus carneus	0.01479	2.98		
Dascyllus trimaculatus	0.06030	2.85	6,7,21	
Diodon liturosus	0.03090	2.89		
Diploprion bifasciatum	0.01778	3.04		
Epibulus insidiator	0.03390	2.91	2,7	
Epinephelus fuscoguttatus	0.01380	3.04	1,7,31	
Epinephelus merra	0.01150	3.10	1,3,7,11	
Epinephelus ongus	0.01860	3.00	1,2,7	
Epinephelus spilotoceps	0.00410	3.35	8	
Fistularia commersonii	0.01120	2.54	32	
Forcipiger flavissimus	0.00104	3.92	19	
Gnathodentex aureolineatus	0.01510	3.11	7,8	
Gomphosus caeruleus	0.00490	2.93	7,21	*G. varius*
Gymnosarda unicolor	0.01660	2.98	33,34	
Gymnothorax meleagris	0.00000	3.00		
Halichoeres cosmetus	0.01000	3.08		
Halichoeres hortulanus	0.01190	3.06	3	
Halichoeres marginatus	0.00526	3.41	3	
Halichoeres scapularis	0.00524	3.38	3	

Halichoeres vrolikii	0.01000	3.08		
Hemicoris batuensis	0.01000	3.06		
Hemigymnus fasciatus	0.01202	3.06		*H. melapterus*
Hemigymnus melapterus	0.01202	3.06		
Hemitaurichthys zoster	0.02188	3.02		
Heniochus diphreutes	0.02188	3.02		
Heniochus pleurotaenia	0.02188	3.02		
Hologymnosus semidiscus	0.01000	3.06		
Kyphosus cinerascens	0.02388	2.94	4	
Labrichthys unilineatus	0.00000	3.00		
Labroides bicolor	0.00447	3.14		
Labroides dimidiatus	0.00600	3.17	1	
Lutjanus biguttatus	0.01445	2.98		
Lutjanus bohar	0.01480	3.07	1,7,34	
Lutjanus gibbus	0.02190	2.96	1,2,4,7,9,35,36	
Lutjanus kasmira	0.01150	3.14	1,4,7,28,34	
Macropharyngodon bipartitus	0.01000	3.06		
Melichthys indicus	0.02512	2.94		
Monotaxis grandoculis	0.03550	2.89	1,4,7,8,9	
Mulloidichthys vanicolensis	0.01480	2.96	4,37	
Myripristis murdjan	0.02090	3.15	2,3	
Myripristis pralinia	0.02340	3.08	1,7	
Myripristis violacea	0.03890	2.92	1,7	
Myripristis vittata	0.01820	3.05		
Naso brachycentron	0.01995	3.00		
Naso brevirostris	0.02090	3.04	1,7,13	

Naso elegans	0.02291	2.97	
Naso fageni	0.01995	3.00	
Naso hexacanthus	0.02950	2.90	7,13
Neoniphon argenteus	0.03240	2.81	1,7
Neopomacentrus cyanomos	0.00000	3.00	
Novaculichthys taeniourus	0.01953	2.91	2
Odonus niger	0.04380	2.91	2
Ostracion meleagris	0.03548	2.81	
Oxycheilinus digramma	0.02271	2.82	2
Oxymonacanthus longirostris	0.00000	3.00	
Paracirrhites arcatus	0.00912	3.07	
Paracirrhites forsteri	0.00912	3.07	
Paraluteres prionurus	0.02188	2.91	
Parapercis hexophthalma	0.00760	3.16	7
Parapercis signata	0.00646	3.10	
Parupeneus macronema	0.00540	3.34	3,11
Pempheris vanicolensis	0.01190	3.03	38
Plectorhinchus vittatus	0.02340	3.02	1,2
Plectroglyphidodon lacrymatus	0.02239	2.99	
Plectropomus laevis	0.00502	3.24	7
Pomacanthus imperator	0.03162	2.91	
Pomacentrus caeruleus	0.02450	2.78	3
Pomacentrus chrysurus	0.02400	3.15	1,7
Pomacentrus indicus	0.02344	2.98	
Pomacentrus pavo	0.03020	2.87	1,7
Pomacentrus philippinus	0.02570	2.85	1,7,21

Priacanthus blochii	0.02814	2.81	2
Priacanthus hamrur	0.02240	2.83	1,7,39,40
Pseudanthias evansi	0.00933	2.97	
Pseudanthias squamipinnis	0.02892	2.65	6
Pseudocheilinus hexataenia	0.01622	2.96	
Pseudocheilinus octotaenia	0.01660	2.95	
Ptereleotris evides	0.00389	3.12	
Pterocaesio pisang	0.00743	3.15	41
Pterocaesio trilineata	0.01150	3.15	1,7
Pterois antennata	0.01148	3.09	
Pygoplites diacanthus	0.09051	2.57	44
Sarda orientalis	0.00977	3.04	
Sargocentron caudimaculatum	0.03910	2.94	2
Sargocentron diadema	0.02040	2.99	1,7,11
Sargocentron microstoma	0.00180	3.85	8
Sargocentron spiniferum	0.02040	3.03	1,4,7
Scarus caudofasciatus	0.01445	3.05	
Scarus festivus	0.01040	3.24	4
Scarus frenatus	0.02166	3.06	1,4
Scarus niger	0.01700	3.18	1,2,7,14
Scarus prasiognathos	0.00794	3.12	
Scarus psittacus	0.02040	3.06	1,3,4,7,8,14
Scarus quoyi	0.02056	3.01	2
Scarus rubroviolaceus	0.01320	3.19	2,4,8
Scarus russelli	0.01445	3.05	
Scarus scaber	0.02780	2.86	3

Scarus tricolor	0.12730	2.33	2		
Scarus viridifucatus	0.01445	3.05			
Scolopsis bilineata	0.01450	3.16	1,7		
Siganus argenteus	0.01510	3.08	1,2,4,7,8		
Siganus corallinus	0.00300	3.53	7,42		
Siganus stellatus	0.04410	2.60	43		
Stethojulis albovittata	0.01280	3.08	11		
Sufflamen bursa	0.03200	2.89			
Sufflamen chrysopterum	0.01530	3.15	8		
Synodus variegatus	0.00410	3.33	1,2,3,7		
Thalassoma amblycephalum	0.02400	2.82			*T. lunare*
Thalassoma hardwicke	0.01350	3.04	3,7,11,21		
Thalassoma lunare	0.02400	2.82	1,2,7,11		
Triaenodon obesus	0.00160	3.36	7		
Variola louti	0.01350	3.06	1,4,7,34		
Wetmorella nigropinnata	0.01995	3.00			
Zanclus cornutus	0.01580	3.27	7,8		
Zebrasoma desjardinii	0.02344	2.97			
Zebrasoma scopas	0.03020	3.01	1,7,8,11,13		

Appendix 9

Table A8. Statistical terms of linear regression analysis on the relationship between $\delta^{15}N$ and $\log_2$ body mass (M) of collected fish species at North Malé Atoll (the Maldives): slope and intercept values of linear regression, minimum and maximum of collected body mass ($\log_2$ transformed), *p* and r^2 values from linear regression, and other species used values of this species. For codes see Table 4.1.

Code	Slope	Intercept	$\log_2 M_{min}$	$\log_2 M_{max}$	*p*	r^2	Substitute for
ACL	-0.594	11.78	5.45	7.53	0.00	0.26	
ACN	0.384	5.67	7.62	8.94	0.26	0.09	
ACT	0.065	9.65	5.03	6.68	0.72	-0.14	
AER	0.265	10.82	6.75	9.35	0.02	0.37	
AML	0.493	8.29	4.59	5.54	0.01	0.61	
ANL	-0.067	13.63	7.25	9.90	0.46	-0.03	
BAU	0.153	10.90	6.82	8.93	0.09	0.44	
BAC	0.806	3.98	8.39	9.15	0.09	0.96	
CAV	0.118	10.54	4.65	7.75	0.31	0.03	
CAX	0.122	10.77	6.25	8.93	0.26	0.04	
CAM	0.220	10.46	8.17	10.59	0.13	0.15	
CEA	0.074	12.30	6.74	9.48	0.22	0.06	
CEMN	0.160	11.56	6.05	9.17	0.18	0.12	
CHF	-1.498	20.68	6.04	6.28	0.30	0.24	CHV
CHM	1.295	2.94	5.27	6.25	0.01	0.35	
CHTF	0.852	6.10	4.48	5.36	0.07	0.80	
CHFA	0.422	7.41	7.81	9.14	0.22	0.76	
CHSD	-0.005	8.03	5.86	8.36	0.98	-0.16	

CHSC	0.035	7.56	6.33	10.47	0.66	-0.02	
CHA	-0.269	12.29	5.09	5.40	0.38	0.38	
CTS	0.124	7.88	6.17	7.72	0.37	0.00	
CTT	0.409	7.20	4.78	6.25	0.08	0.58	
DIL	0.336	7.83	8.65	10.41	0.23	0.39	
FIC	0.033	12.48	8.87	9.78	0.90	-0.95	
GNA	-1.160	20.63	7.32	7.92	0.44	-0.04	
HEZ	0.937	6.24	5.63	6.40	0.00	0.82	
LUB	0.539	8.33	8.05	9.22	0.05	0.87	
LUG	-0.048	13.06	8.11	9.19	0.84	-0.23	
MEI	0.795	3.94	6.99	8.39	0.21	0.16	
MOG	-0.177	12.98	7.80	9.03	0.62	-0.13	
MYB	0.215	10.55	6.62	7.71	0.41	0.27	
MYM	-0.344	14.28	7.13	7.61	0.64	-0.17	
MYVL	0.098	10.93	6.83	7.65	0.71	-0.04	
MYVT	0.881	6.35	6.31	6.76	0.25	0.72	
NAB	3.249	-15.93	7.77	8.39	0.03	1.00	
NAE	0.585	2.39	8.67	10.46	0.10	0.54	
NAH	0.247	8.94	7.49	8.66	0.10	0.52	
ODN	0.020	11.15	8.29	10.38	0.99	-0.33	
PLV	0.265	9.25	9.70	11.08	0.52	-0.11	
POI	0.244	9.77	4.22	5.09	0.68	-0.34	CHG, POCH
POP	1.541	5.40	3.52	3.75	0.55	-0.16	POC
PTP	0.035	11.08	3.99	5.23	0.80	-0.22	PTT
PYD	0.499	7.46	6.50	8.36	0.00	0.25	
SAC	0.065	11.98	7.29	7.66	0.95	-0.99	

SAS	0.466	7.65	8.15	10.59	0.08	0.28
SCF	0.183	7.05	6.34	10.47	0.21	0.10
SCN	0.190	5.99	9.46	12.39	0.36	0.00
SCS	-0.011	8.62	6.79	8.41	0.91	-0.48
VAL	0.039	11.863	6.97	11.15	0.79	-0.78
ZAC	1.129	3.365	6.60	6.95	0.19	0.22
ZES	9.838	-47.110	5.67	5.88	0.33	0.52

Appendix 10

Table A9. One-way ANOVAs results of $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) of seven production source types of coral reefs in North Malé Atoll (Maldives), and from two locations (inner- and outer-atoll sites).$^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. According to Tukey's HSD, non-significant pairs ($p > 0.05$) regardless of location includes 1) $\delta^{13}C$: all pairs between diurnal plankton, nocturnal plankton, sponge and macroalgae; 2) $\delta^{15}N$ $p > 0.05$ pairs: all pairs between algal turf, macroalgae and sponge, nocturnal plankton and detritus pair, and nocturnal plankton and diurnal plankton pair.

Source type (*n* inner- and outer-atoll)	$\delta^{13}C$	$\delta^{15}N$	F_{df} for location difference	
			$\delta^{13}C$	$\delta^{15}N$
Algal turf (6, 6)	-10.80±3.08	4.62±0.29	$F_{1,10} = 0.30^{ns}$	7.51^{*}
Coral (13, 30)	-15.59±1.46	5.75±0.35	$F_{1,41} = 8.43^{**}$	1.92^{ns}
Detritus (11, 8)	-12.99±0.87	6.76±0.45	$F_{1,17} = 3.23^{ns}$	0.52^{ns}
Diurnal plankton (12, 3)	-19.64±2.00	7.34±0.42	$F_{1,13} = 4.31^{ns}$	24.29^{***}
Macroalgae (22, 23)	-19.14±1.75	4.30±0.62	$F_{1,43} = 6.50^{*}$	1.25^{ns}
Nocturnal plankton (3, 3)	-20.19±0.44	7.22±0.25	$F_{1,4} = 5.11^{ns}$	3.88^{ns}
Sponge (8, 13)	-18.38±1.50	4.53±0.91	$F_{1,19} = 3.05^{ns}$	1.24^{ns}
F_{df}	$F_{6,154} = 72.89^{***}$	107.1^{***}		

Appendix 11

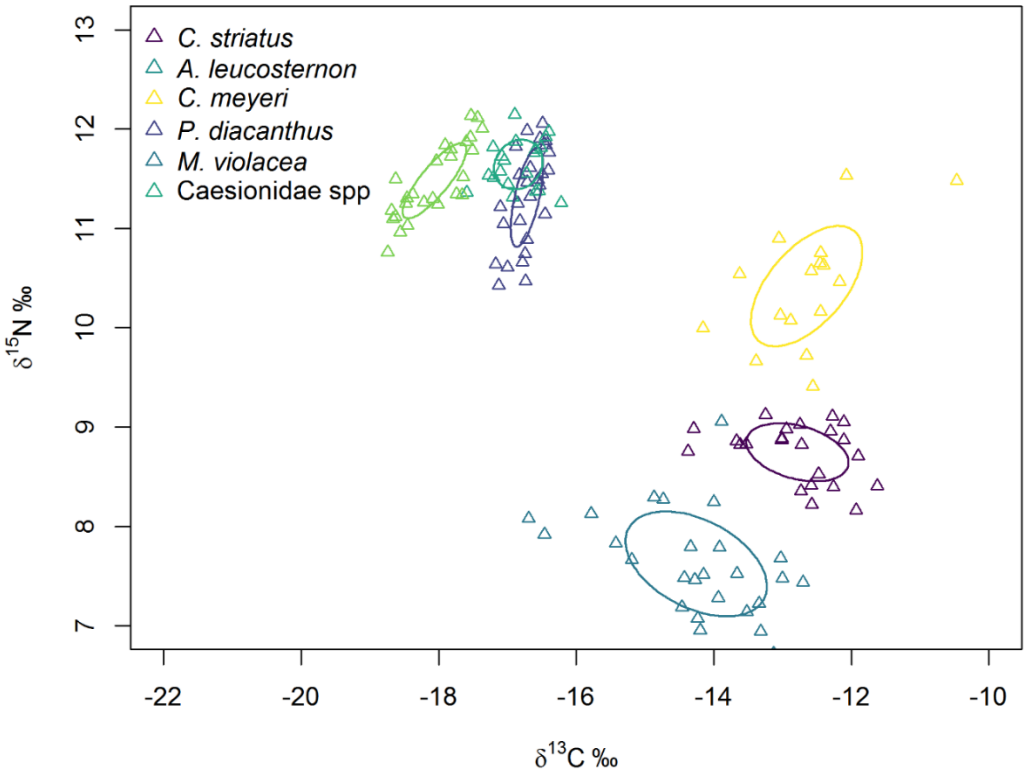

Figure A1. Isotopic niches of the six consumer fish trophic guilds.

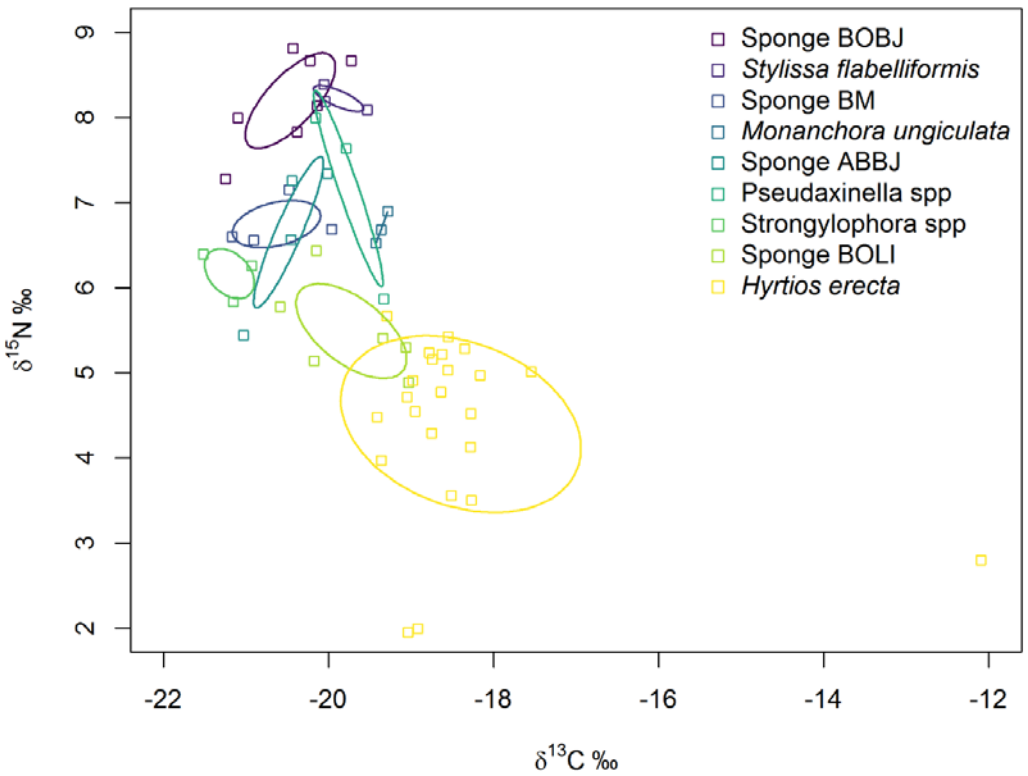

Figure A2. Isotopic niches of all sampled sponges.

Appendix 12

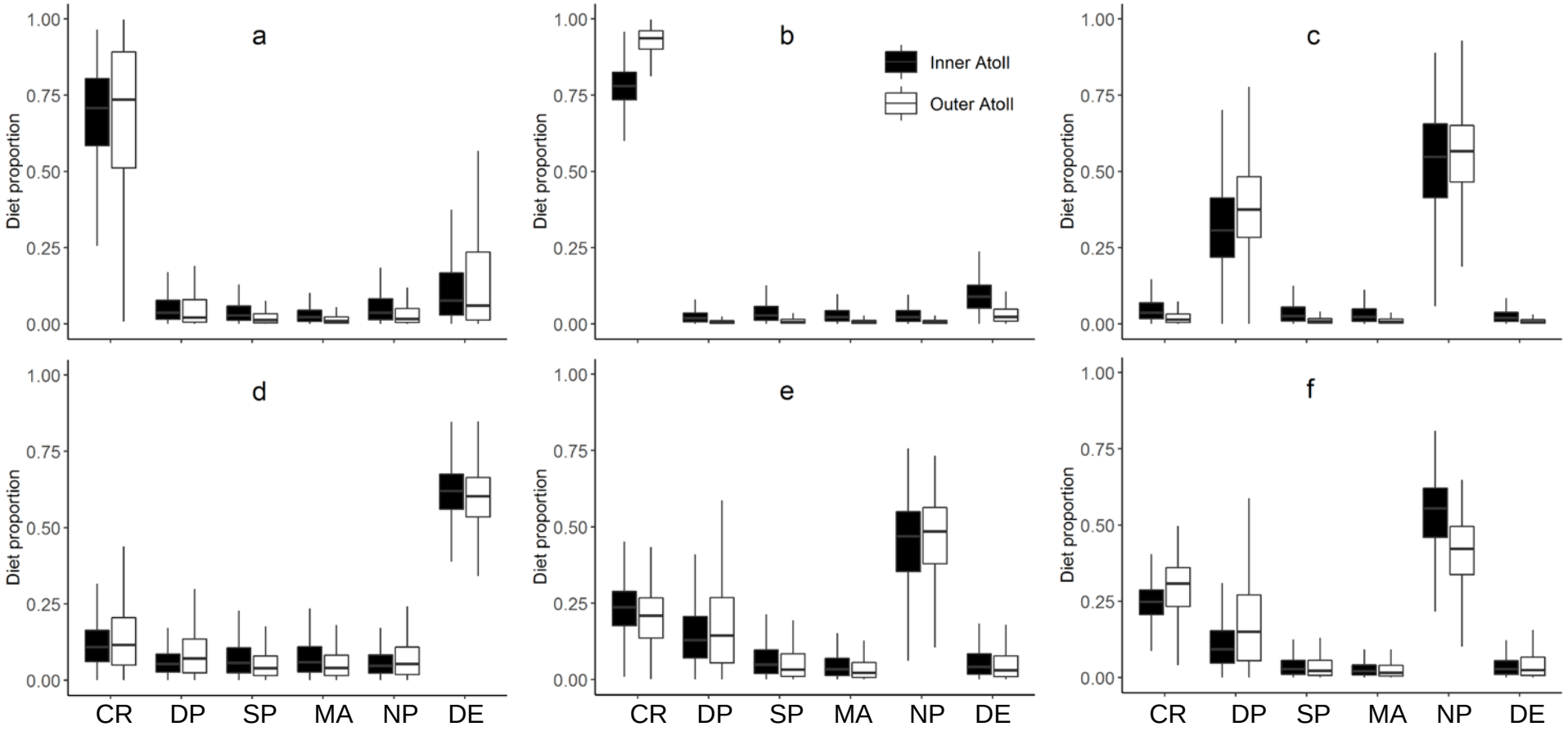

Figure A3. Diet proportion of the corallivore (a), detritivore (b), diurnal planktivore (c), herbivore (d), nocturnal planktivore (e) and spongivore (f) collected from inner- and outer-atoll coral reefs at North Malé Atoll (Maldives). Production source types include CR = corals, DP = diurnal plankton, SP = sponges, MA = macroalgae, NP = nocturnal plankton and DE = detritus.

Appendix 13

Table A10. Mean and standard deviation (SD) values of carbon stable isotope values of five essential amino acids ($\delta^{13}C_{EAA}$): Valine (Val), Leucine (Leu), Threonine (Thr), Phenylalanine (Phe) and Lysing (Lys) of production source types and end members (corals [*Acropora* spp], Copepoda spp, diurnal plankton, benthic algae [*Halimeda opuntia*], sponges [*Hyrtios erecta*], nocturnal plankton and detritus [*Pearsonothuria graeffei*]) and strictly categorised consumer fish (corallivore, detritivore, herbivore, diurnal and nocturnal planktivore and spongivore) at inner- and outer-atoll sites.

Category		$\delta^{13}C_{Val}$	$\delta^{13}C_{Leu}$	$\delta^{13}C_{Thr}$	$\delta^{13}C_{Phe}$	$\delta^{13}C_{Lys}$	Location	*n*
Corals	Mean	-30.21	-27.45	-11.24	-16.48	-19.12	Inner	7
	SD	3.76	1.83	1.83	1.92	1.30		
	Mean	-27.58	-26.28	-11.24	-16.54	-19.00	Outer	9
	SD	3.37	1.97	1.82	0.92	1.20		
Copepoda	Mean	-28.46	-28.15	-19.12	-21.83	-26.22	Inner	3
	SD	3.22	0.83	2.20	2.30	6.28		
	Mean	-26.68	-25.92	-19.19	-20.28	-23.68	Outer	3
	SD	3.46	2.01	2.81	2.08	3.16		
Diurnal plankton	Mean	-27.13	-25.93	-19.41	-21.75	-22.79	Inner	9
	SD	0.82	1.02	1.57	1.36	1.75		
	Mean	-25.17	-24.27	-17.94	-21.03	-20.99	Outer	11
	SD	2.86	2.78	2.17	3.05	1.80		
Benthic algae	Mean	-31.53	-27.83	-20.88	-19.10	-24.33	Inner	8
	SD	2.38	1.69	2.11	2.03	4.12		
	Mean	-31.51	-29.34	-23.16	-20.52	-24.48	Outer	9
	SD	2.04	1.66	1.93	1.37	2.24		
Sponges	Mean	-26.59	-26.99	-23.53	-20.23	-22.69	Inner	7
	SD	1.88	1.60	1.44	-1.61	1.47		
	Mean	-25.53	-25.65	-21.94	-18.42	-21.73	Outer	9
	SD	1.23	1.78	1.93	1.84	1.64		
Nocturnal plankton	Mean	-25.12	-26.64	-17.35	-20.08	-22.37	Inner	3
	SD	2.13	5.49	2.40	1.08	14.44		
	Mean	-25.28	-26.42	-17.82	-20.79	-25.62	Outer	3
	SD	2.33	2.97	3.17	2.64	2.28		
Detritus	Mean	-22.70	-24.06	-13.63	-16.54	-22.15	Inner	8
	SD	2.76	2.57	1.52	1.09	4.27		
	Mean	-21.93	-22.02	-14.33	-16.68	-23.03	Outer	8
	SD	2.35	2.87	1.23	1.68	3.89		

Appendix 14

Table A11. One-way ANOVAs results of $\delta^{13}C$ of the essential amino acids (Val, Leu, Thr, Phe and Lys) of the seven production source types (F_{df} in the bottom row) of coral reefs in North Malé Atoll (Maldives), and from two locations (inner- and outer-atoll sites, shaded area). $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. According to Tukey's HSD, non-significant pairs ($p > 0.05$) regardless of location includes 1) Val: all pairs between diurnal plankton, nocturnal plankton, sponge and copepod, nocturnal plankton and coral pair, and nocturnal plankton and detritus pair; 2) Leu: except diurnal plankton and benthic algae, detritus and benthic algae, detritus and copepod, detritus and corals, and sponge and detritus pairs; 3) Thr: sponge and benthic algae, diurnal plankton and copepod, nocturnal plankton and copepod, and diurnal and nocturnal plankton; 4) Phe: all pairs between copepod, benthic algae and diurnal and nocturnal plankton, sponge and benthic algae, sponge and copepod, sponge and nocturnal plankton, and detritus and coral pairs; 5) Lys: all except pairs between corals and benthic algae, copepod, detritus and nocturnal plankton.

Source type	$\delta^{13}C_{Val}$	$\delta^{13}C_{Leu}$	$\delta^{13}C_{Thr}$	$\delta^{13}C_{Phe}$	$\delta^{13}C_{Lys}$
Corals	$F_{1,14} = 2.16^{ns}$	1.46^{ns}	0.01^{ns}	0.01^{ns}	0.04^{ns}
Copepoda	$F_{1,4} = 0.42^{ns}$	3.17^{ns}	0.00^{ns}	0.75^{ns}	0.39^{ns}
Diurnal plankton	$F_{1,18} = 3.92^{ns}$	2.88^{ns}	2.88^{ns}	0.43^{ns}	5.09^{*}
Benthic algae	$F_{1,15} = 0.00^{ns}$	3.42^{ns}	5.45^{*}	2.91^{ns}	0.01^{ns}
Sponge	$F_{1,14} = 1.87^{ns}$	2.42^{ns}	3.29^{ns}	4.21^{ns}	1.47^{ns}
Nocturnal plankton	$F_{1,4} = 0.01^{ns}$	0.12^{ns}	0.04^{ns}	0.19^{ns}	4.34^{ns}
Detritus	$F_{1,14} = 0.36^{ns}$	2.24^{ns}	1.03^{ns}	0.04^{ns}	0.19^{ns}
F_{df} (all sources)	$F_{6,90} = 20.56^{***}$	9.34^{***}	71.31^{***}	16.72^{***}	6.98^{***}

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