

Trophic Interactions of Marine Sponges

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Thesis abstract

Marine communities in the Anthropocene are changing rapidly with potentially severe consequences for ecosystem functioning. Recently, there has been increased interest in the ecological role of sponges, particularly on coral reefs, driven by evidence that sponges may be less affected by this period of environmental change than other benthic organisms. The Sampela reef system in the Wakatobi Marine National Park, Indonesia, is an example of a reef that has shifted to sponge dominance following a decline in hard corals and an increase in sponge density. Previous research suggests that the Sampela reef system may support a greater abundance of spongivorous fishes relative to surrounding reefs, however, uncertainties remain regarding spongivore identity and predated sponges. In addition, little is known about how shifts towards sponge dominance affect the trophic structure of reefs. The primary aim of my thesis was to investigate sponge trophic interactions to gain insight into the way sponge-dominated reefs of the future might function. This information is essential to predict the broader functional consequences of increasing sponge dominance on reefs in the Anthropocene.

In my first data chapter, I measured the functional impact of spongivorous fishes by quantifying sponge biomass consumption on Wakatobi reefs. Video analysis identified 33 species from 10 families of reef fish grazing on *Xestospongia* spp., although 95% of bites were taken by only 11 species. Gut content analysis indicated that *Pygoplites diacanthus* and *Pomacanthus imperator* were obligate spongivores and *Pomacanthus xanthometopon*, *Zanclus cornutus* and *Siganus punctatus* regularly consumed sponges. *In situ* feeding observations revealed that sponges from the family Petrosiidae are preferred by *P. diacanthus* and *Z. cornutus*. Spongivores were estimated to consume 46.6 ± 18.3 g sponge 1000 m^{-2} of reef day^{-1} and *P. diacanthus* had the greatest predatory impact on sponges. While estimates provided here are conservative and likely underestimate the true magnitude of spongivory on Indo-Pacific coral reefs, this chapter provides the first known estimate of reef wide sponge biomass consumption. Comparisons with published data estimating coral consumption by Chaetodontids in the Pacific suggests that biomass transferred through both pathways is similar in magnitude. Hence spongivory is an important, yet overlooked, trophic pathway on Indo-Pacific reefs.

In my second data chapter, I developed genetic methods to identify sponges from the stomach contents of spongivorous angelfishes sampled in my first chapter. A range of primers and associated predator-blocking primers targeting the 18S rDNA gene were designed and tested on extracts of sponge and spongivore DNA. Sequences were successfully amplified from 14 sponges spanning 6 orders of Porifera, with the majority of samples identified belonging to the order Haplosclerida. This study is the first to successfully sequence sponges from the gut contents of spongivorous fishes. Sequence data indicated that *Pygoplites diacanthus* consumed sponges with considerable chemical defences and

exhibited significant dietary plasticity within the Porifera phylum, similar to observations of angelfishes in the Caribbean and the eastern Pacific.

In my third data chapter, I used stable isotope analysis to investigate differences in consumer niche widths and trophic diversity on the sponge-dominated Sampela reef system in comparison to an adjacent, higher quality reef. I measured the stable isotope ratios of coral reef fish representing different functional feeding groups, prey items and basal carbon sources at both sites. I used isotope data to calculate the trophic position and isotopic niches of each species and performed interspecific and inter-site comparisons. The fish assemblage had a significantly lower mean trophic position at the sponge-dominated site and the majority of species had wider isotopic niches, in accordance with optimal foraging theory which supports expansion in niche widths when per capita prey is low. The fish assemblage sampled at the sponge-dominated site used a significantly lower range of resources, had lower trophic diversity and obtained more carbon from benthic production than fish from the higher quality reef site. Results indicate a simpler trophic structure at the sponge-dominated site characterised by fish with more similar diets. Whilst trophic niche expansion may facilitate population survival in the short term, it can be expected to lead to intensified competition for increasingly scarce resources.

In my final data chapter, I investigated niche partitioning and organic matter contributions to co-occurring temperate sponges. I sampled the stable isotope ratios of five abundant sponge species at 10 m and 30 m at two sites at opposing ends of Doubtful Sound, Fiordland. I also used an ROV to opportunistically sample sponges at depths >50 m and measured stable isotope ratios of picoplankton (<2 μm size phytoplankton), the 2-200 μm fraction of the water column, macroalgae and terrestrial organic matter (TOM), and used isotope mixing models to estimate the contribution of carbon sources to sponge species. Sponges displayed a wide range of $\delta^{15}\text{N}$ values spanning two trophic levels and spatial variation in sponge stable isotope ratios was not reflected by water column resources. There was a high degree of interspecific resource partitioning and comparisons of isotopic niche size suggested more diverse resource use at the Outer Fiord site for *Axinella richardsoni*, *Cymbastela tricalyciformis* and *Raspailia topsenti*. Surprisingly, isotopic mixing models indicated that picoplankton was not a significant carbon source for sponges, which could not be easily explained but may relate to the degradation of dissolved TOM by heterotrophic bacteria. This study found that differential use of the 2-200 μm fraction of the water column and macroalgal derived OM supports the majority of sponges in Fiordland. However, terrestrial production may support some sponges via microbial pathways where marine production is limited.

In summary, species identity and angelfish foraging strategies in the Wakatobi Marine National Park were consistent with known global patterns of spongivory. Observations of feeding behaviour suggested that alternative trophic pathways involving sponge mucus or sponge detritus may also be important for

upper trophic levels. Low densities of spongivores at the Sampela reef system indicate that spongivores may not necessarily benefit from shifts to sponge-dominance that result in low diversity sponge assemblages dominated by one or two competitive species. Whilst sponge consumption may be an important and overlooked trophic pathway on Indo-Pacific reefs, observations from the Sampela reef system demonstrate that the range of trophic pathways originating from the base of the food web is important for maintaining trophic complexity. Hence, sponge-dominated reefs resulting from a decline in corals relative to sponges are less productive and ultimately less stable than coral-dominated reefs.

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Chapter contributions and publications

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Chapter 1: General Introduction

1.1 Global biodiversity decline

The Earth is experiencing species and population losses at such severe rates scientists have declared the beginning of Earth's sixth mass extinction event (Barnosky et al. 2011; Ceballos et al. 2017). The rate of population loss in terrestrial vertebrates is particularly high, with a recent study finding that one-third of the >27,000 terrestrial vertebrate species examined were in decline (Ceballos et al. 2017). Flying insects that perform vitally important functional roles including pollination, nutrient cycling and detritivory are also declining globally (Conrad et al. 2006; Goulson et al. 2008; Hallmann et al. 2017). The biomass of aerial insects in protected areas in Germany has reduced by 76% in 27 years, partly due to agricultural intensification and the associated use of pesticides (Hallmann et al. 2017). Biodiversity, commonly measured as species richness, evenness and heterogeneity (Cardinale et al. 2012) increases ecosystem stability, productivity and directly influences a number of ecosystem services (Cardinale et al. 2012) that are vital to human civilisation (Ceballos et al. 2017). Thus, the loss of biodiversity can be considered a direct threat to the future of mankind (IPBES, 2019).

Historically, pressure on marine environments has been lower than terrestrial environments due to the relative inaccessibility of marine resources (McCauley et al. 2015). However, coastal development and commercial fishing have increased the rate at which marine environments are changing (Jackson et al. 2001; McCauley et al. 2015). In a 2008 assessment of the human impact on marine ecosystems, coastal wildlife habitats were considered most at risk due to the cumulative impacts from land- and ocean-based anthropogenic drivers (Halpern et al. 2008). Land-based drivers include processes that remove vegetation and increase nutrient input into coastal systems, whereas ocean-based drivers include processes that extract resources and alter community composition (Halpern et al. 2008). Species' sensitivity to climate change is thought to be greater in marine than terrestrial systems (Pinsky et al. 2019). Marine ectotherms have narrower thermal safety margins than terrestrial ectotherms and local extirpations resulting from increasing temperatures are twice as common in the ocean than in terrestrial environments (Pinsky et al. 2019). In addition, the rapid reorganisation of communities via changes to species identity and abundance is more prevalent in marine systems as opposed to terrestrial systems (Blowes et al. 2019). Using an extensive database of assemblage time series and independent studies, Blowes et al. (2019) compiled time-series data from >50,000 local assemblages to identify hotspots of biodiversity change. Marine biomes including the tropics, temperate west Atlantic and northwest Australia were all identified as areas that should be prioritised for targeted conservation action based on having the highest rates of species turnover and community restructuring (Blowes et al. 2019). Coral reefs in particular cover < 1% of the global marine environment (Olivotto et al. 2011) but are critical to the survival of approximately a quarter of all marine life and an estimated 450 million people (Wilkinson, 2004; Reaser et al. 2008). Significant community restructuring will shift the functional landscape of these critical habitats and affect their capacity to provide essential goods (e.g. food, wood)

and other services (e.g. coastal protection, climate regulation) (Salm, 1983; Hoegh-Guldberg, 1999; Cardinale et al. 2012).

1.2 Rapid changes in community composition: Regime shifts

One of the best-known examples of significant community restructuring on coral reefs is the regime shifts from coral- to algal-dominated reef states in the Caribbean. This process was identified nearly 40 years ago when a species-specific pathogen led to mass mortality of the grazing urchin *Diadema antillarum* on Jamaican reefs (Lessios, 1988; Hughes, 1994). *Diadema antillarum* mortality, coupled with chronic overfishing of herbivorous fishes, reduced grazing pressure leading to a proliferation of fleshy algae after only 1-2 years (Hughes, 1994). Decades of intense research on herbivorous reef fish assemblages followed (Bellwood et al. 2004) making herbivory the most widely studied functional role on coral reefs (Bellwood et al. 2004). The homogeneous functional group ‘herbivores’ was subdivided based on feeding behaviour, morphology and targeted substrate (Bellwood & Choat, 1990; Bellwood et al. 2004). For example, parrotfishes (family Scaridae) were divided into scrapers (*Scarus*), excavators (*Chlorurus*) and grazers (*Sparisoma*) due to their different impacts on reef substrate (Bellwood & Choat, 1990; Bellwood et al. 2004). More recently, parrotfishes on Caribbean reefs have been delineated into four functional groups (scrapers, excavators, croppers and browsers) based on observations of feeding behaviour, descriptions of individual bites and videos of bite mechanics (Adam et al. 2018). Similarly, detritivorous surgeonfishes (genus *Ctenochaetus*) were originally grouped with other surgeonfishes (Tebbett et al. 2017) until grazing experiments clarified that they were ineffective at removing algal turfs and gut contents were found to be dominated by sediments and detritus (Tebbett et al. 2017). Recognising these differences enables scientists to better predict how changes in assemblage structure may affect ecosystem processes (Bellwood et al. 2019).

Of the benthic invertebrate feeders, corallivores are the best-studied group (Pratchett et al. 2005; Cole et al. 2011) because their reliance on living corals makes them good indicators of habitat degradation (Cole et al. 2008). Corallivores are classified based on their reliance on corals as a resource, with the majority belonging to the Chaetodontidae family (Cole et al. 2008). Obligate corallivores are defined as species that depend on corals for $\geq 80\%$ of their diet (Cole et al. 2008), whereas facultative corallivores may also consume substantial amounts of other organisms including motile invertebrates, sponges, algae and sedentary polychaetes (Harmelin-Vivien & Bouchon-Navaro, 1983; Sano, 1989; Nagelkerken et al. 2009). Obligate corallivores are considered the functional group most threatened by climate-driven habitat loss (Graham et al. 2011), as resource specialisation is key to determining a species’ response to habitat degradation (Layman et al. 2007). Localised extinctions following dramatic declines in coral abundance have already been reported for obligate corallivores and coral-dwelling specialists (Munday, 2004; Brooker et al. 2014). With the exception of herbivores and corallivores, numerous species on coral reefs play relatively unknown and unquantified functional roles (Bellwood

et al. 2019). These significant knowledge gaps need to be addressed and translated into effective management approaches to preserve ecosystem functions on transitioning coral reefs (Bellwood et al. 2019).

1.3 Sponges as potential ‘winners’ on Anthropocene reefs

Heightened interest in the role of sponges on coral reefs has been driven by evidence that sponges may, at least initially, be less affected by this period of unprecedented environmental change (Bell et al. 2013; Bell et al. 2018a; Bell et al. 2018b; Pawlik et al. 2018). Several studies have reported increasing sponge abundance (Ruzicka et al. 2013; McMurray et al. 2015; de Bakker et al. 2017) and transitions to sponge-dominated states have been reported in the Caribbean, Atlantic, Indo-Pacific and the Pacific (reviewed in Bell et al. 2013). In addition to reports of increasing abundance, experimental studies indicate that some sponges may have a greater tolerance for environmental shifts than calcifying fauna (Vicente et al. 2015; Bennett et al. 2017). For example, four Great Barrier Reef (GBR) sponge species exhibited tolerance to ocean warming and acidification predicted under the intermediate Representative Concentration Pathway (RPC) climate change scenario (Bennett et al. 2017). However, under the elevated RPC pathway, significant sponge mortality occurred (Bennett et al. 2017). Interestingly, the interactive effects of ocean acidification (OA) and ocean warming (OW) varied depending on trophic mechanisms (Bennett et al. 2017). Elevated pCO₂ mitigated the effects of temperature stress in phototrophic sponges but exacerbated the effects in heterotrophic sponges (Bennett et al. 2017). It has also been suggested that reefs in the Anthropocene more closely resemble reefs in the geological past (Bellwood et al. 2019). As one of the worlds’ oldest living organisms, sponges have been dominant frame-building organisms in various geological periods (reviewed in Bell et al. 2013) and proliferated after the decimation of bio-calcifying communities at the end of the Triassic period (Delecat et al. 2011). Increasing sponge abundance may have profound impacts on ecosystem functioning (Bell, 2008; Bell et al. 2013; Bell et al. 2018b), yet they remain under-represented in most monitoring programmes and much-needed time-series census studies are lacking for many reef locations (Bell, 2008; Bell et al. 2013; Wulff, 2017). Addressing knowledge gaps regarding sponge functional roles will better enable scientists to predict the broader functional consequences of changing sponge abundance (Bell et al. 2018b).

1.4 Sponge trophic interactions

1.4.1 Spongivores

The majority of sponge predation in temperate waters is carried out by invertebrate feeders (but see Ayling, 1981 for the description of a temperate spongivorous filefish). However, in tropical regions, vertebrate predators (fish and turtles) have the largest predatory impact on sponge assemblages (Meylan, 1988; Randall & Hartman, 1968). The most charismatic sponge specialist is the Hawksbill turtle, *Eretmochelys imbricata*. They have a taxonomically narrow diet, consisting of a few highly siliceous sponge species from the orders Astrophorida, Chondrosida and Hadromerida (Meylan, 1988;

Leon & Bjorndal, 2002; Wulff, 2012; Stringell et al. 2016). Severe Caribbean-wide depletion of *E. imbricata* to <1% former population sizes (McClenachan et al. 2006) has been implicated in increasing sponge abundance on Caribbean reefs (Pawlik et al. 2018), although it is unclear whether sponges predated by spongivorous turtles have increased in abundance. Aside from *E. imbricata*, only a few families of reef fish eat sponges (Randall & Hartman, 1968; Wulff, 2006) and those that do typically only consume part of the sponge; hence mortality is only partial (Wulff, 1994). In addition, many sponges regenerate quickly after predation events, which may have contributed to the assumption that predation has little impact on tropical sponge assemblages (Pawlik, 2011). Randall & Hartman (1968) examined the gut contents of 212 fish species and found sponge remains in only 21 of those species. Sponges made up a substantial percentage of the gut contents of angelfishes of the genus *Holacanthus* (>95%), *Pomacanthus* (>70%), the filefish *Cantherhines macrocerus* (>85%) and the spadefish *Chaetodipterus faber* (>30%). Other spongivorous fishes have been identified from the families Tetraodontidae, Ostraciidae and Scaridae (Randall & Hartman, 1968; Clements & Livingstone, 1983; Wulff, 1994; Dunlap & Pawlik, 1996; Wulff, 1997). Unlike Hawksbill turtles, spongivorous fishes consume a wide range of sponge species in a rotational fashion (Wulff, 1994; Lesser & Slattery, 2013). This feeding behaviour was first noted by Randall & Hartman (1968) who referred to it as the ‘smorgasbord feeding’ approach. They recorded >40 sponge species in the gut contents of 24 *Holacanthus ciliaris* specimens (Randall & Hartman, 1968). Similarly, in Brazil, 34 sponge species were found in the gut contents of three angelfish predators (Andrea et al. 2007) and in the Pacific, spongivorous angelfish consumed 46% of available sponge species (Verdín Padilla et al. 2010). This behaviour was explained by the need to rotate exposed, chemically defended sponge species, thus reducing the effects of secondary metabolites (Wulff, 1994; Wulff, 2006).

Consuming a wide range of sponge species does not necessarily indicate that spongivores select their prey at random. Frequency of occurrence and relative volume of sponge species in the gut contents of spongivorous angelfishes in Randall and Hartman’s 1968 study indicated that *Callyspongia vaginalis*, *Chondrilla nucula*, *Spirastrella coccinea*, *Xestospongia* sp. and *Niphates erecta* are heavily predated by angelfishes. However, preference for particular species cannot be determined without knowledge of relative abundance, as heavily predated species could simply reflect availability in the field (Wulff, 2006). For instance, the most heavily predated species documented by Randall & Hartman (1968), *Callyspongia vaginalis*, is also one of the most abundant species on Caribbean coral reefs (Pawlik et al. 1995; Wulff, 2006).

Gut content analysis is considered by many researchers to be the best method of estimating the feeding preferences of spongivores (Pawlik et al. 2018) however, the destructive sampling of large numbers of reef fishes (e.g. Randall & Hartman 1968) has ethical implications that need to be carefully considered (Pawlik et al. 2018). Instead, palatability assays conducted using a generalist wrasse and pelletised extractions of sponge secondary metabolites have been used to identify sponges with significant

deterrent properties (Pawlik et al. 1995). For example, sponges from the orders Hadromerida, Poecilosclerida and Haplosclerida were palatable to the generalist wrasse *Thalassoma bifasciatum*, whereas sponges from the aspiculate orders Verongiida and Dictyoceratida possessed highly deterrent extracts (Pawlik et al. 1995). Many sponges commonly found in open reef environments (e.g. *Callyspongia*, *Niphates* and *Iotrochota*) were chemically undefended (Pawlik, 2011). These undefended, open reef sponges were also the dominant sponge species recorded in the gut contents of angelfishes sampled by Randall & Hartman (1968) (Pawlik, 1997). This challenged the theory that spongivores are ‘smorgasbord’ feeders, suggesting that instead, they target chemically undefended species to circumvent sponge chemistry (Pawlik, 1997). Open reef, chemically undefended species that incur predation were found to have higher rates of growth and regeneration than chemically defended species (Walters & Pawlik, 2005; Leong & Pawlik, 2010) suggesting a trade-off between investing energy in chemical defences or growth and regeneration (Pawlik, 2011). These observations led to the theory that Caribbean sponges could be partitioned into three ecologically meaningful groups: (1) defended, open reef sponges; (2) undefended but tolerant, palatable sponges; and (3) palatable species that are restricted to cryptic habitats (Pawlik, 2011).

1.4.2 Sponges as consumers

Despite being commonly viewed as simple filter feeders (Maldonado et al. 2012), sponges have a wide range of nutritional strategies (Yahel et al. 2006) that facilitate survival in a range of habitats (Maldonado et al. 2012; Maldonado et al. 2017). Sponges pump large volumes of water, linking benthic and pelagic systems, which can have a profound effect on the overlying water column (Reiswig, 1971; Lesser, 2006). For instance, populations of the giant barrel sponge *Xestospongia muta* can filter a water column 30 m deep every 2.3 to 18 days (McMurray et al. 2014) and some sponges can filter 60 to 900 times their volume per hour (Yahel et al. 2003). Sponges consume both living and non-living (detrital) particles, although the role of detritus in the diets of sponges is relatively unknown (Hadas et al. 2009). Detritus made up approximately half of the particulate organic carbon (POC) consumed by the sponges *Xestospongia muta* and *Negombata magnifica* (Hadas et al. 2009; McMurray et al. 2016) but in other studies, the consumption of detritus was negligible (Yahel et al. 2003). Generally, sponges are limited to feeding on particles <50 µm due to the structure of their aquiferous system (Reiswig, 1971; Pile et al. 1996) and have higher retention efficiencies for picoplankton (0.2 – 2 µm) (Pile, 1997; Ribes et al. 2006), although larger particles may be taken up by surface epithelial cells (Maldonado et al. 2012). Once considered non-selective, several studies have now demonstrated processes of particle selection in marine sponges (Yahel et al. 2006; Hanson et al. 2009; Topçu et al. 2010). For example, Yahel et al. (2006) demonstrated size-independent selective feeding by two species of hexactinellid sponges and suggested that selective feeding likely involves processes of recognition, sorting and transport. Large rare eukaryotic algae and small non-photosynthetic bacteria were efficiently removed but intermediate-

sized *Synechococcus* was less preferred and there were seasonal differences in preferences between species (Yahel et al. 2006).

The recognition that sponges take up dissolved organic carbon (DOC) (Yahel et al. 2003; de Goeij et al. 2008), operationally defined as the fraction of the water column that passes through a GF/F filter with $\pm 0.7 \mu\text{m}$ pore size (Maldonado et al. 2012; de Goeij et al. 2017), has shed light on carbon balance discrepancies (de Goeij et al. 2008). It also led to the recognition of the ‘sponge-loop’ pathway whereby DOC is taken up by sponges, transformed into sponge biomass and eventually shed as cellular detritus, and consumed by detritivores (de Goeij et al. 2008; Rix et al. 2018). Net DOC uptake has been estimated for a number of sponges from tropical, temperate and deep reef systems, and for some sponges, DOC has been estimated to contribute >90% of TOC intake (see de Goeij et al. 2017 for review). On coral reefs, DOC taken up by sponges is released from benthic primary producers eg. corals and macroalgae, representing an additional trophic link between benthic organisms (van Duyl et al. 2011; Rix et al. 2016; Rix et al. 2017). It has also recently been demonstrated that coral- and algal- derived dissolved organic matter are differentially incorporated into the host sponge and its symbionts (Rix et al. 2017), adding another layer of complexity to sponge trophic pathways.

The majority of sponge-hosted symbiotic associations involve microbes but associations with dinoflagellate zooxanthellae are also found among sponges of the genus *Cliona* (Weisz et al. 2010). Some sponges have symbiont densities amounting to 40% sponge tissue volume (Vacelet, 1975; Taylor et al. 2007). Pulse-chase experiments demonstrated that symbiotic zooxanthellae transfer carbon to *Cliona varians* but there was no evidence of nitrogen transfer (Weisz et al. 2010). Similarly, Freeman & Thacker (2011) reported symbiont derived nutrients being transferred into host cells for three tropical sponges with cyanobacterial symbionts, however stable isotope values suggested interspecific differences in carbon and nitrogen integration between host and symbiont community. Symbiotic associations are common in oligotrophic ecosystems such as coral reefs as they allow host species access to additional nutritional pathways that facilitate survival (Freeman et al. 2014).

1.5 Ecological niche

The term ‘ecological niche’ has been central to ecological theory for over a century (Grinnell, 1917; Schoener, 1986). The term was first conceptualised by Grinnell (1917) who described it as the habitat and accompanying behavioural adaptations of a species. In Grinnell’s concept, the ecological niche was a set of behavioural, morphological and physiological adaptations that were represented in all habitat types and filled by ecologically equivalent species (Grinnell 1917; Schoener, 2009). Hutchinson (1957) expanded on the niche concept with a focus on the species rather than the environment. In Hutchinson’s concept, the range of potential environments available to an organism could be visualised as an ‘n-dimensional hypervolume’ with axes that quantitatively describe environmental factors that affect an organism’s performance (Hutchinson, 1957; Hutchinson, 1978; Holt, 2009). The recognition that

species' distributions are also constrained by competition led to the understanding that the fundamental niche is not fully exploited, and species are restricted to a realised niche (Hutchinson, 1978).

Interest in quantifying an organism's ecological niche has grown substantially in the last decade, fuelled by the need to predict ecological responses to environmental change (Holt, 2009). Experimental niche models expose a species to a range of environmental conditions and monitor mortality and fecundity to delineate niche boundaries at which intrinsic growth rate (per capita birth rate minus death rate) is ≥ 0 (Holt, 2009). However, experimental niche models are often limited to small organisms that can be conveniently manipulated at small spatial scales (Holt et al. 2009). Statistical niche models use geographic distribution and environmental variables to produce predictive habitat models that delineate realised niche. For example, Howell et al. (2016) used presence-only data for two types of deep sea sponge aggregations alongside 16 environmental variables to highlight high suitability habitat and inform conservation initiatives. This approach relies upon robust estimates of a species' distribution, which are not always available (Holt et al. 2009), particularly on coral reefs where traditional UVC methods tend to overlook cryptobenthic species (Brandl et al. 2018).

1.6 Stable Isotope Analysis

Over the past decade, stable isotope analysis (SIA) has been proposed as a method to quantify the ecological niche of an organism because it captures both environmental and trophic components of a species' existence, which are represented in multidimensional space (Newsome et al. 2007; Jackson et al. 2011). Carbon isotopes ($^{13}\text{C}/^{12}\text{C}$ ratios) vary among primary carbon sources (primary producers) within a food web due to different inorganic substrate and pathways used in photosynthesis (Smith & Epstein, 1970). Predictable patterns in carbon isotope values (e.g. marine versus terrestrial, inshore versus offshore, benthic versus pelagic), can be used to illustrate reliance on different carbon pathways because of minimal isotopic fractionation (0-1‰) (De Niro & Epstein, 1978) between source and consumer (but see Caut et al. 2009; Busst & Britton, 2016). Nitrogen isotopes ($^{15}\text{N}/^{14}\text{N}$ ratios) indicate the trophic position of a consumer (Post, 2002) because of the predictable stepwise enrichment in ^{15}N per trophic level transfer, generally estimated as 3.5‰ (Post, 2002) but can be as high as 5‰ for some marine herbivores (Mill et al. 2007). In practice, nitrogen enrichment has been shown to decrease with successive trophic levels (Hussey et al. 2014) and sponge $\delta^{15}\text{N}$ values have been shown to vary between co-occurring species by >3.5‰ in polar (Thurber, 2007), temperate (Freeman et al. 2016) and tropical habitats (Freeman et al. 2014; Freeman et al. 2016). Both carbon and nitrogen isotope values of an individual or a population are commonly represented on an isotope biplot, which is useful for depicting relative positions of species within a community (Newsome et al. 2007). Furthermore, intraspecific variation in isotope values can be used as a measure of niche width (Bearhop et al. 2004) provided that isotopic variation in food sources is considered (Newsome et al. 2007). For example, Layman et al. (2007) used intraspecific variation in isotope values to demonstrate the effects of habitat fragmentation

on the trophic ecology of the grey snapper *Lutjanus griseus*. Snapper from fragmented tidal creeks had smaller niche widths than snapper from unfragmented creeks due to a collapse in prey diversity and the disruption of hydrological connectivity (Layman et al. 2007). To quantify niche width, Layman et al. (2007) used the total area of the convex hull (TA), which is the smallest polygon that can be drawn around all individual data points. However, TA is sensitive to sample size, preventing robust comparisons amongst data sets with different sample sizes, as is common in ecological studies (Jackson et al. 2011). To address these methodological issues, Jackson et al. (2011) suggested that niche width be quantified using standard ellipse areas (SEA). A standard ellipse can be understood as a measure of deviation for bivariate ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) data that is comparable to standard deviation for univariate data (Jackson et al. 2011). It contains approximately 40% of the sample data of a group/ species and is less sensitive to sample size than the TA (Jackson et al. 2011). Furthermore, the application of Bayesian inference techniques generates probability distributions of the standard ellipse area allowing for statistical comparisons between different groups (Jackson et al. 2011). In addition, an overlap of SEA among different species indicates similar resource use and can be used as an indicator of interspecific competition (Frisch et al. 2016; Eurich et al. 2019). On coral reefs, these methods have been used to investigate the trophic diversity and trophic position of reef sharks which were found to occupy a similar position in the food chain to mesopredators such as snappers and grouper (Frisch et al. 2016). These findings question the functional role of reef sharks which are frequently assumed to be apex predators even though they have similar diets to teleost fishes (Frisch et al. 2016). Eurich et al (2019) used the isotopic niche (SEA) to illustrate trophic diversity and niche overlap between seven different species of territorial damselfish. Despite generally being classified in the same functional group (territorial algal-turf herbivores) none of the species examined were strict herbivores and some relied mainly on pelagic carbon (Eurich et al. 2019). Both of these studies highlight how SEA can be applied to studies that seek to address functional processes on reef systems.

1.7 Diet characterisation

1.7.1 Visual techniques

Characterising the diet of a predator is key to determining its ecological function (Leray et al. 2015). One of the disadvantages of stable isotope analysis is that it cannot easily be used to identify specific dietary components (Nielsen et al. 2018), particularly in high diversity systems such as coral reefs. Visual techniques (*in situ* observations and gut content analysis) remain some of the best methods of characterising trophic interactions (Nielsen et al. 2018). In the context of sponge predators, long-term observational studies were key to revealing the impact of predation on sponge assemblages in the Antarctic (Dayton et al. 1974; Wulff, 2006) and gut contents have highlighted previously unknown feeding behaviours (Santos et al. 2002). Providing sponge assemblages have been catalogued and characterised (Wulff, 2006) *in situ* observations can lead to species level resolution whereas digestion

can limit identification of stomach contents (Nielsen et al. 2018). Conversely, gut contents provide an unambiguous record of sponges being ingested (Wulff, 2006) where visual observations often fail to distinguish targeted prey (Nagelkerken et al. 2009; Pawlik et al. 2018). Visual observations can also reveal other interesting details about feeding behaviours and provide quantitative estimates of diet (Cole et al. 2011). They are, however, limited by a number of factors. *In situ* observations are complicated in marine systems (Nielsen et al. 2018) often requiring time-intensive approaches (Wulff, 2006) and fish behaviour can be altered by diver presence (Emslie et al. 2018). Gut content that can't be obtained by gastric lavage requires destructive sampling, which is not ethically appropriate for rare species. Gut content samples cannot capture liquid feeding (Nielsen et al. 2018) and are biased towards items that retain structural integrity, thus underestimating soft-bodied prey (Nielsen et al. 2018). Despite these drawbacks, using *in situ* observations and gut content analysis in tandem can provide more detail on the specific diets of reef fish (Nagelkerken et al. 2009) and assist with the categorisation of functional roles.

1.7.2 Molecular techniques

Over the past decade, molecular methods of diet characterisation have been favoured by aquatic and terrestrial researchers (Nielsen et al. 2018) due to their ability to overcome some of the problems associated with visual techniques (Vestheim & Jarman, 2008). Polymerase chain reaction (PCR) is a sensitive technique that can target even small amounts of DNA (Symondson, 2002) providing resolution to highly degraded or rare prey items (Vestheim & Jarman, 2008; Nielsen et al. 2018). DNA extraction and amplification can be used on whole samples when species consume relatively large prey. For example, Dunn et al (2010) used DNA barcoding techniques on muscle tissue retrieved from deep-water sharks improving the rate of data accumulation. DNA barcoding techniques can also be used on gut content homogenates or faecal matter (Deagle et al. 2009; Leray et al. 2015). For example, Deagle et al. (2009) used DNA analysis of faeces to reveal the importance of redbait, jack mackerel and blue mackerel to the diets of Australian fur seals. Visual gut content analysis conducted in tandem with DNA analysis confirmed the same main prey species but yielded much lower species diversity and some groups were unrepresented (eg. cartilaginous fish) (Deagle et al. 2009). Similarly, Leray et al. (2015) used DNA analysis of gut contents of coral-dwelling hawkfishes to demonstrate a high degree of diet partitioning. They identified approximately 300 unique DNA sequences in hawkfish gut contents, significantly increasing knowledge of the number of taxa consumed by this family of reef fishes. Thus, DNA gut content analysis has the potential to vastly improve our understanding of trophic links in marine systems. However, working with degraded DNA also presents a host of methodological challenges including predator DNA swamping, PCR inhibitors eg. bile, faeces and co-amplification of non-target sequences (O'Rorke et al. 2012).

1.8 Study site

1.8.1 The Wakatobi Marine National Park

The first three data chapters of this thesis (Chapters 2-4) were focused in the Wakatobi Marine National Park (MNP), located in The Coral Triangle, Indonesia (Figure 1.1). The Coral Triangle is recognised as the global centre for marine biodiversity and home to over 500 species of coral (Allen & Adrim, 2003). The trajectory of this area reflects the global trajectory of reef systems, with coral cover nearly halving from 42.5% in the 1980s to 22.1% by 2003 (Bruno & Selig, 2007), although not in all areas (e.g. Moritz et al. 2018).

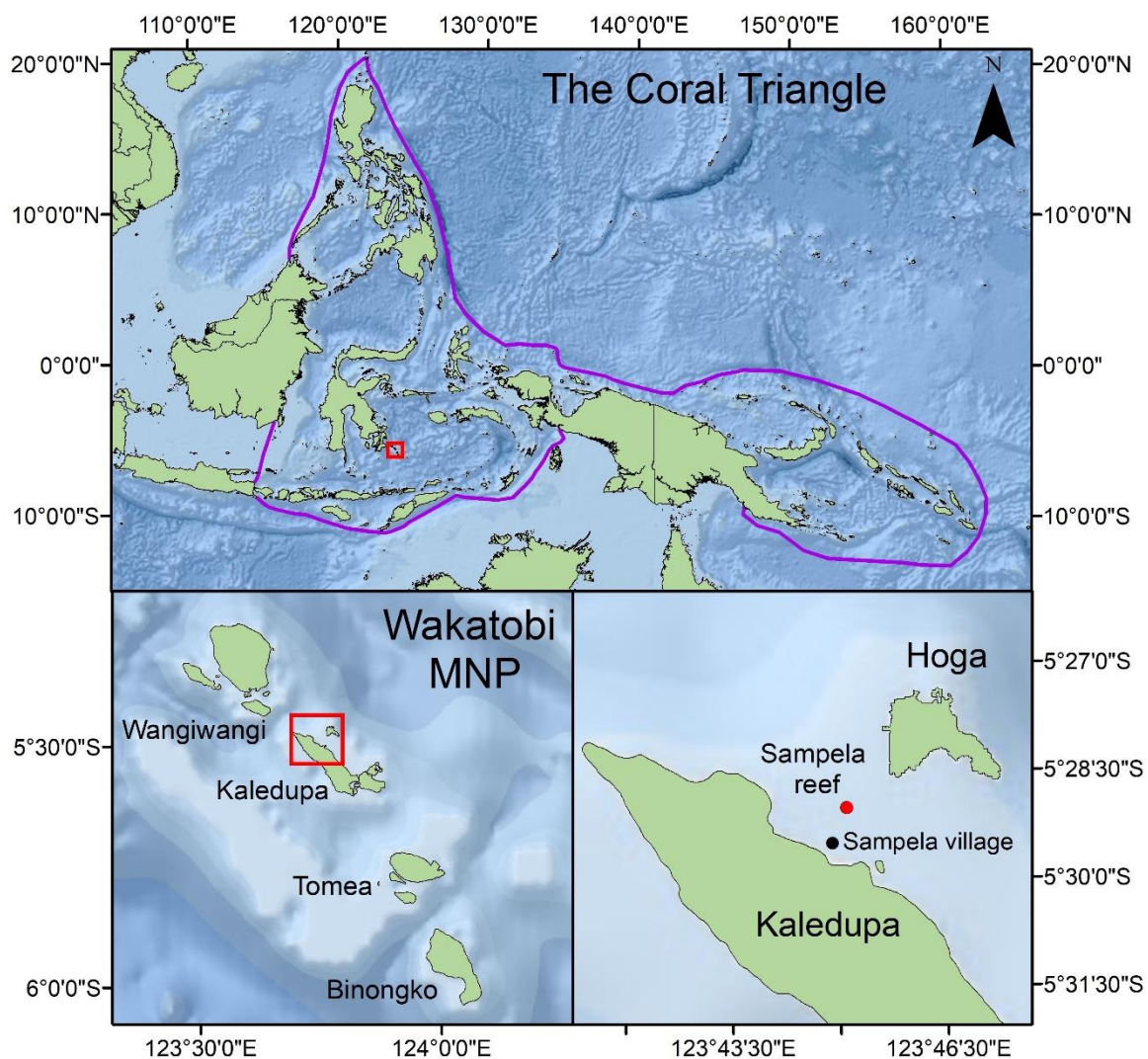


Figure 1. 1 Location of the study area within the Wakatobi Marine National Park, and its position within The Coral Triangle (purple boundary).

The Wakatobi MNP is a World Biosphere Reserve and the third largest, most populated marine national park in Indonesia with approximately 100,000 people residing within the park boundary (Cullen, 2007).

Reefs in the Wakatobi MNP are considered most threatened by destructive fishing practices (e.g. bomb and cyanide fishing) (Allen, 2008; Clifton & Unsworth, 2013), which affects 60% of reefs in the region (Burke et al 2011). A history of poor resource management and high resource pressure has already caused an estimated 50% decline in hard coral cover from 2000-2007 (McMellor & Smith, 2010). The Sampela reef system provides an example for shallow coral reefs in the Anthropocene that shift towards sponge dominance. Sampela reef is impacted by high levels of sedimentation (Crabbe & Smith, 2005; Curtis-Quick, 2013) and its proximity to the Bajo intertidal settlement of Sampela Village means that pressure on the reef system historically has been high. During 2000-2007, hard coral cover declined from ~33% to ~12% (McMellor & Smith, 2010) however sponge density increased (Bell & Smith, 2004), and this site has now transitioned from a coral- to a sponge-dominated reef (Bell & Smith, 2004; Powell et al. 2014). This regime shift has been driven almost exclusively by increases in the abundance of an encrusting phototrophic sponge *Lamellodysedia herbacea*, which accounts for over 40% of sponge abundance (Powell et al. 2014). *Lamellodysedia herbacea* shows tolerance for the poor environmental conditions at Sampela through effective sediment clearance mechanisms and rapid photoacclimation (Biggerstaff et al. 2015; Biggerstaff et al. 2017). Previous work on this reef has indicated a higher abundance of spongivorous fishes relative to surrounding less-degraded reefs (Powell, 2013) indicating that it may support more spongivorous species (Bell et al. 2013). However, uncertainties remain regarding the functional role of some species previously categorised as spongivores (Powell et al. 2014; Bell et al. 2017) and accurate quantification of a species' functional impact is essential to preserve ecosystem processes in the Anthropocene (Bellwood et al. 2019). Research to date has been focused on identifying the drivers of the regime shift (Powell et al. 2014; Biggerstaff et al. 2017). However, considering that sponge-dominated reefs may be a realistic scenario for some future reefs (Bell et al. 2013) it is essential to examine how shifts to sponge dominance affect the trophic structure of reefs.

1.9 Goals and aims

The main aim of this thesis was to investigate sponge trophic interactions to gain insight into how sponge-reefs of the future might function.

The aims of my thesis were:

1. To estimate sponge consumption by spongivorous fishes on Wakatobi reefs and identify predated sponges. I used a combination of video observations and gut content analysis to correctly identify spongivorous fishes, then combined density data with quantitative data on sponge consumption by spongivorous species. This allowed me to address previous functional misclassifications and to compare sponge-spongivore pathways with the coral-corallivore pathways, whilst highlighting predation intensity by different species of reef fish. *In situ* observations of species with the highest predatory impact on sponge assemblages also highlighted any diet preferences for particular sponge species.

2. To develop molecular methods to identify predated sponges from the gut contents of spongivorous angelfishes. This improved the taxonomic resolution for predated sponges by overcoming some of the problems associated with visual observations of sponge predation in a species-rich environment. It also allowed me to compare diet preferences on Indo-Pacific angelfishes with angelfishes from other reef locations.
3. To investigate how habitat quality affects niche widths and trophic diversity in a coral reef fish assemblage. I measured the carbon and nitrogen stable isotope ratios of seven species of reef fish, carbon sources and other benthic prey. This allowed me to examine differences in the trophic position and niche width of individual fish species, and to quantitatively compare trophic structure between sites.
4. To investigate niche partitioning and resource use among co-occurring temperate sponges. In this chapter, I used stable isotope analysis to calculate the isotopic niches of five sponge species at two sites (Outer Fiord and Inner Fiord) at two different depths (10 m and 30 m) in Doubtful Sound, Fiordland. I used isotope data to investigate shifts in resource use over depths and between species. I also used isotope mixing models to estimate the contribution of carbon sources to the diets of each sponge species.

Chapter 2. Biomass transferred from sponges to spongivores equates to the coral-corallivore pathway

2.1 Abstract

Accurate knowledge of the diets of individual species is essential to understand the range of functional roles played by coral reef fishes. Sponges are key members of benthic communities, however, in the Indo-Pacific, an area of exceptionally high sponge diversity, spongivorous fish and predated sponges are relatively unknown. In this chapter, I studied sponge-grazing fishes in the Wakatobi Marine National Park (MNP) using a combination of observational approaches and gut content analysis to quantify sponge consumption. Video analysis identified 33 species from 10 families of reef fish grazing on *Xestospongia* spp. although 95% of bites were taken by only 11 species. Gut content analysis indicated that *Pygoplites diacanthus* and *Pomacanthus imperator* were obligate spongivores and *Pomacanthus xanthurus*, *Zanclus cornutus* and *Siganus punctatus* regularly consumed sponges. Species-specific data on the mean weight (wet weight – 0.01g) of sponge in stomachs combined with spongivore densities provided a reef-wide estimate of sponge consumption for these five spongivorous fishes of 46.6 ± 18.3 g sponge $1000\text{ m}^{-2}\text{ day}^{-1}$. *In situ* observations of feeding behaviour indicated that sponges from the genus *Clathria* and the family Petrosiidae were preferred by *P. diacanthus* and *Z. cornutus*. Despite numerous observations of sponge-grazing, gut contents indicated that *Chaetodon kleinii* and *Forcipiger flavissimus* targeted sponge-associated macrofauna. Similarly, sponge-grazing behaviour exhibited by the detritivore *Ctenochaetus binotatus* could not be explained by gut contents but may indicate a preference for sponge bi-products such as mucus or cellular detritus. This study provides the first known estimate of reef-wide sponge consumption and suggests that in the Wakatobi MNP, biomass transferred between sponges and spongivores may be similar to biomass transferred between corals and corallivores. Hence, spongivory is potentially an important yet overlooked trophic link on Indo-Pacific reefs.

2.2 Introduction

Accurate quantification of a species' functional impact is essential to provide accurate estimates of ecosystem function (Bellwood et al. 2019). Yet, aside from well-studied groups on coral reefs e.g. herbivores and corallivores (Bellwood & Choat, 1990; Bellwood et al. 2004; Pratchett et al. 2005; Cole et al. 2011) our understanding of the range of functional roles performed by individual marine species remains limited. This impacts our ability to make informed recommendations on the preservation of ecosystem functions and services on transitioning coral reefs (Bellwood et al. 2019). Spongivores as a functional group have been relatively overlooked outside of the Caribbean (but see Powell et al. 2015), where sponge biomass is thought to exceed that of corals (Diaz & Rützler, 2001). Reports of increasing sponge abundance from many global locations (Bell et al. 2013; McMurray et al. 2015; Ruzicka et al. 2013; de Bakker et al. 2017) has led to increased interest in the interactions of sponges with other organisms (Wulff, 2012; Pawlik et al. 2018). However, our understanding of the ecological significance of spongivory is restricted by limited data on the taxonomic breadth of spongivores and their preferred prey (Wulff, 2017). Similarly, the predatory impact of spongivores will be influenced by their density and dependence on sponges. Although obligate spongivores have not been explicitly defined in the literature, obligate corallivores are defined as species that rely on corals for $\geq 80\%$ of their diet (Cole et al. 2008), hence a similar definition appears appropriate for spongivores. One of the best-known obligate spongivores is the hawksbill turtle *Eretmochelys imbricata*, which feeds almost exclusively on a narrow range of highly silicified, toxic sponges (Meylan, 1988). Predation pressure from Hawksbill turtles has greatly reduced over the past few decades as turtle populations have been decimated by hunting, habitat loss and poaching (McClenachan et al. 2006; Miller et al. 2019). Reduced predation pressure on sponges from Hawksbill turtles coupled with an increase in space for sponge recruitment due to coral mortality has been linked to an increase in sponge abundance on Caribbean reefs (Pawlik et al. 2018).

Of the vertebrate spongivores still sufficiently abundant on Caribbean reefs, angelfishes (family Pomacanthidae) are somewhat synonymous with spongivory, with at least five species considered obligate spongivores (Randall & Hartman, 1968; Hourigan, et al 1989; Dunlap & Pawlik, 1996; Wulff, 1994; Slattery et al. 2016). Angelfishes are thought to reduce the harmful effects of sponge defensive compounds by foraging on a wide range of sponge species (Randall & Hartman, 1968; Hourigan et al. 1989; Wulff, 1994), a so-called 'smorgasbord' approach (Randall & Hartman, 1968; Wulff, 2006). This is not thought to lead to significant sponge mortality because only part of the sponge is consumed (Wulff, 2006) and those that do experience significant grazing regenerate quickly (Pawlik, 2011). In addition to angelfishes, boxfishes (family Ostraciidae), filefishes (family Monacanthidae) and pufferfishes (family Tetraodontidae) regularly consume sponges as part of an omnivorous diet (Randall & Hartman, 1968; Wulff, 1994). Parrotfishes (family Scaridae) have also been recorded consuming sponges (Dunlap & Pawlik, 1996; Loh & Pawlik, 2009), however, their potential impact on sponge assemblages on reef

systems remains contentious because they are not considered to be regular spongivores (Wulff, 2017). Outside the Caribbean, some butterflyfishes (family Chaetodontidae) have been documented with sponge tissue in their guts in the Central and Indo-Pacific (Hobson, 1974; Sano, 1989; Nagelkerken et al. 2009). However, the volume of sponge found in all species was <20% indicating that sponges were a minor prey item or, for those with <5% volume, potentially ingested incidentally in the pursuit of other prey (Randall & Hartman, 1968; Nagelkerken et al. 2009). The only member of the family Zancidae, the Moorish Idol *Zanclus cornutus*, had gut contents dominated by sponges in Hawaii (>80%: Hobson, 1974). however, in the Gilbert Islands, it consumed mainly algae (Randall, 1955), hence the diets of some species can vary among substantially among geographic locations and habitats. Rabbitfishes (family Siganidae) are generally considered to be grazing or browsing herbivores (Green & Bellwood, 2009; Hoey et al. 2013) however, *Siganus puellus* and *Siganus punctatus* have both been reported to consume substantial quantities of sponge (Sano, 1989; Pitt, 1997; Hoey et al. 2013). This highlights the variation that can exist within a family frequently viewed as a uniform functional group e.g. herbivores (Green & Bellwood, 2009; Hoey et al. 2013).

Indo-Pacific reefs exhibit some of the highest sponge diversity in the world (Bell & Smith, 2004; de Goeij et al. 2017; Rovellini et al. 2019) however, few studies have considered the role of spongivory (but see Powell et al. 2014; Powell et al. 2015). Powell et al. (2014) listed 21 species of spongivorous reef fishes reported from seven studies covering the Indian Ocean, the Indo- and Central Pacific. The abundance of these 21 species was subsequently used as a factor explaining variation in sponge abundance in the Wakatobi Marine National Park (Powell et al. 2014). However, only 11 of the 21 species listed by Powell et al. (2014) had gut content data verifying sponge as ingested prey. Fish may bite at sponges but have no predatory impact (Bell et al. 2017; Pawlik et al. 2018), which seems likely for two surgeonfishes (*Ctenochaetus binotatus* and *Acanthurus pyroferus*) included in the list of spongivores (Powell et al. 2014). *C. binotatus* and *A. pyroferus* are generally considered grazers/detritivores (Green & Bellwood, 2009) that lack the necessary dental morphology to remove sponge tissue (Bellwood et al. 2014). Combining species which likely fulfil different functional roles on reef systems into the category of spongivore could inadvertently bias results (Wulff, 2017). When the functional role of a species remains ambiguous, direct methods of quantification that characterise true diet can prevent functional misclassifications and provide more detail on a specific diet (Nagelkerken et al. 2009; Bell et al. 2017; Nielsen et al. 2018).

Detailed quantitative analysis of stomach contents combined with fish densities can be used to estimate biomass consumption of individual species and functional groups (Harmelin-Vivien & Bouchon-Navarro, 1982; Harmelin-Vivien & Bouchon-Navarro, 1983) and provide direct estimates of the magnitude of a functional process e.g. material consumption per unit area (Bellwood et al. 2019). Estimates of reef wide coral consumption by corallivorous butterflyfish have been calculated using these methods in the Central Pacific (Harmelin-Vivien & Bouchon-Navarro, 1983) and the Red Sea

(Harmelin-Vivien & Bouchon-Navarro, 1982) however there are no published estimates of reef-wide sponge biomass removal by spongivorous fishes. Corallivorous fishes are extremely vulnerable to environmental disturbance due to their heavy reliance on corals (Graham et al. 2011) and localised extinctions have already been reported for some coral specialists (Kokita & Nakazono 2001; Munday, 2004; Brooker et al. 2014) as corals continue to decline (Hughes et al. 2017). By contrast, transitions towards sponge-dominance have been reported from several reefs worldwide (see Bell et al. 2013 for review) and spongivores may benefit from increasing sponge abundance at the expense of corallivores (Bell et al. 2013; Bell et al. 2018b).

In this chapter, I used the same methods as Harmelin-Vivien & Bouchon-Navarro (1982, 1983) with the primary aim of quantifying sponge biomass removed by spongivorous fishes. My aims were: i) to identify sponge-grazing fishes; ii) to measure sponge-grazing fish densities, and iii) to quantify the amount of sponge consumed by sponge-grazing fishes. Considering that the impact of sponge biomass removal will depend on the sponges targeted, I also carried out *in situ* feeding observations with the secondary aim of identifying predated sponges.

2.3 Methods

2.3.1 Study sites

The present study was conducted at four sites within the Wakatobi Marine National Park, the third-largest marine national park in Indonesia (Clifton & Unsworth, 2013) (Figure 2.1). Reefs in the Indo-Pacific region are considered the most threatened in the world (Burke et al 2011), particularly by unsustainable fishing practices (Allen, 2008), which affects 60% of reefs in the region (Burke et al 2011). Four sites were chosen that were separated by at least 3 km, aside from Sampela and B1, that are separated by a channel that is 1.5 km wide and extends to a depth of 40 m, thus limiting the movement of coral reef fish between the Hoga and Kaledupa reef systems.

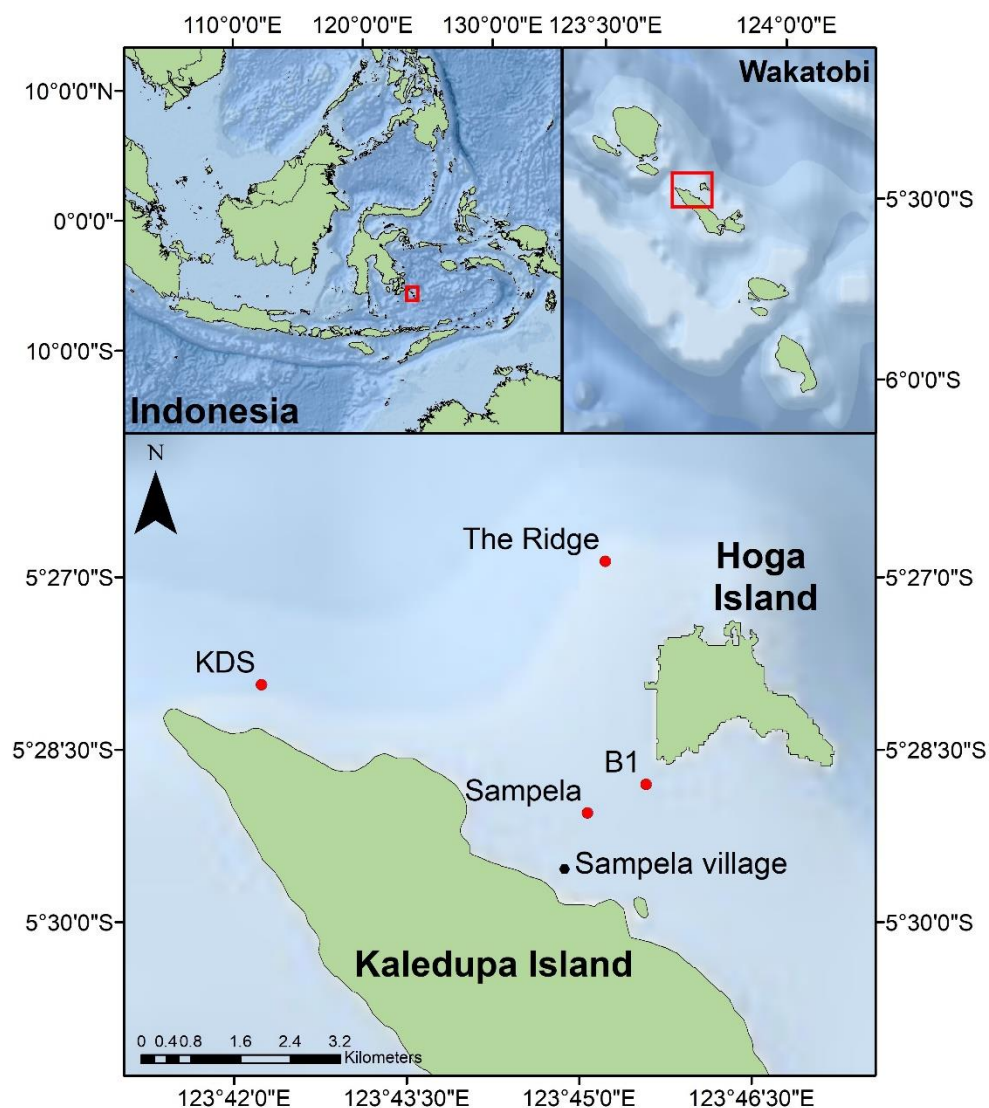


Figure 2. 1 Map of the four study sites and the position of the Hoga/ Kaledupa channel within the Wakatobi Marine National Park and Indonesia.

Both Sampela and Kaledupa Double Spur (KDS) are located on the fringing reef surrounding Kaledupa Island. Sampela is approximately 500 m from the intertidal Bajo settlement of Sampela village at the narrowest part of the channel separating the two islands, whereas KDS is located at the northeast side of Kaledupa Island. B1 is located opposite the Sampela reef site on the west side of Hoga Island and The Ridge is located on a ridge approximately 1 km offshore from Hoga Island. The Sampela reef system is the most degraded of the four sites, experiencing sedimentation rates that are four times higher than the other three reef sites (Crabbe & Smith, 2005) with the lowest abundance of live coral cover ~10% (unpublished monitoring data, Operation Wallacea, 2016). This site has experienced a coral-sponge regime shift (Powell et al. 2014) and has a high abundance of the phototrophic encrusting sponge *Lamellodysedia herbacea* (Powell et al. 2014; Biggerstaff et al. 2015). B1 has moderate coral cover ~20% and both The Ridge and KDS have higher coral cover ~35% (unpublished monitoring data, Operation Wallacea, 2016).

2.3.2 Field surveys of sponge-grazing fishes

To identify sponge-grazing fishes, videos of the giant barrel sponge *Xestospongia* spp. were conducted at all four sites from June to August 2016. Barrel sponges in the Wakatobi MNP are comprised of a species complex including *X. testudinaria*, *X. bergquistia* and an undescribed species (Bell et al. 2014). *Xestospongia* spp. was chosen because an initial trial suggested this genus incurs significant grazing activity (Appendix A) and was present in sufficient densities across the four study sites. Additionally, the Caribbean congener *X. muta* was used by Dunlap & Pawlik (1998) to monitor predation by parrotfishes, suggesting that members of this genus attract predators in a variety of reef locations. A GoPro camera was deployed approximately 1 m away from the sponge and set to record at the end of diving activities to maximise the amount of footage in which diving activities did not disrupt fish behaviour (Emslie et al. 2018). To avoid any effect of depth or microhabitat on fish assemblages (Depczynski & Bellwood, 2004; Stefanoudis et al. 2019), sponges were filmed on the open reef slope (10 – 14 m depending on the site). At the beginning of the video, a measuring stick was placed next to the sponge to generate screenshots from which 2D area of sponge captured in the frame was estimated. All cameras were deployed during the morning (~0800 – 1100) and collected on a subsequent dive in the afternoon, often yielding >2 hours of uninterrupted footage.

2.3.3 Video analysis

The first five minutes of video footage were discarded to account for disturbance from divers leaving the site. The remaining footage was analysed by counting all bites made by fish on *Xestospongia* spp. with bites determined as individual ‘strikes’ on the sponge surface. Fish were identified to species level except for *Ctenochaetus* spp. where it was not always possible to distinguish between *C. binotatus* or *C. striatus* due to poor visibility. However, of the fish that could be identified, >95% of bites were attributed to *C. binotatus*. Distinguishing individual fish that may have fed repeatedly from the same

sponge was not possible using these methods, however, the aim was to measure the grazing intensity of each species rather than individual bite rates. To calculate the area of sponge captured in video footage, screenshots generated from footage containing the scaling device were imported into Image J (Abramoff et al. 2004). The difference in overall grazing intensity (count of bites - all species combined) between sites was tested using negative binomial regression using the MASS package (Venables & Ripley, 2002) for R studio (RStudio Team, 2015) with footage length (minutes) and area of sponge (m²) included as offsets in the model. Fitting both footage length and area as offsets allowed both of these predictors to vary, which was preferable to discarding data by standardising to the shortest video. To determine any differences in fish species contributing to grazing intensity (total bites) at each site, I used PERMANOVA+ add-in to PRIMER version 6.1 (PRIMER-E Ltd, Plymouth, UK). This was done using Type III sums of squares and based on a Bray-Curtis similarity matrix with the unrestricted permutation of raw data and subsequent pair-wise comparisons. Data were checked for multivariate homogeneity of group dispersions using PERMDISP. Data were standardised and square root transformed prior to analysis to reduce the influence of frequent grazers (Clarke and Warwick 2001). The Similarity Percentages routine (SIMPER) was performed to calculate the contribution of each species (%) to the dissimilarity between sites. The more consistently abundant a species is within a site, the more it contributes to intra-site similarity, whereas a species that is consistently abundant at one site but not another is a good discriminating species (Clarke & Warwick 2001).

2.3.4 Sponge-grazing fish densities

Sponge-grazing fish densities were quantified at all sites by underwater visual census (UVC) using six replicate 50 m x 5 m belt transects deployed on the reef slope (8-10 m) with each transect separated by 15 m. For each transect, the lead diver recorded fish data and a second diver followed behind deploying the transect tape to ensure the area covered was undisturbed by diver presence (Emslie et al. 2018). Fourteen species of sponge-grazing fishes were recorded by UVC (Table 2.2). This included nine out of 11 species recording >95% of all bites on *Xestospongia* spp. (Table 2.1). The other two species (*Heniochus singularius* and *Scarus flavipectoralis*) were not recorded because they had not yet been identified as sponge-grazers (not all of the video footage had been analysed at the time of the UVC). Four additional species of Pomacanthidae (*Centropyge vroliki*, *Pomacanthus imperator*, *Pomacanthus xanthurus* and *Pomacanthus sexstriatus*) were also recorded because they were observed in initial videos of sponge-grazing activity (Appendix A) and previously observed grazing on sponges (Powell et al. 2015). A difference in the density of sponge-grazing fishes between sites was analysed using a one-factor ANOVA, with test assumptions verified by Shapiro-Wilk test of normality and Levene's test of the equality of variances. To determine any differences in the community structure of sponge-grazing fishes between sites, I used PERMANOVA+ (as above) using Type III sums of squares and based on a Bray-Curtis similarity matrix with the unrestricted permutation of raw data and subsequent pair-wise comparisons. Data were checked for multivariate homogeneity of group dispersions using PERMDISP.

Data were square root transformed prior to analysis and SIMPER was carried out to identify species responsible for the differences in assemblage structure between sites.

2.3.5 Gut content analysis

To quantify the amount of sponge consumed by sponge-grazing fishes, five species (*Ctenochaetus binotatus*, *Chaetodon kleinii*, *Pygoplites diacanthus*, *Zanclus cornutus*, *Forcipiger flavissimus*) identified from the *Xestospongia* spp. video footage as frequent sponge grazers were spear-fished at The Ridge and Sampela over two weeks in July 2017 by local spearfishers (see Table 2.3 for sample sizes). Fish were collected at both sites, but data were combined due to small sample sizes. One *Pomacanthus imperator* was purchased from fishers at Sampela, and *Pomacanthus xanthometopon* (n = 3) and *Siganus punctatus* (n = 5) were purchased from fishers at the Ridge. These species were not observed in videos of *Xestospongia* spp., but they were observed feeding on other sponges in an initial trial (Appendix A). Fish were placed on ice immediately after spearing and kept at -20°C until dissection. During dissection, fish were weighed (0.1g) and total length (mm), standard length (mm) and gape size (mm) were recorded. The entire digestive tract was removed, preserved at -20°C in 96% ethanol to prevent further digestion. Preserved digestive tracts were returned to Wellington and dissected at the Victoria University Coastal Ecology Laboratory (VUCCEL). During dissection, contents from the stomach (or entire digestive tract for *C. binotatus* and *S. punctatus*) were examined under a dissecting microscope and separated into identifiable and unidentifiable portions. For *P. diacanthus*, *Pomacanthus* spp., *S. punctatus* and *Z. cornutus*, food items in the identifiable portion were separated into 13 major categories (Table 2.3), blotted to remove gastric juices and weighed (wet weight- 0.01g) to estimate diet proportions. Due to low stomach/ digestive tract volumes, the diet composition of *C. kleinii*, *F. flavissimus* and *C. binotatus*, was estimated visually via relative volumetric quantity using a stereomicroscope (after Nielsen & Johnson, 1992). Samples of sponges recovered from gut contents were stored in separate microcentrifuge tubes at -20°C in 96% ethanol for subsequent DNA extraction (Chapter 3). All dissecting instruments were ethanol cleaned and rinsed three times with double distilled water (DDW) between stomachs to prevent cross-contamination.

Levin's standardised diet breadth (Levin, 1968) was calculated by first estimating niche breadth according to the equation:

$$B = \frac{1}{\sum p_j^2}$$

where B is diet breadth and p is the proportion of overall diet made up by the food category (j). This can then be standardised and expressed on a scale of 0 – 1 according to the following:

$$BA = \frac{B - 1}{n - 1}$$

where BA is the standardised niche breadth, B is the measure of niche breadth and n is the number of food categories. Low values indicate a specialised diet based on few dietary items.

2.3.6 Sponge consumption

To estimate sponge consumption, I used the mean weight (wet weight – 0.01g) of sponge in the gut contents of each species and assumed that stomachs are filled at least twice daily (Harmelin & Vivien, 1983). This amount was multiplied by the total number of fish of each species observed at each site and scaled to 1000 m⁻².

2.3.7 Feeding observations

2.3.7.1 Food availability

To determine the proportion of available food resources, ten 1 m² divided quadrats were placed randomly along a 100 m stretch of reef slope at B1, Sampela and The Ridge. Pictures were taken of each 25 cm² divided section to determine benthic cover. Photo quadrats were analysed using CPCe (Kohler & Gill, 2006) with 60 points randomly assigned per divided picture yielding 960 points per 1 m² quadrat. The number of points was determined following repeated measures analysis conducted on a subset of quadrats which showed that 60 points recorded significantly more benthic categories than 5, 10, 20 and 30 points, but not significantly fewer than 120 points (Appendix B, Table 4). Points were assigned to the following major categories: hard coral, sponge, soft coral, macroalgae, turf algae, CCA, rock covered with sand, rock covered with algae, dead coral, sand, detritus (detrital macroalgae or seagrass), rubble, other (e.g. starfish, urchins) and undetermined. To determine differences in benthic community structure between sites, quadrat data were analysed using the same PERMANOVA+ design as described above (see 2.3.4). A difference in sponge abundance between sites was analysed using a one-factor ANOVA, with test assumptions checked using the Shapiro-Wilk test of normality and Levene's test of the equality of variances.

2.3.7.2 Observations

The sponge grazers *Pygoplites diacanthus*, *Zanclus cornutus* and *Coradion melanopus* were chosen for an *in situ* study to identify predated sponges. Feeding observations were conducted throughout the day from 07:00 to 16:00 at Sampela, B1, and the Ridge, during 2016 and 2017. Replicate 3-minute observations were carried out based on the methodology outlined by Berumen et al. (2005). Briefly, data collection commenced after a 3-minute acclimation period, or after the fish took its first bite, and were aborted if the fish fled or sought refuge from the diver (Berumen et al. 2005). The total number of bites was recorded on the following benthic categories: sponge, hard coral, soft coral, macroalgae, detritus (detrital macroalgae or seagrass), other invertebrates and rock (rock with algae, rock with sand, turf algae, CCA and rubble). Subcategories of rock were recorded during observations, but these were combined into the major category 'rock' for selectivity analyses because targeted organism could not be accurately determined. Sponges were identified to the lowest taxonomic level *in situ* and samples

were taken to aid with identification. A minimum of 25 observations was made for each species, except for *C. melanopus*, which was found in low densities (Table 2.2) and was only observed at B1 (n = 6).

Selectivity for each benthic category was assessed by calculating Manly selection functions (w_i) using the package adehabitatHS (Manly et al. 2002) for R studio, which compares the proportional use of each benthic category (u_i) with the proportional availability of that benthic category (p_i) (Manly et al. 1993) according to the following:

$$w_i = u_i / p_i$$

Data were based on a Type II design where resources are equally available for all individuals observed and use of each substrate type is measured for each fish (Manly et al. 2002). For fish feeding studies, use relates to the number of bites on each substrate type in a given period (Berumen et al. 2005). Bonferroni-corrected 95% confidence intervals were calculated around each selection function with positive selectivity determined by selection functions with confidence intervals that were >1 . Positive selection functions indicate that a prey item was being used significantly more than would be expected given availability whereas selection ratios that are < 1 indicate avoidance (Manly et al. 2002).

To highlight selectivity of specific sponge species, sponges in benthic quadrats were identified to the lowest taxonomic level and ranked in order of percentage contribution to overall sponge abundance to determine preference/avoidance of spongivores. Sponges were considered preferred if the percentage of bites $>$ percentage abundance + 5% and avoided if the percentage of bites $<$ percentage abundance + 5%. The total number of species present in each quadrat was also recorded and a one-factor ANOVA was used to test for a difference in total species recorded between sites.

2.4 Results

2.4.1 Video observations

In approximately 50 hours of video footage, 28,477 bites were recorded on *Xestospongia* spp. across four sites. In total, 33 species from 10 families of coral reef fish were identified grazing on *Xestospongia* spp. (Table 2.1). Grazing intensity (total bites) was significantly different between sites with barrel sponges at Sampela experiencing fewer bites than barrel sponges at The Ridge ($Z = 2.85$, $p = 0.004$) and barrel sponges at B1 ($Z = 2.59$, $p = 0.009$), but not barrel sponges at KDS. *Xestospongia* spp. at Sampela experienced 12.2 ± 2.6 bites $\text{min}^{-1} \text{m}^{-2}$ whilst *Xestospongia* spp. at the Ridge experienced 34.9 ± 10.8 bites $\text{min}^{-1} \text{m}^{-2}$ (mean \pm SE) (Figure 2.2).

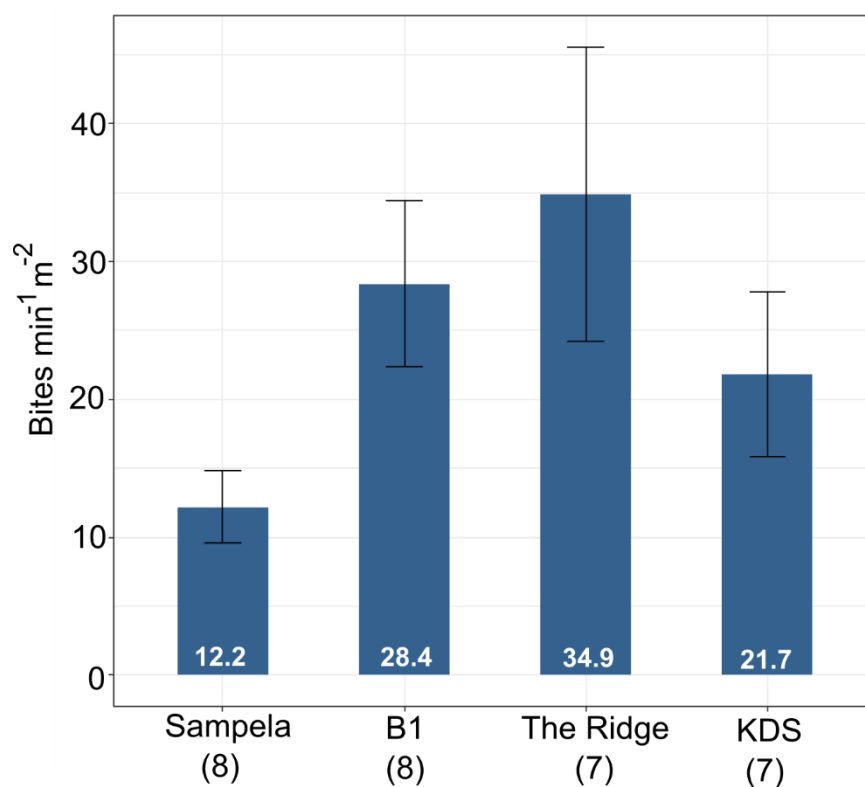


Figure 2. 2 Grazing intensity of fishes (measured as bites $\text{min}^{-1} \text{m}^{-2}$ [mean \pm SE]) on *Xestospongia* spp. filmed at four sites in the Wakatobi MNP. Number in parentheses is the number of *Xestospongia* spp. filmed at each site.

Despite the large number of species observed grazing on *Xestospongia* spp., over half of the total bites recorded were taken by *Ctenochaetus* spp. and *Chaetodon kleinii* and 95% were taken by 11 of the 33 species recorded (Table 2.1).

Table 2. 1 Total number of bites taken by 33 reef fish species filmed grazing on *Xestospongia* spp. at four sites in the Wakatobi MNP. Frequency of occurrence in videos (FO %) calculated from the total number of *Xestospongia* spp. pooled across all sites (n = 30). X indicates fish was observed biting *Xestospongia* spp. at a particular site and * indicates species sampled for gut contents.

Species	Family	Total Bites	Cumulative (%)	FO (%)	Sampela	B1	The Ridge	KDS
<i>Ctenochaetus</i> spp.*	Acanthuridae	10,771	37.8	80	X	X	X	X
<i>Chaetodon kleinii</i> *	Chaetodontidae	7,829	65.3	86.7	X	X	X	X
<i>Zanclus cornutus</i> *	Zanclidae	1,835	71.8	36.7	X	X	X	X
<i>Coradion melanopus</i>	Chaetodontidae	1,687	77.7	26.7		X	X	X
<i>Forcipiger flavissimus</i> *	Chaetodontidae	1,545	83.1	40		X	X	X
<i>Centropyge tibicen</i>	Pomacanthidae	1,167	87.2	13.3	X	X		X
<i>Scarus flavipectoralis</i>	Scaridae	996	90.7	26.7	X	X		X
<i>Pygoplites diacanthus</i> *	Pomacanthidae	595	92.8	20	X	X	X	X
<i>Heniochus singularius</i>	Chaetodontidae	282	93.8	16.7		X	X	
<i>Centropyge bicolor</i>	Pomacanthidae	259	94.7	10	X			X
<i>Centropyge nox</i>	Pomacanthidae	225	95.5	10	X	X	X	
<i>Zebrasoma scopas</i>	Acanthuridae	163	96.1	13.3		X	X	X
<i>Thalassoma lunare</i>	Labridae	156	96.6	23.3	X	X		X
<i>Chaetodon punctatofasciatus</i>	Chaetodontidae	138	97.1	10		X		X
<i>Balistapus undulatus</i>	Balistidae	105	97.5	33.3	X	X	X	X
<i>Centropyge vroliki</i>	Pomacanthidae	102	98.2	10	X			X
<i>Chromis xanthochira</i>	Pomacentridae	102	97.8	10	X		X	
<i>Anampses meleagrides</i>	Labridae	89	98.5	13.3	X	X		X
<i>Scarus niger</i>	Scaridae	83	98.8	13.3		X	X	X
<i>Halichoeres prosopion</i>	Labridae	69	99.0	26.7		X	X	X
<i>Neoglyphidodon melas</i>	Pomacentridae	65	99.2	13.3		X	X	
<i>Diproctacanthus xanthurus</i>	Labridae	51	99.4	6.7		X	X	
<i>Canthigaster valentini</i>	Tetraodontidae	38	99.6	3.3				X
<i>Heniochus pleurotaenia</i>	Chaetodontidae	37	99.7	3.3		X		
<i>Scarus dimidiatus</i>	Scaridae	22	99.8	6.7	X	X		
<i>Pomacentrus adelus</i>	Pomacentridae	13	99.8	3.3	X			
<i>Scarus ghobban</i>	Scaridae	11	99.9	3.3				X
<i>Paracentropyge multifasciata</i>	Pomacanthidae	10	99.9	3.3		X		
<i>Siganus vulpinus</i>	Siganidae	9	99.9	3.3				X
<i>Chaetodon ulietensis</i>	Chaetodontidae	8	99.9	3.3			X	
<i>Sufflamen bursa</i>	Balistidae	6	99.9	10	X		X	
<i>Melichthys vidua</i>	Balistidae	6	99.9	3.3				X
<i>Odonus niger</i>	Balistidae	3	100.0	3.3		X		
Other species grazing on sponges where bites could not be accurately determined:								
<i>Acanthurus pyroferus</i>	Acanthuridae			63.7	X	X	X	X
<i>Acanthurus auranticavus</i>	Acanthuridae			10	X			
<i>Acanthurus nigricauda</i>	Acanthuridae			3.3	X			
<i>Acanthurus thompsoni</i>	Acanthuridae			3.3	X			

Species contributing to grazing intensity were significantly different between sites (pseudoF_{3, 26} = 1.73, p = 0.012) with pairwise tests indicating significant differences between B1 and Sampela (p = 0.001) and B1 and The Ridge (p = 0.038) (Figure 2.3), all other pairwise comparisons showed no significant differences (p > 0.05). The highest average within group similarity was recorded at B1 (49.0%) followed by The Ridge (36.6%), KDS (34.8%) and Sampela (30.7%).

SIMPER showed that the top three species driving the differences in predation between B1 and Sampela (average dissimilarity 68.7%), and B1 and The Ridge (average dissimilarity 63.4%) were *Coradion melanopus*, *Ctenochaetus* spp., and *Chaetodon kleinii*. At B1, *C. melanopus* took 18.8% of bites, opposed to 1% at The Ridge and none were observed at Sampela. At B1, *Ctenochaetus* spp. took 50.4% of bites, opposed to 18.4% at Sampela and 19% at The Ridge. At B1, *C. kleinii* took 7.4% of bites, as opposed to 49.1% at Sampela, 33.7% at The Ridge (Figure 2.3).

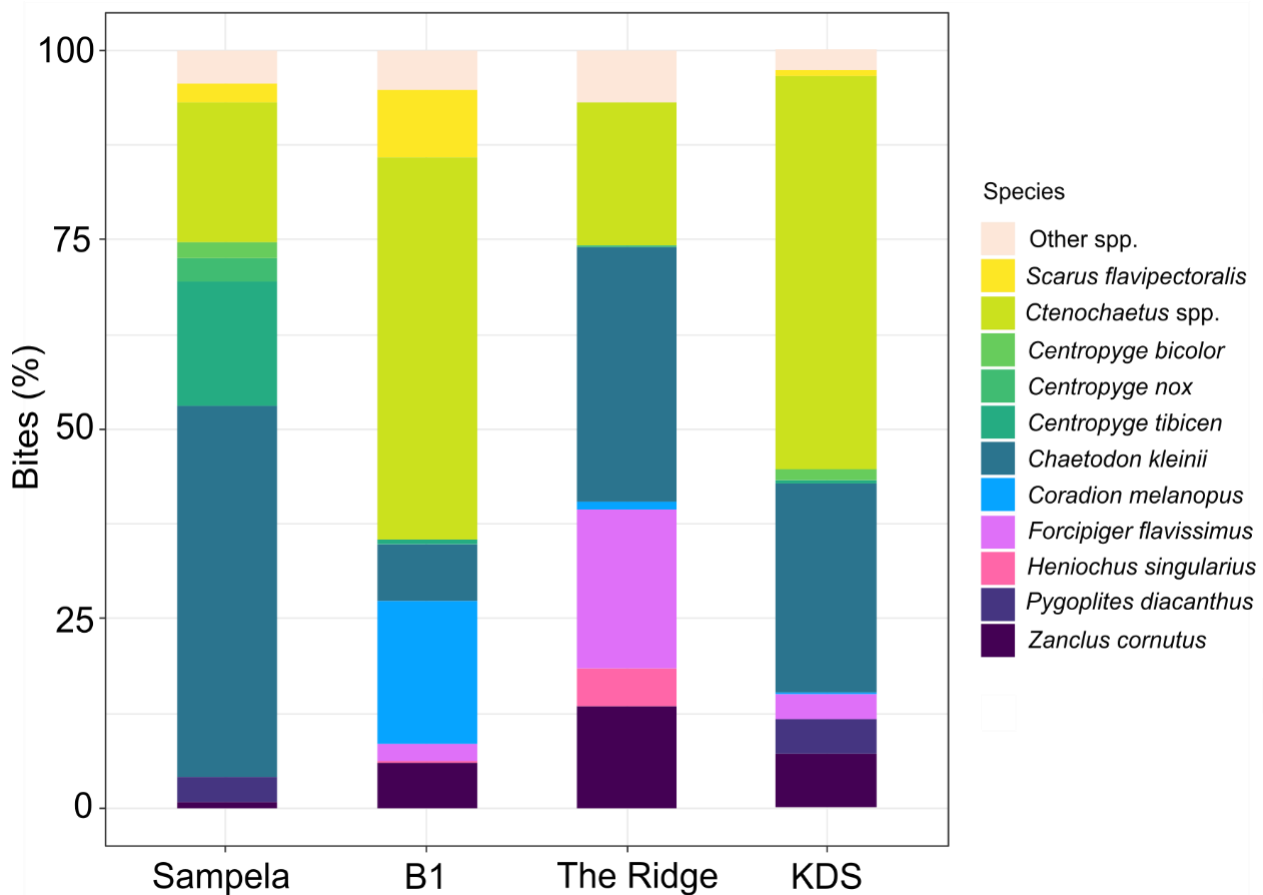


Figure 2. 3 The percentage of total bites on *Xestospongia* spp. taken by 11 species of sponge-grazing fishes filmed at four sites in the Wakatobi MNP.

2.4.2 Sponge-grazing fish densities

Sponge-grazing fish densities were not statistically different between sites with 24 ± 1 (mean \pm SE) fish observed per 250 m². There was also no difference in the densities of the top two species observed grazing on *Xestospongia* spp. (Table 2.1), *Ctenochaetus binotatus* and *Chaetodon kleinii* (Table 2.2). However, there was a difference in sponge-grazing fish assemblage structure between sites (pseudoF_{3, 20} = 3.95, p = 0.001). Pairwise comparisons revealed that the sponge-grazing fish assemblage at Sampela was significantly different to the assemblage at B1 (t = 2.38, p = 0.003), the assemblage at The Ridge (t = 2.93, p = 0.003) and assemblage at KDS (t = 2.19, p = 0.001) and there was also a significant difference in the sponge-grazing fish assemblage between The Ridge and B1 (t = 1.55, p = 0.033) (Figure 2.4).

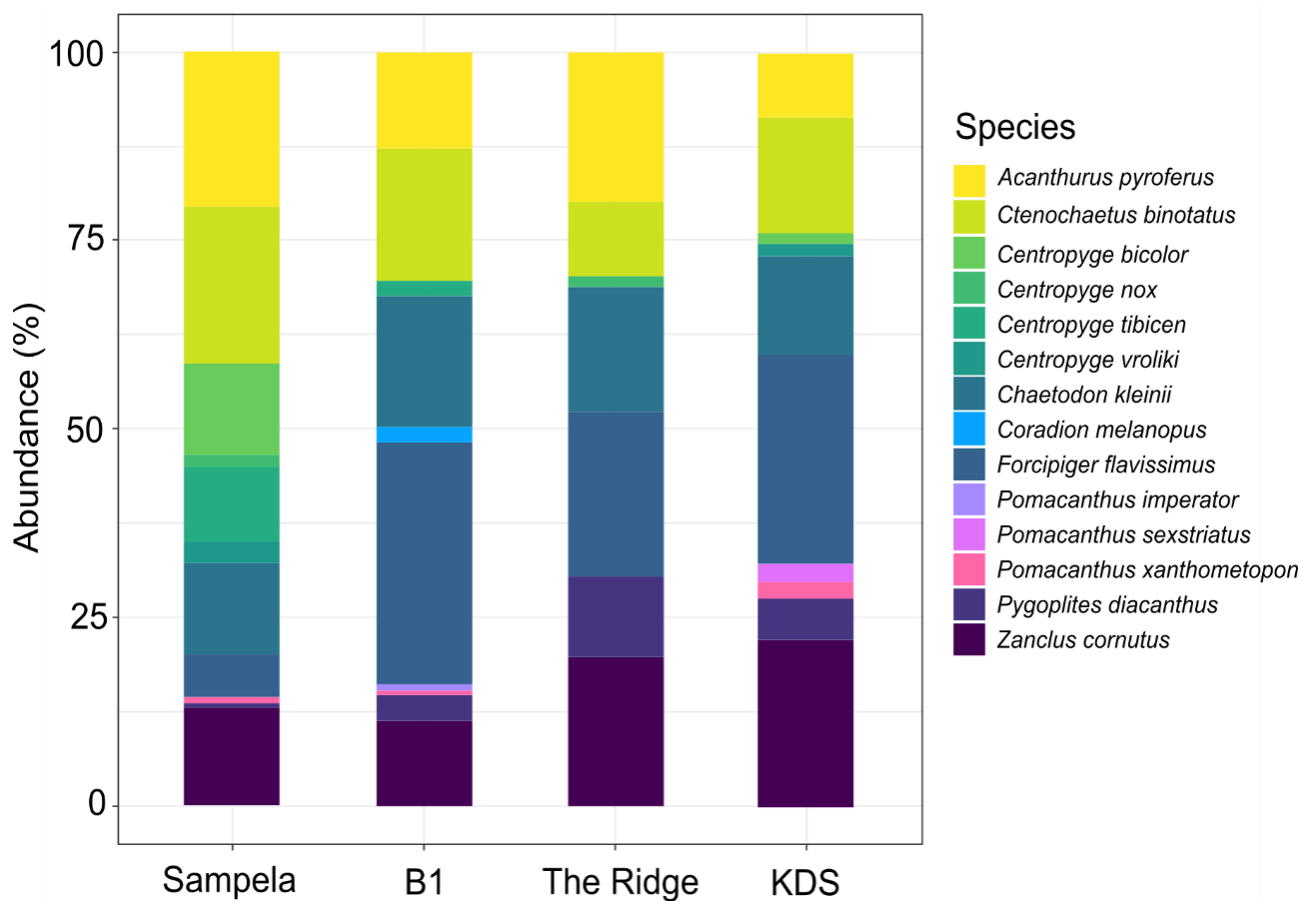


Figure 2. 4 The percentage of total abundance of sponge-grazing fishes surveyed by UVC at each of the four study sites in the Wakatobi MNP.

SIMPER indicated that spatial variation in sponge-grazing fish assemblages was mainly driven by variations in the abundance of *Forcipiger flavissimus*, *Centropyge bicolor* and *Centropyge tibicen* (Table 2.2). At Sampela, densities of *C. bicolor* were 2.8 ± 0.9 fish per 250 m² but *C. bicolor* densities were only 0.3 ± 0.3 fish per 250 m² at KDS and *C. bicolor* was not recorded at The Ridge or B1. Similarly, *C. tibicen* densities were 2.3 ± 0.9 per 250 m² at Sampela, but *C. tibicen* densities were only

0.5 ± 0.3 per 250 m² at B1, and *C. tibicen* was not recorded at The Ridge or KDS. Densities of *F. flavissimus* were much lower at Sampela (1.3 ± 0.5 per 250 m²) than *F. flavissimus* densities at B1 (8 ± 0.9 per 250 m²), The Ridge (5.5 ± 0.6 per 250 m²) and KDS (6.0 ± 1.4 per 250 m²).

Table 2. 2 Sponge-grazing fish densities (mean \pm SE) per 250 m² surveyed by UVC (n = 6) at 4 sites in the Wakatobi MNP.

Family	Species	Sampela	B1	Ridge	KDS
Acanthuridae	<i>Acanthurus pyroferus</i>	4.8 ± 1.1	3.2 ± 0.4	5.0 ± 0.7	1.8 ± 0.7
	<i>Ctenochaetus binotatus</i>	4.8 ± 1.0	4.3 ± 1.0	2.5 ± 0.5	3.3 ± 0.7
Chaetodontidae	<i>Chaetodon kleinii</i>	2.8 ± 0.8	4.3 ± 1.0	4.2 ± 1.5	2.8 ± 0.7
	<i>Coradion melanopus</i>	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	<i>Forcipiger flavissimus</i>	1.3 ± 0.5	8.0 ± 0.9	5.5 ± 0.6	6.0 ± 1.4
	<i>Centropyge bicolor</i>	2.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
Pomacanthidae	<i>Centropyge nox</i>	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
	<i>Centropyge tibicen</i>	2.3 ± 0.9	0.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	<i>Centropyge vroliki</i>	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
	<i>Pomacanthus imperator</i>	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	<i>Pomacanthus sexstriatus</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5
	<i>Pomacanthus xanthurus</i>	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.3
	<i>Pygoplites diacanthus</i>	0.2 ± 0.2	0.8 ± 0.4	2.7 ± 0.6	1.2 ± 0.4
	<i>Zanclus cornutus</i>	3.0 ± 0.5	2.8 ± 0.5	5.0 ± 0.8	4.8 ± 0.5
Zanclidae	<i>Zanclus cornutus</i>	3.0 ± 0.5	2.8 ± 0.5	5.0 ± 0.8	4.8 ± 0.5

2.4.3 Gut content analysis

Obligate spongivores

Pygoplites diacanthus had stomach contents containing >90% sponge (% wet weight), it was also the most specialised species identified by gut content analysis (diet breadth = 0.005) (Table 2.3). Soft coral was found in the stomach of one individual and small amounts of tunicates, and mobile invertebrates including polychaetes and small bivalves were also observed. The one *Pomacanthus imperator* stomach examined had a similarly specialised diet to *P. diacanthus* containing >90% sponge (% wet weight).

Facultative spongivores

Sponge made up approximately half of the contents (% wet weight) of *Pomacanthus xanthurus* stomachs, but soft coral, primarily *Dendronephthya*, was consumed in equal quantities (Table 2.3). Minor order prey items included gastropods, polychaetes and one stomach contained several juvenile starfish. Sponges were also found to be a major prey item of *Z. cornutus* making up approximately 70% of total weighed contents. Other items observed included macroalgae and calcareous sediments. *Siganus punctatus* contained approximately 1/3 sponge (% wet weight), with the most dominant dietary item identified as the red alga *Laurencia* sp. Seagrass was present in all *S. punctatus* stomachs analysed, as were other types of algae and small mobile invertebrates were occasionally observed.

Indirect interactions and sponge as a minor prey item

Chaetodon kleinii had one of the widest diets observed of all the fish observed (Table 2.3). Although observations reported here suggested this species was spongivorous, sponge tissue was not observed, but sponge spicules were seen in 9/12 stomachs. Most of the organic matter in *C. kleinii* appeared to be of soft coral origin and a variety of crustacea were observed. Small volumes of green filamentous algae were also present in the majority of stomachs. *Forcipiger flavissimus* appeared to have a much more specialised diet than *C. kleinii* with the majority of stomachs containing tentacles of *Serpulidae* worms, possibly *Spirobranchus* as distinctive toothed opercula were visible in many samples. Sponge tissue remains were sighted in two stomachs, visually estimated to be <5% of the total volume. Other minor prey items recorded in *F. flavissimus* stomachs included amphipods, isopods, and decapod shrimps. The gut contents of *Ctenochaetus binotatus* were dominated by calcareous sediments and unidentifiable organic matter. Sponge spicules were sighted in 9/12 gut contents but in very low numbers (one or two per gut).

Table 2. 3 Relative gut content (%) of eight species of sponge-grazing reef fish caught in the Wakatobi MNP in 2017. *P. diacanthus* - *S. punctatus* are percent weight (%W) and *C. kleinii* – *C. binotatus* are relative volumetric quantity (%V) due to low volume of stomach/gut contents.

		<i>P. diacanthus</i>	<i>P. imperator</i>	<i>P. xanthometopon</i>	<i>Z. cornutus</i>	<i>S. punctatus</i>	<i>C. kleinii</i>	<i>F. flavissimus</i>	<i>C. binotatus</i>							
	Gut samples (n)	13	1	3	15	5	11	11	10							
	TL range (mm)	122 - 188	102	204 - 272	82 - 150	236 - 285	82 - 104	122 - 158	122- 136							
	Levin's diet breadth	0.005	0.004	0.046	0.036	0.095	0.097	0.032	N/A							
		% W	% F	% W	% W	% F	% W	% F	% W	% F	% V	% F	% V	% F	% V	% F
Annelida	Polychaete	<1	69	<1	<1	33	1	53	<1	60	15	92	75	100		
Crustacea	Amphipod			<1			<1	13	<1	60	14	58	7	27		
	Copepod									20	4	50				
	Decapod						<1	7	<1	20	<1	17	9	18		
	Isopod						<1	7			4	33	2	9		
	Bryozoa	Bryozoan						1	20							
Porifera	Sponge	94	100	96	53	100	73	100	32	100	<1	75	2	18	<1	70
Chordata	Ascidian	<1	38	1	<1	33	3	73	17	100						
Echinodermata	Asteroidea				<1	33										
	Ophiuroidea								<1	20						
Cnidaria	Hydrozoa										2	42				
	Anthozoa - Hard coral				<1	100	<1	13			2	33				
	Anthozoa - Soft coral	5	8	3	46	100	10	7	<1	20	51	83	<1	9	<1	50
Mollusc	Gastropod				<1	33	<1	20			<1	17				
	Bivalve	<1	46				1	60	<1	60	<1	17	1	18		
Algae	Calcareous algae				<1	33	1	73	1	20						
	Red macroalgae								42	100						
	Green filamentous	<1	46								4	58			5	80
	Green turfing								<1	20						
	Brown macroalgae						2	60	<1	40						
Seagrass	Seagrass								6	80					4	90
Eggs	Eggs						<1	6			3	50	<1	9		
Sediments	Calcareous deposits	<1	15				6	67	1	60					90	100
Fluid	Fluid/ mucus												2	9		

2.4.4 Sponge consumption

Estimates of sponge consumption were highest at the two outer reef sites, with 73.3 ± 21.5 g sponge $1000 \text{ m}^{-2} \text{ day}^{-1}$ (mean \pm 95% CI) consumed at The Ridge and 52.9 ± 25.6 g sponge $1000 \text{ m}^{-2} \text{ day}^{-1}$ consumed at KDS (Table 2.4). The lowest estimate was at Sampela with 27.6 ± 15.3 g sponge $1000 \text{ m}^{-2} \text{ day}^{-1}$ and 32.5 ± 10.9 g sponge 1000 m^{-2} was consumed at B1. The reef-wide estimate of sponge consumption by five species of spongivorous reef fish was 46.6 ± 18.3 g sponge $1000 \text{ m}^{-2} \text{ day}^{-1}$ (mean \pm 95% CI).

Table 2. 4 Estimates of sponge consumption (g $1000 \text{ m}^{-2} \text{ day}^{-1}$) for five species of spongivorous reef fish calculated from mean sponge weight in gut contents and fish densities. NB. *Siganus punctatus* was not included in the UVC of sponge-grazing fishes, therefore density was conservatively estimated from video observations.

Site	Spongivore	Total Fish in 1500 m^2	Sponge weight (g) mean \pm 95% CI	Sponge consumption (g $1000 \text{ m}^{-2} \text{ day}^{-1}$) mean \pm 95% CI
Sampela	<i>P. diacanthus</i>	1	2.05 ± 0.38	2.7 ± 0.5
	<i>P. imperator</i>	0	2.83	0.0 ± 0.0
	<i>P. xanthometopon</i>	1	4.68 ± 4.54	6.2 ± 6.1
	<i>Z. cornutus</i>	18	0.39 ± 0.10	9.4 ± 2.4
	<i>S. punctatus</i>	2	3.49 ± 2.35	9.3 ± 6.3
B1	<i>P. diacanthus</i>	5	2.05 ± 0.38	13.7 ± 2.5
	<i>P. imperator</i>	1	2.83	3.8
	<i>P. xanthometopon</i>	1	4.68 ± 4.54	6.2 ± 6.1
	<i>Z. cornutus</i>	17	0.39 ± 0.10	8.8 ± 2.3
	<i>S. punctatus</i>	0	3.49 ± 2.35	0.0 ± 0.0
The Ridge	<i>P. diacanthus</i>	16	2.05 ± 0.38	43.7 ± 8.1
	<i>P. imperator</i>	0	2.83	0.0 ± 0.0
	<i>P. xanthometopon</i>	0	4.68 ± 4.54	0.0 ± 0.0
	<i>Z. cornutus</i>	30	0.39 ± 0.10	15.6 ± 4.0
	<i>S. punctatus</i>	3	3.49 ± 2.35	14.0 ± 9.4
KDS	<i>P. diacanthus</i>	7	2.05 ± 0.38	19.1 ± 3.5
	<i>P. imperator</i>	0	2.83	0.0 ± 0.0
	<i>P. xanthometopon</i>	3	4.68 ± 4.54	18.7 ± 18.2
	<i>Z. cornutus</i>	29	0.39 ± 0.10	15.1 ± 3.9
	<i>S. punctatus</i>	0	3.49 ± 2.35	0.0 ± 0.0

2.4.5 Feeding selectivity of *Pygoplites diacanthus*, *Zanclus cornutus* and *Coradion melanopus*

2.4.5.1 Food availability

There were significant differences in benthic community structure between sites (pseudo $F_{2, 29} = 7.34$, $p = 0.001$) with pairwise tests indicating that all sites were significantly different to one another (all pairwise comparisons, $p = 0.001$) (Figure 2.5). The two benthic categories contributing the most to the dissimilarity between Sampela and the other two sites were rock with sand and sand, both of which were greater at Sampela (Table 2.5). The two benthic categories contributing the most to the dissimilarity between The Ridge and B1 were soft coral, which was higher at The Ridge than B1, and rubble, which was higher at B1 than The Ridge.

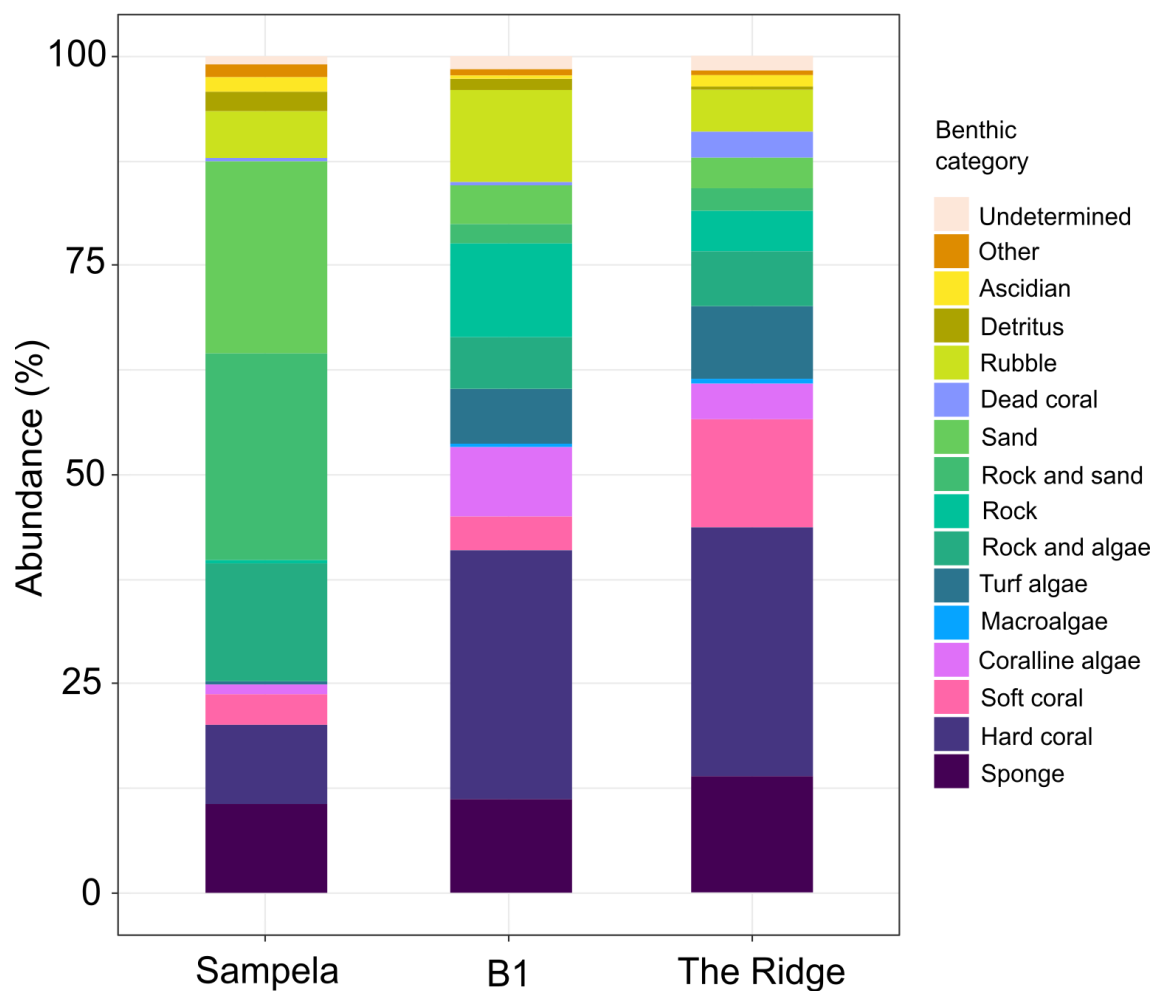


Figure 2. 5 The percentage cover of 16 benthic categories assessed by ten 1 m² divided quadrats at three sites (Sampela, B1 and The Ridge) in the Wakatobi MNP.

Table 2. 5 Benthic categories percentage cover (mean \pm SE) estimated from ten 1 m² divided quadrats placed randomly along a 100 m stretch of reef slope (10-14m) at three sites in the Wakatobi MNP.

Benthic category	Sampela	B1	The Ridge
Sponge	10.6 \pm 2.9	11.3 \pm 2.3	13.8 \pm 2.4
Hard coral	9.5 \pm 1.8	29.7 \pm 4.0	29.7 \pm 6.0
Soft coral	3.8 \pm 1.5	3.9 \pm 1.7	13.0 \pm 4.4
Coralline algae	1.3 \pm 0.7	8.4 \pm 1.0	4.1 \pm 0.9
Dead coral	0.3 \pm 0.2	0.4 \pm 0.2	3.2 \pm 2.9
Rock	0.5 \pm 0.2	11.1 \pm 2.5	4.7 \pm 1.8
Rock and sand	24.7 \pm 4.4	2.2 \pm 0.5	2.7 \pm 0.8
Rock and algae	14.1 \pm 2.8	6.2 \pm 2.3	6.6 \pm 1.9
Macroalgae	0.0 \pm 0.0	0.4 \pm 0.3	0.6 \pm 0.3
Turf algae	0.4 \pm 0.3	6.5 \pm 1.5	8.7 \pm 1.9
Sand	22.8 \pm 4.2	4.8 \pm 1.1	3.6 \pm 1.6
Detritus	2.3 \pm 0.9	1.2 \pm 0.4	0.2 \pm 0.1
Rubble	5.6 \pm 2.2	11.0 \pm 4.7	5.1 \pm 2.1
Ascidian	1.7 \pm 1.0	0.5 \pm 0.2	1.5 \pm 0.4
Undetermined	1.5 \pm 0.1	0.8 \pm 0.2	0.6 \pm 0.2
Other	0.9 \pm 0.2	1.7 \pm 0.5	1.8 \pm 1.0

There was no difference in the overall percentage cover of sponges amongst sites ($F_{2, 27} = 0.43$, $p = 0.66$) (Table 2.5), which covered on average $11.9\% \pm 1.4\%$ of the substrate. There was a significant difference in the number of species recorded at each site ($F_{2, 29} = 5.08$, $p = 0.013$). The number of species recorded was highest at The Ridge (9.2 ± 1.3 m⁻²) and it was significantly higher than Sampela (5.3 ± 0.5 m⁻²) ($p = 0.14$) but not significantly higher than B1 (6.2 ± 0.8 m⁻²). There was no difference in the number of species observed at Sampela and B1. When sponges were ranked by abundance, different species were dominant at each site (Table 2.6, A-C). The sponge assemblage at Sampela was dominated by the encrusting phototrophic sponge *Lamellodysedia herbacea* (39.9%), the giant barrel sponge *Xestospongia* spp. (24.0%) and the encrusting orange sponge *Protosuberites* sp. (10.5%) (Table 2.6, B). At B1, the sponge assemblage was dominated by the massive sponge *Agelas clathrodes* (30%), the giant barrel sponge *Xestospongia* spp. (14.4%) and the tubular sponge *Theonella swinhoei* (13.9%) (Table 2.6, A). The sponge assemblage at The Ridge was dominated by the encrusting sponge *Chalinula milnei* (37.5%), the tubular *Callyspongia aerizusa* (14.4%) and *Verongula* sp. (9.6%) (Table 2.6, C).

2.4.5.2 Feeding observations

Pygoplites diacanthus positively selected sponges at Sampela ($w_i = 5.15$, $CI = 2.57 - 7.73$) and the Ridge ($w_i = 5.51$, $CI = 2.63 - 8.4$) but not at B1 ($CI < 1$). A preference for *Xestospongia* spp. was indicated by *P. diacanthus* at all sites (Table 2.6, A-C). It also appeared to prefer some species of *Clathria* (Figure 2.6, D&F), *Petrosia* (Figure 2.6, G&H) and *Sphaciospongia* sp. (Figure 2.6B). *Zanclus cornutus* positively selected sponges at the Ridge ($w_i = 5.42$, $CI = 1.85 - 8.99$) but not at Sampela or B1 ($CI < 1$).

At B1, *Z. cornutus* spent the majority of time grazing on rock surfaces under overhangs where it was difficult to determine substrate type. At Sampela, most of the fish observed took only a few bites from a substrate type before moving to another area however, two were engaged in focused feeding on *Xestospongia* spp. with one individual recording 83 bites in the three-minute observation period. At The Ridge, *Z. cornutus* demonstrated a preference for sponges from the genus *Petrosia* and *Xestospongia* spp. (Figure 2.6, E) (Table 2.6, A-C). *C. melanopus* exhibited significant dietary selectivity for sponges at B1 ($w_i = 7.60$, CI = 7.39 - 7.81). The six individuals observed fed almost exclusively from *Xestospongia* spp. (Table 2.6, A) and only two bites recorded on rock and one on hard coral.

Table 2. 6 Relative abundance (% cover) of sponge species and percent bites taken by three species of sponge-grazing fishes at three sites (B1, Sampela and The Ridge) in the Wakatobi MNP. Sponges were considered preferred if the percentage of bites > percentage abundance + 5% and avoided if the percentage of bites < percentage abundance - 5%.

A) B1		Abundance (%)	Bites (%)			
Rank	Species		<i>P. diacanthus</i>	<i>Z. cornutus</i>	<i>C. melanopus</i>	
1	<i>Agelas clathrodes</i>	30.0	12.7	0	0	Avoided by all
2	<i>Xestospongia</i> spp.	14.4	32.4	0	100	Preferred by <i>C. melanopus</i> and <i>P. diacanthus</i>
3	<i>Theonella swinhoei</i>	13.9	1.4	0	0	Avoided by all
4	<i>Aaptos suberitoides</i>	10.7	0	0	0	Avoided by all
5	<i>Protosuberites</i> sp.	6.2	0	0	0	Avoided by all
6	<i>Chalinula milnei</i>	5.9	0	0	0	Avoided by all
7	<i>Lamellodysedia herbacea</i>	5.1	0	0	0	Avoided by all
8	<i>Niphates</i> sp.	3.5	0	0	0	
9	<i>Clathria</i> sp.1	2.8	0	6.7	0	
10	<i>Aaptos</i> sp.	1.7	0	0	0	Avoided by all
23	<i>Callyspongia aerizusa</i>	NR	2.8	0	0	
24	<i>Petrosia corticata</i>	NR	1.4	0	0	
25	<i>Clathria reinwardtii</i>	NR	43.7	0	0	Preferred by <i>P. diacanthus</i>
Total bites			71	15	280	
Fish biting sponges/total observed			5/9	5/9	6/6	
Percentage of bites on unidentified sponges			5.6	93.3		

B) Sampela		Bites (%)			
Rank	Sponge species	Abundance (%)	<i>P. diacanthus</i>	<i>Z. cornutus</i>	
1	<i>Lamellodysedia herbacea</i>	39.9	0	1	Avoided by both
2	<i>Xestospongia</i> spp.	24.0	59	75.1	Preferred by both
3	<i>Protosuberites</i> sp.	10.5	0	0	Avoided by both
4	<i>Hyrtios erecta</i>	6.9	5.0	0	Avoided by <i>Z. cornutus</i>
5	<i>Axinyssa</i> sp.	3.5	0	0	
6	<i>Myrmekioderma granulatum</i>	2.9	0	0	
7	<i>Clathria</i> sp.1	2.5	9	3.6	Preferred by <i>P. diacanthus</i>
8	Unknown massive sponge	1.9	0	0	
9	Orange encrusting sp.2	1.5	0	0	
10	Pink branching sp.2	1.3	0	0	
19	<i>Acanthella cavernosa</i>	0.2	0	2.1	
22	<i>Spheciospongia</i> sp.	NR	8	0	Preferred by <i>P. diacanthus</i>
Total bites			200	193	
Fish biting sponges/total observed			13/17	8/20	
Percentage of bites on unidentified sponges			19	18.2	

C) The Ridge		Bites (%)			
Rank	Species	Abundance (%)	<i>P. diacanthus</i>	<i>Z. cornutus</i>	
1	<i>Chalinula milnei</i>	37.5	0	0	Avoided by both
2	<i>Callyspongia aerizusa</i>	14.4	2.9	2.7	Avoided by both
3	<i>Verongula</i> sp.	9.6	0	0	Avoided by both
4	<i>Clathria</i> sp. 1	7.8	0	0	Avoided by both
5	Black encrusting sp. 2	6.0	0	0	Avoided by both
6	<i>Hyrtios erecta</i>	3.3	0	0	
7	<i>Melophlus sarasinorum</i>	2.7	5.7	0	
8	White spikey branching	2.0	0	0	
9	Brown unknown	1.6	0	0	
10	<i>Lamellodysedia herbacea</i>	1.5	0	0	
48	<i>Xestospongia</i> spp.	NR	7.6	62.2	Preferred by both
49	<i>Petrosia corticata</i>	NR	31.4	13.5	Preferred by both
50	<i>Petrosia</i> sp.	NR	26.7	21.6	Preferred by both
Total bites			105	74	
Fish biting sponges/total observed			6/6	4/4	
Percentage of bites on unidentified sponges			25.7	0	

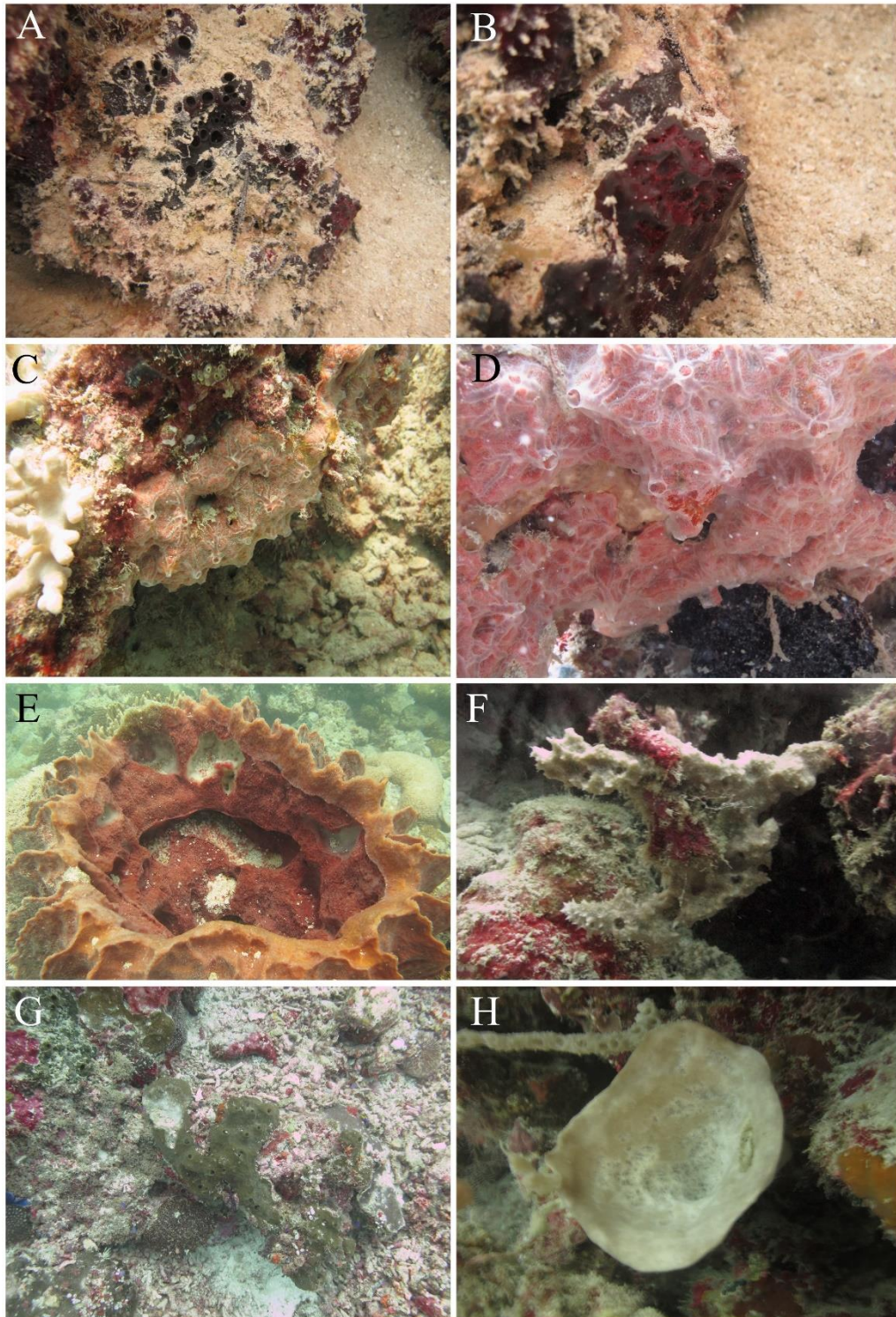


Figure 2. 6 A. *Spheciospongia* sp. at Sampela. B. Close up of bites on *Spheciospongia* sp. made by *P. diacanthus*. C. *Clathria* sp. at Sampela. D. Close up of bites on *Clathria* sp. made by *P. diacanthus*. E. *Xestospongia* spp. at Sampela predated by *P. diacanthus* and *Z. cornutus*. F. *Clathria reinwardtii* at B1 predated by *P. diacanthus*. G. Exposed inner tissue of *Petrosia corticata* at the Ridge, predated by *P. diacanthus* and *Z. cornutus*. H. *Petrosia* sp. at the Ridge, predated by *P. diacanthus* and *Z. cornutus*.

2.5 Discussion

Diet characterisation is fundamental to understand the range of functional roles played by reef fishes (Leray et al. 2015) and this information is essential to preserve key ecosystem functions on transitioning coral reefs (Bellwood et al. 2019). The present study provides the first reef-wide estimate of sponge biomass consumption on coral reefs. Five spongivorous fishes were identified through a combination of video observations and gut content analysis and were estimated to consume 46.6 ± 18.3 g sponge $1000\text{ m}^{-2}\text{ day}^{-1}$. Sponges dominated the diet of the angelfish *Pygoplites diacanthus* which had the greatest predatory impact on sponges. Estimates provided here are conservative. Nevertheless, predation of this magnitude may have sublethal indirect impacts on sponges such as reduced fecundity and physiological condition.

2.5.1 Sponge consumption

Quantitative data on the role of sponges in the diets of sponge-grazing fishes combined with density data made it possible to estimate sponge consumption at each location and provide reef-wide estimates of sponge biomass consumption. Considering that these are the first known estimates of reef-wide sponge consumption, comparisons with studies from other regions are not possible. However, following the same methodology as Harmelin-Vivien & Bouchon-Navarro (1983) allows for a comparison with coral consumption in the Central Pacific. The maximum coral consumption reported was 62.5 g coral $1000\text{ m}^{-2}\text{ day}^{-1}$ at the outer barrier reef slope (Harmelin-Vivien & Bouchon-Navarro, 1983), which is similar to the estimates of daily sponge consumption reported for the outer reef sites in this study (mean 52.9 g – 73.3 g sponge 1000 m^{-2}). This suggests that in the Indo-Pacific, biomass transferred from sponges to spongivores is of a similar magnitude to biomass transferred through the coral - corallivore pathway, although this comparison is limited by large geographic differences. Nevertheless, data presented in this chapter suggests that spongivory may be an important but relatively overlooked trophic link on Wakatobi reefs.

Although biomass transferred from the benthos to upper trophic levels via the processes of corallivory and spongivory appears to be similar, there appear to be many more species of corallivorous fishes than there are spongivorous fishes. Cole et al (2008) compiled the literature on corallivorous fishes, documenting 128 corallivores, 41 of which were considered obligates. By contrast, approximately 50 species have been documented as spongivores (Hiatt & Strasbourg, 1960; Randall & Hartman, 1968; Hobson, 1974; Clements & Livingstone, 1983; Sano, 1989; Wulff, 1994; Dunlap & Pawlik, 1996; Wulff, 1997; Pitt, 1997; Dunlap & Pawlik, 1998; Eagle & Jones, 2004; Nagelkerken et al. 2009; Verdín Padilla et al. 2010; Hoey et al. 2013) with only 13 of these considered obligates (Hiatt & Strasbourg 1960; Randall & Hartman, 1968; Hobson, 1974; Hourigan, 1989; Dunlap & Pawlik 1996; Verdín Padilla et al. 2010). Whilst these differences are undoubtedly exacerbated by the more extensive research that has taken place regarding the functional process of corallivory (Pratchett et al. 2005; Cole

et al. 2008; Cole et al. 2011), the family associated with sponge feeding (Pomacanthidae) has fewer species than the Chaetodontidae family associated with coral feeding (86 species and 128 species respectively (Konow & Ferry-Graham, 2013). Additionally, the most voracious sponge predator recorded during this study, *Pygoplites diacanthus*, is monotypic (Coleman et al. 2016). Hence, it seems likely that the coral-corallivore pathway supports a higher diversity of fish species than the sponge-spongivore pathway.

2.5.2 Obligate spongivores

Angelfishes are well-known spongivores in the Caribbean and the Pacific (Randall & Hartman, 1968; Hobson, 1974; Verdín Padilla et al. 2010), with at least five species thought to rely on sponges for the majority of their diet (Randall & Hartman, 1968; Hobson, 1974). The present study confirms that *Pygoplites diacanthus* is also reliant on sponges as stomach contents contained >80% sponge. These findings are consistent with previous observations of feeding behaviour (Powell et al. 2015). Diet breadth was very low for this species, indicating a high degree of trophic specialisation. Scaling up from the individual to the reef-wide level, *P. diacanthus* has the largest predatory impact, accounting for almost half of the total sponge consumption. The one *Pomacanthus imperator* stomach dissected contained >90% sponge suggesting a similar reliance on sponges to *P. diacanthus*. However, the small sample size means that some uncertainty still surrounds the extent of sponge predation by *P. imperator*.

2.5.3 Facultative spongivores

Sponges were the dominant dietary item for *Zanclus cornutus*, although its diet also consisted of algae and other sessile invertebrates, including bryozoans and ascidians. This species had a slightly lower predatory impact than *P. diacanthus*, consuming an estimated 12.2 ± 3.1 g sponge $1000\text{ m}^{-2}\text{ day}^{-1}$ (mean \pm CI), which is likely to be an underestimate, as high feeding rates (approximately 10 bites min^{-1}) suggests that they likely fill their stomach more than twice daily. *Z. cornutus* is found in a substantial range of habitats, from intertidal mangroves (pers. obs) to mesophotic depths (Wagner et al. 2014). There is also evidence that *Z. cornutus* is expanding into temperate waters, possibly as a result of warming (Seo & Lee 2009; Fernández-Rivera Melo et al. 2018). An expansive range coupled with generalist foraging indicates that *Z. cornutus* could be relatively resilient to habitat disturbances.

Gut content data also confirmed *Pomacanthus xanthurus* was a spongivore, but it appeared to have a lower reliance on sponges than *P. diacanthus*, consuming an equal amount of predominantly *Dendronephthya* soft corals with one stomach containing three juvenile starfish. The rabbitfish *Siganus punctatus* also regularly consumed sponges, which were found in the gut contents of all sampled individuals, although in widely varying amounts (2-76%). Similar feeding behaviour has been recorded in the GBR where sponges were the dominant category in juvenile *S. punctatus* and represented ~20% of the diets of adults (Pitt, 1997). It was also noted by Sano (1989) in Japan where sponge was found to constitute 72% of *S. punctatus* gut contents (Sano, 1989). Data presented here indicate that sponges and

the red algae *Laurencia* spp. made up the majority of the diet of adult *S. punctatus* on Wakatobi reefs. A study looking at the diet and distribution of rabbitfishes on the Great Barrier Reef also found that the stomach contents of the rabbitfish *Siganus puellus* was dominated by sponges, suggesting that the diet of some Siganids is more varied than previously recognised (Hoey et al. 2013).

2.5.4 Indirect interactions and sponge as a minor prey item

Butterflyfishes are generally associated with coral feeding, although only 69 out of 128 species are known to directly consume Scleractinian corals (Cole et al. 2008; Konow & Ferry-Graham, 2013) with numerous other prey items reported (Sano, 1989; Nagelkerken et al. 2009). Despite recording >7500 bites on *Xestospongia* spp. sponge tissue remains were not found in *Chaetodon kleinii* stomach contents, although spicules were sighted in 9/12 stomachs. Similarly, *Forcipiger flavissimus* recorded >1500 bites on *Xestospongia* spp. but out of 11 stomachs analysed, only two contained sponge tissue remains both amounting to ~2% of total contents. *Coradion melanopus* was one of the top sponge-grazing fishes identified in video footage despite being found in extremely low densities on Wakatobi reefs. *In situ* feeding observations pointed towards extremely selective feeding on barrel sponges however gut content samples were not collected due to small population size. Other studies in the Indo-Pacific have found small volumes of sponge (<10% volume) in both *C. kleinii* and *F. flavissimus* (Sano, 1989; Nagelkerken et al. 2009) and 18% sponge in the *C. melanopus* congener *C. altivelis* suggesting that sponges are either a minor prey item or incidentally ingested in the pursuit of sponge-associated macrofauna (Nagelkerken et al. 2009). Observations of the internal tissue of *Xestospongia* spp. in the Wakatobi MNP have shown that they are infested with an unidentified white polychaete (family Syllidae) (mean 579 ± 44 g⁻¹ [DW]) (C.M, unpublished data). The digestive tracts of polychaetes found in the cyanobacterial layer of *Xestospongia* spp. were brown/red whilst those retrieved from the inner tissue were white (pers. obs) suggesting that these polychaetes consume host tissue. Spongivorous polychaetes (e.g. *Haplosyllis spongicola* and *Branchiosyllis oculata*) can directly penetrate sponge tissues where canals are small (Martin & Britayev, 1998) and have been found in extremely high densities on both the surface and inside sponges (Pawlik, 1983). This may explain the high grazing rates by Chaetodontids and other invertivores recorded in video footage of *Xestospongia* spp.

Ctenochaetus binotatus likely lacks the necessary dental morphology to remove sponge biomass and appears to ‘brush’ the surface of the sponge with comb-like teeth (Purcell & Bellwood, 1993). Members of this genus are generally considered detritivores (Choat, 2002) however, other studies have indicated that *Ctenochaetus striatus* can also remove large quantities of algal turf (Marshall & Mumby, 2012). Gut content data presented in this chapter support the conclusion that *C. binotatus* is a detritivore as gut contents were dominated by sediments and detritus (defined as opaque flocculent material) (Wilson et al. 2003). Sponge-grazing detritivores may target mucus-bound sediment on sponge surfaces, as some sponges including *Xestospongia* spp. produce mucus as a sediment clearing mechanism (McGrath et al.

2017). Although there have been no published studies regarding the energy content of sponge mucus, coral mucus is known to be energy and nutrient-rich (Wild et al. 2004) hence it is likely that sponge mucus provides similar nutritional benefits. In the only published study of the trophic role of sponge mucus, the sponge-associated holothuroid *Synaptula lamperti* were found to ingest mucus directly from the sponge *Ianthella basta* and incorporate metabolised compounds into body wall tissues (Hammond & Wilkinson, 1985). Hammond & Wilkinson (1985) concluded that sponge mucus represents a new trophic pathway involving sponges not previously identified. The feeding behaviour of detritivorous fishes could also be explained by the ‘sponge loop’ whereby sponges assimilate DOC and return it to the reef as cellular detritus that is readily ingested by sponge-associated detritivorous invertebrates (de Goeij et al. 2013; Rix et al. 2018). Both pathways suggest that sponges mediate a variety of trophic interactions beyond biomass removal via predation by spongivores.

2.5.6 Preferred sponges

To infer preference, relative abundance needs to be quantified to distinguish between being preferred by spongivores or the most abundant in the field (Wulff, 2012). I used percentage cover to quantify sponge abundance and hence infer preference from visual observations of feeding behaviour. However, methods that estimate abundance using different metrics such as volume or biomass are known to give different results (Wilkinson, 1987). Where sponge assemblages are dominated by tubular or erect sponges, volume may be the most appropriate metric as percentage cover would underestimate their contribution (Wulff, 2001). However, estimating sponge volume is time-intensive, particularly on reefs like those in the Wakatobi MNP that host sponge assemblages with diverse morphologies (Bell et al. 2017). Hence, the divided quadrat was chosen to capture larger morphologies that would be missed using smaller quadrats but also with the resolution necessary to capture encrusting species.

Feeding observations indicated that *Clathria* spp. and *Spheciospongia* sp. are preferred by *Pygoplites diacanthus* and sponges from the Petrosiidae family including *Xestospongia* spp., *Petrosia* sp. and *Petrosia corticata* are preferred by both *P. diacanthus* and *Zanclus cornutus*. *Clathria* spp. were considered unpalatable to spongivorous starfishes in Caribbean (Wulff, 1995) and Antarctic waters (Peters et al. 2009) however small volumes (<2%) were found in the stomach contents of spongivorous angelfishes (Randall & Hartman, 1968). Similarly, angelfishes have been recorded with *Spheciospongia vesparium* in gut contents (Randall & Hartman, 1968) and it was also predated by starfish in feeding choice experiments (Wulff, 1995). Petrosiidae sponges have also been recorded in spongivorous fish gut contents (Randall & Hartman, 1968), although only in one Caribbean study. During *in situ* data collection, *Z. cornutus* and *P. diacanthus* were often seen predated the same sponge and exhibit competitive behaviours. Often, one would chase the other away from the sponge and take numerous bites (frequently >15) before the other would return and exhibit the same behaviour. This is somewhat contrary to the ‘smorgasbord’ feeding behaviour described from the Caribbean where angelfishes take

relatively few bites from the same open reef species before moving on to another food item (Randall & Hartman, 1968; Wulff, 1994). It could be explained by the densities of cyanobacteria in the outer tissues of *Xestospongia* spp. and *Petrosia corticata*, as repetitive feeding on exposed inner tissue (Appendix A), could be nutritionally beneficial (Duffy & Paul, 1992) and contain a lower concentration of chemical defences than the outer tissue layer. Some studies have supported the optimal defence theory (Becerro et al. 1998; Schupp et al. 1999), which suggests that chemical defences are concentrated to high-value areas eg. reproductive tissues or regions most at risk of predation (Rhoades & Cates, 1976; Swearingen & Pawlik, 1988). However, not all studies support this hypothesis, with some finding no difference in deterrent properties between extracts from the inner and outer tissue layers of sponges (Swearingen & Pawlik, 1998; Burns et al. 2003).

2.5.7 Limitations of sponge consumption estimates

Estimates provided in this thesis are conservatively based on stomach contents rather than total gut contents and on the assumption that fish fill their stomachs twice daily. Coral material has been estimated to pass through the intestinal tract of the butterflyfish *Chaetodon unimaculatus* between 1 ½ and 2 hours, indicating that stomach may be filled at least six times daily (Cox, 1986; Cole et al. 2011). Considering that reported gut length to body length ratios for *Chaetodon unimaculatus* (Berumen et al. 2011) and *Pomacanthus* spp. (Pérez-España & Abitia-Cárdenas, 1995) are similar, it seems likely that spongivores may also fill their stomachs up to six times daily. Additionally, there are likely other spongivorous fishes who were not included in the estimates of sponge consumption (e.g. triggerfishes, filefishes and pufferfishes), and the functional roles of some prevalent sponge-grazing fishes remain ambiguous due to lack of gut content data. Four species of *Centropyge* (*bicolor*, *nox*, *tibicen*, *vroliki*) were recorded taking numerous bites from *Xestospongia* spp. and Powell et al. (2015) also recorded similar feeding behaviour in the Wakatobi MNP. Sponge was recorded as the dominant dietary item (>40% by volume) in the stomach of *C. vroliki* (Eagle & Jones, 2004) and morphological studies indicate that *Centropyge* spp. are adapted to feed on attached colonial invertebrates (Konow & Bellwood, 2011). However, other studies list *Centropyge* as epilithic algal grazers that are important and numerous herbivores (Green & Bellwood, 2008). Considering the high abundances of *Centropyge* spp. at the sponge-dominated site, future studies should prioritise the gut content analysis of these species to resolve their potential impact on sponge assemblages. Similarly, hawksbill turtles were not considered by this study due to low abundances on Wakatobi reefs (only one hawksbill turtle was sighted during the three-month study period). Hence their predatory impact on sponges in the Hoga/Kaledupa channel was likely negligible.

2.5.8 Conclusion

The study provides the first known estimate of sponge consumption by spongivorous fishes on coral reefs. Results presented here suggest that sponge consumption in the Wakatobi, particularly by the angelfish *Pygoplites diacanthus* and the Moorish Idol *Zanclus cornutus*, was considerable and comparable to estimates of coral consumption in the Central Pacific. By combining visual techniques and gut content analysis, I have addressed previous functional misclassifications and shown that both methods used in tandem provide more detail on the specific diets of sponge-grazing fishes.

Chapter 3. DNA analysis of angelfish stomach contents reveals the diversity of predated sponges

3.1 Abstract

Accurately defining predator-prey relationships is essential to understand the strength of predation as a top-down force in ecosystems. Angelfishes are one of only a few families of spongivorous fishes found on coral reefs. Whilst extensive work on spongivore feeding preference has been conducted in the Caribbean, where angelfishes have a taxonomically wide diet and consume mainly chemically undefended sponges, little is known about sponges predated by angelfishes in the Indo-Pacific. This information is essential to understand the functional role of spongivory on Indo-Pacific reefs. In Chapter 2, sponges were quantified from the stomach contents of three species of Indo-Pacific angelfish (*Pygoplites diacanthus*, *Pomacanthus imperator* and *Pomacanthus xanthometopon*) but could not be visually identified beyond the phylum level. In studies where visual prey identification is problematic, molecular methods of diet tracing can improve the taxonomic resolution of prey. In this chapter, I developed genetic methods that allowed the identification of predated sponges from the stomach contents of spongivorous angelfishes. A range of primers and associated predator-blocking primers targeting the 18S rDNA gene were designed and tested on extracts of sponge and spongivore DNA. One primer set was the most effective at blocking the amplification of spongivore DNA, and sequences were successfully obtained from 14 sponges extracted from stomach contents. Phylogenetic analysis indicated that these sponges spanned six orders of Porifera, with the majority identified in the Order Haplosclerida. There was strong support for the placement of one sponge within a clade containing the Indo-Pacific species *Chalinula hooperi* and another within a clade with sponges from the genus *Petrosia*. Three sponges were categorised as the order Verongiida due to their placement within a clade containing *Pseudoceratina* and *Verongula* sponges. One sponge belonged to a clade made up of Clionaidae and Spirastrellidae sponges, and others were placed into clades containing sponges from the genera *Aaptos* and *Guitarra*, and the family Agelasidae. Results indicated that *Pygoplites diacanthus* had a wide diet within the Porifera phylum and consumed sponges with substantial chemical defences. This study is the first to successfully sequence sponges from the stomach contents of spongivorous angelfishes and has identified several sponges previously unknown to be predated in the Indo-Pacific.

3.2 Introduction

Predators are vitally important in ecosystems as they generate top-down forces that enhance ecosystem stability (Paine, 1966; Paine, 1969). Yet not all predators are functionally equivalent, and in-depth knowledge of a predator's diet can elucidate its ecological function (Leray et al. 2015). In marine systems, visual approaches to diet characterisation, such as diver led surveys, present practical difficulties (e.g. time and depth limits), and the presence of divers can alter fish behaviour (Emslie et al. 2018). By contrast, gut content analysis provides an unambiguous record of ingested prey (Wulff, 2006) but it is biased towards structural components and taxonomic resolution can be hindered by digestion (Nielsen et al. 2018). Over the past decade, molecular methods have emerged as a valuable tool to overcome some of the challenges associated with diet identification (Nielsen et al. 2018). Polymerase chain reaction (PCR) can detect small amounts of DNA, providing greater resolution to diets that are hard to visually characterise because they lack hard parts, are heavily degraded, or are liquid remains (Symondson, 2002; Vestheim & Jarman, 2008; Nielsen et al. 2018). However, using molecular methods to identify prey items from gut content samples also presents a range of complications due to digestive processes (Vestheim & Jarman, 2008).

Gut content DNA can be degraded and fragmented by digestion (Deagle et al. 2009), which may constrain the study to target only short sequence lengths (Vestheim & Jarman, 2008). Short sequence lengths often have fewer appropriate binding sites and can impact taxonomic resolution, which also depends on the sequence coverage of reference databases (O'Rourke et al. 2012). DNA extraction and PCR can also be inhibited by substances found in digestive tracts (Schrader et al. 2012). Gut contents contain a mixed profile of samples and universal primers that target a broad range of organisms may also amplify predator DNA, which is often found in excess and preferentially amplified over degraded prey DNA (Vestheim & Jarman, 2008). Group-specific primers can overcome the problem of predator amplification and have been successfully applied in many studies (Hoss et al. 1992; Jarman et al. 2004), particularly in cases where the group-specific primer is exclusive to an entire kingdom (e.g. plants used to characterise the diet of a herbivore; Jarman et al. 2004). When group-specific primers are not an option, methods that block predator DNA from amplification, including the use of restriction enzymes (Dunsha et al. 2009), PNA clamps (Chow et al. 2010), and predator-blocking primers (Vestheim & Jarman, 2008; Leray et al. 2013; Leray et al. 2015), have all been used successfully, resolving the diets of marine animals including Australian fur seals (Deagle et al. 2009), corallivorous fishes (Leray et al. 2013; Leray et al. 2015) and the spiny lobster (Chow et al. 2010).

Tropical reefs experience greater predation pressure than other marine ecosystems (Paine, 1966) and tropical sponges differ from their temperate counterparts because they encounter significant predation from vertebrate predators (turtles and fish) in addition to invertebrates (Randall & Hartman, 1968; Meylan, 1988; Wulff, 2006). Sponges exhibit life-history strategies, and structural and chemical

defences to deter predation (Hay, 1996; McClintock et al. 2005; Hill et al. 2005), but they are thought to rely mainly on secondary chemistry (Chanas & Pawlik, 1995; Pawlik, 2011). Obligate spongivores can be defined as species that depend on sponges for $\geq 80\%$ of their diet (Cole et al. 2008; Chapter 2), with the majority belonging to the Pomacanthidae family (Randall & Hartman, 1968). Obligate spongivores are thought to reduce the impact of chemical substances by adopting a ‘smorgasbord’ approach to foraging (Randall & Hartman, 1968), as using a wide variety of prey items may reduce the effect of secondary compounds (Wulff, 1994). Evidence for generalist foraging on sponges was reported in early studies of sponge predation in the Caribbean, which cited >40 species of sponge in the gut contents of 24 *Holacanthus ciliaris* specimens and >20 species from other spongivorous angelfishes (Randall & Hartman, 1968). Similarly, in the Mexican Pacific, angelfishes were reported to feed on 46% of the available sponge species (Verdín Padilla et al. 2010) and in Brazil, 34 species were found in the gut contents of three angelfish predators (Andrea et al. 2007). This foraging strategy is not thought to lead to significant sponge mortality as typically only part of the sponge is consumed (Wulff, 1994), and targeted sponges are often found in abundance on reefs (Wulff, 2006; Pawlik, 2018).

To identify palatable sponges and those with significant deterrent properties, some researchers have conducted feeding choice experiments using pelletised extractions of secondary metabolites (Pawlik et al. 1995; Becerro et al. 2003; Peters et al. 2009). Secondary metabolites are extracted from sponges and added to artificial diet cubes, which are then offered to generalist fishes alongside control pellets to determine predator deterrence (Pawlik et al. 1995). Feeding choice experiments conducted in the Caribbean revealed that species of *Agelas*, *Amphimedon*, *Aplysina* and *Ectyoplasia* had highly deterrent properties whereas species of *Callyspongia*, *Niphates* and *Iotrochota* were palatable to generalist fishes (Pawlik et al. 1995; Pawlik, 1997). Results of the feeding choice experiments were applied to data on sponges consumed by spongivorous angelfishes in a study by Randall and Hartman (1968), indicating that the majority of sponges consumed by Caribbean angelfishes were chemically undefended (Pawlik, 2011). These observations and others (see Pawlik, 2011 for review) led to the theory that sponges could be partitioned into three ecologically meaningful groups: (1) defended, open reef sponges; (2) undefended but tolerant, palatable sponges; and (3) palatable species that are restricted to cryptic habitats (Pawlik, 2011). However, assessing palatability using pellet assays is not always reflective of ecological reality (Lesser & Slattery 2013; Wulff, 2017), as there can be extreme differences in palatability between spongivores (Wulff, 2006). For instance, Hawksbill turtles and angelfishes have different diet preferences (Wulff, 2012). Similarly, using a generalist wrasse to infer the broad effectiveness of defensive compounds ignores the fact that specialist spongivores (e.g. angelfishes) can exhibit a higher tolerance for chemical defences (Schupp et al. 1999). For example, crude organic extracts from the Micronesian sponge *Oceanapia* sp. deterred feeding by the spongivore *Pomacanthus imperator* but the deterrent effect was less pronounced than that observed from generalist reef fishes (Schupp et al. 1999). In addition, defences are not always ‘static’; for example, Slattery et al. (2016)

showed that the Caribbean reef sponge *Plakortis angulospiculatus* could increase defensive compounds when transplanted to an area of higher predation.

Only one published study has explicitly considered the role of sponge predators on Indo-Pacific reefs (Powell et al. 2015), revealing that *Hyrtios erectus* (Keller, 1889), *Jaspis splendens* (de Laubenfels, 1954) and *Xestospongia testudinaria* (Lamarck, 1815) were predated by the spongivores *Pygoplites diacanthus*, *Pomacanthus sexstriatus* and *Zanclus cornutus*, respectively (Powell et al. 2015). Other studies have identified sponges as a dietary item for reef fishes using visual observations of feeding behaviour and gut content analysis (Sano, 1989; Eagle & Jones, 2004; Nagelkerken et al. 2009), but no information on sponge species identity was provided. In Chapter 2, *in situ* observations identified an additional 12 sponges that were predated by the spongivores *P. diacanthus* and *Z. cornutus*, with *Clathria*, *Spheciospongia* and Petrosiidae sponges preferred by *P. diacanthus*. However, the full dietary range was difficult to characterise visually because spongivorous fishes are often found at depths exceeding safe diving limits, forage in cryptic habitats and change their behaviour in the presence of divers (pers. obs). In Chapter 2, sponges from the gut contents of three angelfish predators (*P. diacanthus*, *Pomacanthus imperator* and *Pomacanthus xanthurus*) were quantified, but taxonomic resolution beyond the phylum level was limited by the lack of reliable field guides and species-rich environment (Bell & Smith, 2004; Rovellini et al. 2019). Additionally, homogenisation of sponges within the digestive tract hampered visual identification using spicule morphology. To address these problems, and improve the taxonomic resolution of predated sponges, I developed molecular methods to identify predated sponges from the gut contents of spongivorous angelfish from the Indo-Pacific. My specific aims for this chapter were: (i) to test 6 primer sets using DNA extractions from gut content sponges; and (ii) to identify generated sequences to the lowest possible taxonomic level using phylogenetic analysis.

3.3 Methods

3.3.1 Sample collection

Gut content analysis of the Indo-Pacific angelfishes *Pygoplites diacanthus*, *Pomacanthus imperator* and *Pomacanthus xanthometopon* was performed in Chapter 2 as part of a wider study of sponge-grazing fishes. *P. imperator* (n = 1) was bought opportunistically from local fishers at Sampela reef and *P. xanthometopon* was bought opportunistically from local fishers at a site known locally as The Ridge (see Chapter 2, Figure 2.1). *P. diacanthus* was caught by local spearfishers at both Sampela (n = 7) and The Ridge (n = 6). Fish were dissected and stomachs stored in 96% ethanol at -20°C and returned to Wellington for dissection (see Chapter 2 for detail). During gut content analysis, sponges present in the stomachs of spongivorous fishes were visually separated by colour and location in the stomach. Samples of each sponge were retained and stored in 96% ethanol at -20°C in sterile microcentrifuge tubes. All instruments and dissecting tools were ethanol cleaned and rinsed three times with double distilled water (DDW) between stomachs to prevent cross-contamination.

3.3.2 Initial screening of primers

Six primer sets were tested on a subset of 18 sponge samples recovered from six individual *Pygoplites diacanthus* stomachs, with success determined by visualisation on 1% agarose gels. Total genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen) following the protocol of the manufacturer. PCR was conducted in 20 µl reactions consisting of 10 µl MyTaq Red Mix (Bioline), 0.4 µl of each primer (10 µM), ~25 ng of template DNA and a volume of distilled water to reach 20 µl. PCR cycling conditions were: 94°C for 5 minutes; then 35 cycles of 94°C for 30 seconds, 58°C for 55 seconds (COI, COI ext, rnl, ITS2) or 50°C for 55 seconds (18S, 28S), 72°C for 45 seconds and a final extension step of 72°C for 7 minutes. The 18S primer set (Knapp et al. 2015) was the most successful, producing a single clear band of expected length for 13/18 samples. PCR using primers to amplify 28S (Baroin et al. 1988) produced single faint bands for 6/13 samples; no results could be visualised using the primers for COI (Folmer et al. 1994) or COI-ext (Rot et al. 2006); and multiple bands were present when amplifying ITS2 (Adlard & Lester, 1995) and rnl (Lavrov et al. 2008). Successful amplifications using the 18S primer set were sent for purification and sequencing on an ABI 3730 capillary sequencer (Macrogen, South Korea). Generated sequences were run through BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), which verified three samples as >97% similar to sponge sequences held in the database and two samples that were verified as Poriferan, but with considerable uncertainty in identity. Two further samples were identified as crinoid and the remaining six samples were identified as the predator, *P. diacanthus*. 18S is a popular gene used in the amplification and identification of metazoans because it is highly conserved among taxa (O'Rourke et al. 2012). Closer inspection of the 18S primer set revealed that the forward primer was conserved between fish and sponges and the reverse primer had only two mismatches near the end of the primer sequence. Due to

the lack of specificity between fish and sponge, the 18S primers used here were not effective in preventing the amplification of predator DNA, which is often preferentially targeted over degraded prey DNA (Vestheim & Jarman, 2008).

3.3.3 Primer design for blocking predator DNA

All available 18S sequence data for marine sponges were downloaded from the NCBI database using the search terms “18S ribosomal RNA gene”, “Demospongiae” and “Marine”. This yielded >100 sequences that were at least 1725 base pairs (bp) long, which were compiled alongside 18S sequence data that were available for three spongivorous angelfishes, *P. diacanthus*, *P. imperator* and *P. xanathometopon* (Shen & Hsiao, 2016). Sequences were aligned using the ClustalW function in BioEdit version 7.0.5.3 (Hall et al. 1999) and the alignment was searched for sponge-specific primer binding sites that would prevent the amplification of predator DNA. No variable regions of appropriate length (e.g. ~20 bp) for primer design were found. Subsequently, I explored the use of predator-blocking primers which are modified so that they will not prime amplification and can be an effective method for inhibiting the amplification of undesired DNA (Vestheim & Jarman, 2008; O’Rourke et al. 2012). Aligned sequence data and entropy plots were used to identify (1) primer binding sites that contained a conserved region across both sponge and predator DNA (hereafter the universal primer binding site) that flanked a (2) variable region with at least 4 or 5 mismatches between fish and sponge sequences (hereafter the blocking primer binding site) (Figure 3.1). This allowed for a predator-specific blocking primer to overlap with the 3’ end of the universal primer binding site but extend into a predator specific sequence (Vestheim & Jarman, 2008). The predator-blocking primer is then modified with a C3 spacer attached to the terminal 3’ hydroxyl group of the oligonucleotide that prevents elongation during PCR (Vestheim & Jarman, 2008; O’Rourke et al. 2012).

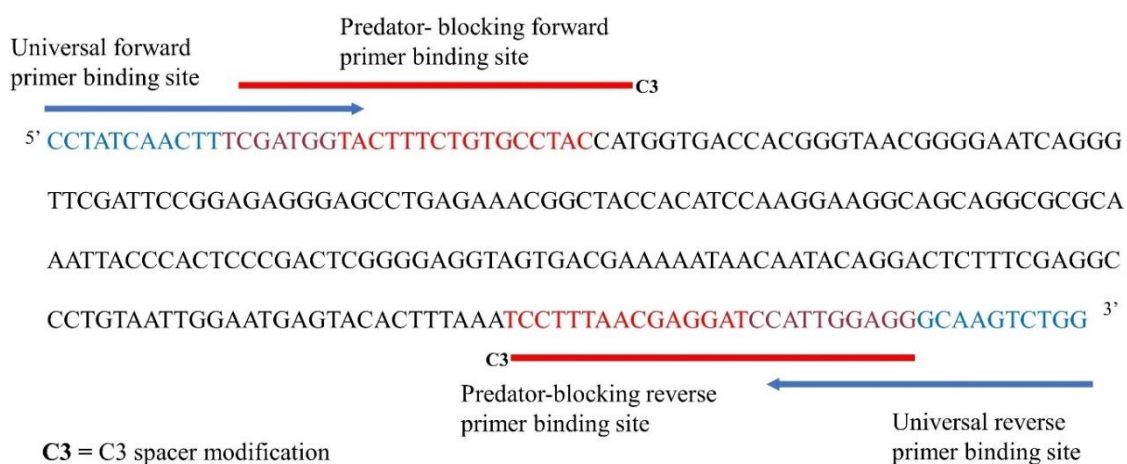


Figure 3. 1 A 255 bp region of *Pygoplites diacanthus* 18S sequence showing the location of primer set 3 (Table 3.2) designed and trialled in this study. C3 denotes C3 spacer modification that prevents elongation during PCR.

Primers that target short sequences of multi-copy DNA are likely to be effective in amplification of DNA degraded by digestion (Vestheim & Jarman, 2008), however short sequences limit the taxonomic resolution of the output, particularly with the 18S target gene, which is highly conserved amongst Eukaryotes and sometimes fails to resolve to the species level (O'Rourke et al. 2012). A range of universal primers and associated predator-blocking primers were designed to target different sequence lengths and tested using the initially screened samples using both sponge and predator controls. Primers had to be between 18-25 bp with an overlap of at least 8 bp between the universal and the blocking primer sets and 4-5 mismatches between predator and prey at the blocking primer site (Vestheim & Jarman, 2008) (Figure 3.1). Blocking primers also had to have a higher melting temperature (T_m) so they will anneal before the universal primers during PCR, fortunately, an increase in T_m is usually achieved by the addition of the C3 spacer (O'Rourke et al. 2012). Two forward and two reverse binding sites were identified on the 18S gene that met most of these conditions (Table 3.1).

Table 3. 1 18S primers designed during this study. 3 denotes C3 spacer modification added to the 3'-end of blocking primers.

Primer name	Position	Sequence (5' - 3')	BP	GC%	T _m (C)
18S_2A_F	Forward universal	GGC TCA TTA AAT CAG TTA T	19	31.6	48.7
18S_2A_F_Block	Forward blocking	AAT CAG TTA TGG TTC CTT TGA TCG C3	25	40.0	62.5
18S_3A_F	Forward universal	CCT ATC AAC TTT CGA TGG	18	44.4	51.6
18S_3A_F_Block	Forward blocking	TCG ATG GTA CTT TCT GTG CCT AC3	23	47.8	62.9
18S_4A_R	Reverse universal	CCA GAC TTG CCC TCC AAT	18	55.6	56.1
18S_4B_R_Block	Reverse blocking	CCT CCA ATG GAT CCT CGT TAA AGG A3	25	48.0	65.8
18S_5A_R	Reverse universal	CTT GGC AAA TGC TTT CGC	18	50.0	53.9
18S_5B_R_Block	Reverse blocking	GCT TTC GCT TTC GTC CGT CTT G3	22	54.5	64.0

3.3.4 Designed primer trials

To verify the applicability of the universal primers (without the addition of predator-blocking primers), PCRs were conducted using predator and sponge DNA. Predator DNA was extracted from two liver samples of *Pygoplites diacanthus* using a DNeasy Blood and Tissue Kit (Qiagen) following the protocol of the manufacturer. Three samples verified by BLAST as Poriferan were used for sponge DNA. As part of the optimisation process, PCR was conducted in 10 µl reactions consisting of 5 µl MyTaq Red Mix (Bioline), 0.2 µl of each universal primer (10 µM), ~25 ng of template DNA and a volume of distilled water to reach 10 µl. Four primer sets were tested (Table 3.2), and gel electrophoresis confirmed that all universal primers amplified both predator and sponge DNA.

Table 3. 2 Four 18S primer sets trialled during this study.

Set	Forward primer	Reverse primer	BP
Set 1	18S_2F GGC TCA TTA AAT CAG TTA T	18S_4R CCA GAC TTG CCC TCC AAT	492
Set 2	18S_2F GGC TCA TTA AAT CAG TTA T	18S_5R CTT GGC AAA TGC TTT CGC	893
Set 3	18S_3F CCT ATC AAC TTT CGA TGG	18S_4R CCA GAC TTG CCC TCC AAT	255
Set 4	18S_3F CCT ATC AAC TTT CGA TGG	18S_5R CTT GGC AAA TGC TTT CGC	656

PCRs were conducted using the same samples and the four primer sets (Table 3.2) with the addition of the associated blocking primers (Table 3.1) at two different concentrations (5 X and 10 X) to determine the most effective combination (Vestheim & Jarman, 2008). The PCR recipe was as described previously for a 20 µl reaction, with the addition of either 0.4 µl (10 X) or 0.2 µl (5 X) of each blocking primer (100 µM). Gel electrophoresis confirmed that the most effective combination was set 2 with blocking primers included at 10 X concentration (no bands could be visualised for predator DNA samples yet sponge DNA samples continued to amplify) (Figure 3.2). Set 1 showed some reduction in predator DNA amplification (faint bands) but no reduction at 5 X concentration. Set 3 completely failed as all samples continued to amplify and faint bands could still be determined from predator samples at both concentrations using set 4.

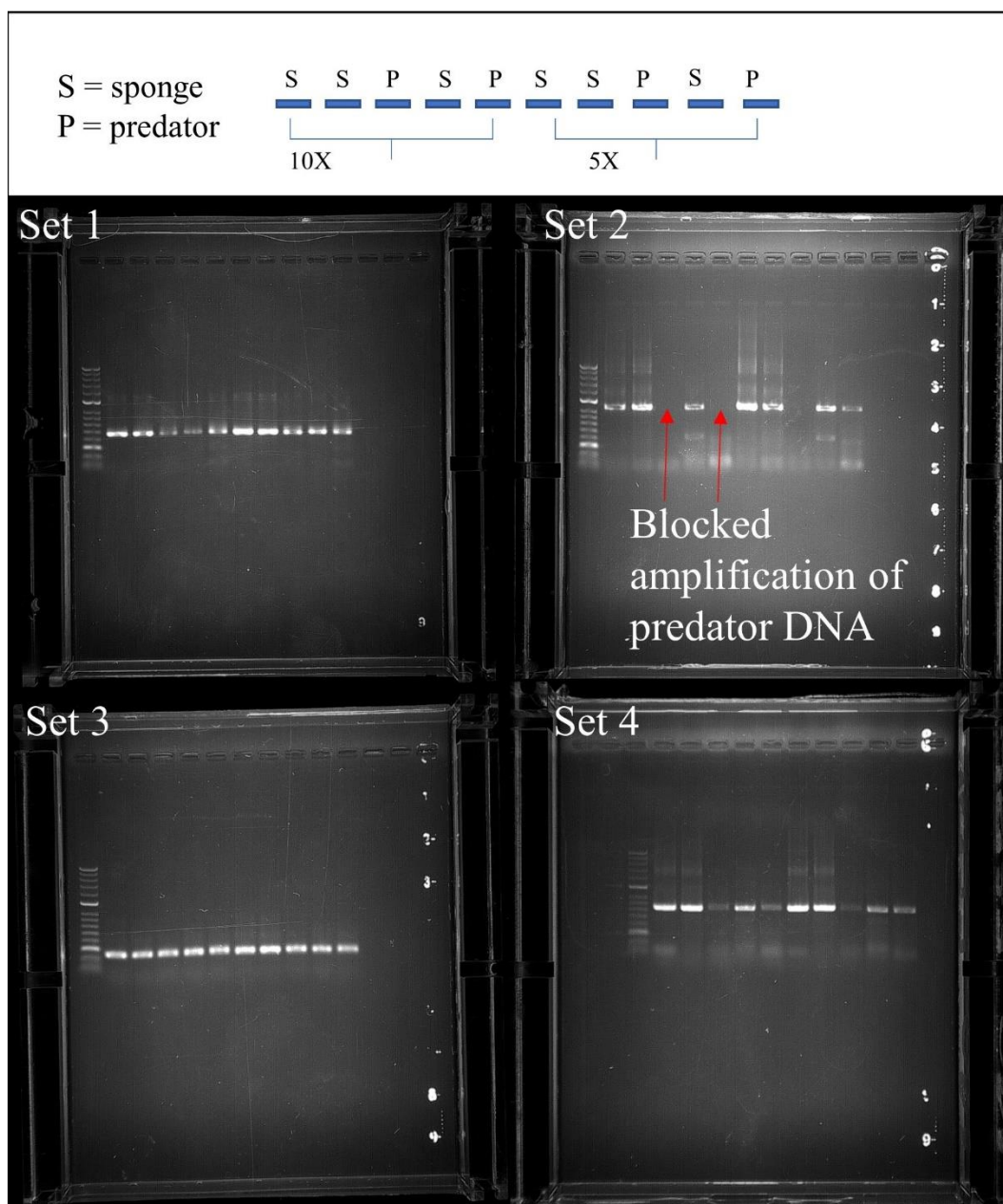


Figure 3. 2 Gel electrophoresis of four primer sets (Table 3.2) designed to block predator DNA from amplification. Predator-blocking primers were tested at 5 X and 10 X concentration (see the top of diagram) on DNA extracts from sponges (S) and the predator (P). Set 2 successfully blocked the amplification of predator DNA at 10 times concentration.

After determining the most efficient predator-blocking primer set, PCR was performed on the remaining sponge samples along with a sponge control, a predator control and a negative control. PCR was conducted in 25 μ l reactions consisting of 12.5 μ l MyTaq Red Mix (Bioline), 0.5 μ l of each universal primer (10 μ m), 0.5 μ l of each blocking primer (100 μ m), ~25 ng of template DNA and a volume of distilled water to reach 25 μ l. 5 μ l of Bovine Serum Albumin (BSA) was added to the reaction to

improve PCR yield. PCR cycling conditions were: 94°C for 5 minutes; the 35 cycles of 94°C for 30 seconds, 48°C for 55 seconds, 72°C for 45 seconds and a final extension step of 72°C for 7 minutes. In total, 32 samples were successfully amplified and sent for purification and sequencing on an ABI 3730 capillary sequencer (Macrogen, South Korea).

3.3.5 Data analysis

Generated sequences were screened for contamination from other targets (e.g. fungus) and for duplicate samples (e.g. the same sponge sequence from the same stomach). Remaining sequences were quality checked using CodonCode Aligner (<http://www.codoncode.com/aligner>) by clipping bases until there were fewer than 3 bases with a phred quality score of less than 20 in a 25 bp window and removing any sequences shorter than 200 bp in length. A demosponge dataset was created using 18S rDNA sequence data downloaded from the GenBank database, the minimum inclusion was 500 bp and downloaded sequences were limited to the 6 orders represented by sequences obtained by this study (Appendix C). All sequences were aligned using ClustalW function in BioEdit version 7.0.5.3 (Hall et al. 1999). A phylogenetic tree was constructed of aligned sequence data using ModelFinder in IQ-TREE multicore version 1.6.11 (Kalyaanamoorthy et al. 2017), which calculates the log-likelihoods of an initial parsimony tree for many different models, choosing the model that minimises the Bayesian information criterion (BIC). The glass sponge *Rhabdocalyptus dawsoni* was used as an outgroup (accession number: AF100949) and branch supports were obtained using the SH-aLRT test (Guindon et al. 2010) and ultrafast bootstrap (Hoang et al. 2018). The best-fit model according to BIC was TIM2+F+I+G4. Branch supports are reported as SH-aLRT support (%) / ultrafast bootstrap support (%) and high support for the existence of a clade was determined by SH-aLRT $\geq 85\%$ / UFBoot $\geq 95\%$.

3.4 Results

Out of the 32 samples sent for sequencing, seven amplified fungus, two amplified crinoid and 23 amplified sponge. Of the 23 sponge sequences, only 14 met the stringent quality control standard outlined above. The identity of one of these samples could not be resolved beyond Porifera and was removed from the dataset. The remaining 13 sponges represented six orders of Porifera (Figure 3.3).

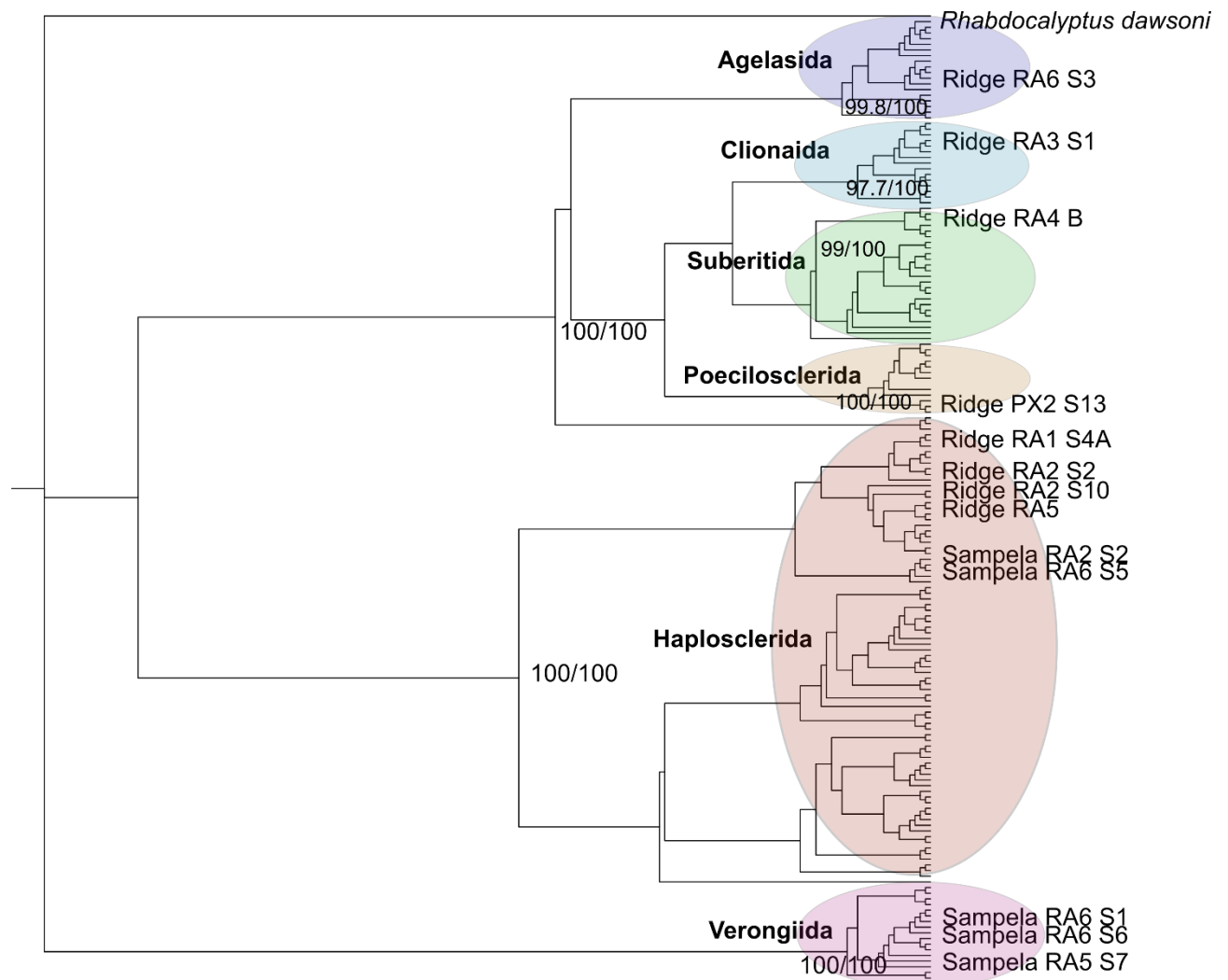


Figure 3. 3 Cladogram showing the placement of 13 sponges within six orders of Porifera dissected from the stomach contents of the angelfishes *Pygoplites diacanthus* (RA) and *Pomacanthus xanthurus* (PX) caught at two sites in the Wakatobi MNP and based on 18S sequence data. The outgroup is the glass sponge *Rhabdocalyptus dawsoni* (AF100949). Branch supports reported as SH-aLRT support (%) / ultrafast bootstrap support (%).

Six sponges were placed in the order Haplosclerida and came from *Pygoplites diacanthus* specimens collected at both Sampela and the Ridge (Figure 3.4). The 18S data provided strong support that one sponge (Ridge RA2 S10) was placed into a clade with the Indo-Pacific sponge *Chalinula hooperi* (Bakus & Nishiyama, 2000) (91.7/100) and another sponge (Ridge RA5) had strong support for placement within a clade of *Petrosia* sponges (98.6/96). The sponge Ridge RA1 S4A also formed a highly supported clade (97.8/99) with the species *Haliclona curacaoensis* (van Soest, 1980) and *Niphates erecta* (Duchassaing & Michelotti, 1864). For the remaining samples in this order, identities could not be resolved beyond Haplosclerida. The Haplosclerida sponges formed four weakly supported major clades. All of the sponges placed in the order Haplosclerida were placed within a weakly supported major clade containing mainly Petrosiidae and Niphatidae sponges, with some interspersed *Haliclona*.

The second order that was represented by more than one sponge was Verongiida and all of these sponges came from stomachs of *P. diacanthus* collected at Sampela (Figure 3.5). There was strong support for all three sponges being placed within a clade containing Pseudoceratinidae and *Verongula* sponges (86.5/97). The remaining sponges were all classified as separate orders and were sequenced from the stomachs of *P. diacanthus* and *Pomacanthus xanthometopon* collected at The Ridge. There was strong support for the placement of one sponge (Ridge RA6 S3) within a clade of Agelasidae sponges (87.5/95) and another sponge (Ridge RA4 B) had strong support for placement within a clade of *Aaptos* sponges from the order Suberitida (98.7/100). The sponge Ridge RA3 S1 had strong support for placement within a clade containing both Clionaidae and Spirastrellidae genera (97.2/99) and the only sponge that was successfully sequenced from the gut contents of *P. xanthometopon* (Ridge PX2 S13) was grouped in the order Poecilosclerida and had strong support for placement into a clade containing *Guitarra* sp. (88.1/100).

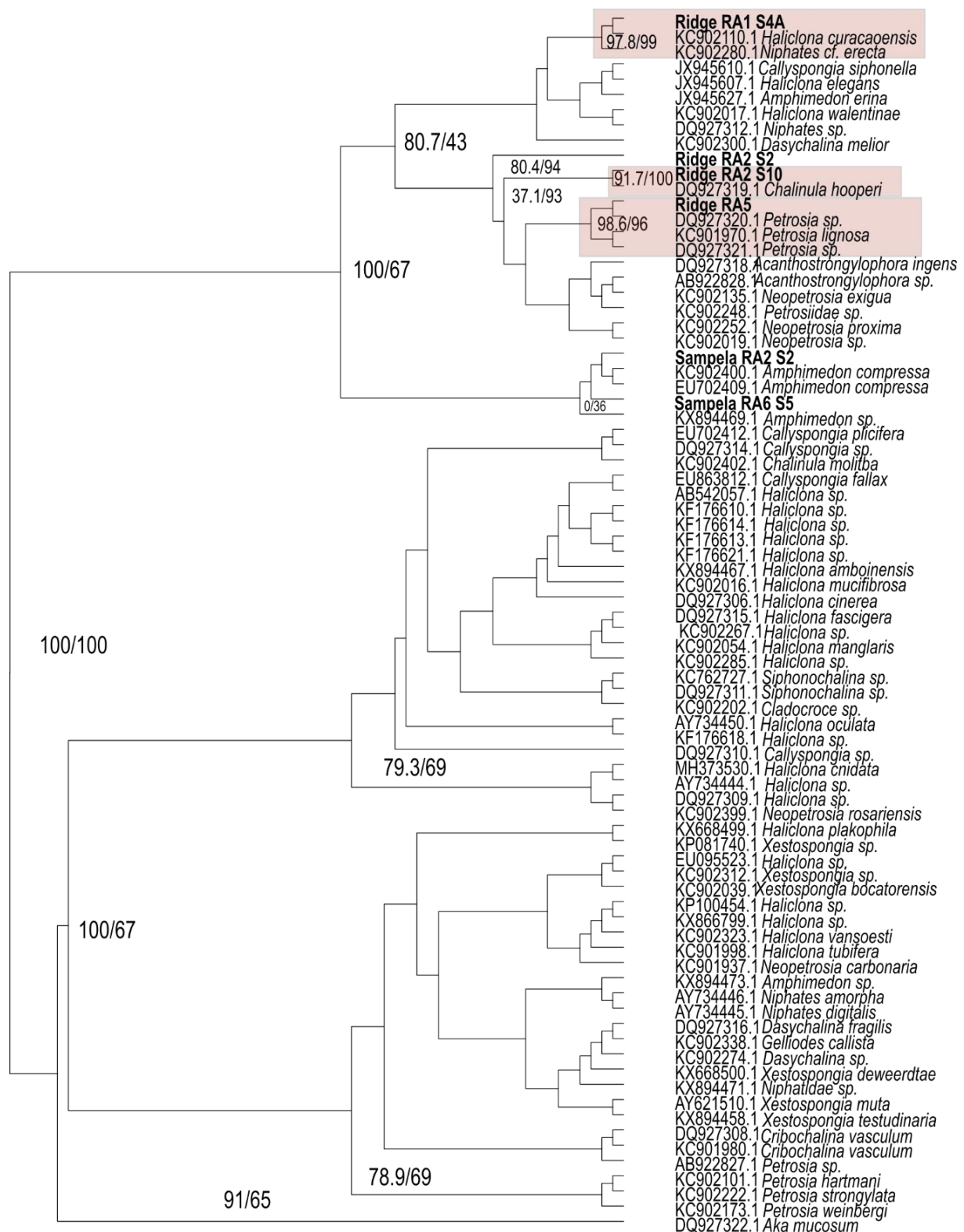


Figure 3. 4 Cladogram showing the placement within the order Haplosclerida of six sponges sampled from the stomach contents of the angelfish *Pygoplites diacanthus* (RA) caught at two sites in the Wakatobi MNP and based on 18S sequence data. Branch supports are reported as SH-aLRT support (%) / ultrafast bootstrap support (%).

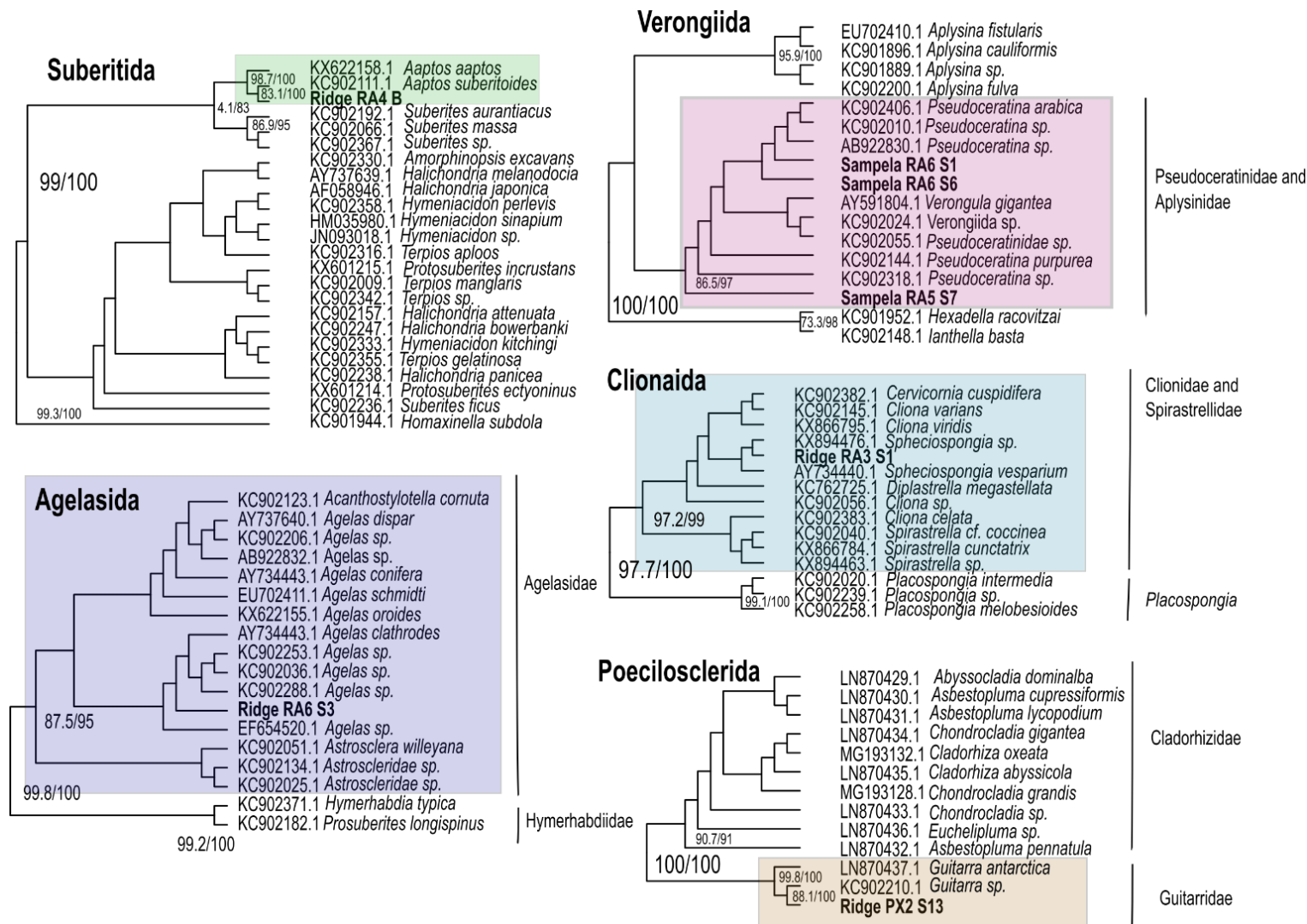


Figure 3. 5 Cladogram showing the placement of seven sponge sequences amplified from the stomach contents of the angelfishes *Pygoplites diacanthus* (RA) and *Pomacanthus xanthometopon* (PX) caught at two sites in the Wakatobi MNR. Branch supports are reported as SH-aLRT support (%) / ultrafast bootstrap support (%).

3.5 Discussion

Resolving predator-prey dynamics is essential to understand the structuring of ecological communities (Leray et al. 2015; Nielsen et al. 2018). This chapter developed molecular methods to identify sponges from the stomach contents of spongivorous angelfishes from the Indo-Pacific. Fourteen sponges produced sequences of high enough quality to be included in the final dataset. These sponges represented 6 orders of Porifera, indicating that *Pygoplites diacanthus* exhibited significant dietary plasticity within the Porifera phylum. Many of the sponges were placed into clades with sponges that exhibit deterrent properties including sponges from the family Agelasidae, the genus *Aaptos* and the order Verongiida, suggesting that *P. diacanthus* consumed sponges with substantial chemical defences.

3.5.1 Taxonomy of predated sponges

From 13 angelfish stomachs, 14 unique sponge sequences were retrieved spanning six orders of Porifera, indicating significant dietary plasticity, similar to the diets of angelfish in the Caribbean (Randall & Hartman, 1968; Hourigan et al. 1989; Wulff, 1994) and the Pacific (Verdín Padilla et al. 2010). The majority of samples were represented by the order Haplosclerida, which are an extremely diverse order of Porifera (van Soest & Hooper, 2002). The Haplosclerida sequences were separated into four weakly supported clades, although one clade was only represented by one species, *Aka mucosum*. The other 3 clades all contained members of multiple families. Many of the genera in this order were not monophyletic, presenting substantial difficulties classifying sponges from this order (Redmond et al. 2013). In a study on the phylogenetics of Demospongiae, Redmond et al (2013) found 5 highly supported clades, one of which (Clade C) is equivalent to the clade in this study where the Haplosclerida samples were situated (Redmond et al. 2013). This clade is made up of mainly Petrosiidae and Niphatidae samples, with some interspersed *Haliclona* (Redmond et al. 2013). Within this group, there was strong support for the placement of one sponge into a clade with the Indo-Pacific sponge *Chalinula hooperi* (Bakus & Nishiyama, 2000) commonly found in the shallow waters of pristine, offshore reefs (Cleary & de Voogd, 2007) that produces substances toxic to corals (Bakus & Nishiyama, 2000). Two congeners *Chalinula nematifera* (de Laubenfels, 1954) and *Chalinula milnei* (de Laubenfels, 1954) that have a documented distribution in the Wakatobi (Chapter 2; Rossi et al. 2015) are also coral-killing sponges that secrete mucus containing toxic substances causing coral mortality (Porter & Targett, 1988). Coral-killing species such as *C. nematifera* that have a wide distribution across Indonesia, high growth rates (Rossi et al. 2015) and the ability to overgrow many species of coral (Turicchia et al. 2018) have the potential to seriously threaten Indo-Pacific reefs. Considering that angelfishes may play an important role in controlling the distribution of these species, angelfish abundances should be closely monitored, as the impacts of coral-killing species could be exacerbated if angelfish populations decline.

There was strong support for the placement of one sponge into a clade containing sponges from the genus *Petrosia*. This supports observations made in Chapter 2 in which *in situ* observations of the

spongivores *Pygoplites diacanthus* and *Zanclus cornutus* revealed a preference for some sponges from the family Petrosiidae. Some species of *Petrosia* have also been shown to be palatable to generalist fishes in the Red Sea (Burns et al. 2003). Another sponge had high support for placement alongside two Caribbean sponges *Niphates erecta* and *Haliclona curacaoensis*. Species of *Haliclona* have been reported from the gut contents of spongivorous fishes in the Caribbean (Randall & Hartman, 1968; Hourigan et al. 1989) and the Pacific (Verdín Padilla et al. 2010). Similarly, sponges from the genus *Niphates* are thought to be palatable to spongivorous fishes and invest heavily in growth to compensate for biomass removal (Pawlik, 2011). For the three other samples in this group, identity could not be resolved beyond the order Haplosclerida.

Three sponges in the Verongiida group had high support for placement into a clade including sponges from the genera *Pseudoceratina* and *Verongula*. Verongiida sponges lack spicules, instead, they are structurally supported by 3D internal scaffolds made of fibrous chitin (Żółtowska- Aksamitowska et al. 2018). Sponges from the genus *Pseudoceratina* are reported to produce diverse secondary metabolites with biological activities including anti-fouling, anti-microbial and anti-cancer properties (reviewed in Żółtowska- Aksamitowska et al. 2018). Some studies have found that sponges in the order Verongiida are avoided by angelfishes and have defensive compounds that deter predation (Pawlik et al. 1995; Burns et al. 2003; Wulff, 2006; Loh & Pawlik, 2014). However, three species of angelfish were observed taking bites from *Pseudoceratina crassa* in the Caribbean (Hourigan et al. 1989) and results presented here indicate that *Pygoplites diacanthus* consumed Verongiida sponges.

There was high support for one sponge belonging to a clade with two species of *Aaptos*. *Aaptos suberitoides* is commonly found on Wakatobi reefs and has been found to produce numerous compounds with antitumor, antimicrobial, and antiviral activity (reviewed in Cleary et al. 2018). Meylan (1998) documented the closely related *Aaptos aaptos* as one of the ten most important sponges predated by the Hawksbill turtle, even though it produces toxic secondary metabolites (Nakamura et al. 1982). Similarly, *Aaptos niger* was reported to form part of the diet of a spongivorous nudibranch and the angelfish *Holacanthus passer* (Verdín Padilla et al. 2010). However, data presented in this chapter represents the first account of a species of *Aaptos* being predated by a spongivorous fish in the Indo-Pacific. There was also strong support for the placement of one sponge into a clade of sponges from the family Agelasidae that are known for producing bromopyrrole alkaloids (Wilson et al. 1999; Lindel et al. 2000) that deter predation (Chanas & Pawlik, 1996). An unknown species of *Agelas* represented the largest volume of sponge in the gut contents of the angelfish *Holacanthus ciliaris* and was also predated by a filefish (Randall & Hartman, 1968). Hence some species in this family appear to attract predation, even with substantial chemical defences.

There was strong support for one sponge belonging to a clade containing the bio-eroding families Clionaidae and Spirastrellidae. This also supports observations made in Chapter 2 where *Pygoplites*

diacanthus was recorded taking bites from *Spechiospongia* sp. (Chapter 2, Figure 2.6). It is also consistent with observations from the Caribbean, which have recorded bio-eroding sponges from the genera *Spirastrella*, *Cliona* and *Spechiospongia* in angelfish gut contents (Randall & Hartman, 1968). The only sponge that could be amplified from the gut contents of *Pomacanthus xanthometopon* had strong support for placement into a clade of sponges from the genus *Guitarra* (Carter, 1874) from the Guitarridae family. To date, there are 15 described species of *Guitarra*, but none have a documented distribution in the Indo-Pacific (<https://www.marinespecies.org>). Highlighting rare or previously unknown species can be an advantage of using gut contents to assess predated sponges (Wulff, 2006). For example, the sponge *Iotrochota birotulata* was commonly found in the gut contents of the spongivorous urchin *Eucidaris tribuloides* in Brazil despite having no known local distribution (Santos et al. 2002). Finally, the identity of one sample could not be resolved beyond the higher classification of Porifera. This is likely due to poor data coverage for sponges from the Indo-Pacific, one of the most speciose regions of Porifera on the planet (Maldonado et al. 2017). Resolving the taxonomy of predated sponges using molecular methods will be easier in future studies, as global barcoding initiatives increase the availability of sponge sequences.

3.5.2 Predator-blocking primers

Using molecular methods in diet tracing studies can help to overcome some of the problems associated with visual identification including capturing rare or highly digested items, or liquid components (Nielsen et al. 2018). Yet, working with mixed samples presents an entirely new range of problems including predator DNA swamping (Vestheim & Jarman, 2008), presence of PCR inhibitors (e.g. faeces and bile, Penn et al. 2016) and choosing appropriate regions to amplify degraded DNA (Vestheim & Jarman, 2008). The most successful primer set designed during this study (Set 2) completely blocked the amplification of predator DNA when blocking primers were included at 10 times concentration. Vestheim & Jarman (2008) also found that blocking primers were most effective at removing krill DNA from diet samples when they were included at 10-fold molar excess. When this primer set was applied to the original trial samples, predator DNA that previously amplified was successfully blocked and sponge DNA that was present in the samples amplified. Blocking primers have been successfully applied to help identify the diet of Antarctic krill (Vestheim & Jarman, 2008), a coral predator (Leray et al. 2013) and Australian fur seals (Deagle et al. 2009). However, this study represents the first known attempt to identify predated sponge samples recovered from the gut contents of spongivorous fish.

Although the primers designed in this study prevented the amplification of predator DNA, they amplified two groups of non-target sequences, fungus and crinoids. Seven of the DNA sequences were verified by basic local alignment search tool (BLAST) to be a fungus. Some marine sponges have been found to possess significant fungal diversity (Gaino et al. 2014), although it seems more likely that these samples were part of the predator's gut microbial community, the composition of which is understood

to be heavily influenced by diet (Muegge et al. 2011; Clements et al. 2014). In addition to fungus, one crinoid sequence was amplified and sequenced. This could be non-targeted predation, as crinoids in the Wakatobi are regularly seen attached to sponges (pers. obs.); however, the same feeding behaviour has been recorded in video observations (unpublished data) indicating that crinoids are likely a minor prey item for *Pygoplites diacanthus*. The amplification of non-target sequences highlights the difficulties in designing primers that target an entire phylum but exclude other non-sponge prey, parasites or gut microbiota (O’Rorke et al. 2012; Vestheim & Jarman, 2008). This study targeted the 18S gene because of prior amplification success but future studies characterising diet using gut content samples are likely to take advantage of next-generation sequencing (NGS) and metabarcoding techniques (O’Rorke et al. 2012; Pompanon et al. 2012). NGS allows the sequencing of many species from the same sample providing a high degree of resolution on diverse diets, and also reduces the influence of non-target sequences that can be screened from the dataset using NGS methods (O’Rorke et al. 2012; Pompanon et al. 2012).

3.5.3 Conclusion

This study is the first to successfully sequence sponges from the gut contents of spongivorous fish and has yielded several genera previously unknown to be predated in the Indo-Pacific. As one of the most important groups of spongivores on coral reefs, resolving the diet preferences of angelfishes is an essential step on the way to determining the ecological importance of spongivory. Using molecular methods of diet characterisation, this study yielded significant information on the diversity and taxonomy of predated sponges, overcoming problems associated with observational approaches such as cryptic feeding behaviour and unidentifiable gut contents.

Chapter 4. Niche width expansion and a loss of trophic diversity on a sponge-dominated reef in The Coral Triangle

4.1 Abstract

Coral reefs are extremely productive, diverse ecosystems. Yet, widespread shifts in benthic community structure due to habitat degradation are affecting organic matter transfer between trophic levels, altering food web structure and dynamics. Optimal foraging theory suggests the niche width of a species increases in response to a decrease in per capita prey, as they adjust their foraging behaviour to include non-preferred food. Trophic specialists with less flexible diets are the most susceptible to environmental disturbance and may be disproportionately affected, leading to a system dominated by generalists. In this chapter, I investigated how changes in resource availability affect niche widths and trophic diversity in a coral reef fish assemblage. I used stable isotope analysis and sampled coral reef fish representing functional feeding groups, prey items and basal carbon sources at a sponge-dominated reef system and an adjacent, higher quality reef site. The fish assemblage at the sponge-dominated site had a significantly lower mean trophic position in comparison to the higher quality site. Four out of seven fish species had wider isotopic niches at the sponge-dominated site, although the isotopic niche of the detritivore was significantly smaller. The obligate corallivore had a significantly wider isotopic niche at the sponge-dominated site and increased proximity to the isotopic niche of the detritivore suggests that it supplemented its diet with detritus. Patterns of niche width expansion observed by the specialists (the spongivore and the corallivore) at the sponge-dominated site were not reflected by their main food sources (sponges and corals). The fish assemblage sampled at the sponge-dominated site used a significantly lower range of resources ($\delta^{13}\text{C}$ range) and obtained more carbon from benthic production than at the high-quality site. Trophic diversity was lower and functional redundancy higher, suggesting that fish at the sponge-dominated site had more similar diets. Low coral and macroalgal cover, and relative isolation from oceanic upwelling, suggest that the sponge-dominated site was mainly fuelled by detrital production. Observed differences in trophic structure between the two sites are likely due to fewer organic matter pathways supporting secondary production at the sponge-dominated site, which ultimately leads to a simplification of trophic structure. Whilst trophic niche expansion may facilitate the survival of populations in the short term, it is expected to lead to intensified competition for increasingly scarce resources.

4.2 Introduction

Coral reefs are one of the most productive ecosystems on the planet (Odum & Odum, 1955; Hughes et al. 2003; Atkinson & Falter, 2003). They support extremely high biodiversity (Hoeksema, 2017) and provide numerous benefits to coastal communities including food, coastal protection and livelihoods (Bryant et al. 1998; Diaz et al. 2015). Yet, persistently high levels of natural and anthropogenic disturbance have caused widespread changes to benthic community structure on a global scale (Hughes et al. 2003), transforming food web structure and dynamics (Bellwood et al. 2004; Graham et al. 2014; Pratchett et al. 2014). Coral reef fish assemblages are exceptionally diverse (Brandl et al. 2016) and perform critical ecosystem functions including herbivory (Bellwood et al. 2004) and the transfer of pelagic nutrients to benthic communities (Hobson, 1991), but they are also extremely vulnerable to disturbance (Emslie et al. 2018). Species are not affected randomly by disturbance (Smith & Knapp, 2003), and those that share vulnerable traits are disproportionately affected (Mouillot et al. 2013). Resource specialisation will likely determine a species' response and susceptibility to perturbations (Layman et al. 2007; Graham et al. 2011). On coral reefs, obligate corallivores that are heavily dependent on corals for food and habitat are predicted to be the most vulnerable to extinction from climate disturbances (Graham et al. 2011), whereas those with more plastic niches are expected to compete more effectively in degraded habitats (Clavel et al. 2011). Some amount of habitat degradation may even be beneficial for some functional groups in the short term (Rogers et al. 2018) through the provision of alternative food (Kramer et al. 2014) or a reduction in competition. Understanding how species and communities respond to habitat degradation is critical to predicting the capacity of future coastal environments to provide crucial goods and services (Sale et al. 2014).

Optimal foraging theory predicts that individuals choose prey in a way that maximises their overall rate of energy gain (Pyke et al. 1977; Brooker et al. 2014). Therefore, a decrease in per capita prey is expected to lead to an increase in niche width (Rossi et al. 2015) as consumers include sub-optimal prey in their diet following a decline in preferred food (Rossi et al. 2015; Calizza et al. 2017). This can lead to increased competition for resources and the exclusion of poor competitors (Calizza et al. 2017). Diet switching to sub-optimal items may have a cost at the individual level e.g. loss of physiological condition (Berumen et al. 2005) and the population level by reducing the fecundity (Jones & McCormick, 2002), growth (Feary et al. 2009) and trophic position of consumers (Hempson et al. 2015). For example, a decrease in the trophic position of the coral grouper *Plectropomus maculatus* on the Great Barrier Reef was linked to a shift from pelagic to benthic prey following widespread coral bleaching and mortality (Hempson et al. 2015). Increased generalism may also have a cost at the community level, as disturbance-mediated shifts towards generalist-dominated communities lead to functional homogenisation (Olden et al. 2004), a state where persisting species all perform similar ecological roles (Clavel et al. 2011; Mouillot et al. 2013). Community reorganisation in favour of

generalists following environmental disturbance has been documented in many ecosystems worldwide including coral reefs (Munday, 2004), seagrass meadows (Calizza et al. 2017) and temperate forests (Valladares et al. 2012). This is expected to have various impacts on ecosystems depending on which ecosystem functions are lost (Graham et al. 2011; Bellwood et al. 2019).

Food webs are most stable at high levels of complexity (Johnson et al. 2014), which poses a challenge for researchers trying to link environmental change with the functioning of consumers (Middelburg, 2014). Gut content analysis is typically used to quantify the relative abundance of prey thus determining predator-prey relationships and niche breadth (Levin, 1968; Layman et al. 2007). However, gut content analysis can be biased towards structural items at the expense of soft-bodied taxa (Nielsen et al. 2018) and it only provides a dietary snapshot (Pinnegar & Poulin, 1999). It also does not provide information on assimilated products, which may differ substantially from bulk ingested matter (Nielsen et al. 2018). Stable isotope analysis (SIA) is a useful tool for elucidating trophodynamics in marine systems and relies on the basic premise that isotopes fractionate as a result of physical, chemical and biological processes (Middelburg, 2014). It incorporates dietary information from longer time periods (Pinnegar & Poulin, 1999) and can be performed on producers and consumers, providing an end-to-end reconstruction of food webs (Middelburg, 2014). Nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$ noted $\delta^{15}\text{N}$) are used to estimate trophic position because the lighter isotope (^{14}N) is lost at a faster rate than the heavy isotope (^{15}N) during excretion and assimilation (Fry, 2006). This leaves the consumer enriched in ^{15}N by an estimated 2‰ - 4‰ relative to its diet (De Niro & Epstein, 1981; Minagawa & Wada, 1984). Hence, $\delta^{15}\text{N}$ values can be used to estimate food chain length and the trophic positions of consumers (Minagawa & Wada, 1984). Carbon isotopes are used to determine sources of nutrition because different photosynthetic pathways (C3 or C4) result in variation in $\delta^{13}\text{C}$ values (O’Leary, 1988). Carbon is not thought to fractionate to the same extent as nitrogen (0-1 ‰) (De Niro & Epstein, 1981; Post, 2002) hence $\delta^{13}\text{C}$ values of consumers generally reflect the main source of carbon consumed. Bivariate isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) have been conceptualised as the ‘isotopic niche’ (Bearhop et al. 2004; Newsome et al. 2007) that fits Hutchinson’s ecological niche concept (Hutchinson, 1957; Hutchinson, 1978) because it represents both scenopoetic (habitat) and bionomic (resource) axes (Hutchinson, 1978; Newsome et al. 2007). Intraspecific variation in isotope ratios can be used as a measure of niche width (Bearhop et al. 2004) because it indicates among-individual differences in diet, e.g. generalist or specialist consumers (Bearhop et al. 2004; Jackson et al. 2011). The rise of the isotopic niche as a proxy for trophic niche (Newsome et al. 2007) allows researchers to measure trophic specialisation among species in a community and investigate how dynamics change in response to different environmental conditions.

From 2002 to 2007, the Sampela reef system in the Wakatobi Marine National Park (MNP) experienced >50% decline in hard coral cover and a concurrent increase in sponge density (McMellor & Smith,

2010; Powell et al. 2014). This sponge assemblage at Sampela is dominated by *Lamellodysedia herbacea*, an encrusting phototrophic species with adaptations including rapid photo-acclimatisation and effective sediment clearance that allow it to proliferate under poor environmental conditions (Biggerstaff et al. 2015; Biggerstaff et al. 2017). Research on the Sampela reef system has mainly centred on identifying the main drivers of change (Powell, et al. 2014; Biggerstaff et al. 2015; Biggerstaff et al. 2017) rather than assessing the consequences to trophic structure and functioning. Given that sponge-dominated reefs may be a potential future scenario for some reef systems (Bell et al. 2013) it is essential to understand how changes to benthic community composition affect trophic structure and functioning.

In this chapter, I used stable isotope analysis to investigate how changes in resource availability affect niche widths and trophic diversity in a coral reef fish assemblage. I sampled the stable isotope ratios of seven coral reef fish species each representing a different functional feeding group, associated prey and basal carbon sources at a low quality, sponge-dominated reef and an adjacent higher quality reef site. My specific aims were: i) to compare the trophic position and $\delta^{13}\text{C}$ values of fish between reefs and species; ii) to compare isotopic niche size of fish species between reefs; iii) to investigate isotopic discrimination and spatial variation in basal carbon sources and benthic prey; iv) to compare the relative proportion of benthic and pelagic organic matter contributing to fish diets at both sites; and, v) to compare trophic structure between reefs using Layman community metrics derived from stable isotope data.

4.3 Methods

4.3.1 Study sites

The Wakatobi Marine National Park (see Chapter 2, Figure 2.1) is Indonesia's third-largest marine national park and is located within the coral triangle, a region supporting extremely high marine biodiversity (Allen, 2008; Veron et al. 2009). Reefs in Southeast Asia are considered the most threatened in the world (Burke et al. 2011). From 2002-2007, live coral cover on the reefs surrounding Kaledupa and Hoga island declined by 50% (McMellor & Smith, 2011). The Sampela reef system is located 500 m from the intertidal Bajo settlement at Kaledupa Island that is home to >1000 people, hence pressure on this reef system from destructive fishing practices, pollution and coral mining historically has been high. From 2002 to 2007, live coral cover at this site declined from $32.6 \pm 2.0\%$ to $12.2 \pm 1.6\%$ (McMellor & Smith, 2010) but since 2007, live coral cover has remained relatively stable and at the time of this survey was estimated as $9.5\% \pm 1.6\%$ (Chapter 2). This site has sedimentation rates estimated at four times higher than other reef sites in the area (Crabbe & Smith, 2005; Curtis-Quick, 2013), has high abiotic cover and low structural complexity. The comparison site was located on an adjacent reef system on the island of Hoga at a site known locally as The Ridge (hereafter described as the high-quality site). Like the sponge-dominated site, this reef also experienced a 50% decline in live coral cover between 2002 and 2007 (McMellor & Smith, 2010) and stabilised at approximately 30% (Curtis-Quick, 2013). At the time of this study, live coral cover was three times higher than the sponge-dominated site ($29.7\% \pm 6.0\%$) (Chapter 2). This site also has higher structural complexity, lower abiotic cover and lower sedimentations rates (Crabbe & Smith, 2005; Powell et al. 2014). Both reef systems are separated by a channel that is approximately 1 km wide and extends to a depth of 40 m, limiting the movement of coral reef fish between reef systems.








4.3.2 Sample collection

4.3.2.1 Fish

Fish collections were carried out on both reef systems by local Bajau spearfishers over three weeks during July and August 2017. Seven coral reef fish species representing different functional feeding groups were sampled at both sites. Species were identified as representing a specific functional feeding group based on *in situ* observations, gut content data (Chapter 2) and literature references (Table 4.2). Adult fishes in a similar size range were targeted at both locations to avoid variation in isotopic ratios related to dietary ontogeny or size-based metabolic processes (de la Moriniere et al. 2003; Carassou et al. 2008), therefore one-way ANOVAs were used to test for any differences in sizes (TL mm) between sites (Appendix D, Table 2). Fish collections were opportunistic, spearing a maximum of two individuals of each species per trip. All samples were collected under permit and sample sizes were limited to 5-10 individuals per site due to ethical considerations (Appendix D, Table 1). Fish were frozen at -20°C until dissection when a section of anterior dorsal muscle tissue was collected and stored at -

20°C. Muscle tissue was sampled as it presents more homogenous isotopic values compared to other tissues (Pinnegar & Polunin. 1999), more closely reflects the animal's diet (Grey et al. 2009) and represents nitrogen and carbon isotope values assimilated over a few months (Tieszen et al. 1983).

Table 4. 1 Seven coral reef fish species targeted by this study and major recorded prey items (>10% gut contents) from this study (Chapter 2) and literature references (all images licenced under Creative Commons BY-C 3.0).

Species	Common name (Family)	Functional group (Major recorded prey)	References
<i>Zanclus cornutus</i> 	Moorish Idol (Zanclidae)	Facultative spongivore/ Omnivore (Sponges, calcareous sediments, algae)	Chapter 2; Powell et al. 2015
<i>Pygoplites diacanthus</i> 	Regal angelfish (Pomacanthidae)	Obligate spongivore (Sponges)	Chapter 2; Powell et al. 2015
<i>Forcipiger flavissimus</i> 	Longnose butterflyfish (Chaetodontidae)	Invertivore (Sessile polychaetes, decapod shrimp)	Chapter 2; Nagelkerken et al. 2009
<i>Ctenochaetus binotatus</i> 	Two-spot bristletooth (Acanthuridae)	Detritivore (Organic detritus/ sediments)	Chapter 2; Randall & Clements, 2001.
<i>Chaetodon kleinii</i> 	Klein's butterflyfish (Chaetodontidae)	Facultative corallivore (Hard coral, copepods, hydroids, polychaetes, isopods, amphipods, algae)	Chapter 2; Nagelkerken et al. 2009
<i>Chaetodon lunulatus</i> 	Oval butterflyfish (Chaetodontidae)	Obligate corallivore (Hard coral)	Pratchett et al. 2005; Nagelkerken et al. 2009;
<i>Neoglyphidodon nigroris</i> 	Black-and-gold chromis (Pomacentridae)	Planktivore (Salps, tunicates, algae)	Eurich et al. 2019; Allen, 1975.

4.3.2.2 Carbon sources and prey

Suspended particulate organic matter (SPOM) was sampled to represent the base of the pelagic food web (Briand et al. 2016; Carassou et al. 2008). Five litres of sub-surface seawater were collected using prewashed containers (Whatman GF/F filtered seawater, nominal pore size 0.7 μm). Water samples were pre-filtered through a 200 μm mesh to remove larger particles such as zooplankton and salps, before filtering onto GF/F pre-combusted filters (450°C, 5 hours) under low pressure (5 in. Hg vacuum) using a vacuum pump to avoid the lysis of fragile cells. Fragments of *Porites lutea*, *Pavona varians* and *Pocillopora verrucosa* were collected because of adequate abundances at each site. Coral fragments were blasted with GF/F filtered seawater using a water pick and the resulting solution, representing coral tissue, mucus and symbionts, was filtered onto pre-combusted GF/F filters papers under low pressure using a vacuum pump. Macroalgal cover on Wakatobi reefs is low (Table 4.1) and the only algae found at both sites was a green turfing alga that was scraped from the benthos. A brown macroalga *Dictyota* sp. was collected at the sponge-dominated site and the calcareous *Halimeda* sp. at the high-quality site. The detritivorous sea cucumber *Synaptula* sp. was collected at both sites to represent the base of the detrital food web (detritus + 1 trophic position) as detritus is hard to isolate in marine systems (McMahon et al. 2016). Samples of the sponges *Xestospongia* sp., *Hyrtios erecta*, *Lamellodysedia herbacea* and *Clathria* sp. were collected because of adequate populations at each site and due to observations of spongivore activity (Chapter 2). *L. herbacea* was collected because of its role in a coral – sponge regime shift (Powell et al. 2014). Three to five replicates were collected for each item and all samples were frozen at -20°C until processing (Appendix D, Table 1).

4.3.3 Sample processing

All samples were prepared and processed at the National Institute for Water and Atmospheric Research (NIWA). Carbon sources and potential prey items were acidified to remove carbonates. Filter papers (retaining coral tissue and SPOM) were placed on acid-resistant trays in a desiccator with concentrated hydrochloric acid (HCl) left under a fume hood overnight (approx. 16 hours). After fuming, filters were rinsed with double distilled water (DDW) dried at 60°C and stored with desiccant in plastic sealed containers. For the remaining prey items, samples were placed in falcon tubes and treated with 10% HCl for 24 hours. Acid was added in 24-hour intervals until samples stopped effervescing at which point samples went through three wash cycles (rinsed with DDW and centrifuged at 2500 rpm for 15 minutes) before the resulting samples were oven-dried at 60°C. A subset of paired acidified and non-acidified samples was prepared to test for any effect of acid treatment on nitrogen isotope values (Bunn et al. 1995; Pinnegar & Polunin, 1999; Kennedy et al. 2005). Paired t-tests and one non-parametric Wilcoxon Rank-Sum test showed that acidification had no significant effect on $\delta^{15}\text{N}$ values (Appendix D, Table 3).

Fish muscle tissue was pre-frozen at -80°C for two hours and then freeze-dried overnight. Lipids were not removed from fish tissue samples as bulk tissue mass C: N ratios were < 3.5 indicative of pure protein and the absence of lipids (Post et al. 2007). All samples were homogenised using a mortar and pestle and instruments were ethanol cleaned and rinsed three times with DDW between samples. Samples were weighed into tin capsules (0.5-0.6 mg fish, 2-4 mg sponge, 0.5 mg acidified sponge, 4 mg algae, 0.5-0.7 mg acidified algae) and a quarter of each filter papers were prepared for coral and phytoplankton samples. Samples were analysed on a DELTA VPlus isotope ratio mass spectrometer (IRMS) (Thermo Fischer Scientific, Bremen, Germany). CO₂ and N₂ reference gas standards were introduced to the mass spectrometer with every sample analysis. ISODAT (Thermo Fisher Scientific, Bremen) software was used to calculate $\delta^{15}\text{N}$ values against atmospheric air, and $\delta^{13}\text{C}$ values relative to the international standard Carrara Marble NSB-19 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA). This was further calibrated against the original Pee Dee Belemnite (PDB) limestone standard and then corrected for 17O. Carbon isotope data were corrected via a two-point normalisation process using NIST 8573 (USGS40 L-glutamic acid; certified $\delta^{13}\text{C} = -26.39 \pm 0.09$ ‰) and NIST 8542 (IAEA-CH-6 Sucrose; certified $\delta^{13}\text{C} = -10.45 \pm 0.07$ ‰). A two-point normalisation process using NIST 8573 (USGS40 L-glutamic acid; certified $\delta^{15}\text{N} = -4.52 \pm 0.12$ ‰) and IAEA-N-2 (ammonium sulphate; certified $\delta^{15}\text{N} = +20.41 \pm 0.2$ ‰) was applied to $\delta^{15}\text{N}$ data. DL-Leucine (DL-2-Amino-4-methylpentanoic acid, C₆H₁₃NO₂, Lot 127H1084, Sigma, Australia) was run every ten samples to check analytical precision and enable drift corrections to be made if necessary. NIST 8547 (IAEA-N1 ammonium sulphate; certified $\delta^{15}\text{N} = +0.43 \pm 0.04$ ‰) and USGS65 Glycine (certified $\delta^{13}\text{C} = -20.29 \pm 0.04$ ‰; certified $\delta^{15}\text{N} = 20.68 \pm 0.06$ ‰) were run daily to check isotopic accuracy with two laboratory standards L Proline and homogenized squid run as an additional precision check. Repeat analysis of standards produced data accurate to within better than 0.15‰ for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, and precision of better than 0.22‰ for $\delta^{15}\text{N}$ and 0.24‰ for $\delta^{13}\text{C}$.

Ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were expressed relative to internal standards. The relative abundances of stable isotopes were expressed according to the following:

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$$

where X is ^{15}N or ^{13}C and R is the ratio $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$.

4.4 Data analysis

4.4.1 Spatial and interspecific variation in trophic position and $\delta^{13}\text{C}$ values

Trophic position (TP) was calculated according to the scaled equation by Hussey et al. (2014), which accounts for the narrowing of nitrogen isotope fractionation ($\Delta^{15}\text{N}$) at higher trophic positions (Caut et al. 2009; Hussey et al. 2014).

$$\text{TP} = \frac{\log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{base}}) - \log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{consumer}})}{K} + \text{TP baseline}$$

Where $\delta^{15}\text{N}_{\text{lim}}$ is the saturating isotope limit as TP increases (21.93‰) and K is the rate at which $\delta^{15}\text{N}_{\text{consumer}}$ approaches $\delta^{15}\text{N}_{\text{lim}}$ per trophic level increase (0.14). The TP baseline (TP = 2) was represented by the detritivorous primary consumer *Synaptula* sp. because of the minimal variation in $\delta^{15}\text{N}$ values in samples of that species at both sites (Appendix D, Table S1).

I tested for differences in trophic position and $\delta^{13}\text{C}$ values between sites and species with permutational analysis of variance using the PERMANOVA+ add-in to PRIMER version 6.1 (PRIMER-E Ltd, Plymouth, UK) with fish size (TL mm) included as a covariate. This was done using Type I (sequential) sums of squares and based on a Euclidean distance matrix with 999 permutations of the residuals under a reduced model. Pairwise comparisons were used to identify intraspecific differences between reefs and interspecific differences within reefs. Data were checked for multivariate homogeneity of group dispersions using PERMDISP.

4.4.2 Isotopic niches of coral reef fishes

To examine differences in the isotopic niche (a generalised proxy for trophic niche) of fish species between reefs, I used the SIBER package for R (Jackson et al. 2011) to calculate the standard ellipse of each species at both sites (Figure 4.1). The standard ellipse is a measure of deviation for bivariate data comparable to standard deviation for univariate data (Jackson et al. 2011). In δ space, the standard ellipse is located by the means of X and Y and the shape and size are determined by the associated covariance matrix (Jackson et al. 2011). It contains approximately 40% of the sample data and as such the standard ellipse area (SEA) represents the core niche area of a species (Jackson et al. 2011). Standard ellipses are less sensitive to sample size variation than other traditional measures of niche width e.g. the total area of the convex hull (TA) (Layman et al. 2007), which increases with sample size (Jackson et al. 2011).

To determine if any significant differences in isotopic niche size existed between reef systems, I used Bayesian inference techniques (10^4 iterations) described by Jackson et al (2011) to generate probability

distributions of the SEA_c (c = small sample size corrected). Differences in SEA_c size were considered significant when $\geq 95\%$ of posterior draws for one group/species was smaller than the other (critical p -value ≤ 0.05). Bayesian inference techniques allow researchers to make direct statistical comparisons otherwise not afforded by point estimates of dispersion (Jackson et al. 2011). The main assumption of using these statistical techniques is that data approximate a multivariate normal distribution (MVN) (Jackson et al. 2011) so prior to analysis groups were checked for MVN using Royston's Multivariate Normality Test that uses Shapiro-Wilk's W statistic.

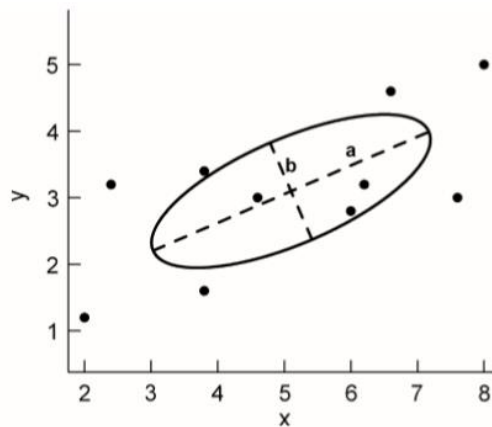


Figure 4. 1 Example standard ellipse from Batschelet, (1981) and reproduced in Jackson et al. (2011). Black circles are sample data, the black ellipse is the standard ellipse containing 40% of the sample data. Semi-major axis (a) and semi-minor axis (b) are shown on the diagram.

4.4.3 Carbon sources and benthic prey

To examine differences in isotope values between carbon sources and benthic prey, I used PERMANOVA+ with Type III sums of squares and based on a Euclidean distance matrix constructed using $\delta^{15}N$ and $\delta^{13}C$ data with 999 permutations of the residuals under a reduced model. This provided an overall picture of isotopic discrimination between sampled species/taxa and was used to check for large differences in metabolic pathways between species within groups (e.g. sponges). Individual source items were grouped into major taxa (SPOM, corals, sponges, algae) and reanalysed using the same PERMANOVA design to assess isotopic discrimination in major groups and between site variation in isotope values. For groups with appropriate replication (corals, sponges, detritus), I investigated differences in isotopic niche size (SEA_c) between sites (see section 4.2.2) to account for any differences observed in the isotopic niches of fish between sites.

4.4.4 Pelagic and benthic carbon contributions to coral reef fish diets

To compare the proportional contribution of pelagic and benthic sources of carbon to fish consumers between sites I used the SIMMR package (Stable Isotope Mixing Models in R) (Parnell et al. 2016) to run dual isotope mixing models. SIMMR works by fitting a Bayesian model with Markov Chain Monte Carlo algorithm (MCMC) producing simulations of possible values for each carbon source based on the available data. Models were run separately for both sites to adjust for site-specific values (Appendix D, Table 16). For the pelagic carbon signature, I used the mean \pm standard deviation (SD) of SPOM with samples pooled between sites because the isotope values of SPOM did not vary significantly between sites (Appendix D, Table 15). For the benthic carbon signature, I used the mean \pm SD of corals and detritus +1TP (pooled) with site-specific values due to a significant difference in isotopic values of detritus +1TP between reefs (Appendix D, Table 15). Turf algae were not included in the benthic signature because of low $\delta^{15}\text{N}$ values. Trophic enrichment factors used in the mixing models were $\Delta^{15}\text{N}$ 3.4 ± 0.98 ‰ and $\Delta^{13}\text{C}$ 0.39 ± 1.3 ‰ (Post, 2002). These enrichment factors were estimated from two primary consumers (filter-feeding bivalves and grazing snails) covering littoral and pelagic food webs (Post, 2002). Values cited are the mean \pm SD enrichment factors of both aquatic food webs and wide variation in these values propagates the same degree of error through proportional contribution estimates. Pelagic production was corrected for + 2 trophic positions because it was assumed that fish would either consume filter-feeding organisms or phytoplankton feeders (e.g. copepods). Benthic production was corrected for + 1 trophic position because corals are consumed directly by coral-feeding fishes and detritus was sampled using a proxy detritivorous sea cucumber and thus already corrected for + 1 trophic position.

4.4.5 Quantitative comparison of trophic structure

The isotopic values of fish were used to quantitatively compare trophic structure between sites using the community-wide metrics proposed by Layman et al. (2007) (hereafter community metrics). These community metrics are calculated based on the spread and extent of bivariate isotope data (Jackson et al. 2011). The range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values provide respective information on food chain length and diversification of resource use. Mean distance to the centroid (CD) is the average Euclidean distance of each species to community centroid (bivariate mean of all data points) and an indicator of trophic diversity. Mean nearest neighbour distance (NND) and the standard deviation of nearest neighbour distance (SDNND) are both metrics that describe trophic redundancy (Layman et al. 2007). It is important to note that in this context, trophic redundancy relates to multiple species having more similar diets rather than suggesting similar functional roles. NND indicates the proximity of species in isotope space and is measured as the average Euclidean distance to each species' nearest neighbour with low values indicating a large proportion of species with similar trophic preferences. SDNND is a measure of evenness of spatial density and packing (Layman et al. 2007; Jackson et al. 2011) with low values representing a more uniform distribution of species. I used Bayesian inference techniques proposed by

Jackson et al. (2011) to propagate sampling error in the mean isotope ratio of community members thus providing a measure of uncertainty in community metrics and allowing statistical comparison of isotopic values between sites (Jackson et al. 2011).

4.5 Results

4.5.1 Spatial and interspecific variation in trophic position and $\delta^{13}\text{C}$ values

The trophic position of fish was influenced by site (pseudoF_{1,68} = 41.97, p = 0.001), species (pseudoF_{6,68} = 126.18, p = 0.001) and size (pseudoF_{1,68} = 11.64, p = 0.002) (Appendix D, Table 4). Fish from the sponge-dominated site had an overall lower mean trophic position than the high-quality site (2.67 ± 0.04 and 2.80 ± 0.04 respectively [mean \pm SE]) (Figure 4.2).

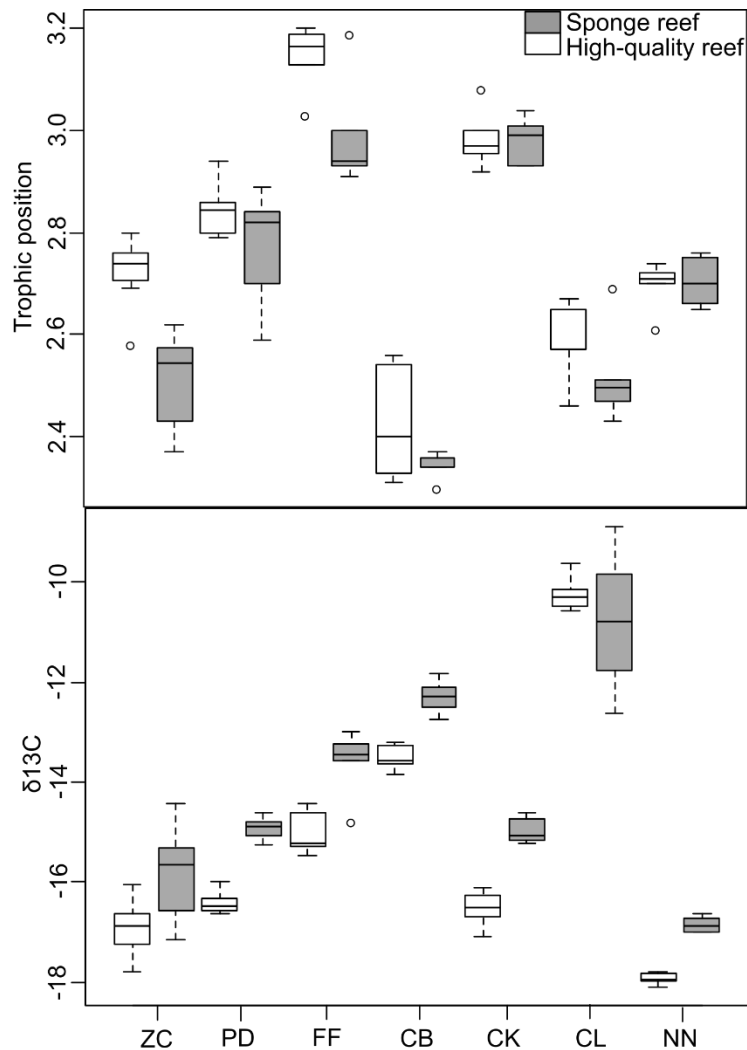


Figure 4. 2 Boxplots of trophic position and $\delta^{13}\text{C}$ values of seven species of reef fish compared between two reef systems of contrasting quality in the Wakatobi MNP, Indonesia. ZC = *Zanclus cornutus*, PD = *Pygoplites diacanthus*, FF = *Forcipiger flavissimus*, CB = *Ctenochaetus binotatus*, CK = *Chaetodon kleinii*, CL = *Chaetodon lunulatus*, NN = *Neoglyphidodon nigroris*.

At the high-quality site, the invertivore *Forcipiger flavissimus* had the highest trophic position (3.15 ± 0.03 [mean \pm SE]) and the detritivore *Ctenochaetus binotatus* had the lowest (2.43 ± 0.05 [mean \pm SE]) (Figure 4.2). Pairwise tests showed that only *Zanclus cornutus* and *Neoglyphidodon nigroris* had similar trophic positions, all other species were significantly different to one another (all pairwise comparisons $p < 0.05$) (Appendix D, Table 6). At the sponge-dominated site, *Z. cornutus* and *Chaetodon lunulatus*, *Pygoplites diacanthus* and *N. nigroris* and *F. flavissimus* and *Chaetodon Kleinii* had similar trophic positions, all other species were significantly different to one another (all pairwise comparisons $p < 0.05$). The highest trophic position was shared by *F. flavissimus* (2.99 ± 0.05 [mean \pm SE]) and *C. kleinii* (2.98 ± 0.02 [mean \pm SE]) and the lowest was occupied by *C. binotatus* (2.34 ± 0.01 [mean \pm SE]).

Between-site pairwise tests indicated that the trophic positions of *Z. cornutus* and *F. flavissimus* were significantly lower at the sponge-dominated site ($p = 0.001$ and $p = 0.03$ respectively) (Figure 4.2) (Appendix D, Table 8). However, size data indicated that *Z. cornutus* caught at the sponge-dominated site were significantly smaller than the high-quality site (Appendix D, Table 2), $105 \text{ mm} \pm 5 \text{ mm}$ and $141 \text{ mm} \pm 5 \text{ mm}$, respectively (mean \pm SE) ($F = 26.78$, $p < 0.001$). Therefore, the PERMANOVA analysis was performed again removing *Z. cornutus* from the dataset and trophic position remained significantly lower at the sponge-dominated site (pseudo $F_{1, 56} = 11.63$, $p < 0.001$).

Fish $\delta^{13}\text{C}$ values did not covary with size (pseudo $F_{1, 55} = 1.47$, $p = 0.23$) so it was removed from the model (Appendix D, Table 5). There were significant differences in $\delta^{13}\text{C}$ values between sites (pseudo $F_{1, 69} = 41.97$, $p = 0.001$). Fish from the sponge-dominated site were ^{13}C -enriched compared to fish from the high-quality site ($-14.27\text{‰} \pm 0.32\text{‰}$ and $-15.39\text{‰} \pm 0.37\text{‰}$ [mean \pm SE]) (Figure 4.2). Species also had a significant effect on $\delta^{13}\text{C}$ values (pseudo $F_{6, 69} = 184.38$, $p = 0.001$) and there was a significant interaction detected between site and species (pseudo $F_{6, 69} = 4.46$, $p = 0.001$). At the high-quality site, *Z. cornutus*, *P. diacanthus* and *C. kleinii* had comparable $\delta^{13}\text{C}$ values, all other species were significantly different to one another (all pairwise comparisons $p < 0.05$) (Appendix D, Table 7). At the sponge-dominated site, *P. diacanthus* and *C. kleinii*, and *C. binotatus* and *C. lunulatus* had comparable $\delta^{13}\text{C}$ values, all other species were significantly different to one another (all pairwise comparisons $p < 0.05$). All species were significantly enriched in ^{13}C at the sponge-dominated site (all pairwise comparisons $p < 0.05$) aside from *Z. cornutus* and *C. lunulatus* (Appendix D, Table 8).

4.5.2 Isotopic niches of coral reef fishes

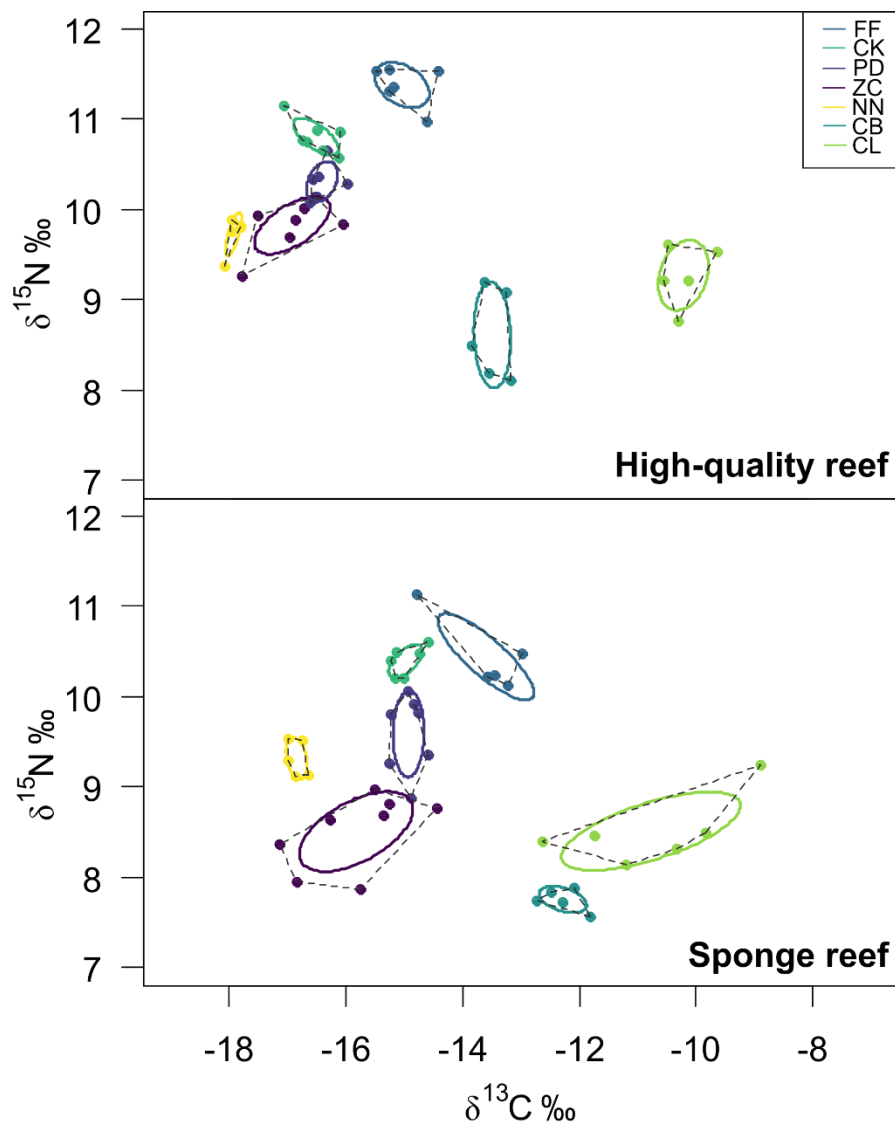


Figure 4. 3 Isotopic niches (standard ellipse area - SEAc) of seven species of reef fish each representing a different functional feeding group sampled at two sites in the Wakatobi MNP, Indonesia. SEAc represented by solid ellipses and dashed polygon is the isotopic total area (TA). ZC = *Zanclus cornutus*, PD = *Pygoplites diacanthus*, FF = *Forcipiger flavissimus*, CB = *Ctenochaetus binotatus*, CK = *Chaetodon kleinii*, CL = *Chaetodon lunulatus*, NN = *Neoglyphidodon nigroris*.

The corallivore *Chaetodon lunulatus* had a statistically larger isotopic niche at the sponge-dominated site ($\text{SEA}_B > 95\%$, $p < 0.05$) (Appendix D, Table 9). *Zanclus cornutus*, *Pygoplites diacanthus* and *Forcipiger flavissimus* also had larger isotopic niches at the sponge-dominated site and were close to the critical p-value ($\text{SEA}_B > 92\%$, $p = 0.07$). The detritivore *Ctenochaetus binotatus* had a statistically smaller isotopic niche at the sponge-dominated site ($p < 0.05$) (Figure 4.3).

4.5.3 Carbon sources and benthic prey

Source isotope values were significantly different between sites (pseudoF_{1, 69} = 12.07, p = 0.002) and sources (pseudoF_{11, 69} = 100.71, p = 0.001) (Figure 4.4) (Appendix D, Table 10). Pairwise tests were conducted for each site separately due to differences in algal types collected. Green turf algae had the lowest $\delta^{15}\text{N}$ values of any source item and it was isotopically separated from *Dictyota* sp. at the sponge-dominated site (p(MC) = 0.006) and *Halimeda* sp. (p(MC) = 0.004) at the high-quality site (Appendix D, Table 11/12). *Dictyota* overlapped with both *Hyrtios* and *Porites* with no significant differences in isotope values. SPOM and *Lamellodysedia herbacea* were the most ^{13}C depleted sources sampled. They showed no difference in isotope values at either site.

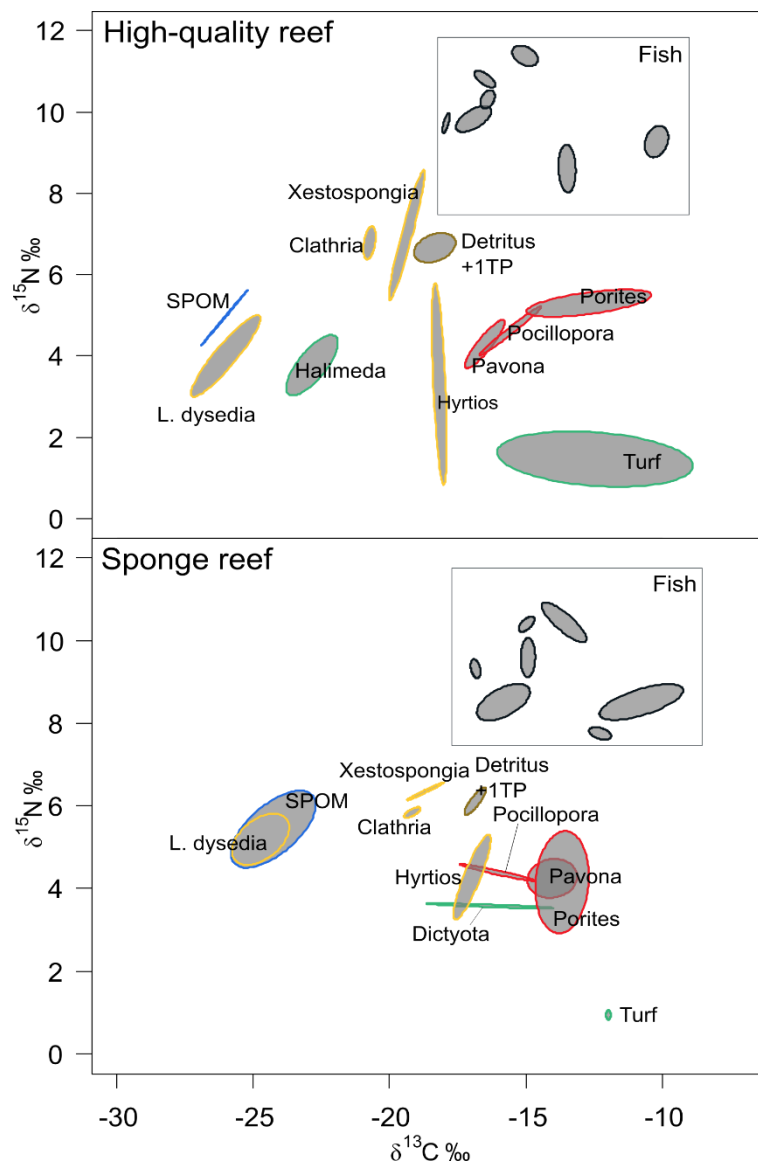


Figure 4. 4 Isotopic niches (standard ellipse area - SEAc) of carbon sources and potential prey collected at two sites in the Wakatobi MNP, Indonesia. Insert is Figure 4.3.

All three corals showed little difference in $\delta^{15}\text{N}$ values but more variation in $\delta^{13}\text{C}$ values (Figure 4.4). At the high-quality site, *Pavona* and *Pocillopora* had similar isotope values but *Porites* was isotopically separated ($p(\text{MC}) = 0.012$ and $p(\text{MC}) = 0.045$) whereas at the sponge-dominated site *Pavona* and *Porites* had similar isotope values but *Pocillopora* was isotopically separated ($p(\text{MC}) = 0.032$ and $p(\text{MC}) = 0.041$). Sponges were ^{13}C depleted relative to corals although *Hyrtios* had similar isotope values to *Pavona* at the high-quality site and *Pocillopora* at the sponge-dominated site. The sponge species had a wide range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and, except for *Xestospongia* and *Clathria*, they were isotopically distinct. Detritus (+1TP) was isotopically distinct at the sponge-dominated site but had similar isotope values to *Xestospongia* at the high-quality site.

Source items were grouped into major taxa (algae, sponges, corals and SPOM) with *L. dysedia* removed from the analysis because $\delta^{13}\text{C}$ values were $\sim 7\text{‰}$ lower relative to the spongivores and due to being isotopically distinct from other sponges. One sample of *Hyrtios* from the high-quality site was also removed due to a very low $\delta^{15}\text{N}$ value, potentially caused by the inclusion of outer tissue containing symbionts, known to cause depleted ^{15}N in sponges (Freeman et al. 2014). Both groups of algae that were not replicated between sites were also removed. There were significant differences between major taxa (pseudo $F_{4, 47} = 87.58$, $p = 0.001$) and between sites (pseudo $F_{1, 47} = 5.50$, $p = 0.01$) (Appendix D, Table 13). Pairwise tests showed that all major sources had significantly different isotope ratios to one another (Figure 4.5) (Appendix D, Table S14). The only sources that showed a difference in isotope values between sites were detritus (+1TP) ($t = 4.20$, $p(\text{MC}) = 0.002$) and sponges ($t = 1.98$, $p(\text{MC}) = 0.041$) (Appendix D, Table 15). Bayesian analysis of SEA_C indicated that the isotopic niche sizes of corals and sponges were similar at both sites. There was a $>90\%$ probability that the isotopic niche of detritus (+1TP) was smaller at the sponge-dominated site.

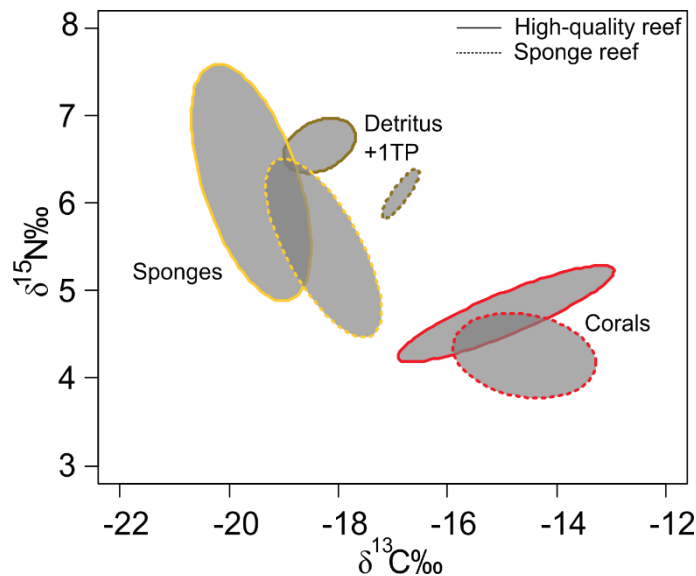


Figure 4. 5 Isotopic niches (standard ellipse area - SEA_C) of sponges, corals and detritus +1TP collected at two sites in the Wakatobi MNP, Indonesia.

4.5.4 Pelagic and benthic carbon contributions to coral reef fish diets

All species showed a higher proportional use of benthic carbon than pelagic carbon at both sites (Figure 4.6). Pelagic contributions were higher and benthic contributions lower at the high-quality site for the majority of species, except for *Forcipiger flavissimus*, for which there was considerable uncertainty in proportion estimates and *Ctenochaetus binotatus* which, showed little difference in values between sites. At both sites, the species with the largest contribution of pelagic carbon was the planktivore *Neoglyphidodon nigroris* ($32\% \pm 9\%$ (HQ) and $25.6 \pm 8\%$ (LQ [mean \pm SD])) whereas *C. binotatus* had the highest contribution of benthic carbon ($89\% \pm 8\%$ (HQ) and $90 \pm 7\%$ [mean \pm SD]).

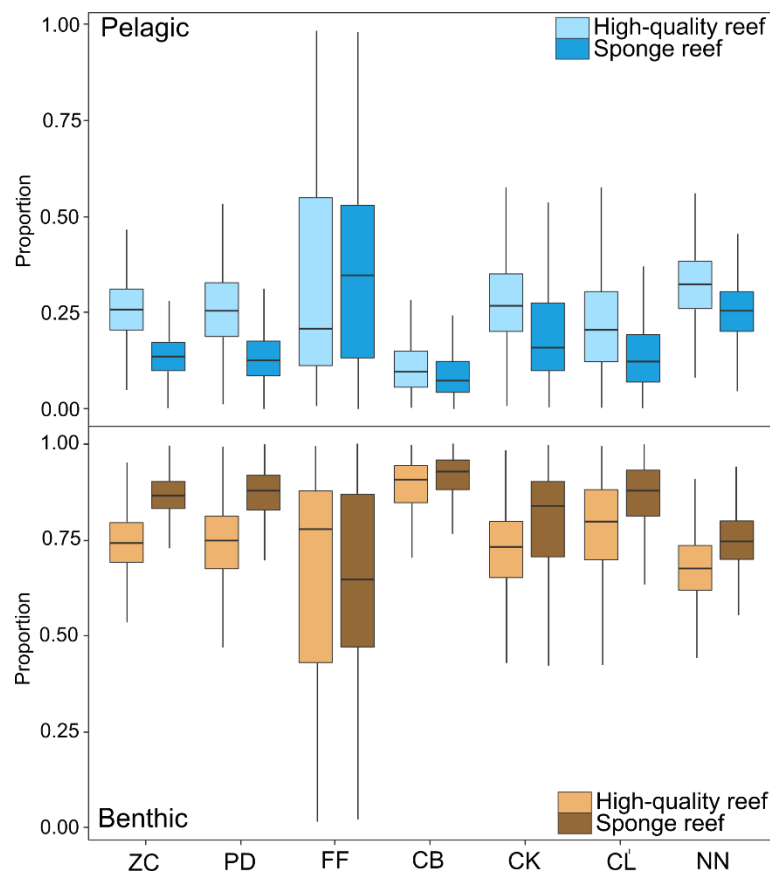


Figure 4. 6 Relative contributions of pelagic and benthic carbon to the diet of seven coral reef fish species representing functional feeding groups sampled at two sites in the Wakatobi MNP, Indonesia. Boxes represent the credible interval of 50% and error bars the credible interval of 95%. ZC = *Zanclus cornutus*, PD = *Pygoplites diacanthus*, FF = *Forcipiger flavissimus*, CB = *Ctenochaetus binotatus*, CK = *Chaetodon kleinii*, CL = *Chaetodon lunulatus*, NN = *Neoglyphidodon nigroris*.

4.5.5 Quantitative comparison of trophic structure

Bayesian analysis of community metrics showed that the sponge-dominated site had lower trophic diversity (>80% probability lower CD), significantly lower diversity of basal resources (>95% probability smaller $\delta^{13}\text{C}$ range) and higher trophic redundancy than the high-quality site (>95% probability smaller SDNND; >77% probability smaller NND) (Figure 4.7).

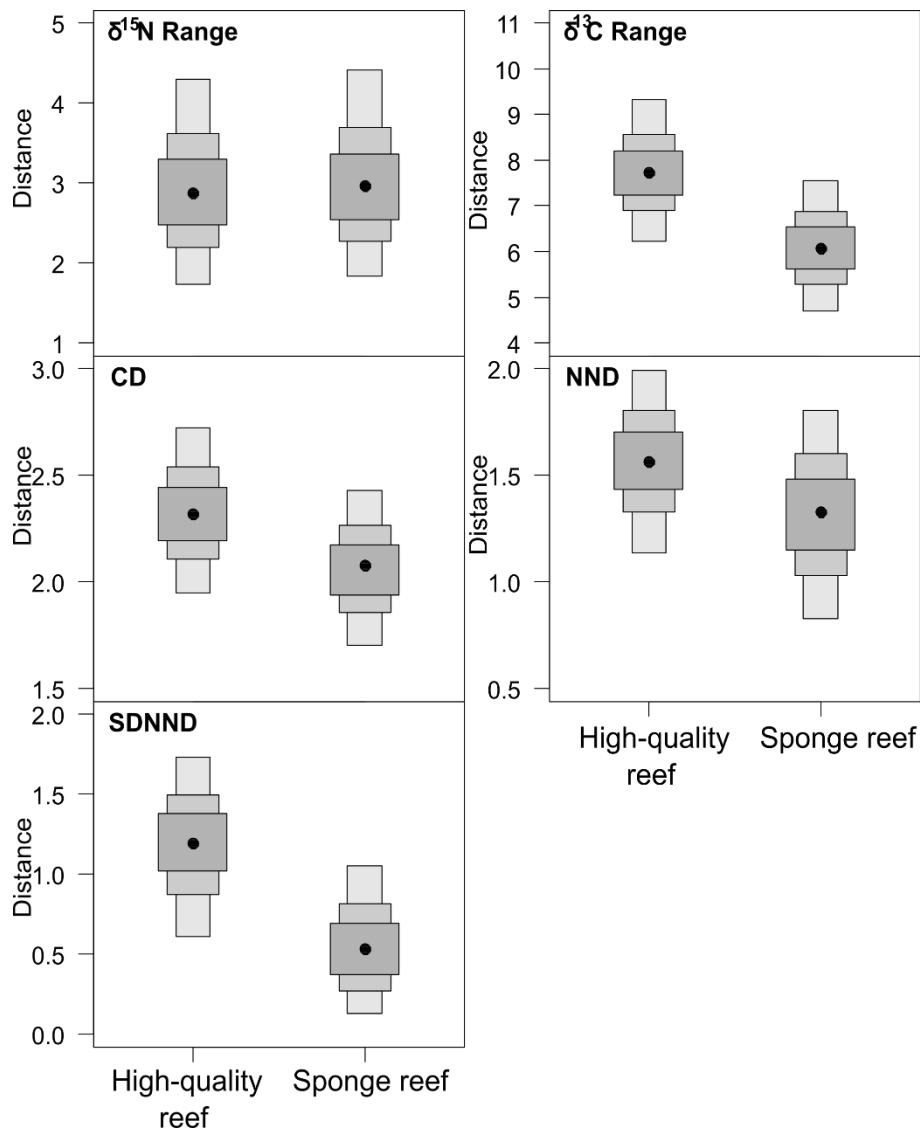


Figure 4. 7 Bayesian results of the Layman community metrics for two sites (higher-quality and sponge-dominated) sampled in the Wakatobi MNP Indonesia. Black circles represent the mode and boxes correspond to the 50%, 75% and 95% credible intervals. $\delta^{15}\text{N}$ Range = Range of $\delta^{15}\text{N}$ values. $\delta^{13}\text{C}$ Range = Range of $\delta^{13}\text{C}$ values. CD = Mean distance to the centroid. NND = Mean nearest neighbour distance. SDNND = Standard deviation of nearest neighbour distance.

4.6 Discussion

A deeper understanding of how individual species and entire communities respond to environmental change is key to predicting the future functioning of coral reefs (Sale et al. 2014). In this chapter, I examined how changes in resource availability affect foraging strategies and trophic diversity in a coral reef fish assemblage. Results indicated that the majority of fish at the sponge-dominated site responded to a low-quality environment by expanding their diet, increasing diet similarity and reducing trophic diversity at the community level. This can be expected to reduce community stability as resource partitioning decreases, eroding species co-existence.

4.6.1 Spatial and interspecific variation in trophic position and $\delta^{13}\text{C}$ values

Reduction of food chain length and mean trophic position of consumers are indicators of habitat degradation (Pauly & Watson, 2005; Layman et al. 2007; Hempson et al. 2017). In my study, the mean trophic position of fish (species pooled) on the sponge-dominated reef was significantly lower than on the high-quality reef. This could indicate an increased reliance on benthic carbon sources as benthic carbon tends to exhibit lower $\delta^{15}\text{N}$ values than pelagic carbon (Hempson et al. 2017; Eurich et al. 2019). For example, Hempson et al. (2017) found that the trophic position of the coral grouper *Plectropomus maculatus* decreased as a result of diet switching from planktivorous damselfishes to algal-feeding damselfishes. In this study, between-site pairwise tests indicated that *Zanclus cornutus* and *Forcipiger flavissimus* both had significantly lower trophic positions at the sponge-dominated reef. Individuals of *Z. cornutus* sampled at the sponge-dominated site were significantly smaller than individuals sampled at the high-quality site, therefore spatial differences in trophic position could be the result of dietary ontogeny (de la Morinière. 2003) or size-related processes which affect $\delta^{15}\text{N}$ tissue values (Carassou et al. 2008). *F. flavissimus* occupied the highest trophic position at the high-quality site, but at the sponge-dominated site, it had the same trophic position as *Chaetodon kleinii*, indicating a comparatively shorter food chain. Lower trophic positions may reflect diet switching to suboptimal prey at the sponge-dominated site (Rossi et al. 2015; Calizza et al. 2017), as *F. flavissimus* is known to have one of the widest diets of all butterflyfish (Harmelin-Vivien & Bouchon-Navarro, 1983). Whilst this adaptability may assist survival in the short term, over longer time scales a reduction in food quality could reduce fecundity, growth rates and maturity (Jones & McCormick, 2002; Graham et al. 2007) due to a loss of physiological condition (Berumen et al. 2005).

With the exception of *Chaetodon lunulatus* and *Zanclus cornutus*, fish $\delta^{13}\text{C}$ values were significantly depleted at the high-quality site indicating greater reliance on oceanic production (Fry et al. 1982; de la Morinière et al. 2003; Wyatt et al. 2012) likely caused by increased proximity to the open ocean. Wyatt et al. (2012) observed the same spatial pattern in the $\delta^{13}\text{C}$ values of herbivores, carnivores and planktivores at Ningaloo Reef, but not with detritivores or corallivores (Wyatt et al. 2012). This was explained by the dominance of reef-derived material in the diets of both feeding groups across a back

reef to open ocean gradient (Wyatt et al. 2012) and may explain why *C. lunulatus* and *Z. cornutus* did not show the same depletion in ^{13}C as the other fish at the high-quality site. Similarly, Carassou et al. (2008) attributed higher $\delta^{13}\text{C}$ and lower $\delta^{15}\text{N}$ in parrotfish tissues to the role of detritus in their diets therefore higher $\delta^{13}\text{C}$ values could be an indication of increased consumption of detritus at the sponge-dominated site. Interestingly at the high-quality site, the species with similar $\delta^{13}\text{C}$ values were *Z. cornutus*, *Pygoplites diacanthus* and *Chaetodon kleinii*, with trophic positions increasing respectively (2.72 ± 0.03 , 2.85 ± 0.02 and 2.98 ± 0.02 [mean \pm SE]). This supports the conclusion from Chapter 2 that the trophic ecologies of these three species are tightly linked to sponges, with *Z. cornutus* and *P. diacanthus* consuming sponge tissue and *C. kleinii* targeting sponge-associated fauna.

4.6.2 Isotopic niches of coral reef fishes

Optimal foraging theory predicts that individuals choose prey in a way that maximizes their overall rate of energy gain (Pyke et al. 1977; Brooker et al. 2014). Hence in habitats where preferred food is limited, consumers adjust their foraging strategy to include non-preferred prey (Berumen et al. 2005), which leads to wider trophic niches (Rossi et al. 2015). The isotopic niche of the corallivore *Chaetodon lunulatus* was statistically larger on the sponge-dominated reef and three further species (*Forcipiger flavissimus*, *Zanclus cornutus* and *Pygoplites diacanthus*) had isotopic niches that were close to being statistically larger ($p = 0.07$) although sample sizes may have been too low to detect statistical significance. Nevertheless, this indicates a decrease in resource specialisation at the sponge-dominated site for the majority of consumers. Similar patterns of niche width expansion have been observed in tropical streams altered by agricultural practices (Parreira de Castro et al. 2016) and in degraded *Posidonia* meadow beds (Calizza et al. 2017) indicating the potential for habitat disturbance to lead to generalist-dominated communities (Clavel et al. 2011).

On Indo-Pacific reefs, *Chaetodon lunulatus* is considered to be an obligate corallivore (Nagelkerken et al. 2009) that depends on coral for $\geq 80\%$ of its diet (Cole et al. 2008). Unlike other specialist corallivores that have sharply declined in abundance and suffered localised extinction following coral loss (Kokita & Nakazono 2001; Munday, 2004; Brooker et al. 2014), this study suggests that *C. lunulatus* can adopt more generalist foraging behaviour in coral-poor habitats. This success could be attributed to flexible prey choice within the Scleractinian order (Berumen et al. 2006; Pratchett et al. 2005) yet there was no difference in the size or location the isotopic niche of corals between sites suggesting that *C. lunulatus* supplemented its diet with another source of carbon. Increased proximity to the isotopic niche of the detritivore *Ctenochaetus binotatus* indicates that detrital carbon is more important for *C. lunulatus* at the sponge-dominated site. This is consistent with the findings of McMahon et al. (2016) who showed that microbially re-worked detritus was an important secondary source of carbon for a suite of reef species, including the obligate corallivore *Chaetodon trifascialis*. Although the majority of species sampled had larger isotopic niches at the sponge-dominated site, the detritivore *C. binotatus* had a

statistically smaller isotopic niche. This was consistent with detritus (+1 TP), which also had less variable isotopic values at the sponge-dominated site, possibly indicating a lower diversity of sources contributing to the detrital pool than at the high-quality site.

Intraspecific and interspecific competition for food affects foraging strategies (Dill, 1983) and hence could have an impact on isotopic niche size. Inspection of monitoring data collected from six sites in the Hoga/Kaledupa channel for 2017 (including the two sites sampled during this study) indicated that there was no statistical difference in the density of *Chaetodon lunulatus* or obligate corallivores between the sponge-dominated site and the higher quality site (Operation Wallacea unpublished monitoring data, 2017; Appendix D, Table 17). In Chapter 2, densities of *Pygoplites diacanthus* and *Forcipiger flavissimus* were lower at the sponge-dominated site but here was no difference in the densities of *Zanclus cornutus* or *Chaetodon binotatus* indicating no obvious relationship between intraspecific competition and isotopic niche size. Similarly, predation risk was not considered during this study although it is known to affect the foraging strategies of fishes (Dill, 1983). Predation risk could be higher at the sponge-dominated site due to reduced structural complexity and fewer refuge holes providing protection from predators (Rodgers et al. 2014), although this would generally be expected to reduce movement and access to resources, which would decrease niche width (Calizza et al. 2013).

4.6.3 Carbon sources and benthic prey

There was considerable variation in prey and carbon source values, with only one overlap in isotopic niche observed between sponges and detritus + 1TP at the high-quality site. The detrital signature was inferred from a proxy detritivore, *Synaptula* sp., following McMahon et al (2016) who also used a detritivorous sea cucumber to characterise detritus on coral reefs (McMahon et al. 2016). Most of the *Synaptula* sp. collected were found on the outside of sponges and some *Synaptula* sp. are known to associate with some species of Porifera (Hammond & Wilkinson, 1985). Similar isotopic values of the two organisms could be the result of *Synaptula* sp. consuming sponge mucus (Hammond & Wilkinson, 1985) or both groups ingesting similar particles (>50 µm) from surface epithelial cells (Maldonado et al. 2012).

The $\delta^{13}\text{C}$ values for SPOM were low (mean -25.13‰) however, similar values have been obtained from coastal waters in the Caribbean (Freeman et al. 2014; van Duyl et al. 2018) and photosynthetically derived carbon can lead to carbon values as low as -27‰ (Levin et al. 2002). Sponges displayed a wide range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and, aside from *Xestospongia* and *Clathria*, sponges were isotopically discriminated, suggesting distinct carbon and nitrogen cycling processes (Freeman et al. 2014). The phototrophic sponge *Lamellodysedia herbacea* had similar isotopic values to SPOM and was significantly more depleted in ^{13}C than the heterotrophic sponges, indicative of nutrition derived from phototrophic symbionts (Fiore et al. 2010). When heterotrophic sponges were grouped, they were ^{15}N -

enriched and ^{13}C -depleted relative to corals. The same spatial variation in isotopic values was also observed between the spongivore (*Pygoplites diacanthus*) and the corallivore (*Chaetodon lunulatus*), however, both specialists were enriched by more than 1‰ in ^{13}C than their main food source. Carbon isotopic fractionation has been shown to vary as a function of diet quality (Busst & Britton, 2016) and diets with high C: N ratios lead to larger $\Delta^{13}\text{C}$ (Caut et al. 2009; Busst & Britton, 2016). This may account for the large differences in $\delta^{13}\text{C}$ values between the specialists and their prey observed in this case.

Turf algae had the lowest $\delta^{15}\text{N}$ values of all sources and they were approximately 2‰ lower than both *Dictyota* sp. and *Halimeda* sp. This could be due to the method of collection in which algal turfs were scraped from the benthos which likely retained other organisms including benthic diatoms and cyanobacteria. This would be consistent with other studies that have sampled microphytobenthos (diverse assemblages of photosynthetic diatoms, cyanobacteria, flagellates and green algae) in coastal systems have also reported equally low $\delta^{15}\text{N}$ values (McLeod et al. 2009). Due to the low $\delta^{15}\text{N}$ values of turf algae and insufficient data for macroalgae for both sites, algae were not included as part of the benthic signature in isotope mixing models. However, as algae generally have higher $\delta^{13}\text{C}$ values than SPOM (Fry & Sherr, 1984; Fry, 2006) it is unlikely the exclusion of algae would have an impact on proportion estimates.

4.6.4 Organic matter contributions and community structure

Pelagic and benthic-derived food differ in stable isotope ratios (Fry et al. 1982) and differences in proportional use are reflected in the stable isotope values of consumers (van Duyl et al. 2018; Eurich et al. 2019). Mixing models estimated a larger contribution of benthic carbon versus pelagic carbon for all species at both sites, which was likely caused by low $\delta^{13}\text{C}$ values of SPOM (range: - 23‰ to -26.9‰) relative to fish $\delta^{13}\text{C}$ values (range: - 8.9‰ to -18.1‰). As mentioned, isotopic fractionation can vary as a result of diet quality and environment (McCutchan et al. 2003; Caut et al. 2009; Busst & Britton, 2016). Therefore, the trophic enrichment factors applied may underestimate carbon fractionation taking place from SPOM to secondary consumers. Accordingly, this discussion is limited to relative contribution rather than specific values. Spatial variation the isotope ratios of pelagic and benthic organic matter was accounted for by using site-specific values in isotope mixing models (Phillips et al. 2014). Most fish showed an increased reliance on pelagic carbon at the high-quality site, likely the result of greater proximity to upwelling (Wyatt et al. 2012). Pelagic nutrients are generally considered higher quality than reef derived nutrients due to lower C: N ratios (Atkinson & Falter, 2003; Wyatt et al. 2012). Relative isolation from upwelling and low coral and low macroalgal cover indicate that detrital production could be the main carbon source fuelling secondary production at the sponge-dominated site. Quantitative analysis of community structure revealed that consumers on the sponge-dominated site exploited a significantly lower range of resources ($\delta^{13}\text{C}$ range). The sponge-dominated site had

lower trophic diversity (CD) and higher trophic redundancy than the high-quality site suggesting a simplified trophic structure and that consumers have more similar diets. Whilst supplementing dietary requirements with detrital carbon has been described as nutritionally beneficial (Nelson & Wilkins, 1988), research utilising compound-specific stable carbon isotope ratios showed that a single source of carbon dominated the diets of benthic foraging reef fish and predators, indicating tight links between primary producers and consumers (McMahon et al. 2016). Hence, a reduction in the range and availability of primary producers can destabilise ecosystems by disrupting resource compartmentalisation among co-existing species (Calizza et al. 2017).

4.6.5 Study limitations

This study used a comparative approach to compare differences in trophic structure and consumer niche widths between a sponge-dominated reef and an adjacent, higher quality “reference site”. The selection of reference sites is inevitably subjective and high spatial and temporal variability on coral reefs cannot easily be encompassed by the use of a single reference site. However, due to the high cost of stable isotope analysis, the decision was made to sample two sites fully and represent more species at the assemblage level, than sample at a wider range of sites but with fewer species represented. This enabled a thorough comparison between these two sites but inevitably limits any extrapolation from this one sponge-dominated site to all sponge-dominated reefs. Future studies with access to other reef systems that have shifted to sponge-dominance should increase replication at the site level to better understand the trophic consequences of shifts to sponge dominance.

4.6.6 Conclusion

Understanding how populations and communities persist under different environmental conditions is a critical step towards predicting the capacity of future coastal environments to provide crucial goods and services. In this chapter, I used stable isotopes and Bayesian modelling to investigate how habitat quality affects foraging strategies and trophic diversity in a coral reef fish assemblage. I have shown that the majority of species, including the obligate corallivore, had wider trophic niches at the sponge-dominated site. Fish at the sponge-dominated site also used a significantly lower range of resources, had lower trophic diversity and obtained more carbon from benthic production than at the high-quality reef site. Results suggest a more simplified trophic structure at the sponge-dominated site characterised by fish with more similar diets.

Chapter 5. Resource partitioning by temperate sponges is driven by differential use of pelagic and benthic organic matter

5. 1 Abstract

Co-occurring species in competitive ecosystems partition resources to facilitate coexistence. For marine sponges, trophic diversity is heightened by selective filtration of suspended particles, associated symbiont communities and the uptake of dissolved organic matter (DOM). Resolving the diversity of nutritional pathways used by sponges is essential to understand how sponge assemblages may react to future changes in carbon sources. In this chapter, I investigated niche partitioning and resource use among co-occurring temperate sponges. My approach was to measure the carbon and nitrogen stable isotope ratios of five abundant sponge species at two depths at two sites on opposing ends of Doubtful Sound, Fiordland. I also measured stable isotope ratios of picoplankton (<2 μm size phytoplankton), the 2-200 μm fraction of the water column, macroalgae and terrestrial organic matter (TOM) and used isotope mixing models to estimate the contribution of carbon sources to sponge species. Sponges displayed a wide range of $\delta^{15}\text{N}$ values spanning two trophic levels. Sponges at the Inner Fiord had higher $\delta^{15}\text{N}$ values than the Outer Fiord and 30 m sponges had higher $\delta^{15}\text{N}$ values than 10 m sponges, spatial differences that were not reflected by water column resources. There was a high degree of interspecific resource partitioning with the majority of isotopic niche overlaps observed at the Inner Fiord site at 10 m. Comparisons of isotopic niche size suggested more diverse resource use at the Outer Fiord site for *Axinella richardsoni*, *Cymbastela tricalyciformis* and *Raspailia topsenti*. Neither picoplankton nor TOM contributed significantly to the diets of most sponges, rather the 2-200 μm fraction of the water column was important for *Raspailia topsenti*, *Axinella richardsoni* and *Tethya* sp. at the Inner Fiord with the proportional contribution of macroalgal-derived organic matter increasing at the Outer Fiord. Interestingly, macroalgal-derived organic matter was the most important carbon source for both *Cymbastela tricalyciformis* and *Latrunculia fiordensis* at the Outer Fiord site. These species were found in low abundances at the Inner Fiord where macroalgal coverage was also low. The only evidence of TOM assimilation was by two sponges sampled opportunistically by remotely operated underwater vehicle (ROV) at 60 m at the Inner Fiord, suggesting either direct consumption of picoplankton or participation in a chemosynthetic food web driven by microbial decomposition of TOM. This study has demonstrated a high degree of resource partitioning between co-existing temperate sponges driven by differential use of pelagic and benthic organic matter. However, TOM may support some sponges via microbial pathways where marine production is limited.

5. 2 Introduction

Understanding how species co-exist, especially when they appear to rely on a common resource, has been a central theme in ecology for nearly a century (Grinnell, 1917; Schoener, 1986). The term ‘ecological niche’ was first popularised by Grinnell (1917) who described it as the habitat and accompanying behavioural adaptations of a species. His concept focused on the environment and suggested that ecological equivalents could be found in different locations implying invariant rules and the existence of unoccupied niches (Schoener, 2009). Hutchinson (1957) advanced the concept by suggesting that the ecological niche could be quantitatively described in space as an ‘n dimensional hypervolume’ (or fundamental niche) described by scenopoetic (environmental components of niche space) and bionomic (trophic components of niche space) axes (Hutchinson, 1957; Hutchinson, 1978; Schoener, 2009). However, interspecific competition for resources means that often the fundamental niche is not fully exploited, and species are restricted to a realised niche (Hutchinson, 1978). For some scientists, the focus shifted to understanding the evolution of interspecific differences in resource use (MacArthur & Levins, 1967) as closely-related species cannot coexist indefinitely if niche overlap is high (Gause, 1934; MacArthur & Levins, 1967; Schoener, 2009) and specialisation is often necessary to avoid extinction (MacArthur & Levins, 1967). Differential resource use mediates species co-existence and regulates diversity (Schoener, 1986). Resolving resource partitioning is thus essential to understand how communities may react to future change in resource availability (Middelburg, 2014).

Temperate subtidal reefs support a diverse array of suspension feeders that are reliant on similar food resources suggesting a degree of resource partitioning (Stuart & Klumpp, 1984). Marine sponges provide a significant trophic link between benthic and pelagic systems through active suspension feeding (Lesser, 2006). They can consume a range of particle sizes (Maldonado et al. 2012), although they are most efficient at filtering picoplankton (0.7 – 2 µm) due to the structure of their aquiferous system (Reiswig, 1971; Pile et al. 1996). Once considered non-selective, (Maldonado et al. 2012), several studies have demonstrated the ability of sponges to selectively retain some particles over others (Yahel et al. 2006; Weisz et al. 2007; Maldonado et al. 2012). For example, Hanson et al. (2009) demonstrated that *Callyspongia* sp. optimised nutritional intake by consistently selecting against low DNA (LDNA) bacteria that have lower carbon and nutrient contents than high DNA (HDNA) bacteria. In addition to heterotrophic selectivity, partnerships with symbionts allow host sponges to engage in alternative metabolic pathways (Taylor et al. 2007), particularly under nutrient-limited conditions (Freeman & Thacker, 2011, Freeman et al. 2014). These metabolic interactions can be phototrophic or chemotrophic depending on the symbionts, available substrate and oxygen conditions (Vacelet et al. 1995; Taylor et al. 2007). For example, some deep-sea sponges have been shown to harbour methanotrophic symbionts and possess carnivorous feeding structures (Vacelet et al. 1995). Dissolved organic carbon (DOC), operationally defined as the organic carbon that passes through a GF/F filter paper (nominal pore size 0.7 µm) (Maldonado et al. 2012), has also been identified as a key nutritional

resource for sponges (Yahel et al. 2003; de Goeij et al. 2013; McMurray et al. 2016). A series of isotopic tracer experiments (de Goeij et al. 2013; Rix et al 2016; Rix et al 2017) showed that sponges assimilate DOM and return it to the benthos as particulate detritus in a process called ‘the sponge loop’ (de Goeij et al. 2013; Rix et al 2018). However, the ubiquity of the sponge loop remains undetermined as none of the nine emergent sponge species studies by McMurray et al. (2018) produced significant quantities of detritus (McMurray et al. 2018). Both coral- and algal-derived DOM have been shown to support the sponge holobiont (Rix et al. 2016; Rix et al. 2017), however, the internal routing of these two carbon sources varies between the sponge host and its associated microbial community (Rix et al 2017).

Stable isotope analysis (SIA) has emerged as a powerful tool for ecologists investigating niche partitioning and organic matter cycling (Fry, 2006; Middelburg, 2014). Nitrogen isotopes are commonly used to depict trophic position due to the predictable (but see Mill et al. 2007; Hussey et al. 2014) stepwise enrichment from the source to the consumer of approximately 2-4‰ (Minagawa & Wada, 1984; Fry, 1988). Carbon isotopes generally show less variation per trophic level transfer (around 0 - 1‰) (but see Caut et al. 2009; Busst & Britton, 2016) and can be used to identify organic matter sources that vary in carbon isotope values due to different photosynthetic pathways (Post, 2002; Bearhop et al. 2004). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values can be used to visualise a species in δ -space and thus describe its isotopic niche (Chapter 4; Newsome et al. 2007; Jackson et al. 2011), a generalised proxy for trophic niche (Catry et al. 2016). These techniques have been used to identify niche separation among co-occurring sponges in tropical and temperate systems (Thurber, 2007; Freeman et al. 2014; Freeman et al. 2016). For example, Freeman et al. (2014) used SIA to show that high microbial abundance (HMA) sponges occupy a unique niche space compared to low microbial abundance (LMA) sponges. However, host species identity was found to drive stable isotope signatures more than symbiont abundance (Freeman et al. 2014).

Resolving the relative contribution of different sources of organic matter (OM) to an organism’s diet relies on isotopic discrimination between sources (Parnell et al. 2010; Philips et al. 2014). Benthic and pelagic sources of OM differ in stable isotope ratios, as pelagic sources tend to exhibit lower $\delta^{13}\text{C}$ values and higher $\delta^{15}\text{N}$ values than benthic primary producers (Fry, 2006; van Duyl et al. 2011). Suspended detrital (non-living) particles from benthic and pelagic sources are consumed by sponges (Hadas et al. 2009; McMurray et al. 2016). For example, detritus was estimated to constitute 20% of the diet of the giant barrel sponge *Xestospongia muta*, approximately double the contribution of living particulate organic carbon (LPOC) (McMurray et al. 2016). Primary producers e.g. marine algae also release DOC into the water column as a photosynthetic biproduct (Wild et al. 2004; Wild et al. 2010) that is then taken up by the sponge holobiont (Rix et al. 2016; Rix et al. 2017). Both detrital and dissolved OM are generally thought to reflect the isotopic signature of the source (Benner et al. 1997; Fry et al. 1998), hence bulk isotope values of sources can be used as a proxy to infer both detrital and dissolved OM from primary producers (van Duyl et al. 2011; van Duly et al. 2018).

Van Duyl et al. (2011) used isotopic discrimination between sources and fatty acid analysis to show that coral cavity sponges in the Caribbean rely on reef-derived OM from coral mucus and crustose coralline algae (CCA) rather than pelagic production. In a later study, van Duyl et al. (2018) showed that co-occurring sponges inhabiting an atoll in the Caribbean relied on different proportions of benthic- and pelagic-derived OM. Recently, it has been proposed that the high proportion of heterotrophic sponge species in the Caribbean could be explained by an ability to consume refractory DOC originating from terrestrial environments considered to be unavailable to microbes (Pawlik et al. 2016). However, there is no evidence that sponges assimilate refractory compounds directly from terrestrial OM (TOM), which are typically high molecular weight (HMW) compounds and generally considered resistant to degradation (Fasching et al. 2014). If sponges can utilise a portion of refractory TOM, it could explain global patterns of sponge abundance (Pawlik et al. 2016) and would highlight an essential, yet overlooked, link between the terrestrial, marine pelagic and marine benthic systems. TOM can be differentiated from marine OM by its high C: N ratio (McLeod et al. 2007), hence SIA can be used to investigate the relative contribution of TOM, pelagic and benthic production to the diet of sponges in coastal systems.

Substantial gradients in the composition and availability of OM (Wing et al. 2012) make Fiordland in Southwest New Zealand an ideal system to study OM cycling. Heavy rainfall transports large quantities of TOM into the fiords where it makes up a substantial fraction of carbon source pools (McLeod & Wing, 2009), with the relative input of marine OM (phytoplankton and macroalgae) increasing along a seaward gradient (McLeod et al. 2007). TOM has been shown to support upper trophic levels via the chemoautotrophic activities of bacterial symbionts that fix dissolved CO₂ (McLeod et al. 2007; Rodgers et al. 2008; McLeod et al. 2009) although smaller inputs of marine OM likely support the majority of macroinvertebrate consumers (McLeod et al. 2009). Sponges are most abundant between 40 - 60 m depth with abundance increasing from the head of the fiords to mid and outer locations (Bell, unpublished data), consistent with patterns of marine production (Wing et al. 2014). However, even where marine production is limited, sponges cover ~6.5% of the substrate (Bell, unpublished data) suggesting that sponges can make use of diverse sources of OM on temperate reefs in the fiords.

In this chapter, I investigated niche partitioning and resource use among co-occurring temperate sponges. Specifically, my aims were: i) to investigate spatial variation in carbon and nitrogen stable isotope ratios of sponges and water column resources; ii) to assess interspecific niche partitioning and shifts in resource use from 10 m to 30 m by calculating the isotopic niche (a proxy for trophic niche) of each species; iii) to investigate shifts in the diversity of resources used by each species; and, iv) to calculate organic matter contributions to sponge diets.

5.3 Methods

5.3.1 Study Sites

Fiordland in southwest New Zealand is an extensive coastal forest incised by deep fiords, contained within the Te Wāhipounamu World Heritage Area. It is characterised by heavy rainfall (6-8 m annually) (Gibbs et al. 2000) and densely vegetated, mountainous terrain. Doubtful Sound is the deepest of the 14 fiords in the park and the second-longest, with three distinct arms (Figure 5.1). The Inner Fiord site was 40 km from the open sea and in some parts only 1 km wide. In this narrow part of the fiord, freshwater input is vastly increased by an outflow from the Manapouri hydroelectric power station, which can release an average $450 \text{ m}^3 \text{ s}^{-1}$ freshwater into Deep Cove (McLeod et al. 2010). In parts of the fiord, this has extended the low salinity layer (LSL) to 5 m depth (Boyle et al. 2001). The Outer Fiord site was 1 km from the mouth of the fiord.

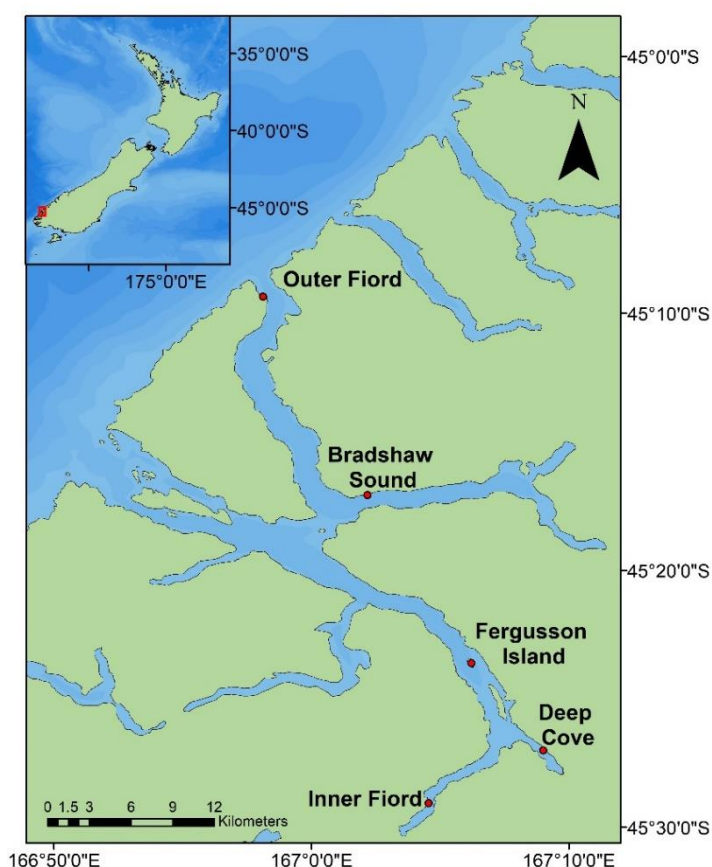


Figure 5. 1 Map showing locations of sample sites in Doubtful Sound, Fiordland visited during February 2019 on the MV Southern Winds. Sponges were collected the Inner Fiord and Outer Fiord at opposing ends of Doubtful Sound, with three additional deep sponges collected by ROV at Fergusson Island. Organic matter samples were collected at the Inner Fiord and Outer Fiord, and an intermediate site in Bradshaw Sound.

5.3.2 Sample collection

All samples were collected during a one-week voyage in February 2019. Water column samples were collected from the Inner Fiord and Outer Fiord (Figure 5.1) at 10 m and 30 m using a 5 litre Niskin bottle, with one additional sample gathered opportunistically from 80 m at the Inner Fiord site. All samples were screened (200 μm), then filtered into different size fractions to represent two different food sources for sponges. The screened sample was filtered on to a pre-combusted Whatman GFM filter paper (nominal pore size 2 μm) to obtain the 2-200 μm size fraction. The resulting filtrate (<2 μm) was filtered on to a pre-combusted Whatman GF/F filter paper (nominal pore size 0.7 μm) to obtain the 0.7-2 μm (picoplankton) size fraction. Samples of the most abundant macroalgae were collected via SCUBA at the Inner Fiord and Outer Fiord sites (10-15 m), and an intermediate site in Bradshaw Sound (Figure 5.1). *Ulva* sp. was the only macroalga found at the Inner Fiord so five samples were collected from this site and five from Bradshaw Sound. Five samples of *Ecklonia radiata* and *Codium* sp. were collected from both the Outer Fiord site and Bradshaw Sound. Terrestrial organic matter (TOM) was collected at all three sites by sieving surface water for vascular plant detritus (Appendix E, Table 2 for sample size info). Macroalgal samples and leaf litter were rinsed with filtered seawater (<0.7 μm) to remove sediments and epibiota, then rinsed with double distilled water (DDW) and stored at -20°C.

Five sponge species were collected via SCUBA at the Inner Fiord and Outer Fiord from 10 m and 30 m. I aimed to collect five samples of each species from each depth, however, replication varies due to variation in abundances (see Appendix E, Table 1 for sample size info). Five additional sponge samples of unknown species were also collected opportunistically using an ROV, two from a depth of 60 m at the Inner Fiord and three from a depth of 55 m at a site near Fergusson Island in the main channel (Figure 5.1). Once collected, sponges were placed in buckets with filtered seawater for 2 hours to remove ambient seawater, then a section of sponge tissue was dissected and stored at -20 °C. This time interval was deemed sufficient based on pumping rates for other New Zealand temperate sponges (Perea-Blázquez et al. 2012). For most species, there was either no observable difference in ectosome and choanosome (e.g. *Axinella richardsoni*), or the specimen was too thin to separate tissue types (e.g. *Cymbastela tricalyciformis*). However, for *Tethya* sp. and *Latrunculia fiordensis*, tissue from the choanosome was collected for isotopic analysis. *C. tricalyciformis* is often found with an unidentified species of zoanthid (*Epizoanthus* sp.) embedded in its tissue; these were removed with sterile tweezers during processing. Dissecting instruments were cleaned with 70% EtOH and rinsed with DDW between samples.

5.3.3 Sample processing

Frozen samples were returned to Wellington and processed for stable isotope analysis at the National Institute of Water and Atmospheric Research (NIWA). All filter papers were dried at 60°C, then acidified overnight by fumigation in a desiccator with concentrated hydrochloric acid (HCl) to remove

carbonates. They were then rinsed again with DDW and dried at 60°C. Sponges were tested for the presence of carbonates by doing an HCl acid drop test on small subsamples of each species. Effervescing was noted for some species; therefore, all sponges were acidified according to the protocol outlined in Chapter 4. Simultaneous carbon and nitrogen analyses were carried out on the same acidified sample, as initial tests showed there was no effect of acidification on $\delta^{15}\text{N}$ values (see Chapter 4). Macroalgae and TOM were similarly tested for the presence of carbonates, but none were detected, so these samples were analysed unacidified for stable isotopes. Organic matter sources and sponges were dried at 60°C and homogenised into a fine powder using a mortar and pestle. Samples were weighed into tin boats for analysis (2.5-7 mg sponges, 2 mg macroalgae, 2 mg for TOM $\delta^{13}\text{C}$, 8 mg for TOM $\delta^{15}\text{N}$). Due to extremely high C: N ratios, TOM samples were analysed twice to obtain values for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Picoplankton filter papers were cut into ¼ and folded into tin capsules and GFM filter papers were hole punched (x 5) with resulting circles placed into tin capsules. Samples were analysed on a DELTA VPlus isotope ratio mass spectrometer (IRMS) (See Chapter 4 for a detailed description of methods). Ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were expressed relative to the internal standards relative to the international standard Carrara Marble NSB-19 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) for carbon and atmospheric nitrogen for nitrogen. Stable isotope values for carbon and nitrogen are expressed as delta (δ) values, according to the equation:

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

5.4 Data Analysis

5.4.1 Spatial variation in the stable isotope ratios of sponges and water column resources

To investigate spatial variation in the isotope ratios of sponges (all species combined) and water column resources, I used PERMANOVA + add-in to PRIMER version 6.1 (PRIMER-E Ltd, Plymouth, UK). This was done separately for the isotope ratios of sponges, picoplankton and the 2-200 fraction of the water column, using Type III sums of squares and based on a Euclidean distance matrix of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with the factors “site” and “depth” included in the model. Test assumptions were checked using PERMDISP.

5.4.2 Isotopic niches of sponge species

To investigate interspecific resource partitioning, I also used PERMANOVA+ (as above) to construct Euclidean distance matrices of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each site and depth with the factor “species” included in the model. Models were run separately for each site and depth due to slight differences in

species composition (e.g. *Latrunculia fiordensis* was not present at the Inner Fiord at 10 m and *Raspailia topsenti* was not present the Outer Fiord at 10 m). Pairwise tests were used to confirm isotopic separation between species.

To compare the diversity of resources used between depths and sites, I estimated the isotopic niche (a generalised proxy for trophic niche) by calculating the standard ellipse of each sponge species at each site using the SIBER package (Stable isotope Bayesian Ellipses in R) (Jackson et al. 2011). The standard ellipse contains approximately 40% of the sample data and can be considered as a measure of deviation for bivariate data comparable to standard deviation for univariate data that is less sensitive to sample size than other traditional measures of niche width (Jackson et al. 2011; Chapter 4). To compare the size of ellipses between depths and sites, I used Bayesian inference techniques (10^4 iterations) to generate probability distributions of the standard ellipse area adjusted for small sample size (SEA_c) and inferred statistical significance when $\geq 95\%$ of posterior draws for one species/group was smaller than the other. This is a robust method of comparing isotopic niche size between groups even when sample sizes differ (Jackson et al. 2011). Species with small ellipses (low intraspecific variation in stable isotope ratios) use a narrow range of resources and those with large ellipses (high intraspecific variation in stable isotope ratios) use a higher diversity of resources. I also calculated the overlap in SEA_c between groups as a shared percentage of isotopic space with species relying on similar resources expected to have a higher percentage overlap. The main assumption of these statistical techniques is that data approximates a multivariate normal distribution (MVN) (Jackson et al. 2011) thus groups were checked for MVN using the Henze-Zirkler multivariate normality test (Appendix E, Table 12).

5.4.3 Organic matter contributions to sponge diets

The key to the successful application of isotope mixing models is discrimination between carbon sources (McLeod et al. 2007; Parnell et al. 2010). I, therefore, used PERMANOVA+ (as above) on a resemblance matrix based on Euclidean distance with subsequent pairwise tests to check for between-source differences in isotope values. To estimate the contributions of TOM, picoplankton, the 2-200 μm size fraction and macroalgal-derived OM to sponge diets, I used the SIMMR package (Stable Isotope Mixing Models in R) (Parnell, 2016) which uses the Markov chain Monte Carlo (MCMC) algorithm to estimate possible values for each carbon source based on the data. Initial estimates are usually poor and discarded as part of the burn-in phase. Subsequent estimates are stored and used for the posterior distribution (the best estimates of the dietary proportions based on the data). The isotope mixing model package SIMMR requires three parameters: (1) the bivariate isotope data of sponges; (2) the means \pm standard deviation of carbon sources; and (3) trophic enrichment factors (TEF) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. I ran separate models for each site and adjusted the input values for picoplankton, the 2-200 μm size fraction, and macroalgae, to reflect spatial variation in these values (Appendix E, Table 18). For each species, I pooled samples between depths to increase the sample size to $n = 10$ at each site for

most species, apart for *Cymbastela tricalyciformis* (Inner Fiord) and *Raspailia topsenti* (Outer Fiord) where sample sizes were limited by abundance. There is little information on specific TEFs for sponges (Freeman & Thacker, 2011), therefore I adopted TEF values from Post (2002) of $\Delta^{15}\text{N}$ $3.4 \pm 0.98\text{‰}$ and $\Delta^{13}\text{C}$ $0.39 \pm 1.3\text{‰}$. These factors were estimated from two primary consumers (filter-feeding bivalves and grazing snails) covering littoral and pelagic food webs (Post, 2002) and are hence appropriate for this study.

5.4 Results

5.4.1 Spatial variation in the stable isotope ratios of sponges and water column resources

There were significant differences in the isotope values of sponges between the Inner Fiord and Outer Fiord (pseudoF_{1, 81} = 4.52, p = 0.015) (Appendix E, Table 3) driven by significant differences in sponge $\delta^{15}\text{N}$ values (pseudoF_{1, 81} = 8.05, p = 0.005), which were higher at the Inner Fiord in comparison to the Outer Fiord ($9.83\text{‰} \pm 0.20\text{‰}$; $9.08\text{‰} \pm 0.21\text{‰}$ [mean \pm SE]) (Figure 5.2). There was no difference in sponge $\delta^{13}\text{C}$ values between sites. There were also significant differences in the isotope ratios of sponges between depths (pseudoF_{1, 81} = 3.23, p = 0.046), which was also driven by differences in sponge $\delta^{15}\text{N}$ values (pseudoF_{1, 81} = 4.64, p = 0.039), which were higher at 30 m than at 10 m ($9.69\text{‰} \pm 0.19\text{‰}$; $9.17\text{‰} \pm 0.23\text{‰}$ [mean \pm SE]). There was no difference in sponge $\delta^{13}\text{C}$ values between depths.

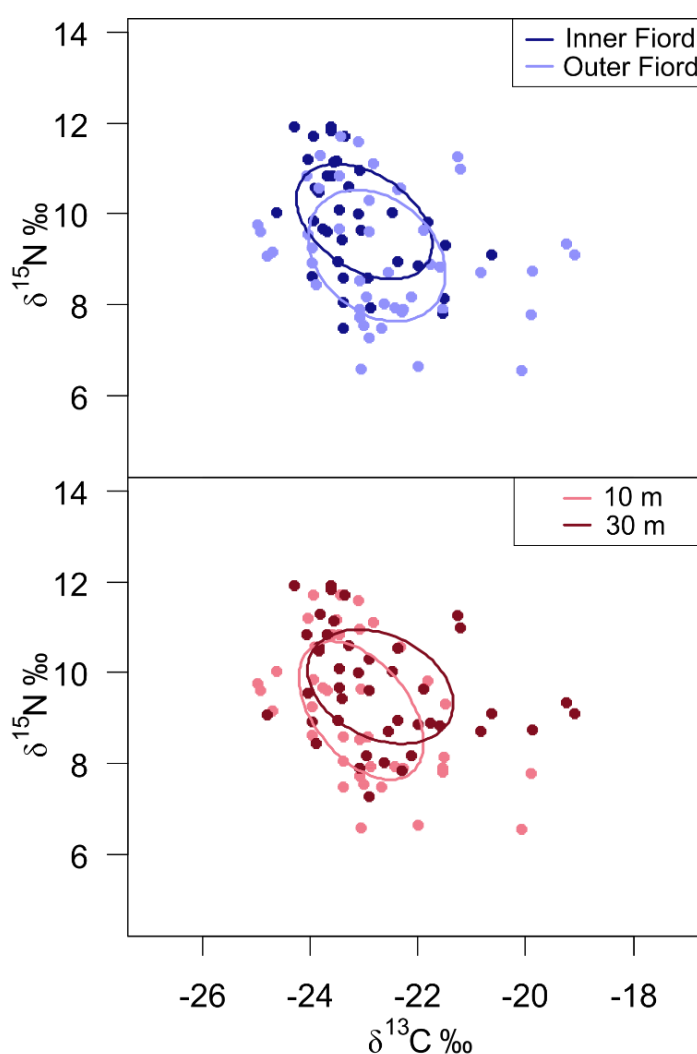


Figure 5. 2 The isotopic niches (standard ellipse area - SEAc) of sponges (all species combined) grouped by site (Inner Fiord and Outer Fiord) and depth (10 and 30 m) collected from Doubtful Sound in Fiordland in February 2019.

For the 2-200 μ fraction of the water column, stable isotope ratios were significantly different between sites (pseudoF_{1, 14} = 5.88, p = 0.007), driven by $\delta^{13}\text{C}$ values, which were lower at the Inner Fiord than the Outer Fiord ($-25.15\text{‰} \pm 0.75\text{‰}$; $-24.10\text{‰} \pm 0.21\text{‰}$ [mean \pm SD]) (Figure 5.3). There was also a significant difference between depths (pseudoF_{1, 14} = 3.49, p = 0.048) (Appendix E, Table 6). Pairwise tests indicated that there was no difference in isotope ratios between depths at the Inner Fiord, but at the Outer Fiord, there was a significant difference between 10 m and 30 m (t = 7.22, p = 0.001), driven by $\delta^{15}\text{N}$ values which were lower at 30 m compared to 10 m ($7.87\text{‰} \pm 0.24\text{‰}$; $6.15\text{‰} \pm 0.36\text{‰}$ [mean \pm SD]). There was no difference in the stable isotope ratios of picoplankton between sites or depths (Appendix E, Table 7).

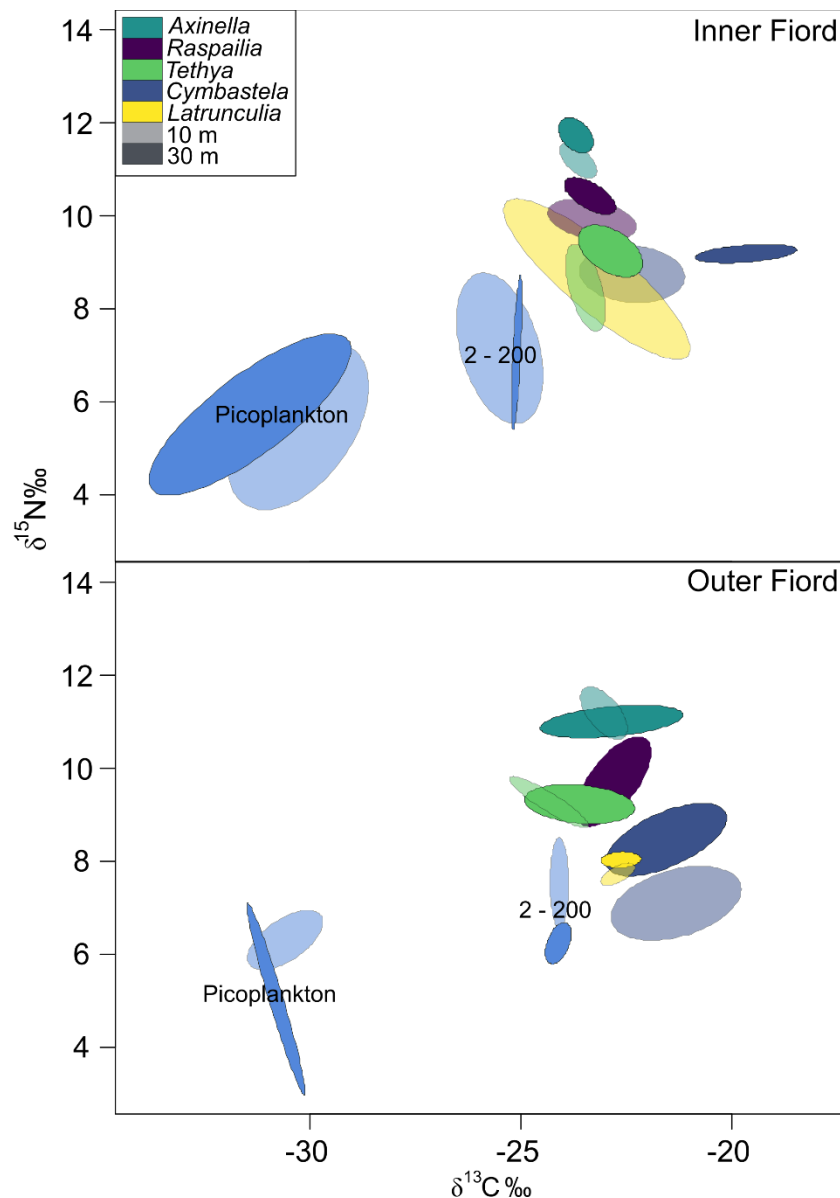


Figure 5. 3 Isotopic niches (standard ellipse area - SEAc) of sponge species, picoplankton and the 2-200 μm size fraction of the water column collected from Doubtful Sound in Fiordland grouped by site (Inner Fiord and Outer Fiord) and depth (10 and 30 m).

5.4.2 Isotopic niches of sponge species

5.4.2.1 Interspecific niche partitioning

At the Inner Fiord at 10 m, *Latrunculia fiordensis* occupied a similar position to *Raspailia topsenti* (shared 3.7% total SEAc), *Cymbastela tricalyciformis* (shared 19.3 % total SEAc) and *Tethya* sp. (shared 16.2% total SEAc) but was isotopically separated from *Axinella richardsoni* ($p = 0.02$) (Figure 5.3). There was also no difference in the placement of *C. tricalyciformis* and *Tethya* sp. (shared 8.1% total SEAc) but other species were isotopically distinct (all pairwise comparisons $p < 0.05$) (Appendix E, Table 8). At the Inner Fiord at 30 m, all species were isotopically distinct (all pairwise comparisons $p < 0.05$) (Appendix E, Table 9).

At the Outer Fiord at 10 m, all species were isotopically distinct (all pairwise comparisons $p < 0.05$) (Appendix E, Table 10). At the Outer Fiord at 30 m, *R. topsenti* occupied a similar position to *Tethya* sp. (23.8% total SEAc) as did *C. tricalyciformis* and *L. fiordensis* (8.4 % total SEAc). All other species were isotopically distinct (all pairwise comparisons $p < 0.05$) (Appendix E, Table 11).

5.4.2.2 Resource use between depths and sites

At the Inner Fiord site, both *Cymbastela tricalyciformis* and *Tethya* sp. had significantly different isotope values between 10 m and 30 m ($p = 0.03$ and $p = 0.05$ respectively) (Figure 5.3). There was a $>95\%$ probability that the isotopic niche of *C. tricalyciformis* was larger at 10 m and a $>85\%$ probability that the isotopic niche of *Raspailia topsenti* was larger at 10 m. For both *Tethya* sp. and *Axinella richardsoni* isotope niche size was similar between depths (Appendix E, Table 14).

At the Outer Fiord site, there were no differences in isotopic niche placement between depths (all pairwise comparisons $p < 0.05$). There was a $>85\%$ probability that the isotopic niche of *A. richardsoni* was larger at 30 m, but for all other species, isotope niche size was similar between depths (Appendix E, Table 14).

When samples were pooled between depths, *A. richardsoni* was the only species that demonstrated a statistical difference in isotopic niche size between the two sites, having a statistically larger isotopic niche at the Outer Fiord ($>99\%$ probability) (Figure 5.4). Both *C. tricalyciformis* and *R. topsenti* had a $\geq 85\%$ probability of having a larger isotopic niche at the Outer Fiord site (Appendix E, Table 15).

5.4.3 Organic matter contributions to sponge diets

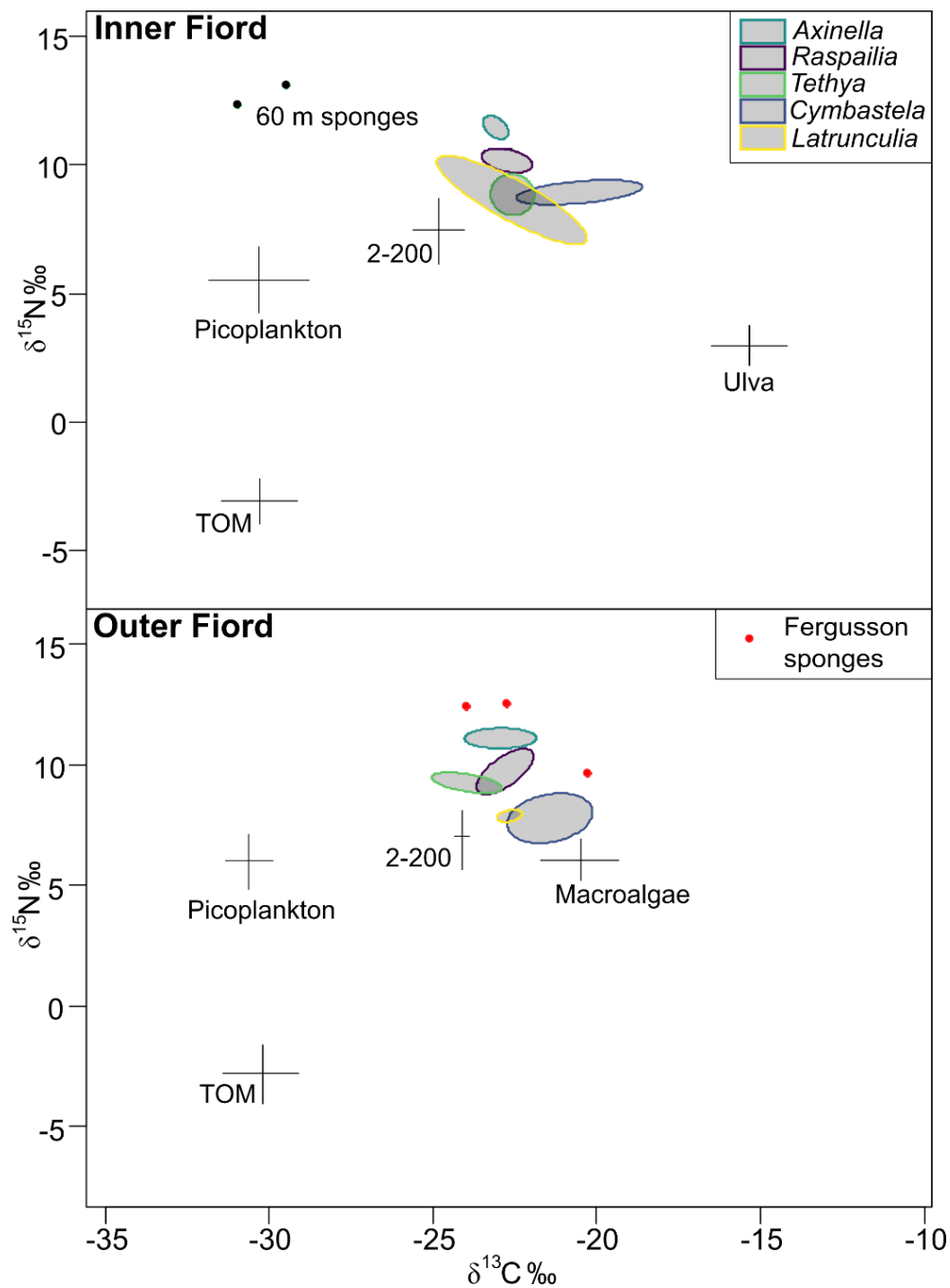


Figure 5. 4 Isotope biplots of carbon sources (mean \pm SD) and isotopic niches (standard ellipse area - SEAc) of sponge species (samples pooled between depths) collected from Doubtful Sound in Fiordland at the Inner Fiord and Outer Fiord. NB: Fergusson sponges (three individual unknown species collected from 55 m at Fergusson Island) and 60 m sponges (Inner Fiord) were collected by ROV.

Isotope values were significantly different between carbon sources (pseudoF_{5,68} = 226.06, p = 0.0001) and pairwise tests showed that all sources were significantly different to one another (Figure 5.4) (Appendix E, Table 17). The source with the lowest $\delta^{15}\text{N}$ values was TOM ($\delta^{15}\text{N}$, $-2.77\text{‰} \pm 1.12\text{‰}$ [mean \pm SD]). Both picoplankton and TOM had similarly low $\delta^{13}\text{C}$ values ($-30.14\text{‰} \pm 1.12\text{‰}$ and $-29.71\text{‰} \pm 1.13$, respectively [mean \pm SD]), however, picoplankton had a higher average $\delta^{15}\text{N}$ values ($5.48\text{‰} \pm 1.19\text{‰}$ [mean \pm SD]). Picoplankton and the 2-200 μm fraction had distinct carbon and nitrogen ratios, and the 2-200 fraction had significantly higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than picoplankton. *Ulva* sp. had lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$ values than *Codium* sp. or *Ecklonia radiata*. Both *Codium* sp. and *E. radiata* had similar $\delta^{13}\text{C}$ values and were only separated by $\delta^{15}\text{N}$, with *E. radiata* having marginally higher $\delta^{15}\text{N}$ values than *Codium* sp. ($\delta^{15}\text{N}$, $6.62\text{‰} \pm 0.99\text{‰}$ and $5.45\text{‰} \pm 0.37\text{‰}$ respectively [mean \pm SD]). Due to having similar carbon and nitrogen isotopic values, *E. radiata* and *Codium* sp. were pooled for subsequent mixing model analysis representing the macroalgal-OM signature at the Outer Fiord site.

Aside from the two deep-water sponges retrieved from 60 m at the Inner Fiord (Figure 5.4), sponges showed limited use of picoplankton (mean $>15\%$) or TOM (mean $>5\%$) (Figure 5.5). The mixing model for the Inner Fiord estimated that the 2-200 μm size fraction of the water column was an important OM source for *Raspailia topsenti* ($70\% \pm 9\%$ [mean \pm SD]), *Axinella richardsoni* ($63\% \pm 20\%$ [mean \pm SD]) and *Tethya* sp. ($50\% \pm 10\%$ [mean \pm SD]). Macroalgal- derived organic matter was most important to *Cymbastela tricalyciformis* ($38\% \pm 10\%$ [mean \pm SD]) (Appendix E, Table 19). At the Outer Fiord, macroalgal- derived OM was an important carbon source for both *C. tricalyciformis* ($74\% \pm 8\%$ [mean \pm SD]) and *Latrunculia fiordensis* ($59\% \pm 7\%$ [mean \pm SD]). *R. topsenti* also relies on macroalgal-derived OM for an estimated ($48\% \pm 16\%$ [mean \pm SD]) and less on the 2-200 μm size fraction of the water column at this site ($36\% \pm 20\%$ [mean \pm SD]). *Tethya* sp. relies on macroalgal OM ($39\% \pm 17\%$ [mean \pm SD]) and the 2-200 fraction ($37\% \pm 12\%$ [mean \pm SD]) in roughly equal proportions. Only *A. richardsoni* shows little change in resource use between the two sites with the 2-200 μm size fraction estimated to contribute ($62\% \pm 19\%$ [mean \pm SD]) (Appendix E, Table 20).

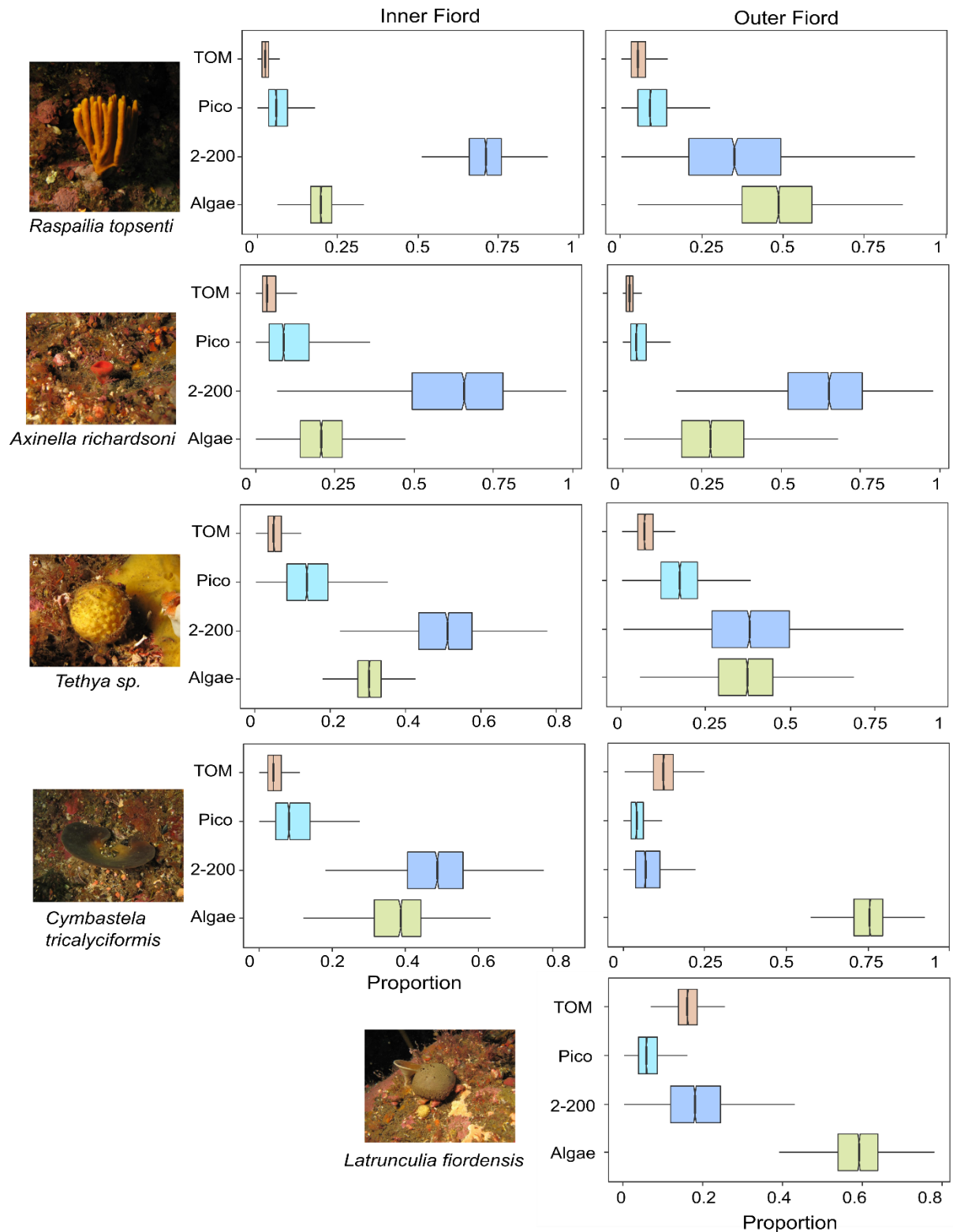


Figure 5. 5 Isotope mixing model posterior estimates of the proportional contribution of each carbon source to the diet of five sponge species at two different sites in Doubtful Sound, Fiordland. Boxes represent the credible interval of 50% and error bars the credible interval of 95%.

5.5 Discussion

Differential resource use is fundamental for maintaining biodiverse communities (Schoener, 1986). In this chapter, I have shown that resource partitioning is high among temperate, co-occurring sponges, suggesting that differential resource use facilitates co-existence and also limits distribution. Despite the dominance of TOM in the OM pool, the only evidence of its assimilation was from two sponges collected at 60 m at the inner site, whereas the 10 m and 30 m sponges relied mainly on marine production. Isotope mixing models did not identify picoplankton as a significant carbon source for sponges, which could not be easily explained, but may relate to the degradation of dissolved TOM (dTOM) by heterotrophic bacteria. Marine OM supports the majority of sponges in Fiordland but there is evidence that terrestrial production may support some sponges where marine OM is limited.

5.5.1 Spatial and interspecific variation in isotope ratios

Fiordland sponges displayed a wide range of $\delta^{15}\text{N}$ values, from 6.57‰ (*Cymbastela tricalyciformis*) to 13.13‰ (unidentified sp., Inner Fiord). Assuming trophic enrichment to be ~3.4‰ (Post, 2002), these values span two trophic levels. This is consistent with other studies that have measured sponge nitrogen isotope values, some of which have found $\delta^{15}\text{N}$ values to span up to three trophic levels (Bergmann et al. 2009). This could indicate specialisation on particular size fractions of suspended particulate organic matter (SPOM) (Thurber, 2007; Mintenbeck et al. 2007) or the possible influence of nitrogen cycling within the sponge holobiont (Southwell et al. 2008), although no information has been published regarding the microbial communities of the species sampled in this study. Sponge $\delta^{15}\text{N}$ values presented in this chapter are higher than the values presented for tropical sponges in Chapter 4. This is consistent with other studies that have found temperate sponges have higher $\delta^{15}\text{N}$ values than tropical sponges (Freeman et al. 2016). This may indicate reduced reliance on symbiont-derived nutrients (Freeman & Thacker, 2011; Freeman et al. 2016) as HMA sponges tend to exhibit lower $\delta^{15}\text{N}$ values than LMA sponges (Freeman et al. 2016). It could also relate to water temperature as lower temperatures are associated with higher fractionation (Sackett et al. 1965).

Increasing sponge $\delta^{15}\text{N}$ values with depth has also been observed in the Arctic (Bergmann et al. 2009), the Antarctic (Mintenbeck et al. 2007) and the sub-Antarctic Indian Ocean (Puccinelli et al. 2018). However, in these studies, the depth range spanned hundreds of metres whereas, in this study, sponges were ^{15}N -enriched from 10 m to 30 m depth. An increase in sponge $\delta^{15}\text{N}$ values as a function of depth has been linked to isotopic changes in SPOM that take place as particles sink through the water column (Altabet, 1987; Mintenbeck et al. 2007). Microbial degradation of SPOM leads to ^{15}N enrichment as the lighter isotope (^{14}N) is lost (Fry, 2006; Mintenbeck et al. 2007). Small particles filtered by sponges sink at a low velocity with likely greater exposure to microbial activity, leading to higher $\delta^{15}\text{N}$ values (Mintenbeck et al. 2007; Parzanini et al. 2019). Thus, higher $\delta^{15}\text{N}$ sponge values at depth are thought to reflect the process of SPOM ^{15}N enrichment (Mintenbeck et al. 2007). That being the case, we would

expect to see a corresponding increase in SPOM water column $\delta^{15}\text{N}$ values at 30 m, but the opposite was observed at the Outer Fiord site where the 2-200 μm size fraction had a lower $\delta^{15}\text{N}$ value at 30 m. An increase in SPOM $\delta^{15}\text{N}$ values alongside sponge $\delta^{15}\text{N}$ values, as a function of increasing depth, has been observed in some (Puccinelli et al. 2018), but not all studies (Bergmann et al. 2009; Sitnikova et al. 2016). Another possible explanation is that 30 m sponges are feeding on a higher proportion of re-suspended benthic particulate matter, which due to microbial reworking, is expected to be more enriched in ^{15}N than SPOM (Mintenbeck et al. 2007; Bergmann et al. 2009). However, calm conditions in deeper waters of the inner fiords suggest that benthic resuspension through water movement is unlikely. Additionally, a high concentration of dTOM in surface waters limits light availability at 30 m, which changes redox conditions, thus affecting processes of nitrification and denitrification (Solomon et al. 2015). Both processes have been shown to take place within the sponge holobiont (Southwell et al. 2008; Hoffmann et al. 2009; Fiore et al. 2013) and can be expected to influence sponge $\delta^{15}\text{N}$ values (Southwell et al. 2008).

5.5.2 Isotopic niches of sponge species

Minimal overlap in isotopic niches (all observed <20% total SEA_C) indicates that biogeochemical cycling of carbon and nitrogen is highly variable between species (Freeman et al. 2016; Freeman et al. 2020). Species identity has been shown to contribute substantially to the variance in isotopic values in studies on tropical (Freeman et al. 2014; Freeman et al. 2020) and temperate reef systems (Freeman et al. 2016), with species often separated in isotope space (Thurber, 2007; Freeman et al. 2016; Freeman et al. 2020). This indicates a high degree of metabolic diversity that could be caused by selective feeding or species-specific metabolic processes occurring within the sponge holobiont that affect carbon and nitrogen cycling (Freeman et al. 2016). The most significant overlaps were found between *Latrunculia fiordensis* and *Tethya* sp. and *Cymbastela tricalyciformis* at the Inner Fiord, indicating similar resource use patterns. However, the sample size for *L. fiordensis* was very small due to low abundance ($n = 3$) and there was substantial variation in stable isotope ratios between these three samples. Aside from these overlaps, there was only one small overlap between *C. tricalyciformis* and *Tethya* sp. hence resource partitioning was high between sponge species at the Inner Fiord site despite depleted resources. In contrast to Inner Fiord, the niche of *L. fiordensis* was much smaller at the Outer Fiord, indicating a narrow range of consumed particles; it also overlapped with *C. tricalyciformis*. Low abundances observed for both of these species at the Inner Fiord site suggests that their distribution may be limited by food availability.

The only species to show any significant differences in niche placement between depths were *C. tricalyciformis* and *Tethya* sp. at the Inner Fiord. *C. tricalyciformis* had higher $\delta^{13}\text{C}$ values at 30 m suggesting it may use different resources at this depth, although the sample size for this species was also small ($n = 3$) due to low abundances. Pooling samples between depths indicated that three out of

four species where comparisons could be made (sample sizes ≥ 5) had larger niches at the Outer Fiord site indicating more diverse resource use, likely reflecting increased marine productivity towards the mouth of the fiords (Wing et al. 2014).

5.5.3 Organic matter use by Fiordland sponges

The carbon sources (picoplankton, the 2 – 200 μm size fraction, TOM and macroalgae) sampled in this study had distinct isotope values, which increases the discriminatory power of mixing models (Phillips et al. 2014). Values obtained for macroalgae and TOM were similar to values reported from Fiordland by other studies (McLeod et al. 2007; McLeod et al. 2009; Wing et al. 2012). Only one study has sampled size-fractionated SPOM in Fiordland during a phytoplankton bloom (Wing et al. 2012). However, Wing et al. (2012) only reported isotopic values from the 20-200 μm size fraction (-21.6 ± 0.2 $\delta^{13}\text{C}$ [mean \pm SE]) and filter papers were not acidified, so meaningful comparisons between the values of the two studies cannot be made. The only other study that used size-fractionated SPOM to investigate sponge diets was conducted on a tropical reef (van Duyl et al. 2011). Van Duyl et al. (2011) reported no significant differences in mean $\delta^{13}\text{C}$ values between the three size fractions analysed, which contrasts with this study, as picoplankton were $\sim 6\text{‰}$ more depleted than the 2-200 μm size fraction. Additionally, Fiordland picoplankton was extremely ^{13}C depleted (mean -30.14‰) relative to $\delta^{13}\text{C}$ values (mean approximately -23‰) reported by van Duyl et al. (2011) for a similar particle size range (0.7-1.2 μm).

One possible explanation for the ^{13}C -depleted picoplankton values is that it represents heterotrophic bacteria degrading dTOM. The isotopic value of the bulk DOM pool will reflect the relative input of sources (Guillemette et al. 2016). Therefore, in the fiords, DOM $\delta^{13}\text{C}$ values likely reflect TOM $\delta^{13}\text{C}$ values which makes up a significant proportion of the OM pool, particularly in the inner fiord (McLeod & Wing, 2009). TOM is dominated by high molecular weight (HMW) compounds that are generally assumed to be resistant to microbial degradation due to their molecular structure and high C: N content (McKnight et al. 1998). However, several recent studies have challenged this assumption (Ward et al. 2013; Fasching et al. 2014; Fitch et al. 2018) showing that bacteria produce extracellular enzymes that hydrolyse or oxidise the complex structures present in dTOM into more labile carbon (Fitch et al. 2018). This can either be quickly respired and lost from the system as CO_2 or assimilated into bacterial biomass (Kirchman, 2013). Additionally, in sufficient light conditions, dTOM can also be degraded by photo-oxidation, which also leads to the production of more labile DOC (Kirchman, 2013; Fitch et al. 2018). The picoplankton $\delta^{13}\text{C}$ values presented here suggests that dTOM is incorporated into bacterial biomass (Guillemette et al. 2016) assuming that carbon fractionation from bulk TOM to dTOM and from dTOM to bacteria is minimal (Guillemette et al. 2016).

If, as suggested, the picoplankton signature represents the biomass of heterotrophic bacteria degrading dTOM, then it would be expected that $\delta^{13}\text{C}$ values would be transferred up the food chain via the

microbial loop (Azam et al. 1983) and via sponges that preferentially consume picoplankton (Reiswig, 1971; Pile et al. 1996). However, the isotope mixing model outputs presented in this chapter suggested that sponges sampled at 10 m and 30 m did not consume a substantial amount of picoplankton. This is in contrast to the majority of sponge feeding studies which indicate that sponges are most efficient at retaining picoplankton (Pile et al. 1999; Maldonado et al. 2012). This could be explained if the isotopic fractionation of carbon from picoplankton to sponge is greater than the value of $0.39 \pm 1.3\text{‰}$ applied in the mixing models. There is little information about the fractionation of carbon and nitrogen from different dietary sources for sponges (Freeman et al. 2011) and in some circumstances, carbon fractionation can be much higher than conventional literature estimates (Busst & Britton, 2016). However, similar enrichment factors have been used by other researchers to investigate sponge feeding using isotopic mixing models e.g. $1 \pm 1\text{‰}$ (van Duyl et al. 2011; Freeman et al. 2011), $0.5 \pm 0.5\text{‰}$ (van Duyl et al. 2018) and one of these studies also found that phytoplankton and bacterioplankton are not the main food source for some sponges (van Duyl et al. 2011). Considering that two sponges sampled at 60 m at the Inner Fiord had similar $\delta^{13}\text{C}$ values to picoplankton and TOM, the enrichment factors applied were likely adequate, but the 10 m and 30 m sponges did not metabolise dTOM-degrading bacteria. Picoplankton may be dominated by dTOM-degrading bacteria, but it is likely made up of other particles which are less abundant but may be utilised preferentially by sponges. Size-fractionated SPOM will contain multiple types of organisms, all with distinct metabolic pathways, so the values presented here will represent the dominant organism (Hansman & Sessions, 2016). It is possible that sponges are consuming specific picoplankton particles, but that the signature of these particles is swamped by the dominant organism.

Mixing model results suggest that *C. tricalyciformis* and *L. fiordensis* rely on macroalgal-derived OM for <50% of their nutritional requirements. Macroalgal OM could be ingested by sponges as dissolved compounds (Rix et al. 2017) or as particulate detrital material (McMurray et al. 2016), which would likely exhibit similar $\delta^{13}\text{C}$ values but higher $\delta^{15}\text{N}$ values relative to bulk tissue as a result of microbial reworking. The ability of sponges to assimilate algal DOM has been demonstrated by isotopic tracer experiments (Rix et al. 2017) and through fatty acid analysis (de Goeij et al. 2008; van Duyl et al. 2012) and other studies have used isotopic values of macroalgal tissue to infer the assimilation of algal products (van Duyl et al. 2011; van Duyl et al. 2018). For example, stable isotope analysis of organic matter sources indicated that reef-derived DOM from coral mucus and coralline algae contributed significantly to the diet of cavity sponges in the Caribbean (Van Duyl et al. 2011). Similarly, isotopic values of sponges on the Saba Bank indicated that they rely on both benthic- and pelagic-derived OM (van Duyl et al. 2018). These studies assume that DOC closely reflects the isotopic value of its source material (Fry et al. 1998; Benner et al. 1997), although this has been shown to vary by $\pm 2\text{‰}$ for some sources (Hullar et al. 1996). Methods employed in this chapter account for spatial variability in organic

matter source values and incorporate error associated with isotope fractionation, therefore, can be considered robust estimates of source contributions and associated uncertainty (Parnell et al. 2010).

The only evidence of TOM assimilation by Fiordland sponges was from two sponges sampled at 60 m the Inner Fiord site. These sponges had extremely low $\delta^{13}\text{C}$ values (-29‰ to -30‰) that were comparable to picoplankton and TOM. This suggests either they consume picoplankton directly, or that they host a microbial community containing chemoautotrophic bacteria and indirectly access products of TOM degradation. Similar $\delta^{13}\text{C}$ values have been observed for chemoautotrophic bacterial mats (Levin et al. 2002) and the chemoautotrophic bivalve *Solemya parkinsonii* found in the fiords (McLeod et al. 2007). Chemoautotrophic bacteria fix carbon fuelled by energy derived from sulphate oxidation, a metabolic pathway (form I Rubisco) discriminates against $^{13}\text{CO}_2$ producing $\delta^{13}\text{C}$ values ranging from -27‰ to -37‰ (Levin et al. 2002). Typically, high microbial abundance sponges, and other invertebrates with chemoautotrophic endosymbionts, have low $\delta^{15}\text{N}$ values (Freeman et al. 2014; van Duyl et al. 2018; McLeod et al. 2007). However, in deep, freshwater environments, ^{13}C depleted sponges that receive carbon from methanotrophic and/or chemoautotrophic bacteria have been found both with extremely ^{15}N -depleted and ^{15}N -enriched tissues (Sitnikova et al. 2016), likely resulting from different microbial metabolic pathways (Southwell et al. 2008; van Duyl et al. 2018). Therefore, enriched ^{15}N values could be the result of sponge-hosted nitrification, which has been shown to occur with an isotopic fractionation factor of 11‰ (Southwell et al. 2008) or denitrification which leads to higher $\delta^{15}\text{N}$ values (Parzanini et al. 2019). Only the deep sponges sampled at the inner site had extremely low $\delta^{13}\text{C}$ values, the sponges collected from 55 m at Fergusson Island had similar $\delta^{13}\text{C}$ values to the 10 and 30 m sponges. This is likely due to differences in hydrodynamic conditions between the two sites. Fergusson Island is in the middle of the main channel where wind and waves induce the vertical mixing of stratified water leading to greater nutrient transport across depths (Wing & Jack, 2014). At the inner site, however, waters are highly stratified with a shallow photic zone leading to nutrient limitation at depth. Nutrient limited conditions often cause invertebrates to form associations with microbial symbionts to access the products of autotrophic metabolism (Freeman et al. 2014). These associations allow sponges to inhabit seemingly inhospitable environments and expand across ecological niches in marine ecosystems (Freeman et al. 2020).

5.5.4 Study limitations

This survey was conducted over a week-long expedition in February; hence it was limited to a two site comparison and temporal variation in isotope values was not considered. Nitrogen and carbon isotope values of sponges are estimated to represent the last 1-2 months dietary assimilation (Freeman & Thacker, 2011; Simister et al. 2013; van Duyl et al. 2018) and phytoplankton blooms are known to occur in the fiords (Wing et al. 2012), which can increase $\delta^{13}\text{C}$ values (Miller et al. 2012) and affect mixing model estimates. However, assuming that the time frame for dietary assimilation is

approximately equivalent between sponge species, the difference between the lowest and highest carbon values of individual sponges collected from 10 and 30 m was approximately 6‰ which supports differential resource use, even in the event of a phytoplankton bloom. Similarly, spatial variation in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of SPOM has previously been cited as low in this system (Wing & Jack, 2012).

5.5.5 Conclusion

Understanding the diversity of resources utilised by marine sponges is essential to predict the impact of shifts in organic matter subsidies and resource availability. This study has demonstrated that marine sponges have diverse trophic strategies that facilitate coexistence under different gradients of organic matter composition and availability. For the 10 m to 30 m sponges, this was mainly driven by differential use of the 2-200 fraction of the water column and macroalgal-derived OM, with little evidence of TOM assimilation. However, in the highly stratified, nutrient-limited waters at the Inner Fiord site, TOM was utilised by 60 m sponges through microbially-mediated pathways.

Chapter 6. General Discussion

6.1 Summary of key findings

The primary aim of my thesis was to investigate sponge trophic interactions to gain insight into how sponge-reefs of the future might function. In summary, my main findings were: i) Far from being negligible, sponge biomass consumption on Wakatobi reefs was comparable to coral consumption in the Pacific, and the species with the greatest predatory impact was the angelfish *Pygoplites diacanthus*; ii) *Pygoplites diacanthus* had a wide diet within the Porifera phylum, similar to angelfishes in the Caribbean and the Pacific, and consumed sponges with substantial chemical defences; iii) fish sampled at a sponge-dominated reef had lower mean trophic positions and the majority of species had wider isotopic niches than fish from an adjacent, higher quality reef. They also used a significantly lower range of carbon sources, had lower trophic diversity and obtained more carbon from benthic production; and iv) niche partitioning amongst co-occurring sponges on temperate reefs was high, facilitated by differential use of the 2-200 μm fraction of the water column and macroalgal- derived organic matter. Here, I discuss my findings with respect to current thinking on marine ecosystems in the Anthropocene.

6.2 Trophic consequences of sponge dominance

The Sampela reef system provides an analogue for what may occur on some shallow coral reefs in the future that shift towards sponge dominance due to a decline in corals relative to sponges, as opposed to an increase in sponges relative to corals (Bell et al. 2018b). The distinction between these two types of sponge-dominant reef states is relevant because qualitative modelling suggests that they will lead to different outcomes of different ecosystem components (e.g. macroalgae, grazers, phytoplankton etc) (Bell et al. 2018b). Although there is evidence that some sponges, particularly *Lamellodysedia herbacea*, may be increasing in abundance at Sampela (Biggerstaff, 2016), benthic quadrat data presented in this thesis suggested that sponge abundance was similar at Sampela, B1 and The Ridge, but assemblage composition differed between sites (Chapter 2). For spongivores, changing sponge dominance as a result of declining corals generally yielded ambiguous responses in qualitative modelling scenarios, whereas changing sponge dominance as a result of increasing sponge abundance led to some positive outcomes (Bell et al. 2018b). Observations from the Sampela reef system may shed some light on the ambiguous responses predicted for spongivores in situations where sponge-dominated reef states are caused by declining corals relative to sponges.

It has been suggested that increasing sponge-dominance may support a greater abundance of spongivorous fishes (Bell et al. 2013). Previous studies have suggested that there is a higher density of spongivorous fishes on the sponge-dominated site relative to surrounding reefs (Powell, 2013; Powell et al. 2014). This contrasts with observations from this thesis which showed that sponge consumption was lowest on the sponge-dominated site, due to low densities of spongivorous fishes (Chapter 2). The contrasting outcomes of Powell et al. (2014) and this thesis can be explained by differences in the

species categorised as spongivores. Here, sponge-grazing fishes were determined to be either obligate or facultative spongivores through the quantification of sponge in gut contents. Whereas in the 2014 study, Powell et al. included several species I have found to have no quantifiable predatory impact on sponge assemblages. Additionally, Powell et al. (2014) included detritivorous surgeonfishes (genus *Ctenochaetus*) and epilithic algal grazers (genus *Acanthurus*) that may be numerous at Sampela because it is degraded relative to surrounding reefs, with a high percentage of rock covered with algal turfs and sediment (Tebbett et al. 2017; Chapter 2). Low densities of spongivores on the sponge-dominated reef could be explained by sponge assemblage composition. At Sampela, the sponge assemblage is dominated by *Lamellodysedia herbacea* (Powell et al. 2014; Biggerstaff et al. 2015; Chapter 2), which accounts for approximately 40% of total sponge abundance but does not appear to be preferred or readily consumed by spongivores (Chapter 2). As previously discussed in Chapter 2, using percentage cover to assess sponge abundance does not adequately capture large, emergent species, and Sampela also has a large population of barrel sponges (McGrath, 2018), the biomass of which likely exceeds *L. dysedia*. Nevertheless, Powell et al. (2014) used counts of sponge patches to assess sponge abundance and also reported that *L. dysedia* dominated the sponge assemblage at Sampela. In Chapter 3, DNA analysis did not identify *L. herbacea* in the gut contents of spongivorous angelfishes (Chapter 3). Similarly, the grazing rate on *L. herbacea* was low in comparison to *Xestospongia* spp. and *Petrosia corticata* (Chapter 2; Appendix A). The only spongivorous fish filmed taking bites from of *L. dysedia* was *Zanclus cornutus*, although tissue removal could not be verified by video analysis. In Chapter 4, stable isotope analysis of *Z. cornutus* and *Pygoplites diacanthus* muscle tissue did not support *L. dysedia* as a substantial food source for spongivores as it was $\sim 7\%$ ^{13}C depleted relative to muscle tissue (Chapter 4). The majority of reefs that have transitioned to sponge dominance following widespread coral mortality usually involve the increases of one or two competitive species (Williams, et al., 1999; Aronson, et al., 2002; Powell et al. 2014). Perhaps the ambiguous response of spongivores in qualitative modelling scenarios reflects continuing uncertainties regarding future sponge assemblage composition and the diet preferences of spongivores.

The success of spongivores on future sponge-dominated reef states may depend on the capacity for spongivores to adapt their diet preferences. Some obligate spongivores that consume a narrow range of sponge species e.g. Hawksbill turtles (Meylan, 1988; Pawlik et al. 2018) may be less resilient to changes to sponge assemblage composition than angelfishes that consume a wide range of species within the Porifera phylum (Chapter 3; Randall & Hartman, 1968; Hourigan et al. 1989; Verdín Padilla et al. 2010). Results from Chapter 4 indicated that *Pygoplites diacanthus* had a wider isotopic niche at the sponge-dominated site in contrast to the adjacent, higher-quality reef site suggesting some degree of dietary plasticity. However, niche width expansion due to a reduction in preferred prey is also expected to lead to greater interspecific competition with other spongivores (e.g. *Zanclus cornutus*) and generalists and could be another reason why some spongivores may not necessarily benefit from future

sponge-dominated reef states. Interestingly, although diet similarity was higher in the fish assemblage on the sponge-dominated reef, there was no evidence that *P. diacanthus* and *Z. cornutus* were using more of the same resources, which would have been shown by a greater overlap in isotopic niches. This contrasts with other studies that have also reported trophic niche width increases of consumers in response to decreasing resources. For example, interspecific competition strength increased in macroinvertebrate assemblages in low coverage seagrass meadows where trophic niche widths also increased (Calizza et al. 2017). The difference between the findings of Calizza et al. (2017) and this study could relate to consumer vagility which adds greater spatial complexity to trophic interactions (Duffy et al. 2007). Layman et al. (2007) found that the niche width of the grey snapper *Lutjanus griseus* decreased in response to increasing tidal creek fragmentation and subsequent reductions in potential prey taxa. Different niche width responses to a decrease in available prey between my study and Layman et al. (2007) likely relate to the foraging constraints caused by a disruption to hydrological connectivity in fragmented tidal creeks (Layman et al. 2007).

Although I have presented evidence in this thesis that sponge consumption is similar in magnitude to coral consumption as an energy pathway on Indo-Pacific reefs, it appears to support few species directly and is likely a relatively minor pathway supporting secondary production. The results of the comparative study in Chapter 4 highlight the importance of a wide range of organic matter pathways in the maintenance of resource specialisation and trophic diversity. The importance of producer diversity has been demonstrated in terrestrial plants, where long-term experiments have shown that loss of plant diversity propagates through food webs, decreasing arthropod species richness and shifting trophic structure towards herbivore dominance (Haddad et al. 2009). In benthic marine communities, higher producer diversity increased the stability of a diverse, productive consumer community, whereas producer biomass did not affect stability or diversity (Ramus & Long, 2016). Additionally, on coral reefs, biodiversity hotspots with larger background species pools (i.e. greater resource specialisation) were found to be more sensitive to a loss of coral diversity than reefs with lower species pools (Holbrook et al. 2015). Increased diversity within guilds of primary producers enhances the temporal stability of organic matter subsidies to consumers (Tilman & Downing, 1994). Hence, sponge-dominated reef such as Sampela that have fewer organic matter pathways supporting secondary production will ultimately be less stable and less productive than coral dominated reefs.

6.3 Indirect trophic pathways

Aside from the ‘true spongivores’ that remove sponge tissue e.g. *Pygoplites diacanthus*, *Zanclus cornutus*, there was extensive sponge-grazing observed by detritivores (e.g. *Ctenochaetus binotatus* and *Acanthurus* spp.) raising questions about the nature of this interaction. Gut content analysis confirmed that the diet of *C. binotatus* is dominated by reef sediments and detritus (Chapter 2; Tebbett et al. 2017). Nevertheless, the extent of grazing activity on *Xestospongia* spp. across a range of sites suggests that

this feeding behaviour may confer some nutritional benefit to detritivores. There are two possible explanations for sponge-grazing by detritivores: (1) the sponge loop and (2) mucus production (de Goeij et al. 2013; McGrath et al. 2017).

6.3.1 The sponge loop

In the ‘sponge loop’, dissolved organic carbon is taken up by sponges (Yahel et al. 2003; de Goeij et al. 2013) from diverse sources including benthic primary producers (e.g. algae and corals) (Rix et al. 2016; Rix et al. 2017), metabolised, and eventually released as particulate detritus, which is subsequently ingested by detritivorous invertebrates (de Goeij et al. 2013; Rix et al. 2018). To date, there is no evidence that sponge detritus is consumed directly by detritivorous fishes, but the shedding of sponge cells as detritus could be a possible explanation for the sponge-grazing behaviour of *C. binotatus*. Yet, the ubiquity of the sponge loop has not been proven beyond observations of cryptic, encrusting sponges (McMurray et al. 2018; Bell et al. 2018b). For example, McMurray et al. (2018) found no evidence of detritus production in 8 emergent abundant Caribbean sponge species, including the Caribbean barrel sponge *Xestospongia muta*. Instead, some sponges (low microbial abundance sponges) were net consumers of detritus (McMurray et al. 2018). Similarly, Wooster et al. (2019) found no evidence that *Xestospongia testudinaria* in the Red Sea released DOC that had been metabolised back to the benthos in the form of detritus. Therefore, it seems unlikely that *C. binotatus* graze on *Xestospongia* spp. because they are consuming sponge detritus. The extent to which the sponge loop supports higher trophic levels across global reef ecosystems is currently unknown (Bell et al. 2018b). Given the potential relevance to the future functioning of sponge-dominated reefs, this should be considered a priority research area for sponge ecologists (Bell et al. 2018b).

6.3.2 Mucus production

In turbid habitats, sediments accumulate on the surface of marine sponges, the effects of which can be sub-lethal e.g. reduced pumping rates affecting respiration or feeding (Tjensvoll et al. 2013), or lethal e.g. smothering (Wulff, 1997). In response to sedimentation, some sponges have been documented to produce mucus as a sediment-clearing mechanism (Bannister et al. 2012; Biggerstaff, 2017; McGrath et al. 2017). This response appears to be relatively widespread, for example, both McGrath et al. (2017) and Biggerstaff (2017) observed mucus production in every sponge following experimentally manipulated sediment additions to both *Xestospongia testudinaria* and *Lamellodysedia herbacea*. Additionally, in a census of *X. testudinaria* at the sponge-dominated site of Sampela, 99% of sponges were observed with mucus-bound sediment on their external surface (McGrath et al. 2017). Aside from sediment clearance, little is known about the ecological role of sponge mucus on coral reefs (McGrath et al. 2017). Several fish species are known to consume coral mucus (Cole et al. 2008), which is energy-rich (Benson & Muscatine, 1974; Wild et al. 2004) and also released by corals as a sediment clearance mechanism, increasing the organic content of suspended particles (Wild et al. 2004). Hence, detritivores

such as *C. binotatus* may be attracted to grazing on sediment-bound mucus on sponge surfaces because it is nutritionally beneficial. To my knowledge, only one study appears to have considered the trophic role of sponge mucus (Hammond & Wilkinson, 1985). The sea cucumber *Synaptula lamperti* ingested organic exudates (mucus) released by the sponge *Ianthella basta* (Hammond & Wilkinson, 1985). Given the extensive mucus production noted for some sponges (McGrath et al. 2017), sponge mucus may play an important role in organic matter cycling in the surrounding environment.

6.4 Sponge trophic roles

Recently, Lesser & Slattery (2020) suggested that sponges on shallow coral reefs may encounter food limitation on future coral reefs due to increased water stratification caused by increasing sea surface temperature (SST) and decreasing salinity. Stabilized surface waters would prevent vertical mixing hence limiting the transport of deep-water nutrients and decreasing net primary productivity (NPP) with ramifications for filter-feeding organisms such as sponges (Lesser & Slattery, 2020). Ultimately this would lead to a decline in sponge abundance, countering the hypothesis that sponges could be climate change ‘winners’ on future coral reefs (Bell et al. 2013; Lesser & Slattery, 2020). Under the conditions outlined by Lesser & Slattery (2020), photoautotrophic species may reasonably be expected to dominate future sponge assemblages on coral reefs as they are less reliant on heterotrophy and receive carbon (and to some extent nitrogen) from symbionts (Erwin & Thacker, 2008; Freeman et al. 2011; Fiore et al. 2013). Additionally, photoautotrophic sponges appear to have increased tolerance to thermal stress (Bennett et al. 2017) indicating a potential advantage over heterotrophic sponges.

In Chapter 5, the conditions in the inner fiords in Doubtful Sound were similar to those described by Lesser & Slattery (2020), as large freshwater inputs and low wave exposure lead to highly stratified surface waters (Wing & Jack, 2014). However, I found no evidence of photoautotrophic species in the 10 and 30 m sponges collected from the inner fiords, likely due to low light penetration caused by topographic shading and a high concentration of dissolved tannins in the water column (Wing & Jack, 2014). Instead, evidence of autotrophic metabolic pathways via chemosynthesis was indicated by the stable isotope ratios of two 60 m sponges, although it cannot be ruled out that they were directly consuming dTOM-metabolising bacteria. Nevertheless, the differences in isotope ratios observed between the shallow sponges and the two 60 m sponges likely relate to nutrient limitation caused by isolation from oceanic productivity and a highly stratified water column. Interestingly, the 10 and 30 m sponges did not show the same ability to assimilate TOM through microbially-mediated pathways, particularly through the direct consumption of dTOM-degrading bacteria, which appeared to be highly abundant in the picoplankton fraction of the water column. Pawlik et al. (2016) suggested that sponges in the Caribbean may be able to make use of refractory DOC from terrestrial sources that are unavailable to microbes and that this may explain the presence of predominantly heterotrophic species on Caribbean reefs. Observations made in this thesis suggest that sponges are likely unable to process refractory DOC

directly, probably due to a lack of necessary enzymes to metabolise complex structures present in TOM (Fitch et al 2018). Similarly, refractory DOC may not necessarily be unavailable to microbes, as the isotopic value of picoplankton presented in Chapter 5 strongly suggested that this fraction of the water column in the fiords was dominated by heterotrophic bacteria degrading terrestrial DOM. Lack of evidence supporting the consumption of dTOM-degrading bacteria by 10 and 30 m sponges could not be easily explained but may relate to the poor nutritional quality of the resource. TOM has extremely high C: N ratios (>15) (McLeod et al. 2007) and several studies have indicated that sponges are limited by the availability of nitrogen (Hadas et al. 2009; Lesser et al. 2019). Some studies have shown negative selectivity for low DNA bacteria with lower carbon and nutrient contents than high DNA (HDNA) bacteria (Hanson et al. 2009). Therefore, the shallow sponges may actively select against abundant dTOM-degrading bacteria, which could be a poor-quality food source due to high C: N ratios (Busst & Britton, 2016).

Stable isotope values of heterotrophic sponges presented in Chapter 4 were similar to the values obtained for the detrital proxy *Synaptula* sp. indicating they have similar diets. These observations add to the growing body of knowledge that detritus (non-living particulate organic carbon) plays more of a role in the diets of some marine sponges than is currently appreciated. Measuring the uptake of detritus by sponges can be complicated by the release of sponge detritus (faecal pellets and shed cells) (Hadas et al. 2009) hence, few studies have quantified the consumption of detritus by marine sponges (Maldonado et al. 2012). Detritus constituted 1/3 of organic carbon ingested by the Red Sea sponge *Negombala magnifica* (Hadas et al. 2009), 20% of the diet of the giant barrel sponge *Xestospongia muta* (McMurray et al. 2016) and >34% of the diet of *Xestospongia testudinaria* in the Red Sea (Wooster et al. 2019). *Xestospongia* spp. have a wide distribution across Wakatobi reefs, but they are found in high densities at the sponge-dominated site of Sampela (Bell et al. 2014) which also appears to be a site fuelled by detrital production (Chapter 4). Perhaps one of the reasons that *Xestospongia* spp. appear to be successful in this otherwise low-quality, highly sedimented environment is that detritus is an important component in the diet of these species.

Stable isotope mixing models in Chapter 5 suggested that Fiordland sponges have diets that are dominated by the 2-200 µm fraction of the water column and macroalgal-derived organic matter. However, one of the limitations with this approach is that the macroalgal contributions cannot be distinguished between DOC released by algae or algal detritus. Yet, even direct techniques e.g. In-Ex methods cannot determine the source of DOC and/or detritus consumed by sponges, only isotopic tracer experiments can directly trace organic material from sources to sponges, and subsequent uptake by detritivores (de Goeij et al. 2013; Rix et al. 2017; Rix et al. 2018).

6.5 Different methods of diet characterisation

In this thesis, I used three different methods of diet characterisation: (1) visual observations, (2) gut content analysis and (3) stable isotope analysis. Using all three methods was paramount to providing a more nuanced picture of the trophic ecologies of sponge-grazing fishes, particularly when one method provided a different outcome (Chapter 2). For example, in the case of *Chaetodon kleinii*, visual observations indicated this species was a spongivore, but gut content analysis revealed mainly mobile invertebrates and soft coral (Chapter 2). However, the proximity of the isotopic niche to the obligate spongivore (Chapter 4) supports the observations that the trophic ecology of this species is tightly linked to sponges, perhaps through the consumption of sponge-associated fauna. Nagelkerken et al. (2009) also applied these three methods to the diets of butterflyfishes in Indonesia (including *C. kleinii*). They also found that each method often indicated a different diet and that all three necessary to provide more detail on a specific diet (Nagelkerken et al. 2009). Nagelkerken et al. (2009) also asserted that predator and prey stable isotope ratios reflected commonly observed enrichment factors. However, I would argue that this is not the case, as the majority of coral feeding Chaetodontids had mean $\delta^{13}\text{C}$ values of approximately -12.5‰ yet the mean $\delta^{13}\text{C}$ value of corals was approximately -15‰ (Nagelkerken et al. 2009). This indicates carbon trophic enrichment of roughly 2.5‰, larger than the commonly cited 0-1‰ (DeNiro & Epstein, 1981) or 0.39 ± 1.3 ‰ (Post, 2002) used in this thesis (Chapter 4; Chapter 5).

6.6 Trophic enrichment

Recently, researchers have been critical of the use of constant trophic enrichment factors (Caut et al. 2008; Caut et al. 2009; Bond & Diamond, 2011; Busst & Britton, 2016) for ignoring variations related to size, tissue type, nutritional stress, excretory mechanisms and feeding rates (Mill et al. 2007). Similarly, prey types have a notable influence on trophic enrichment (Caut et al. 2009). In Chapter 4, I measured the stable isotope ratios of two reef specialists and their respective food sources, the obligate spongivore *Pygoplites diacanthus* (Chapter 2) and the obligate corallivore, *Chaetodon lunulatus* (Pratchett et al. 2005; Nagelkerken et al. 2009). The difference in nitrogen isotope values recorded between *P. diacanthus* and sponges was ~ 4.1 ‰ and between *C. lunulatus* was ~ 4.4 ‰, towards the upper estimate of the trophic enrichment factors for nitrogen (Post, 2002). Nitrogen enrichment >3.4 ‰ has been explained by the ‘isotopic routing’ of small amounts of animal material that may act as a dietary supplement (Gannes et al. 1997; Mill et al. 2007). This could be an explanation for *P. diacanthus* as DNA dietary analysis conducted in Chapter 3 identified crinoid DNA from the stomach contents of one individual (Chapter 3). In addition, gut content analysis conducted in Chapter 2 recorded small volumes of bivalves and polychaetes from the stomach contents of *P. diacanthus* (Chapter 2). Similarly, Nagelkerken et al. (2009) recorded small volumes of gastropods in the stomach contents of *C. lunulatus*. However, the difference in carbon isotope values recorded between *P. diacanthus* and *C. lunulatus* and their respective food sources was ~ 4.1 ‰ and ~ 4.4 ‰, which is much higher than traditional literature estimates of carbon enrichment (De Niro & Epstein, 1981; Post, 2002). In a laboratory-based feeding

experiment, temperate omnivores had $\delta^{13}\text{C}$ enrichment factors ranging from 2‰ – 5.6‰ and nitrogen enrichment factors ranging from 2 – 6.9‰ with low protein diets always resulting in the highest discrimination factor for each tissue (Busst & Britton, 2016). The protein quality hypothesis suggests that low-quality protein sources will lead to higher enrichment factors and that as dietary nitrogen concentration increases, more amino acids are catabolised hence discrimination factors are smaller in high protein diets (Macko et al. 1986; Busst & Britton, 2016). My observations suggest that spongivores and corallivores may also have trophic enrichment factors that are substantially different from traditional literature estimates. Long-term controlled feeding studies using a diet with a known isotopic ratio are the best way to calculate trophic enrichment factors (Sweeting et al. 2007; Bond & Diamond, 2011; Busst & Britton, 2016), however, this approach is impractical in remote, tropical study sites. As an alternative, wild studies have used stomach content material as a proxy for diet when the diet is well known (Mill et al. 2007), which has the potential to alleviate the uncertainty associated with isotopic enrichment (Guelinckx et al. 2008).

6.7 Research limitations

6.7.1 Field observations

Visual observations of spongivore feeding activity were constrained by safe diving limits that included a maximum diving depth of 18 m. Unfortunately, a lot of activity by prominent spongivores including *Pygoplites diacanthus* and *Pomacanthus* spp. tends to exceed this depth limit, potentially due to higher sponge abundance at depth (Bell et al. 2018b). Similarly, aside from *Pygoplites diacanthus* and *Zanclus cornutus*, other spongivores (*Pomacanthus* spp.) and potential spongivores (e.g. *Coradion melanopus*) are found in low densities hence they were not frequently observed by divers or in video footage. Low densities also meant that it was unethical to carry out destructive sampling of some species to obtain gut content data and restricted the sample sizes of others e.g. *Pomacanthus imperator* and *Pomacanthus xanthurus* (Chapter 2). Developing methods that do not require destructive sampling e.g. using DNA analysis of faecal matter (Deagle & Tollit, 2007; Deagle et al. 2009) or taking fin clips for stable isotope analysis (Wang et al. 2017) would be a promising advance on future studies of coral reef trophic ecology. These possibilities were discussed during thesis planning but ultimately, they were rejected because they would have required a large investment in method development and presented lots of uncertainties regarding data yield.

6.7.2 Invertebrate spongivores

This study was limited to vertebrate sponge predators which are thought to have the biggest predatory impact on sponge assemblages on shallow coral reefs (Randall & Hartman, 1968; Meylan, 1988). However, tropical sponges also have a range of invertebrate predators. Generalist macro-invertebrate grazers that have been documented consuming sponges in the Western Atlantic include the starfish *Oreaster reticulatus* (Wulff, 1995; Wulff, 2017) and three predatory urchins, *Lytechinus variegatus*,

Tripneustes ventricosus, and *Eucidaris tribuloides* (Reiswig, 1973). *Eucidaris tribuloides* individuals sampled in Brazil had large enough quantities of sponge in their gut content to indicate deliberate feeding rather than incidental ingestion (Santos et al. 2002). Sponge spicules were found in the faecal content in 5/23 species of opisthobranchs living on the Mexican Pacific coast (Verdín Padilla et al. 2010). The false limpet *Tylodina fungina* and the nudibranch *Tayuva lilacina* (formerly *Discodoris ketos*) were highly specialised on only one species of sponge whilst the widest diet was maintained by *Doriprismatica sedan* (formerly *Glossodoris sedan*) consuming 16 species of sponge (Verdín Padilla et al. 2010). In a study investigating the lipid biochemistry of nudibranchs in Vietnam, Zhukova (2014) identified an abundance of very-long-chain fatty acids (so-called demospongiac acids) as a unique feature of the fatty acid profiles of nudibranchs. The presence of demospongiac acids was explained by specialised feeding on sponges by nudibranchs (Zhukova, 2014). The sponge-dwelling brittlestar *Ophiothrix lineata* is almost exclusively associated with the Caribbean sponge *Calyspongia vaginalis* (Henkel & Pawlik, 2014). *O. lineata* consumes the larvae of its host without providing any discernible benefit (e.g. sediment removal), indicating this interaction ranges from commensalism to parasitism depending on the reproductive status of *C. vaginalis* (Henkel & Pawlik, 2014). Predation on sponges at different life cycle stages (e.g. larvae, juvenile) has rarely been considered by sponge ecologists (but see Henkel & Pawlik, 2014; Mariani & Uriz, 2001) but may play a role in structuring sponge assemblages. Analysis of the intestinal tracts of sponge-inhabiting shrimp from the Indo-Pacific, the Caribbean and the Indian Ocean have characterised several species as parasites due to the prevalence of spicules and spongin in gut contents (Ďuriš et al. 2011). Similarly, the polychaete *Branchiosyllis oculata* readily feeds on sponge tissue (Pawlik, 1983) and *Xestospongia* spp. in the Wakatobi MNP are infested with an unidentified white polychaete (family Syllidae) (pers. obs). This link between these inquilines and upper trophic levels is poorly understood and, to my knowledge, studies are yet to directly link sponge-associated fauna to the feeding activities of coral reef fishes.

6.8 Future research directions

6.8.1 Adaptation to sponge predation

Research presented in this thesis suggests that spongivorous angelfishes consume sponges with substantial chemical defences (Chapter 3). Phylogenetic analysis placed sponges predated by angelfishes in clades with sponges that are known to possess substantial chemical defences e.g. Agelasidae, *Aaptos* and *Chalinula*. However, it should be noted that interspecific variation in deterrent properties has been documented for some genera (e.g. *Haliclona*). Hence, being grouped in a clade with chemically defended species does not unequivocally indicate that predated sponges were similarly defended. Yet, for some sponges that were grouped in clades represented by sponges appear to be consistently chemically defended (e.g. *Agelas*, *Aaptos*) (Loh & Pawlik, 2014), it seems likely that they share the same defensive properties. The presence of chemically defended sponges in angelfish

stomachs is an interesting observation because it thought that Caribbean angelfishes mainly consume chemically undefended, palatable sponges (Pawlik, 2011; Pawlik et al. 2018). Whereas other researchers contend that angelfishes feed rotationally on many sponges, including highly defended species (Wulff, 1994; Hourigan et al. 1989; Lesser & Slattery, 2013). Without dietary proportion data, the extent of predation on chemically defended species by Indo-Pacific angelfishes remains unknown. Similarly, there is no evidence that Indo-Pacific sponges fall into the same ecological categories noted by Pawlik (2011) and little published information on the chemical defences of Indo-Pacific sponges. One interesting avenue of research would be to test whether the Caribbean sponge categories (defended, open reef sponges; undefended but tolerant, palatable sponges; and palatable species that are restricted to cryptic habitats) are true of Indo-Pacific sponges. This would assist in determining the role of spongivory as a top-down control on sponge assemblages. Palatability assessments should be conducted using both generalist and specialist predators to account for differences in their tolerance of chemical defences (Schupp et al. 1999). Additionally, sponges that are deemed palatable and defended by feeding choice experiments should be verified using field observations of live sponges as assessing palatability using pellets may not always be reflective of ecological reality (Lesser & Slattery 2013; Wulff, 2017).

The presence of chemically defended sponges in angelfish stomachs raises some interesting questions about post-ingestive processes that allow the consumption of highly potent prey. To date, most adaptations for sponge predation considered by ecologists have been limited to morphology e.g. jaw and gut length (Konow & Bellwood, 2011) and behaviour e.g. ‘smorgasbord’ feeding (Randall & Hartman, 1968; Wulff, 1994) or avoidance (Pawlik, 2018). To date, no researchers have considered digestive mechanisms (beyond morphology) e.g. gut microbiome, that angelfishes may possess that allow them to specialise on sponges. However, one study has shown that angelfishes have a novel intestinal microbiome containing anaerobic bacteria (Satoh et al. 2014). Conversely, spongivorous opisthobranchs have attracted significant attention for their ability to detoxify and/or excrete harmful chemicals (Cimino & Ghiselin, 2009; da Cruz et al. 2012). Some nudibranchs can even sequester defensive metabolites from sponges, enhancing their defensive strategies (da Cruz et al. 2012). For example, the nudibranch *Hypselodoris cantabrica* requires the defensive metabolite nakafurance-9 directly from its prey, the sponge *Dysedia fragilis*, which it accumulates in higher concentrations than *D. fragilis*, making *H. cantabrica* better defended than its prey (da Cruz et al. 2012). An appreciation of the post-ingestive processes of terrestrial herbivores had been a crucial step towards defining herbivory in terrestrial systems (Clements et al. 2014). Hence, it is likely that addressing these knowledge gaps could contribute significantly to our understanding of spongivory in tropic marine systems.

6.9 Concluding remarks

To conclude, this thesis has shown that the identity of spongivores in the Wakatobi Marine National park, and wide range of sponges consumed by the most important sponge predator *Pygoplites diacanthus*, are consistent with known global patterns of spongivory. Using the Sampela reef system as an example for Anthropocene shallow coral reefs that transition to sponge dominance due to a decline in corals, I have shown that they provide fewer organic matter pathways for secondary production resulting in a less complex trophic structure characterised by fish with more similar diets. Similarly, observations from this thesis suggest that obligate spongivores are unlikely to benefit from shifts to sponge dominance when they result in low diversity sponge assemblages on low diversity reefs. However, sponges mediate alternative trophic pathways that are currently under-appreciated in reef food web models, likely due to considerable uncertainties surrounding the ubiquity of these pathways.

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Appendices

Appendix A

Trial video observations of sponge-grazing

Petrosia corticata at the Ridge (with feeding scars)

During the 148 minutes of footage recorded on the exposed inner tissue of *Petrosia corticata*, 888 bites were recorded giving a grazing intensity rate of $112.6 \text{ min}^{-1} \text{ m}^{-2}$. The majority of bites were directed at an area of exposed choanosome and sponge tissue was observed being removed by all species. The most voracious predator was *Zanclus cornutus* which took 59.5% of bites, followed by *Pygoplites diacanthus* taking 25.5% and *Siganus punctatus* took 6.6%. Other species observed taking bites included *Pomacanthus imperator*, *Heniochus singularius*, *Centropyge nox* and *Scarus ghobban* (terminal phase). *Pomacanthus imperator* took the highest no. of bites in a single feeding event (34).

Lamellodysedia herbacea at Sampela (no feeding scars)

In the 130 minutes of footage recorded on *L. dysedia*, 68 bites were observed on the sponge surface giving a total grazing intensity rate of $4.4 \text{ min}^{-1} \text{ m}^{-2}$. *Scarus flavipectoralis* juveniles took the most numerous bites (45.6%), followed by *Ctenochaetus binotatus* (29.4%). *Zanclus cornutus* and *Centropyge kleinii* took 8 and 7 bites respectively and 2 bites were made by *Centropyge tibicen*. Although bites were recorded on sponge surface, there was no evidence of sponge tissue being removed in the video.

Aaptos suberitoides at Sampela (with feeding scars)

In the 230 minutes of footage recorded on *Aaptos suberitoides*, 133 bites were recorded giving a grazing intensity rate of $4.6 \text{ min}^{-1} \text{ m}^{-2}$. The most numerous bites recorded by the angelfish *Centropyge bicolor* (76.7%) followed by the juvenile parrotfish *Scarus flavipectoralis* (9.8%). $\leq 5\%$ of bites were recorded by *Scarus niger*, *Centropyge tibicen* and *Zanclus cornutus*. *Canthigaster valentini*, *Chaetodon kleinii*, *Halichoeres prosopeion* and *Sufflamen bursa* all took only one bite in the whole video. Although bites were recorded on sponge surface, there was no evidence of sponge tissue being removed in the video.

Green tubular sponge (*Theonella* sp?) at KDS (with feeding scars)

In the 33 minutes of footage recorded on the green tubular sponge, a total of 385 bites were recorded by six species. The majority of bites were taken by *Pygoplites diacanthus* (295) and were directed towards an exposed bit of choanosome which made up approximately 4.8% of total sponge area. This area of exposed inner tissue had a grazing intensity rate from *Pygoplites diacanthus* of $265.8 \text{ bites min}^{-1} \text{ m}^{-2}$ whereas the undisturbed sponge surface had a grazing intensity rate of $10.3 \text{ bites min}^{-1} \text{ m}^{-2}$. *Zanclus cornutus* took the second-highest number of bites (47) all of which were directed towards the white exposed inner tissue giving a grazing intensity rate of $74.8 \text{ bites min}^{-1} \text{ m}^{-2}$. *Pomacanthus xanthurus*

was also sighted feeding from the sponge, taking 4 bites on the white exposed inner tissue. Other species noted were *Centropyge tibicen*, *Chaetodon kleinii* and *Ctenochaetus binotatus*, taking 25, 9 and 4 bites respectively, all from the undisturbed sponge surface.

Xestospongia spp. at Buoy 1

During the 96 minutes of video footage recorded on *Xestospongia* sp. at Buoy 1, 2423 bites were recorded giving a grazing intensity rate of $61.4 \text{ min}^{-1} \text{ m}^{-2}$. *Ctenochaetus binotatus* took the most numerous bites (62.5%) followed by *Coradion melanopus* (21.3%), *Zanclus cornutus* (5.1%) and *Forcipiger flavissimus* (4.5%). Eight additional species: *Chaetodon kleinii*, *Thalassoma lunare*, *Anampses meleagrides*, *Balistapus undulatus*, *Neoglyphidodon melas*, *Scarus niger* (terminal phase), *Odonus niger* and *Halichoeres prosopion* were all observed taking <2% of total bites recorded.

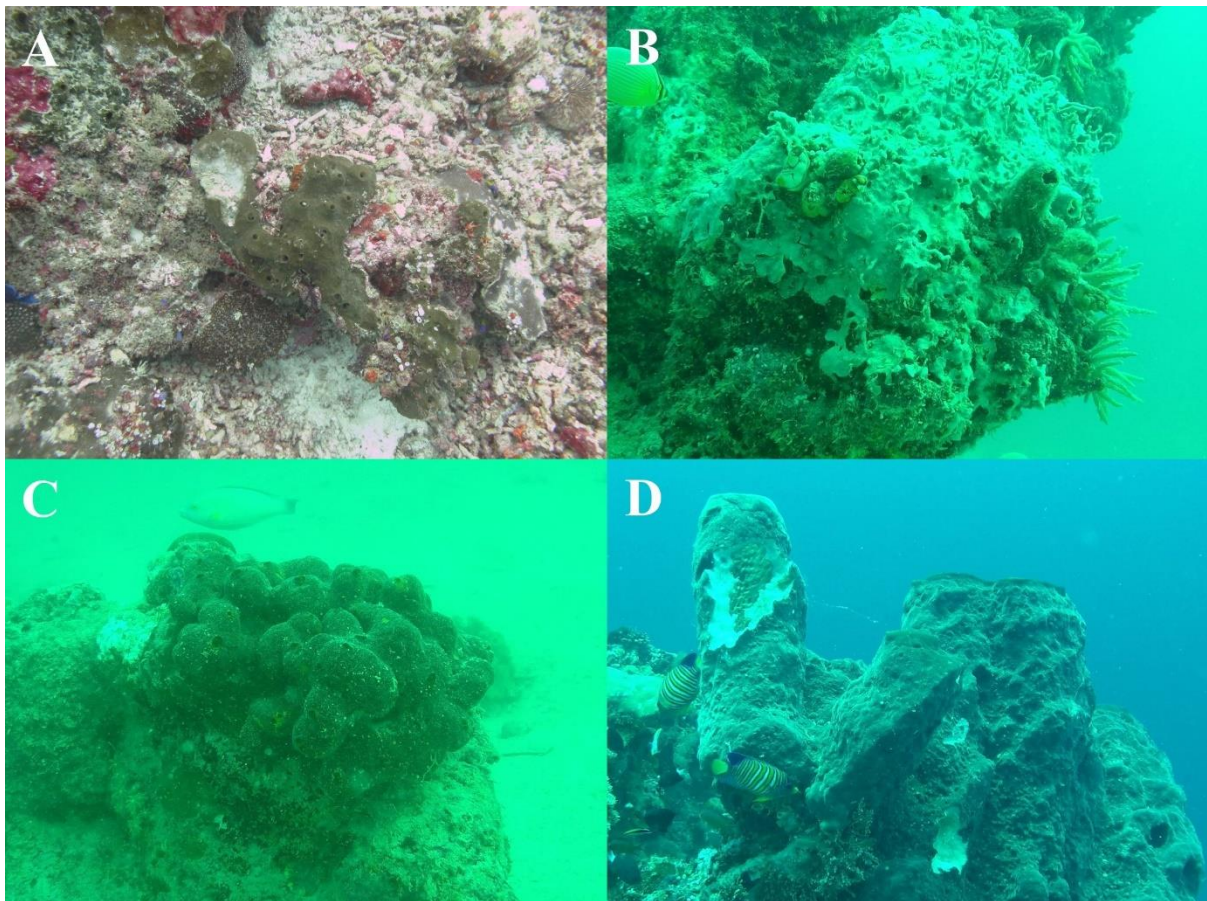


Figure 1. Screenshots of: A = *Petrosia corticata*; B = *Lamellodysedia herbacea*; C = *Aaptos suberitoides*; D = Green tubular sponge.

Appendix B

Table 1. Negative binominal modelling of fish predation (total bites) between sites in the Wakatobi MNP with the 2D area of sponge (m^2) captured in the frame and the length of footage (min^{-1}) fitted as offsets in the model. Note, Sampela was used as the reference for the factor to which the other sites were compared.

Coefficients	Estimate	SE	Z value	Pr(> z)
Intercept	-10.801	0.2511	-43.02	<2e-16
B1	0.9199	0.3549	2.592	0.0095
Ridge	1.0476	0.3676	2.85	0.0044
KDS	0.5753	0.3674	1.566	0.1174
Dispersion parameter		1.992		
		SE	0.486	
AIC		456.01		
2 x log-likelihood		446.01		

Table 2. PERMANOVA (main test) of the effect of site on the identity of fish taking bites from *Xestospongia* spp. in the Wakatobi Marine National Park, Indonesia.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	3	11042	3680.5	1.73	0.012	999
Res	26	55262	2125.2			
Total	29	66304				
PERMDISP on group factor 'Site', F = 1.38, p = 0.47						

Table 3. PERMANOVA (main test) of the effect of site on sponge-grazing fish densities in the Wakatobi Marine National Park, Indonesia.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	3	5719.8	1906.6	3.95	0.001	999
Res	20	9643.5	482.17			
Total	23	15363				
PERMDISP on group factor 'Site', F = 3.1, p = 0.11						

Table 4. Repeated measures analysis on a subset (n = 12) of quadrats to determine the appropriate number of points used in CPCe analysis. A = main test and B = pairwise tests. 60 points recorded significantly more benthic categories than 5, 10, 20 points and there was a very close to significant result with 30 points. There was no difference in the number of benthic categories recorded between 60 and 120 points.

A	df	Sum Sq	Mean Sq	F	Pr(>F)
Points	5	130.61	26.122	62.32	<0.001
Residuals	55	23.06	0.419		

B	Estimate	Std. Error	z value	Pr(> z)	
120points - 10points == 0	3.0833	0.2643	11.665	< 0.001	***
20points - 10points == 0	1.4167	0.2643	5.36	< 0.001	***
30points - 10points == 0	2.1667	0.2643	8.197	< 0.001	***
5points - 10points == 0	-0.4167	0.2643	-1.576	0.61419	
60points - 10points == 0	2.9167	0.2643	11.035	< 0.001	***
20points - 120points == 0	-1.6667	0.2643	-6.305	< 0.001	***
30points - 120points == 0	-0.9167	0.2643	-3.468	0.00694	**
5points - 120points == 0	-3.5	0.2643	-13.242	< 0.001	***
60points - 120points == 0	-0.1667	0.2643	-0.631	0.98878	
30points - 20points == 0	0.75	0.2643	2.837	0.05178	.
5points - 20points == 0	-1.8333	0.2643	-6.936	< 0.001	***
60points - 20points == 0	1.5	0.2643	5.675	< 0.001	***
5points - 30points == 0	-2.5833	0.2643	-9.773	< 0.001	***
60points - 30points == 0	0.75	0.2643	2.837	0.05178	.
60points - 5points == 0	3.3333	0.2643	12.611	< 0.001	***

Table 5. PERMANOVA (main test) of the effect of site on benthic community composition in the Wakatobi Marine National Park, Indonesia.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	2	8375.6	4187.8	9.77	0.001	999
Res	27	11571	428.54			
Total	29	19946				

PERMDISP on group factor 'Site', F = 1.13, p = 0.39						
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Appendix C

Table 1. Demosponge dataset created using 18S rDNA sequence data downloaded from the GenBank database and used in phylogenetic analyses.

Group	Accession	Definition
Outgroup	AF100949	<i>Rhabdocalyptus dawsoni</i> 18S ribosomal RNA gene, complete sequence
Agelasida	KC902123	<i>Acanthostylotella cornuta</i> voucher 0CDN8730-X small subunit 18S ribosomal RNA gene, partial sequence
	AY737640	<i>Agelas dispar</i> 18S ribosomal RNA gene, complete sequence
	KC902206	<i>Agelas</i> sp. USNM 1133824 small subunit 18S ribosomal RNA gene, partial sequence
	AB922832	<i>Agelas</i> sp. ARY-006 gene for 18S ribosomal RNA, partial sequence
	AY734443	<i>Agelas conifera</i> 18S ribosomal RNA gene, complete sequence
	EU702411	<i>Agelas schmidt</i> i 18S ribosomal RNA gene, complete sequence
	KX622155	<i>Agelas oroides</i> voucher TAU_ Po.25569 18S ribosomal RNA gene, partial sequence
	AY769087	<i>Agelas clathrodes</i> 18S ribosomal RNA gene, partial sequence
	KC902253	<i>Agelas</i> sp. n. USNM 1133819 small subunit 18S ribosomal RNA gene, partial sequence
	KC902036	<i>Agelas</i> sp. USNM 1204819 small subunit 18S ribosomal RNA gene, partial sequence
	KC902288	<i>Agelas</i> sp. n. USNM 1133823 small subunit 18S ribosomal RNA gene, partial sequence
	EF654520	<i>Agelas</i> sp. UCMPWC1026 small subunit ribosomal RNA gene, partial sequence
	KC902051	<i>Astrosclera willeyana</i> voucher 0CDN5435-R small subunit 18S ribosomal RNA gene, partial sequence
	KC902134	Astroscleridae sp. BMOO-04629 small subunit 18S ribosomal RNA gene, partial sequence
	KC902025	Astroscleridae sp. BMOO-09934 small subunit 18S ribosomal RNA gene, partial sequence
	KC901906	<i>Amphinomia sulphurea</i> voucher 0M9H2288-F small subunit 18S ribosomal RNA gene, partial sequence
	KC902371	<i>Hymerhabdia typica</i> voucher BELUM: Mc4588 small subunit 18S ribosomal RNA gene, partial sequence
	KC902182	<i>Prosuberites longispinus</i> voucher BELUM: Mc7173 small subunit 18S ribosomal RNA gene, partial sequence
Clionaida	KC902382	<i>Cervicornia cuspidifera</i> voucher 0M9G1351-I small subunit 18S ribosomal RNA gene, partial sequence
	KC902145	<i>Cliona varians</i> voucher 0M9G1439-C small subunit 18S ribosomal RNA gene, partial sequence
	KX866795	<i>Cliona viridis</i> voucher Po.25908 18S ribosomal RNA gene, partial sequence
	KX894476	<i>Spheciospongia</i> sp. DTTH-2018 voucher SPV 18S ribosomal RNA gene, partial sequence
	AY734440	<i>Spheciospongia vesparium</i> 18S ribosomal RNA gene, complete sequence
	KC762725	<i>Diplastrella megastellata</i> isolate 1_9 18S ribosomal RNA gene, partial sequence

	KC902056	<i>Cliona</i> sp. SI06x31 small subunit 18S ribosomal RNA gene, partial sequence
	KC902383	<i>Cliona celata</i> voucher BELUM: Mc5497 small subunit 18S ribosomal RNA gene, partial sequence
	KC902040	<i>Spirastrella</i> cf. <i>coccinea</i> USNM 1133785 voucher USNM_1133785 small subunit 18S ribosomal RNA gene, partial sequence
	KX866784	<i>Spirastrella cunctatrix</i> voucher Po.25527 18S ribosomal RNA gene, partial sequence
	KX894463	<i>Spirastrella</i> sp. SPS.1 18S ribosomal RNA gene, partial sequence
	KC902020	<i>Placospongia intermedia</i> voucher SI06x153 small subunit 18S ribosomal RNA gene, partial sequence
	KC902239	<i>Placospongia</i> sp. USNM 1133726 small subunit 18S ribosomal RNA gene, partial sequence
	KC902258	<i>Placospongia melobesioides</i> voucher 0M9H2592-K small subunit 18S ribosomal RNA gene, partial sequence
Suberitida	KX622158	<i>Aaptos</i> voucher TAU_ Po.25875 18S ribosomal RNA gene, partial sequence
	KC902111	<i>Aaptos suberitoides</i> voucher 0CDN8805-G small subunit 18S ribosomal RNA gene, partial sequence
	KC902192	<i>Suberites aurantiacus</i> voucher MNRJ15701 small subunit 18S ribosomal RNA gene, partial sequence
	KC902066	<i>Suberites massa</i> voucher BELUM: Mc4528 small subunit 18S ribosomal RNA gene, partial sequence
	KC902367	<i>Suberites</i> sp. 0M9H2772-G small subunit 18S ribosomal RNA gene, partial sequence
	KC902330	<i>Amorphinopsis excavans</i> voucher 0CDN9237-Y small subunit 18S ribosomal RNA gene, partial sequence
	Y737639	<i>Halichondria melanodocia</i> 18S ribosomal RNA gene, complete sequence
	AF058946	<i>Halichondria japonica</i> 18S ribosomal RNA gene, partial sequence
	KC902358	<i>Hymeniacidon perlevis</i> voucher BELUM: Mc4938 small subunit 18S ribosomal RNA gene, partial sequence
	HM035980	<i>Hymeniacidon sinapium</i> isolate Hymsin4-ZG3230 18S ribosomal RNA gene, partial sequence
	JN093018	<i>Hymeniacidon</i> sp. HymSP-TOTAL 18S ribosomal RNA gene, partial sequence
	KC902316	<i>Terpios aploos</i> voucher 0CDN3602-Y small subunit 18S ribosomal RNA gene, partial sequence
	KX601215	<i>Protosuberites incrustans</i> voucher ZMAPOR20543 18S ribosomal RNA gene, partial sequence
	KC902009	<i>Terpios manglaris</i> voucher SI06x252 small subunit 18S ribosomal RNA gene, partial sequence
	KC902342	<i>Terpios</i> sp. MNRJ15821 small subunit 18S ribosomal RNA gene, partial sequence
	KC902157	<i>Halichondria attenuata</i> voucher BELUM: Mc4732 small subunit 18S ribosomal RNA gene, partial sequence
	KC902247	<i>Halichondria bowerbanki</i> voucher BELUM: Mc4003 small subunit 18S ribosomal RNA gene, partial sequence
	KC902333	<i>Hymeniacidon kitchingi</i> voucher BELUM: Mc3332 small subunit 18S ribosomal RNA gene, partial sequence

	KC902355	<i>Terpios gelatinosa</i> voucher BELUM: Mc3315 small subunit 18S ribosomal RNA gene, partial sequence
	KC902238	<i>Halichondria panicea</i> voucher BELUM: Mc4070 small subunit 18S ribosomal RNA gene, partial sequence
	KX601214	<i>Protosuberites ectyoninus</i> voucher UBAPOR01 18S ribosomal RNA gene, partial sequence
	KC902236	<i>Suberites ficus</i> voucher BELUM: Mc4322 small subunit 18S ribosomal RNA gene, partial sequence
	KC901944	<i>Homaxinella subdola</i> voucher BELUM: Mc5438 small subunit 18S ribosomal RNA gene, partial sequence
Poecilosclerida	LN870429	<i>Abyssocladia dominalba</i> partial 18S rRNA gene, specimen voucher ZMBN103444
	LN870430	<i>Asbestopluma cupressiformis</i> partial 18S rRNA gene, specimen voucher ZMBN103450
	LN870431	<i>Asbestopluma lycopodium</i> partial 18S rRNA gene, specimen voucher ZMBN103462
	LN870434	<i>Chondrocladia gigantea</i> partial 18S rRNA gene, specimen voucher NTNU15204
	MG193132	<i>Cladorhiza oxeata</i> 18S ribosomal RNA gene, partial sequence
	LN870435	<i>Cladorhiza abyssicola</i> partial 18S rRNA gene, specimen voucher ZMBN103470
	MG193128	<i>Chondrocladia grandis</i> 18S ribosomal RNA gene, partial sequence
	LN870433	<i>Chondrocladia</i> (Meliiderma) sp. n. A JTH-2015 partial 18S rRNA gene, specimen voucher BMNH2015.6.4.4
	LN870436	<i>Euchelipluma</i> sp. n. A JTH-2015 partial 18S rRNA gene, specimen voucher P0108.75DR15
	LN870432	<i>Asbestopluma pennatula</i> partial 18S rRNA gene, specimen voucher ZMBN103468
	LN870437	<i>Guitarra antarctica</i> partial 18S rRNA gene, specimen voucher ECOQUIM786e
	KC902210	<i>Guitarra</i> sp. 0CDN7391-S small subunit 18S ribosomal RNA gene, partial sequence
Haplosclerida	KC902110	<i>Haliclona curacaoensis</i> voucher USNM_1133790 small subunit 18S ribosomal RNA gene, partial sequence
	KC902280	<i>Niphates cf. erecta</i> USNM 1133780 voucher USNM_1133780 small subunit 18S ribosomal RNA gene, partial sequence
	JX945610	<i>Callyspongia siphonella</i> voucher MCZ: DNA106625 18S ribosomal RNA gene, partial sequence
	JX945607	<i>Haliclona elegans</i> voucher MCZ: DNA106275 18S ribosomal RNA gene, partial sequence
	JX945627	<i>Amphimedon erina</i> voucher MCZ: DNA106220 18S ribosomal RNA gene, partial sequence
	KC902017	<i>Haliclona walentinae</i> voucher MNRJ15645 small subunit 18S ribosomal RNA gene, partial sequence
	DQ927312	<i>Niphates</i> sp. NIWAKD148 18S ribosomal RNA gene, complete sequence
	KC902300	<i>Dasychalina melior</i> voucher 0CDN9312-H small subunit 18S ribosomal RNA gene, partial sequence
	DQ927319	<i>Chalinula hooperi</i> voucher POR17651 18S ribosomal RNA gene, complete sequence
	DQ927320	<i>Petrosia</i> sp. NIWAKD1068 18S ribosomal RNA gene, complete sequence

	KC901970	<i>Petrosia lignosa</i> voucher 0CDN9353-Z small subunit 18S ribosomal RNA gene, partial sequence
	DQ927321	<i>Petrosia</i> sp. NIWAKD1020 18S ribosomal RNA gene, complete sequence
	DQ927318	<i>Acanthostrongylophora ingens</i> voucher POR17500 18S ribosomal RNA gene, complete sequence
	AB922828	<i>Acanthostrongylophora</i> sp. AKK2-001 gene for 18S ribosomal RNA, partial sequence
	KC902135	<i>Neopetrosia exigua</i> voucher 0CDN8774-V small subunit 18S ribosomal RNA gene, partial sequence
	KC902248	Petrosiidae sp. 0CDN2354-S small subunit 18S ribosomal RNA gene, partial sequence
	KC902252	<i>Neopetrosia proxima</i> voucher USNM_1133725 small subunit 18S ribosomal RNA gene, partial sequence
	KC902019	<i>Neopetrosia</i> sp. USNM 1133759 small subunit 18S ribosomal RNA gene, partial sequence
	KC902400	<i>Amphimedon compressa</i> voucher USNM_1133713 small subunit 18S ribosomal RNA gene, partial sequence
	EU702409	<i>Amphimedon compressa</i> 18S ribosomal RNA gene, complete sequence
	KX894469	<i>Amphimedon</i> sp. DTTH-2018 voucher AMC 18S ribosomal RNA gene, partial sequence
	EU702412	<i>Callyspongia plicifera</i> 18S ribosomal RNA gene, complete sequence
	DQ927314	<i>Callyspongia</i> sp. NIWAKD1668 18S ribosomal RNA gene, complete sequence
	KC902402	<i>Chalinula molitba</i> voucher CD06x1 small subunit 18S ribosomal RNA gene, partial sequence
	EU863812	<i>Callyspongia fallax</i> 18S ribosomal RNA gene, partial sequence
	AB542057	<i>Haliclona</i> sp. Sa080513 gene for 18S rRNA, partial sequence
	KF176610	<i>Haliclona</i> sp. BC1 18S ribosomal RNA gene, partial sequence
	KF176614	<i>Haliclona</i> sp. BC1 18S ribosomal RNA gene, partial sequence
	KF176613	<i>Haliclona</i> sp. BC1 18S ribosomal RNA gene, partial sequence
	KF176621	<i>Haliclona</i> sp. BC1 18S ribosomal RNA gene, partial sequence
	KX894467	<i>Haliclona amboinensis</i> voucher HAA.1 18S ribosomal RNA gene, partial sequence
	KC902016	<i>Haliclona mucifibrosa</i> voucher MNRJ15705 small subunit 18S ribosomal RNA gene, partial sequence
	DQ927306	<i>Haliclona cinerea</i> voucher POR14138 18S ribosomal RNA gene, complete sequence
	DQ927315	<i>Haliclona fascigera</i> voucher NIWAKD150 18S ribosomal RNA gene, complete sequence
	KC902267	<i>Haliclona</i> sp. BMOO-07065 small subunit 18S ribosomal RNA gene, partial sequence
	KC902054	<i>Haliclona manglaris</i> voucher USNM_1133711 small subunit 18S ribosomal RNA gene, partial sequence
	KC902285	<i>Haliclona</i> sp. BMOO-07059 small subunit 18S ribosomal RNA gene, partial sequence
	KC762727	<i>Siphonochalina</i> sp. 2-A 18S ribosomal RNA gene, partial sequence
	DQ927311	<i>Siphonochalina</i> sp. POR14630 18S ribosomal RNA gene, complete sequence

	KC902202	<i>Cladocroce</i> sp. 0CDN9562-C small subunit 18S ribosomal RNA gene, partial sequence
	AY734450	<i>Haliclona oculata</i> 18S ribosomal RNA gene, complete sequence
	KF176618	<i>Haliclona</i> sp. BC7 18S ribosomal RNA gene, partial sequence
	DQ927310	<i>Callyspongia</i> sp. POR14635 18S ribosomal RNA gene, complete sequence
	MH373530	<i>Haliclona cnidata</i> voucher S1_2015 18S small subunit ribosomal RNA gene, partial sequence
	AY734444	<i>Haliclona</i> sp. sp5 18S ribosomal RNA gene, complete sequence
	DQ927309	<i>Haliclona</i> sp. NIWAKD550 18S ribosomal RNA gene, complete sequence
	KC902399	<i>Neopetrosia rosariensis</i> voucher USNM_1133857 small subunit 18S ribosomal RNA gene, partial sequence
	KX668499	<i>Haliclona plakophila</i> voucher USNM:1254650 18S ribosomal RNA gene, partial sequence
	KP081740	<i>Xestospongia</i> sp. JV-2014 isolate XSPRA12 18S ribosomal RNA gene, partial sequence
	EU095523	<i>Haliclona</i> sp. DS-2007a 18S ribosomal RNA gene, partial sequence
	KC902312	<i>Xestospongia</i> sp. USNM 1204834 small subunit 18S ribosomal RNA gene, partial sequence
	KC902039	<i>Xestospongia bocatorensis</i> voucher SI04x40 small subunit 18S ribosomal RNA gene, partial sequence
	KP100454	<i>Haliclona</i> sp. NBL-2014 18S ribosomal RNA gene, partial sequence
	KX866799	<i>Haliclona</i> sp. Po.25929 18S ribosomal RNA gene, partial sequence
	KC902323	<i>Haliclona vansoesti</i> voucher SI06x152 small subunit 18S ribosomal RNA gene, partial sequence
	KC901998	<i>Haliclona tubifera</i> voucher SI06x97 small subunit 18S ribosomal RNA gene, partial sequence
	KC901937	<i>Neopetrosia carbonaria</i> voucher SI06x50 small subunit 18S ribosomal RNA gene, partial sequence
	KX894473	<i>Amphimedon</i> sp. AMQ 18S ribosomal RNA gene, partial sequence
	AY734446	<i>Niphates amorpha</i> 18S ribosomal RNA gene, partial sequence
	AY734445	<i>Niphates digitalis</i> 18S ribosomal RNA gene, partial sequence
	DQ927316	<i>Dasychalina fragilis</i> voucher POR14455 18S ribosomal RNA gene, complete sequence
	KC902338	<i>Gelliodes callista</i> voucher 0CDN9710-O small subunit 18S ribosomal RNA gene, partial sequence
	KC902274	<i>Dasychalina</i> sp. 0CDN9201-L small subunit 18S ribosomal RNA gene, partial sequence
	KX668500	<i>Xestospongia deweerdae</i> voucher USNM:1254645 18S ribosomal RNA gene, partial sequence
	KX894471	Niphatidae sp. NIS 18S ribosomal RNA gene, partial sequence
	AY621510	<i>Xestospongia muta</i> 18S ribosomal RNA gene, complete sequence
	KX894458	<i>Xestospongia testudinaria</i> voucher XES.1 18S ribosomal RNA gene, partial sequence
	DQ927308	<i>Cribochalina vasculum</i> voucher NIWAKD538 18S ribosomal RNA gene, complete sequence
	KC901980	<i>Cribochalina vasculum</i> voucher 0M9G1420-I small subunit 18S ribosomal RNA gene, partial sequence

	AB922827	<i>Petrosia</i> sp. ARY-005 gene for 18S ribosomal RNA, partial sequence
	KC902101	<i>Petrosia</i> aff. <i>hartmani</i> USNM 1204818 voucher USNM_1204818 small subunit 18S ribosomal RNA gene, partial sequence
	KC902222	<i>Petrosia strongylata</i> voucher 0CDN9767-Z small subunit 18S ribosomal RNA gene, partial sequence
	KC902173	<i>Petrosia weinbergi</i> voucher 0M9G0152-Z small subunit 18S ribosomal RNA gene, partial sequence
	DQ927322	<i>Aka mucosum</i> voucher NIWAKD535 18S ribosomal RNA gene, complete sequence
Verongiida	EU702410	<i>Aplysina fistularis</i> 18S ribosomal RNA gene, complete sequence
	KC901896	<i>Aplysina cauliformis</i> voucher USNM_1204836 small subunit 18S ribosomal RNA gene, partial sequence
	KC901889	<i>Aplysina</i> sp. USNM 1204828 small subunit 18S ribosomal RNA gene, partial sequence
	KC902200	<i>Aplysina fulva</i> voucher USNM_1153593 small subunit 18S ribosomal RNA gene, partial sequence
	KC902406	<i>Pseudoceratina arabica</i> voucher 0CDN9812-Y small subunit 18S ribosomal RNA gene, partial sequence
	KC902010	<i>Pseudoceratina</i> sp. P02x141 small subunit 18S ribosomal RNA gene, partial sequence
	AB922830	<i>Pseudoceratina</i> sp. P02x141 small subunit 18S ribosomal RNA gene, partial sequence
	AY591804	<i>Verongula gigantea</i> 18S ribosomal RNA gene, complete sequence
	KC902024	Verongiida sp. 0CDN8087-Y small subunit 18S ribosomal RNA gene, partial sequence
	KC902055	Pseudoceratinidae sp. BMOO-08168 small subunit 18S ribosomal RNA gene, partial sequence
	KC902144	<i>Pseudoceratina purpurea</i> voucher 0CDN8109-X small subunit 18S ribosomal RNA gene, partial sequence
	KC902318	<i>Pseudoceratina</i> sp. USNM 1204854 small subunit 18S ribosomal RNA gene, partial sequence
	KC901952	<i>Hexadella racovitzai</i> voucher BELUM: Mc5090 small subunit 18S ribosomal RNA gene, partial sequence
	KC902148	<i>Ianthella basta</i> voucher G02x165 small subunit 18S ribosomal RNA gene, partial sequence

Table 2. Sequence data from predated sponges

<p>Ridge RA1 S4A</p> <p>TGCAGAAGGTCCCGACTTTACGGAAGGGATGTATTTATTAGATCCAAAACCAATGCCGAGGCTT TCGGTCTCGGGAAGCTGGTGATTCATGATAACTGCTCGAATCGCACGGCCTTCGCGCCGGCGAT GATTCATTCAAGTTTCTGCCCTATCAACTTTCGATGGTAGGGTATTGGCCTACCATGGTCGCAAC GGGTAACGGAGAATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAA GGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACTCGGGGAGGTAGTGACAATAAATAACA ATGCCGGCCTCTTGAGTAGGCCGGCAATTGGAATGAGTACAATCCAAACCCCTTAACGAGGAAC AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAG TTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTCGGAGCCGACCGGGCGGTCTGTTCGAGAGACAC GCACTGCTCGGTGGCTCTTCCTCTCGGAGGCCCTGGCTGCCCTTAAGTGGAGCGGGCAGGGGAT TCGGGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCGGTGCTTGGATACATTAGCA TGGAATAATGGAATAGGGCTTGGGTCTATTTTGTGGTTTTCGGGACCGAGGTCATGGTTAAG AGGGACAGTTGGGGGCATTTCGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATGGAAGACG AACAACCTGCGAA</p>

Ridge RA2 S2

AATCAGTTATAGTTTTATTTGATGGTGCCCCACTACATGGATAACCGTGGTAATTCTAGAGCTAA
TACATGCAGAAGGTCCCGACTTTTCGGAAGGGATGTATTTATTAGATCCAAAACCAATGCCGAG
GCTTCGGTCTCGGGAAGCTGGTGATTTCATGGTAACTGCTCGAATCGCACGGCCTTCGCGCCGGC
GATGATTCATTCAAGTTTCTGCCCTATCAACTTTTCGATGGTAGGGTATTGGCCTACCATGGTCGC
AACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATC
CAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACTCGGGGAGGTAGTGACAATAAATA
ACAATGCCGGCCTCTTGAGTAGGCCGGCAATTGGAATGAGTACAATCCAAACCCCTTAACGAGG
AACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTA
AAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTCGGAGCCGACCGGGCGGTCTGTGAGAGA
CACGCACTGCTCGGTGGCTCTTCCTCTCGGAGGCCCTGGCTGCCCTTGACTGGAGCGGGCAGGG
GATTCGGGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCGGTGCTTGATACATTA
GCATGGAATAATGGAATAGGGCTTGGGTCCTATTTTG

Ridge RA2 S10

GCTCCGGCCTCGGGAAGCTGGTGATTTCATGGTAACTGCTCGAATCGCACGGCCTTCGCGCCGGC
GATGATTCATTCAAGTTTCTGCCCTATCAACTTTTCGATGGTAGGGTATTGGCCTACCATGGTCGC
AACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATC
CAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACTCGGGGAGGTAGTGACAATAAATA
ACAATGCCGGCCTCTTGAGTAGGCCGGCAATTGGAATGAGTACAATCCAAACCCCTTAACGAGG
AACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTA
AAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTCGGAGCCGACCGGGCGGTCTGTGAGAGA
CACGCACTGCTCGGTGGCTCTTCCTCTCGGAGGCCCTGACTGCCCTTGACTGGAGCGGGCAGGG
GATTCGGGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCGGTGCTTGATACATTA
GCATGGAATAATGGAATAGGGCTTGGGTCCTATTTTGTTGGTTTTTCGGGACCGAGGTCATGGTT
AAGAGGGACAGTTGGGGGCATTTCGTATTCAATTGTCAGAGGTGAAATTCCTTGATTTATGGA

Ridge RA3 S1

TCATTCAAATTTCTGCCCTATCAACTTTTCGATGGTACGGTAGTGGCCTACCATGGTTGCCACGGG
TGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCTAGGA
AGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGATAAATAACAATG
CCGGGCTTTCTTAGTC

Ridge RA4 B

TTATAGTTTATGTGATGGTTTCTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGC
AGAAAGTCCCGACTTTTTGGAAGGGATGTATTTATTAGATCCAAAACCAAGTGCAGCTTCGGCT
CGGTTTCTTGGTGATTTCATGATAACTGCTCGAACCCTATGGCCCTTGCGCCGACGGTGCTTCATT
CAAATTTCTGCCCTATCAACTTTTCGATGGTACGGTAGTGGCCTACCATGGTTGCAACGGGTGAC
GGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGC
AGCAGGCGCGCAAATTACCCAATCCCGACTCGGGGAGGTAGTGACAATAAATAACAATGCCGG
GCTATTGTAGTCTGGCAATTGGAATGAGTACAATCTAAACCCCTTAACGAGGAACAATTGGAGG
GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAG
TTAAAAAGCTCGTAGTTGGATTTTCGGGGCAGCCTGCCTGGTCCGCCGCAAGGCGAGTACTGGTC
AGCCGCCCTTCCTCTCGAAAGCCCCGACTGCTCTTCACTGCAGTGGTCCGGTAGTTCGGGACGTT
TACTTTGAAAAAATTAGAGTGTTCAAGGCAGGCTATCGCCTGAATACATTAGCATGGAATAATG
GAAGAGGACCTCGGTCCTATTTTGTTGGTTTCTAGGGCCGAAGTAATGATTAAGAGGGACAGTT
GGGGGCATTCGTATTTAATTGTCAGAGGTGAAATTCCTTGATTTATGAAAGACGAAGTAG

Ridge RA5

GCAAGGCCTTTGCGCGGGCGATGATTCATTCAAGTTTCTGCCCTATCAACTTTTCGATGGTAGGGT
ATTGGCCTACCATGGTCGCAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCT
GAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACTCGGG
GAGGTAGTGACAATAAATAACAATGCCGGCCTCTTGAGTAGGCCGGCAATGGGAATGAGTACA
ATCCAAACCCCTTAACGAGGAACAATGGGAGGGCAAGTCGGGTGCCGGCGGCCGCGGTAATTC

CAGTTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGTTGGTAGTTGGATTTCGGAGCCG
ACCGGGCGTTCGGTCCAAAGACACGCACTGCTCGGTGGCTTTTCTTTGGGAGGCCCTGGCTGC
CTTTCAGTGGAGCGGGCAGGGGATTTCGGGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCA
GGCCGGTGCTTGGATACATTAGCATGGAATAATGGAATAGGGCTTGGGTCCTATTTTGTGGTTT
TCGGGACCGAGGTC

Ridge RA6 S3

GGGATAACCGTAGTAATTCTAGAGCTAATACATGCAGAAAGTCCCGACTTCTTGGAAGGGATGT
ATTTATTAGATCCAAAACCAGCGCGAGTTTCTTCGGGGGCTCGGTTCATGGTGATTTCATGATA
ACTGCTCGAATCGCATGGCCTTGCGCCGGCGATGATCCATTCAAATTTCTGCCCTATCAACTTTC
GATGGCAAGGTAGTGGCTTGCCATGGTGACAACGGGTGACGGAGAATTAGGGTTTCGATTCCGG
AGAGGGAGCCTGAGAAACGGCTACCACATCCGAGGAAGGCAGCAGGCGCGCAAATTACCCAAT
CCCGACTCGGGGAGGTAGTGACAATAAATAACAATGCTGGGCTATCGTAGTCTGGCAATTGGAA
TGAGTACAATCTAAACCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGC
GGTAATTCCAGTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTC
GGGGCAGCCCGGCTGGTCCGTCGCAAGGCGAGTACTGGTCGGCCGCCCTTCTCTCGAAAGCCC
CGACTGCTCTTCACTGTAGTGGTCGGGGAGTTTCGGGACGTTTACTTTGAAAAAATTAGAGTGTTC
AAGGCAGGCCATCGCCTGAATACATTAGCATGGAATAATGGAAGAGGACCTCGGTCTATTTTG
TTGGTTTCTAGGGCCGAAAGTAATGATTAAGAGGGACAGTTGGGGGCATTCGTATTTAATTGTC
AGAAGGTGAAAATTCT

Sampela RA2 S2

GCTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCAGAAGGTCCCGACTCTCGGAAGG
GATGTATTTATTAGATCCAAGACCAATGCCGGGATCTCGGTCCCGGAAGCTGGTGATTCATGG
TAACTGCTCGAATCGCACGGCCCTCGCGCCGGCGATGATTCAAGTTTCTGCCCTATCAACT
TTCGATGGTAGGGTATTGGCCTACCATGGTCGCAACGGGTGACGGAGAATTAGGGTTTCGATTCC
GGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCC
AATCCCGACTCGGGGAGGTAGTGACAATAAATAACAATGCCGGCCTCTTGAGTAGGCCGGCAA
TTGGAATGAGTACAATCCAACCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGC
AGCCGCGGTAATTCCAGTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTT
GGATTTTCGGAGCCGACCGGGCGGTCTGTGAGAGACACGCACTGCTCGGTGGCTCTTCTCTCG
GAGGCCCTGGCTGCCCTTAACTGGAGCGGGCAGGGGATTTCGGGACGTTTACTTTGAAAAAATTA
GAGTGTTCAAAGCAGGCCGGTGCTTGGATACATTAGCATGGAATAATGGAATAGGGCTTGGGTC
CTATTTTGTGGTTTTTCGGGACCGAGGTCATGGTTAAGAGGGACAGTTGGGGGCATTCGTATTCA
ATTGTCAGAGGTGAAATTCTTGAATTTATGGAAGACGAACATG

Sampela RA5 S7

TCTGATGGTACCTCCTACTCGGATAACCGTGGTAATTCTAGAGCTAATACATGCAGCAAGTCCC
GACTTCTGGAAGGGACGTATTTATTAGATACAAAACCAATGCAGCTTCGGCTGGTCCCTTGGTG
ATTCATGATAAAGTCTCGAATCGCATGGCCTTGCGCTGGCGATGGTTCATTCAAGTTTCTGCCCT
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Sampela RA6 S1

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Sampela RA6 S5

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Sampela RA6 S6

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CTGCAA

Sampela RA7 S5

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Ridge PX2 S13

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Appendix D

Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (mean \pm SD) of sources, benthic prey items and consumers (fish) collected at two sites in the Wakatobi Marine National Park, Indonesia.

Sources and benthic prey	High-quality reef system			Sponge-dominated reef system		
	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N
Green turf algae	1.46 ± 0.47	-12.48 ± 2.51	3	0.82 ± 0.08	-12.23 ± 0.06	3
<i>Dictyota</i> sp.				3.58 ± 0.05	-16.33 ± 1.63	3
<i>Halimeda</i> sp.	3.77 ± 0.52	-22.85 ± 0.67	3			
SPOM	4.00 ± 0.70	-26.02 ± 0.89	3	5.43 ± 0.65	-24.25 ± 1.09	3
<i>Synaptula</i> sp.	6.66 ± 0.31	-18.35 ± 0.65	5	6.11 ± 0.28	-16.86 ± 0.34	5
<i>Pavona varians</i>	4.29 ± 0.42	-16.51 ± 0.51	3	4.25 ± 0.33	-14.04 ± 0.64	3
<i>Pocillopora verrucosa</i>	4.62 ± 0.43	-15.57 ± 0.79	3	4.39 ± 0.14	-16.05 ± 0.96	3
<i>Porites lutea</i>	5.3 ± 0.23	-12.7 ± 1.61	3	4.15 ± 0.87	-13.68 ± 0.68	3
<i>Xestospongia</i> sp.	6.97 ± 1.11	-19.38 ± 0.46	3	6.35 ± 0.15	-18.67 ± 0.47	3
<i>Hyrtios erectus</i>	3.31 ± 1.73	-18.18 ± 0.19	3	4.27 ± 0.72	-16.98 ± 0.48	3
<i>Clathria</i> sp.	6.78 ± 0.28	-20.74 ± 0.16	3	5.84 ± 0.09	-19.16 ± 0.21	3
<i>Lamellodysedia herbacea</i>	4.94 ± 0.48	-26.06 ± 0.6	3	5.19 ± 0.43	-24.73 ± 0.73	3
Consumers						
<i>Zanclus cornutus</i>	9.83 ± 0.29	-16.93 ± 0.59	7	8.51 ± 0.41	-15.83 ± 0.89	8
<i>Pygoplites diacanthus</i>	10.31 ± 0.2	-16.41 ± 0.35	6	9.59 ± 0.43	-14.93 ± 0.24	7
<i>Forcipiger flavissimus</i>	11.38 ± 0.22	-15.04 ± 0.42	6	10.44 ± 0.41	-13.61 ± 0.7	5
<i>Ctenochaetus binotatus</i>	8.61 ± 0.5	-13.5 ± 0.27	5	7.75 ± 0.13	-12.29 ± 0.35	5
<i>Chaetodon kleinii</i>	10.81 ± 0.19	-16.52 ± 0.35	7	10.4 ± 0.16	-14.98 ± 0.26	6
<i>Chaetodon lunulatus</i>	9.27 ± 0.33	-10.22 ± 0.37	5	8.51 ± 0.38	-10.78 ± 1.36	6
<i>Neoglyphidodon nigroris</i>	9.73 ± 0.21	-17.93 ± 0.12	5	9.32 ± 0.2	-16.85 ± 0.16	5

Table 2. One-way ANOVA to test for differences in fish size (TL mm) between sites.

Species	F	Sig.
<i>Z. cornutus</i>	26.78	<0.001
<i>P. diacanthus</i>	0.587	0.46
<i>F. flavissimus</i>	0	0.984
<i>C. binotatus</i>	2.103	0.185
<i>C. kleinii</i>	2.577	0.137
<i>C. lunulatus</i>	2.577	0.143
<i>N. nigroris</i>	0.071	0.797

Table 3. Parametric paired-samples t test and non-parametric Wilcox rank sum on the effect of acidification on $\delta^{15}\text{N}$ values on sponges and algae collected in the Wakatobi Marine National Park, Indonesia.

	Taxa	n	Acidified	Non-Acidified	T	P
Parametric paired samples T test						
Sponge	<i>L. herbacea</i>	6	5.19 ± 0.28	5.07 ± 0.43	0.62944	0.56
	<i>Xestospongia</i>	6	6.8 ± 0.62	6.66 ± 0.79	0.92193	0.40
	<i>sp.</i>					
	<i>Clathria</i> sp.	6	6.25 ± 0.35	6.31 ± 0.55	-	0.62
					0.53573	
Algae	Green turf algae	6	1.19 ± 0.47	1.14 ± 0.46	0.25959	0.81
Wilcox rank sum test						V
p						
Sponge	<i>Hyrtios erecta</i>	6	3.96 ± 1.37	3.74 ± 1.24	19	0.094

Table 4. PERMANOVA (main test) of the effect of size (total length), site and species on the trophic position of fish collected in the Wakatobi Marine National Park, Indonesia.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Length	1	6.2032E-2	6.2032E-2	11.641	0.002	996
Site	1	0.22363	0.22363	41.968	0.001	997
Species	6	4.0343	0.67239	126.18	0.001	999
Si x Sp	6	6.5726E-2	1.0954E-2	2.0558	0.078	997
	68	0.36234	5.3286E-3			
	82	4.748				
PERMDISP on factor 'Site' F: 0.24749 df1: 1 df2: 81 P(perm) = 0.634						
PERMDISP on factor 'Species' F: 3.4128 df1: 6 df2: 76 P(perm) = 0.09						

Table 5. PERMANOVA (main test) of the effect of size (total length), site and species on the $\delta^{13}\text{C}$ values of fish collected in the Wakatobi Marine National Park, Indonesia.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Length	1	0.4642	0.4642	1.4761	0.239	998
Site	1	25.581	25.581	81.345	0.001	997
Species	6	60.41	60.41	192.1	0.001	999
	6	1.516	1.516	4.8209	0.003	998
	68	0.3144	0.3144			
	82					
PERMDISP on factor 'Site' F: 0.36872 df1: 1 df2: 81 P(perm): 0.65						
PERMDISP on factor 'Species' F: 0.55889 df1: 6 df2: 76 P(perm): 0.801						

Table 6. Interspecific comparisons of Trophic Position (TP) of seven reef fish species collected at two sites in the Wakatobi Marine National Park, Indonesia.

Pairwise comparisons	HQ reef system			Sponge reef system		
	t	p (perm)	perms	t	p(perm)	perms
<i>Z. cornutus</i> , <i>P. diacanthus</i>	3.5107	0.002	1249	5.0343	0.001	5024
<i>Z. cornutus</i> , <i>F. flavissimus</i>	11.074	<0.001	1699	8.4111	0.001	1279
<i>Z. cornutus</i> , <i>C. binotatus</i>	5.4148	0.002	788	3.9372	0.006	1282
<i>Z. cornutus</i> , <i>C. kleinii</i>	7.7287	<0.001	1698	11.36	<0.001	2157
<i>Z. cornutus</i> , <i>C. lunulatus</i>	3.1059	0.011	579	1.45E-02	0.989	2891
<i>Z. cornutus</i> , <i>N. nigroris</i>	0.6492	0.522	582	4.2213	0.002	1285
<i>P. diacanthus</i> , <i>F. flavissimus</i>	8.7702	0.002	460	3.4578	0.003	792
<i>P. diacanthus</i> , <i>C. binotatus</i>	7.9266	0.001	462	8.6398	0.001	791
<i>P. diacanthus</i> , <i>C. kleinii</i>	4.6164	0.002	1707	4.4145	0.001	1251
<i>P. diacanthus</i> , <i>C. lunulatus</i>	6.5186	0.003	336	4.6651	0.003	1706
<i>P. diacanthus</i> , <i>N. nigroris</i>	4.7296	0.002	462	1.3186	0.224	787
<i>F. flavissimus</i> , <i>C. binotatus</i>	12.949	0.002	462	12.552	0.008	126
<i>F. flavissimus</i> , <i>C. kleinii</i>	5.068	0.002	1699	0.28167	0.854	245
<i>F. flavissimus</i> , <i>C. lunulatus</i>	12.882	0.003	336	7.8839	0.003	462
<i>F. flavissimus</i> , <i>N. nigroris</i>	12.55	0.002	462	5.2856	0.007	126
<i>C. binotatus</i> , <i>C. kleinii</i>	11.235	0.002	789	28.083	0.002	336
<i>C. binotatus</i> , <i>C. lunulatus</i>	2.464	0.044	91	4.0567	0.005	462
<i>C. binotatus</i> , <i>N. nigroris</i>	4.7118	0.007	126	14.463	0.009	126
<i>C. kleinii</i> , <i>C. lunulatus</i>	10.458	0.002	581	11.429	0.002	336
<i>C. kleinii</i> , <i>N. nigroris</i>	9.3723	0.001	791	9.9145	0.003	336
<i>C. lunulatus</i> , <i>N. nigroris</i>	2.6401	0.030	91	4.2279	0.009	461

Table 7. Interspecific comparisons of pairwise comparisons of $\delta^{13}\text{C}$ values of seven reef fish species collected at two sites in the Wakatobi Marine National Park, Indonesia. Non-significant pairwise tests in bold font.

Pairwise comparisons	HQ reef system			Sponge reef system		
	t	p (perm)	perms	t	p(perm)	perms
<i>Z. cornutus</i> , <i>P. diacanthus</i>	2.0066	0.066	332	2.5716	0.026	423
<i>Z. cornutus</i> , <i>F. flavissimus</i>	6.5734	0.001	474	4.6999	0.001	506
<i>Z. cornutus</i> , <i>C. binotatus</i>	12.089	0.002	469	8.377	0.002	507
<i>Z. cornutus</i> , <i>C. kleinii</i>	1.5922	0.149	206	2.2342	0.048	292
<i>Z. cornutus</i> , <i>C. lunulatus</i>	22.462	0.002	447	8.4296	0.001	698
<i>Z. cornutus</i> , <i>N. nigroris</i>	3.7355	0.003	302	2.4949	0.033	289
<i>P. diacanthus</i> , <i>F. flavissimus</i>	7.0139	0.003	219	4.6758	0.006	339
<i>P. diacanthus</i> , <i>C. binotatus</i>	19.086	0.004	294	15.648	0.002	337
<i>P. diacanthus</i> , <i>C. kleinii</i>	0.6172	0.561	218	0.37343	0.734	188
<i>P. diacanthus</i> , <i>C. lunulatus</i>	33.783	0.003	322	8.0094	0.001	601
<i>P. diacanthus</i> , <i>N. nigroris</i>	13.03	0.004	258	15.506	0.003	382
<i>F. flavissimus</i> , <i>C. binotatus</i>	7.11	0.002	280	3.7626	0.009	112
<i>F. flavissimus</i> , <i>C. kleinii</i>	6.953	0.003	451	4.4714	0.01	272
<i>F. flavissimus</i> , <i>C. lunulatus</i>	20.105	0.004	310	4.1939	0.008	328
<i>F. flavissimus</i> , <i>N. nigroris</i>	14.915	0.005	303	10.041	0.011	87
<i>C. binotatus</i> , <i>C. kleinii</i>	16.104	0.001	397	14.768	0.003	327
<i>C. binotatus</i> , <i>C. lunulatus</i>	15.986	0.016	113	2.4034	0.055	319
<i>C. binotatus</i> , <i>N. nigroris</i>	33.579	0.014	96	26.702	0.006	111
<i>C. kleinii</i> , <i>C. lunulatus</i>	30.086	0.003	436	7.4562	0.005	319
<i>C. kleinii</i> , <i>N. nigroris</i>	8.6154	0.001	213	14.078	0.001	235
<i>C. lunulatus</i> , <i>N. nigroris</i>	44.499	0.004	109	9.8582	0.004	375

Table 8. Intraspecific comparisons of trophic position (TP) and $\delta^{13}\text{C}$ values for seven reef fish species collected at two sites in the Wakatobi Marine National Park, Indonesia.

	Trophic Position			$\delta^{13}\text{C}$		
	t	P(perm)	perms	t	P(perm)	perms
<i>Z. cornutus</i>	4.38	0.001*	5031	2.772	0.191	468
<i>P. diacanthus</i>	1.57	0.149	1706	11.161	0.002*	564
<i>F. flavissimus</i>	2.83	0.028*	461	4.207	0.008*	244
<i>C. binotatus</i>	1.57	0.208	126	6.13	0.009*	98
<i>C. kleinii</i>	0.19	0.917	1246	8.866	0.001*	626
<i>C. lunulatus</i>	1.36	0.189	336	0.88	0.414	338
<i>N. nigroris</i>	0.19	0.862	126	12.359	0.008*	72

Table 9. Intraspecific comparisons of Bayesian standard ellipse area (SEA_B).

SEAc larger at the low-quality site	Probability	Sig (p)
<i>Zanclus cornutus</i>	92.6	0.071
<i>Pygoplites diacanthus</i>	92.3	0.078
<i>Forcipiger flavissimus</i>	92.8	0.071
<i>Chaetodon lunulatus</i>	97.5	0.025*
<i>Neoglyphidodon nigroris</i>	74.1	0.250
SEAc smaller at the low-quality site		
<i>Ctenochaetus binotatus</i>	96.3	0.037*
<i>Chaetodon kleinii</i>	74.1	0.250

Table 10. PERMANOVA (main test) of the effect of site and source of stable isotope ratios of carbon sources and potential prey collected from two sites in the Wakatobi Marine National Park, Indonesia.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	13.943	13.943	12.074	0.002	999
Source	11	1279.3	116.3	100.71	0.001	999
Si x So	9	24.962	2.7736	2.4018	0.005	998
Res	48	55.43	1.1548			
Total	69	1396.2				
PERMDISP on factor 'Site' F: 1.7987 df1: 1 df2: 68 P(perm)= 0.222						
PERMDISP on factor 'Source' F: 0.65746 df1: 11 df2: 58 P(perm) = 0.902						

Table 11. Between source comparisons of stable isotope ratios of carbon sources and potential prey collected from the high-quality site in the Wakatobi Marine National Park, Indonesia. Bold font indicates isotopic niche overlap between groups.

Groups	t	P(perm)	perms	P(MC)
Turf, <i>Halimeda</i> sp.	6.8413	0.087	10	0.004
Turf, SPOM	8.5363	0.104	10	0.001
Turf, <i>Pavona</i>	3.2406	0.107	10	0.019
Turf, <i>Pocillopora</i>	2.8291	0.106	10	0.033
Turf, <i>Porites</i>	2.2007	0.116	10	0.052
Turf, Detritus+1TP	6.7631	0.022	56	0.001
Turf, <i>Xestospongia</i>	5.4139	0.104	10	0.006
Turf, <i>Clathria</i>	6.6109	0.092	10	0.003
Turf, <i>Hyrtios</i>	3.3572	0.092	10	0.023
Turf, <i>L. dysedia</i>	9.1054	0.094	10	0.001
<i>Halimeda</i> sp., SPOM	3.894	0.082	10	0.009
<i>Halimeda</i> sp., <i>Pavona</i>	10.299	0.101	10	0.002
<i>Halimeda</i> sp., <i>Pocillopora</i>	10.311	0.087	10	0.001
<i>Halimeda</i> sp., <i>Porites</i>	9.7378	0.098	10	0.002
<i>Halimeda</i> sp., Detritus+1TP	9.5866	0.022	56	0.001
<i>Halimeda</i> sp., <i>Xestospongia</i>	5.5534	0.107	10	0.004

<i>Halimeda</i> sp., <i>Clathria</i>	7.0483	0.086	10	0.003
<i>Halimeda</i> sp., <i>Hyrtios</i>	4.1898	0.1	10	0.006
<i>Halimeda</i> sp., <i>L. dysedia</i>	5.1974	0.094	10	0.002
SPOM, <i>Pavona</i>	12.554	0.115	10	0.001
SPOM, <i>Pocillopora</i>	12.529	0.091	10	0.001
SPOM, <i>Porites</i>	11.72	0.111	10	0.002
SPOM, Detritus+1TP	12.616	0.023	56	0.001
SPOM, <i>Xestospongia</i>	7.6016	0.109	10	0.002
SPOM, <i>Pavona</i>	8.7506	0.092	10	0.001
SPOM, <i>Hyrtios</i>	6.5436	0.093	10	0.003
SPOM, <i>L. dysedia</i>	1.1901	0.404	10	0.288
<i>Pavona</i> , <i>Pocillopora</i>	1.5606	0.192	10	0.202
<i>Pavona</i> , <i>Porites</i>	3.9145	0.087	10	0.012
<i>Pavona</i> , Detritus+1TP	5.8521	0.025	56	0.001
<i>Pavona</i> , <i>Xestospongia</i>	4.9375	0.088	10	0.002
<i>Pavona</i> , <i>Clathria</i>	11.594	0.097	10	0.001
<i>Pavona</i> , <i>Hyrtios</i>	1.7929	0.107	10	0.100
<i>Pavona</i> , <i>L. dysedia</i>	16.42	0.088	10	0.001
<i>Pocillopora</i> , <i>Porites</i>	2.7617	0.099	10	0.045
<i>Pocillopora</i> , Detritus+1TP	6.0192	0.015	56	0.001
<i>Pocillopora</i> , <i>Xestospongia</i>	5.1519	0.116	10	0.004
<i>Pocillopora</i> , <i>Clathria</i>	10.176	0.103	10	0.001
<i>Pocillopora</i> , <i>Hyrtios</i>	2.576	0.101	10	0.037
<i>Pocillopora</i> , <i>L. dysedia</i>	15.409	0.105	10	0.001
<i>Porites</i> , Detritus+1TP	7.2041	0.022	56	0.001
<i>Porites</i> , <i>Xestospongia</i>	5.9039	0.095	10	0.002
<i>Porites</i> , <i>Clathria</i>	8.5768	0.106	10	0.001
<i>Porites</i> , <i>Hyrtios</i>	4.2413	0.101	10	0.006
<i>Porites</i> , <i>L. dysedia</i>	12.921	0.086	10	0.001
Detritus+1TP, <i>Xestospongia</i>	1.6132	0.143	56	0.111
Detritus+1TP, <i>Clathria</i>	5.3086	0.018	56	0.003
Detritus+1TP, <i>Hyrtios</i>	3.9311	0.016	56	0.007
Detritus+1TP, <i>L. dysedia</i>	14.69	0.021	56	0.001
<i>Xestospongia</i> , <i>Clathria</i>	1.9067	0.186	10	0.092
<i>Xestospongia</i> , <i>Hyrtios</i>	3.1417	0.101	10	0.027
<i>Xestospongia</i> , <i>L. dysedia</i>	8.4556	0.104	10	0.001
<i>Clathria</i> , <i>Hyrtios</i>	4.204	0.1	10	0.009
<i>Clathria</i> , <i>L. dysedia</i>	11.719	0.095	10	0.001
<i>Hyrtios</i> , <i>L. dysedia</i>	7.308	0.109	10	0.001

Table 12 Between source comparisons of stable isotope ratios of carbon sources and potential prey collected from the low-quality site in the Wakatobi Marine National Park, Indonesia.

Groups	t	P(perm)	perms	P(MC)
Turf, SPOM	17.537	0.093	10	0.001
Turf, <i>Pavona</i>	9.2721	0.092	10	0.002
Turf, <i>Pocillopora</i>	9.3153	0.096	10	0.001
Turf, <i>Porites</i>	5.6712	0.105	10	0.003
Turf, Detritus+1TP	26.492	0.014	56	0.001
Turf, <i>Xestospongia</i>	29.127	0.113	10	0.001
Turf, <i>Clathria</i>	59.307	0.083	10	0.001
Turf, <i>Hyrtios</i>	11.696	0.105	10	0.001
Turf, <i>L. dysedia</i>	26.732	0.088	10	0.001

Turf, <i>Dictyota</i>	5.2335	0.11	10	0.006
SPOM, <i>Pavona</i>	12.235	0.107	10	0.001
SPOM, <i>Pocillopora</i>	8.9887	0.104	10	0.001
SPOM, <i>Porites</i>	10.982	0.111	10	0.001
SPOM, Detritus+1TP	12.476	0.015	56	0.001
SPOM, <i>Xestospongia</i>	7.1958	0.105	10	0.003
SPOM, <i>Clathria</i>	6.8681	0.085	10	0.005
SPOM, <i>Hyrtilos</i>	8.3238	0.095	10	0.001
SPOM, <i>L. dysedia</i>	0.60008	0.575	10	0.696
SPOM, <i>Dictyota</i>	6.8272	0.099	10	0.003
<i>Pavona</i> , <i>Pocillopora</i>	2.9028	0.1	10	0.032
<i>Pavona</i> , <i>Porites</i>	0.49404	0.934	10	0.778
<i>Pavona</i> , Detritus+1TP	8.466	0.028	56	0.001
<i>Pavona</i> , <i>Xestospongia</i>	10.139	0.113	10	0.001
<i>Pavona</i> , <i>Clathria</i>	12.369	0.095	10	0.001
<i>Pavona</i> , <i>Hyrtilos</i>	4.5365	0.104	10	0.002
<i>Pavona</i> , <i>L. dysedia</i>	16.702	0.097	10	0.001
<i>Pavona</i> , <i>Dictyota</i>	2.3152	0.094	10	0.071
<i>Pocillopora</i> , <i>Porites</i>	2.817	0.11	10	0.041
<i>Pocillopora</i> , Detritus+1TP	3.9358	0.018	56	0.002
<i>Pocillopora</i> , <i>Xestospongia</i>	5.2358	0.105	10	0.003
<i>Pocillopora</i> , <i>Clathria</i>	5.9942	0.096	10	0.008
<i>Pocillopora</i> , <i>Hyrtilos</i>	1.2482	0.295	10	0.267
<i>Pocillopora</i> , <i>L. dysedia</i>	11.718	0.108	10	0.002
<i>Pocillopora</i> , <i>Dictyota</i>	0.77916	0.514	10	0.500
<i>Porites</i> , Detritus+1TP	7.0044	0.019	56	0.001
<i>Porites</i> , <i>Xestospongia</i>	7.8262	0.097	10	0.001
<i>Porites</i> , <i>Clathria</i>	8.8248	0.117	10	0.001
<i>Porites</i> , <i>Hyrtilos</i>	4.0831	0.094	10	0.010
<i>Porites</i> , <i>L. dysedia</i>	13.792	0.094	10	0.001
<i>Porites</i> , <i>Dictyota</i>	2.3842	0.087	10	0.054
Detritus+1TP, <i>Xestospongia</i>	5.4725	0.02	56	0.001
Detritus+1TP, <i>Clathria</i>	8.31	0.022	56	0.001
Detritus+1TP, <i>Hyrtilos</i>	4.1091	0.02	56	0.006
Detritus+1TP, <i>L. dysedia</i>	17.819	0.017	56	0.001
Detritus+1TP, <i>Dictyota</i>	3.5161	0.017	56	0.003
<i>Xestospongia</i> , <i>Clathria</i>	2.247	0.08	10	0.078
<i>Xestospongia</i> , <i>Hyrtilos</i>	4.6765	0.105	10	0.004
<i>Xestospongia</i> , <i>L. dysedia</i>	10.832	0.089	10	0.001
<i>Xestospongia</i> , <i>Dictyota</i>	3.6938	0.103	10	0.007
<i>Clathria</i> , <i>Hyrtilos</i>	5.2179	0.099	10	0.002
<i>Clathria</i> , <i>L. dysedia</i>	11	0.096	10	0.001
<i>Clathria</i> , <i>Dictyota</i>	3.8141	0.097	10	0.019
<i>Hyrtilos</i> , <i>L. dysedia</i>	11.147	0.11	10	0.001
<i>Hyrtilos</i> , <i>Dictyota</i>	0.89201	0.603	10	0.458
<i>L. dysedia</i> , <i>Dictyota</i>	8.0507	0.09	10	0.002

Table 13. PERMANOVA (main test) of the effect of site and taxa type on stable isotope ratios of sources grouped into sponges, corals, algae and SPOM collected from two sites in the Wakatobi Marine National Park, Indonesia.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	12.612	12.612	5.499	0.014	999
Taxa	4	803.52	200.88	87.584	0.001	999
Site x Taxa	4	10.456	12.614	1.1397	0.337	999
Res	47	107.8	2.2936			
Total	56	934.85				
PERMDISP on factor 'Site' F: 0.89517 df1: 1 df2: 55 P(perm) = 0.387						
PERMDISP on factor 'Taxa' F: 1.0145 df1: 4 df2: 52 P(perm) = 0.481						

Table 14. Between source comparisons of stable isotope ratios of sources grouped into sponges, corals, algae and SPOM collected from two sites in the Wakatobi Marine National Park, Indonesia.

Groups	t	P(perm)	Unique perms	P(MC)
Algae, SPOM	14.973	0.005	984	0.001
Algae, Corals	5.0027	0.001	998	0.001
Algae, Detritus	12.476	0.002	997	0.001
Algae, Sponges	10.42	0.001	999	0.001
SPOM, Corals	13.396	0.001	998	0.001
SPOM, Detritus	17.603	0.001	995	0.001
SPOM, Sponges	8.7556	0.001	999	0.001
Corals, Detritus	5.9336	0.001	998	0.001
Corals, Sponges	7.7954	0.001	996	0.001
Detritus, Sponges	2.7029	0.003	999	0.003

Table 15. Spatial variation in the stable isotope ratios of sources (sponges, corals, algae and SPOM) collected from two sites in the Wakatobi Marine National Park, Indonesia.

Source	t	P(perm)	Unique perms	P(MC)
Algae	0.46	1	10	0.72
SPOM	2.31	0.128	10	0.08
Corals	0.71	0.53	961	0.51
Sponges	1.98	0.04	970	0.04
Detritus	4.20	0.01	126	>0.01

Table 16. Input parameters for dual-isotope mixing models to estimate the proportional contribution of pelagic and benthic carbon to secondary production at two sites in the Wakatobi Marine National Park, Indonesia. NB Pelagic carbon corrected for +2 TP and benthic carbon corrected for +1 TP.

Site	Pelagic carbon Mean \pm SD	Pelagic TEF Mean \pm SD	Benthic carbon Mean \pm SD	Benthic TEF Mean \pm SD
High-quality	$\delta^{13}\text{C}$ -25.13 ± 1.31 $\delta^{15}\text{N}$ 4.71 ± 0.99	0.78 ± 1.3 ($\Delta^{13}\text{C}$) 6.8 ± 0.98 ($\Delta^{15}\text{N}$)	$\delta^{13}\text{C}$ -16.15 ± 2.32 $\delta^{15}\text{N}$ 5.42 ± 1.06	0.39 ± 1.3 ($\Delta^{13}\text{C}$) 3.4 ± 0.98 ($\Delta^{15}\text{N}$)
Sponge-dominated	$\delta^{13}\text{C}$ -25.13 ± 1.31 $\delta^{15}\text{N}$ 4.71 ± 0.99	0.78 ± 1.3 ($\Delta^{13}\text{C}$) 6.8 ± 0.98 ($\Delta^{15}\text{N}$)	$\delta^{13}\text{C}$ -15.40 ± 1.53 $\delta^{15}\text{N}$ 4.92 ± 1.01	0.39 ± 1.3 ($\Delta^{13}\text{C}$) 3.4 ± 0.98 ($\Delta^{15}\text{N}$)

Table 17. *Chaetodon lunulatus* and obligate corallivores densities (fish per 250m²) recorded by stereo-video on 6 x 50m transects as part of a local monitoring program at a sponge-dominated site and higher quality reef site.

Site	<i>Chaetodon lunulatus</i>	Obligate corallivores
Sponge-dominated	0.7 ± 0.3	1.0 ± 0.4
High-quality	0.3 ± 0.3	1.2 ± 0.5

Appendix E

Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) of sponges collected at two sites and two depths in Doubtful Sound, Fiordland.

Species	Depth	Inner Fiord			Outer Fiord		
		$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N
<i>Raspailia</i>	10	9.92 ± 0.15	-23.31 ± 0.91	5			
<i>topsenti</i>	30	10.43 ± 0.16	-23.35 ± 0.53	5	9.71 ± 0.81	-22.80 ± 0.75	5
<i>Cymbastela</i>	10	8.75 ± 0.13	-22.36 ± 1.01	4	7.10 ± 0.68	-21.31 ± 1.33	5
<i>tricalyciformis</i>	30	9.18 ± 0.14	-19.65 ± 0.84	3	8.46 ± 0.67	-21.56 ± 1.24	5
<i>Axinella</i>	10	11.19 ± 0.33	-23.64 ± 0.38	5	11.19 ± 0.48	-23.03 ± 0.48	5
<i>richardsoni</i>	30	11.73 ± 0.33	-23.69 ± 0.36	5	11.01 ± 0.30	-22.84 ± 1.46	5
<i>Tethya</i> sp.	10	8.45 ± 0.80	-23.47 ± 0.40	5	9.27 ± 0.47	-24.34 ± 0.81	5
	30	9.24 ± 0.48	-22.87 ± 0.66	5	9.23 ± 0.36	-23.61 ± 1.13	5
<i>Latrunculia</i>	10	8.64 ± 1.21	-23.19 ± 1.55	3	7.72 ± 0.20	-22.70 ± 0.35	5
<i>fiordensis</i>	30				8.03 ± 0.14	-22.63 ± 0.41	5

Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) of organic matter sources collected at three sites in Doubtful Sound, Fiordland.

		Inner Fiord			Outer Fiord			Bradshaw Sound			
Organic matter sources	Depth	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N	$\delta^{15}\text{N} \pm \text{SD}$	$\delta^{13}\text{C} \pm \text{SD}$	N	
Marine pelagic	2-200	10	7.15 ± 1.39	-25.50 ± 0.88	5	7.87 ± 0.24	-24.09 ± 0.22	5			
		30	7.06 ± 1.17	-24.79 ± 0.43	3/5	6.15 ± 0.36	-24.10 ± 0.22	3/5			
	Pico plankton	10	5.47 ± 1.54	-30.30 ± 1.44	5	6.30 ± 0.55	-30.60 ± 0.77	5			
		30	5.72 ± 1.21	-32.26 ± 1.64	3/5	5.04 ± 1.44	-30.67 ± 0.67	5			
	Marine benthic	<i>Ulva</i> sp.		2.26 ± 0.22	-15.43 ± 0.96	5			3.59 ± 0.86	-17.37 ± 0.54	5
<i>Codium</i> sp.						5.24 ± 0.27	-22.05 ± 0.68	5	5.66 ± 0.36	-19.91 ± 1.39	5
<i>Ecklonia radiata</i>						7.50 ± 0.40	-19.46 ± 0.86	5	5.75 ± 0.35	-21.07 ± 0.75	5
Terrestrial detritus	TOM		-3.36 ± 0.40	-30.85 ± 1.15	3	-1.20 ± 0.52	-29.17 ± 0.97	3	-3.51 ± 0.75	-30.35 ± 0.87	4

Table 3. PERMANOVA results of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of sponges collected from two depths (10 and 30 m) at two sites (Inner Fiord and Outer Fiord) in Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	14.77	14.77	4.52	0.015	999
Depth	1	10.54	10.54	3.23	0.046	999
Si x De	1	1.19	1.19	0.36	0.66	999
Res	81	264.24	3.26			
Total	84	288.73				

PERMDISP on group factor 'Site', $F = 1.43$, $p = 0.29$

PERMDISP on group factor 'Depth', $F = 0.22$, $p = 0.66$

Table 4. Permutational ANOVA results for $\delta^{15}\text{N}$ values of sponges collected from two depths (10 and 30 m) at two sites (Inner Fiord and Outer Fiord) in Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	13.942	13.942	8.053	0.005	997
Depth	1	8.0372	8.0372	4.6425	0.039	999
Si x De	1	0.4594	0.4594	0.2653	0.62	995
Res	81	140.23	1.7312			
Total	84	160.49				

PERMDISP on group factor 'Site', $F = 0.99$, $p = 0.334$

PERMDISP on group factor 'Depth', $F = 2.25$, $p = 0.136$

Table 5. Permutational ANOVA results for $\delta^{13}\text{C}$ values of sponges collected from two depths (10 and 30 m) at two sites (Inner Fiord and Outer Fiord) in Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	0.823	0.823	0.53758	0.491	999
Depth	1	2.4985	2.4985	1.632	0.227	993
Si x De	1	0.730	0.730	0.47685	0.504	996
Res	81	124.01	1.5309			
Total	84	128.25				

PERMDISP on group factor 'Site', $F = 0.1115$, $p = 0.783$.

PERMDISP on group factor 'Depth', $F = 1.3518$, $p = 0.29$.

Table 6. PERMANOVA results of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the 2-200 fraction of the water column collected from two depths (10 and 30 m) at two sites (Inner Fiord and Outer Fiord) in Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	6.19	6.19	5.88	0.007	999
Depth	1	3.68	3.68	3.49	0.048	999
Si x De	1	3.05	3.05	2.89	0.06	999
Res	14	14.74	1.05			
Total	17	29.47				

PERMDISP on group factor 'Site', $F = 2.57$, $p = 0.11$.

PERMDISP on group factor 'Depth', $F = 1.74$, $p = 0.29$.

Table 7. PERMANOVA results of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of picoplankton collected from two depths (10 and 30 m) at two sites (Inner Fiord and Outer Fiord) in Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	1	0.106	0.106	0.01	0.96	999
Depth	1	2.69	2.69	0.93	0.39	998
Si x De	1	2.92	2.92	1.01	0.39	999
Res	12	34.60	2.88			
Total	15	40.50				

PERMDISP on group factor 'Site', $F = 3.49$, $p = 0.14$

PERMDISP on group factor 'Depth', $F = 0.01$, $p = 0.98$

Table 8. PERMANOVA pairwise comparisons of sponge species collected from 10 m at the Inner Fiord in Doubtful Sound, Fiordland.

Groups	t	P(perm)	Unique perms
<i>Raspailia topsenti</i> vs <i>Cymbastela tricalyciformis</i>	2.1426	0.048*	125
<i>Raspailia topsenti</i> vs <i>Latrunculia fiordensis</i>	1.2612	0.225	56
<i>Raspailia topsenti</i> vs <i>Axinella richardsoni</i>	2.667	0.010*	126
<i>Raspailia topsenti</i> vs <i>Tethya</i> sp.	2.4675	0.012*	126
<i>Cymbastela tricalyciformis</i> vs <i>Latrunculia fiordensis</i>	0.7259	0.601	35
<i>Cymbastela tricalyciformis</i> vs <i>Axinella richardsoni</i>	4.9537	0.012*	126
<i>Cymbastela tricalyciformis</i> vs <i>Tethya</i> sp.	1.7022	0.087	126
<i>Latrunculia fiordensis</i> vs <i>Axinella richardsoni</i>	2.9376	0.019*	56
<i>Latrunculia fiordensis</i> vs <i>Tethya</i> sp.	0.33515	0.883	56
<i>Axinella richardsoni</i> vs <i>Tethya</i> sp.	5.9475	0.016*	126

Table 9. PERMANOVA pairwise comparisons of sponge species collected from 30 m at the Inner Fiord in Doubtful Sound, Fiordland.

Groups	t	P(perm)	Unique perms
<i>Raspailia topsenti</i> vs <i>Cymbastela tricalyciformis</i>	7.50	0.019*	56
<i>Raspailia topsenti</i> vs <i>Axinella richardsoni</i>	3.81	0.01*	126
<i>Raspailia topsenti</i> vs <i>Tethya</i> sp.	2.78	0.021*	124
<i>Cymbastela tricalyciformis</i> vs <i>Axinella richardsoni</i>	10.32	0.025*	56
<i>Cymbastela tricalyciformis</i> vs <i>Tethya</i> sp.	5.32	0.019*	56
<i>Axinella richardsoni</i> vs <i>Tethya</i> sp.	6.19	0.01*	126

Table 10. PERMANOVA pairwise comparisons of sponge species collected from 10 m at the Outer Fiord site Doubtful Sound, Fiordland.

Groups	t	P(perm)	Unique perms
<i>Cymbastela tricalyciformis</i> vs <i>Latrunculia fiordensis</i>	2.20	0.041*	126
<i>Cymbastela tricalyciformis</i> vs <i>Axinella richardsoni</i>	6.04	0.008*	126
<i>Cymbastela tricalyciformis</i> vs <i>Tethya</i> sp.	4.72	0.013*	125
<i>Latrunculia fiordensis</i> vs <i>Axinella richardsoni</i>	9.82	0.004*	126
<i>Latrunculia fiordensis</i> vs <i>Tethya</i> sp.	4.94	0.013*	126
<i>Axinella richardsoni</i> vs <i>Tethya</i> sp.	4.47	0.006*	126

Table 11. PERMANOVA pairwise comparisons of sponge species collected from 30 m at the Outer Fiord in Doubtful Sound, Fiordland.

Groups	t	P(perm)	Unique perms
<i>Raspailia topsenti</i> vs <i>Cymbastela tricalyciformis</i>	2.21	0.023*	126
<i>Raspailia topsenti</i> vs <i>Latrunculia fiordensis</i>	3.19	0.008*	126
<i>Raspailia topsenti</i> vs <i>Axinella richardsoni</i>	1.56	0.110	126
<i>Raspailia topsenti</i> vs <i>Tethya</i> sp.	1.30	0.205	126
<i>Cymbastela tricalyciformis</i> vs <i>Latrunculia fiordensis</i>	1.75	0.123	125
<i>Cymbastela tricalyciformis</i> vs <i>Axinella richardsoni</i>	3.11	0.006*	126
<i>Cymbastela tricalyciformis</i> vs <i>Tethya</i> sp.	2.67	0.029*	126
<i>Latrunculia fiordensis</i> vs <i>Axinella richardsoni</i>	4.30	0.014*	126
<i>Latrunculia fiordensis</i> vs <i>Tethya</i> sp.	2.75	0.031*	126
<i>Axinella richardsoni</i> vs <i>Tethya</i> sp.	2.27	0.035*	126

Table 12. Henze-Zirkler's Multivariate Normality Test results for each group.

Species	Depth	Inner site			Outer site		
		N	HZ	P-value	N	H	P-value
<i>Raspailia topsenti</i>	10	5	0.39	0.15	0		
	30	5	0.25	0.49	5	0.27	0.42
	Both	10	0.41	0.26	5		
<i>Cymbastela tricalyciformis</i>	10	4	0.25	0.42	5	0.19	0.71
	30	3	N/A		5	0.32	0.29
	Both	7	0.29	0.47	10	0.27	0.62
<i>Axinella richardsoni</i>	10	5	0.16	0.82		0.30	0.32
	30	5	0.43	0.10		0.21	0.63
	Both	10	0.25	0.70	10	0.34	0.41
<i>Tethya</i> sp.	10	5	0.13	0.92		0.28	0.39
	30	5	0.26	0.46		0.32	0.29
	Both	10	0.32	0.47	10	0.34	0.41
<i>Latrunculia fiordensis</i>	10	3	N/A			0.25	0.49
	30	0				0.11	0.96
	Both	3	N/A		10	0.29	0.55

Table 13. Estimates of corrected standard ellipse area (SEA_C) and the Bayesian ellipse area (SEA_B) (mode and 95% credible intervals) of sponges sampled at two sites in Doubtful Sound, Fiordland.

	Depth	Inner Fiord			Outer Fiord		
		SEA _c	SEAB	n	SEA _c	SEAB	n
<i>Raspailia topsenti</i>	10	1.38	0.91 [0.37 – 2.72]	5			
	30	0.59	0.42 [0.17 – 1.26]	5	1.89	1.37 [0.56 - 4.11]	5
	Both	1.01	0.826 [0.453 - 1.71]	10			
<i>Cymbastela tricalyciformis</i>	10	2.37	1.29 [0.47 – 4.68]		3.54	2.29 [0.95 – 7.04]	5
	30	0.69	0.28 [0.07 – 1.34]		2.77	1.91 [0.80 – 5.86]	5
	Both	2.45	1.81 [0.894 - 4.53]	7	4.02	3.35 [1.81 - 6.81]	10
<i>Axinella richardsoni</i>	10	0.42	0.29 [0.12 – 0.89]	5	0.79	0.53 [0.23 – 1.66]	5
	30	0.45	0.29 [0.12 – 0.89]	5	1.71	1.13 [0.46 – 3.36]	5
	Both	0.47	0.391 [0.214 - 0.81]	10	1.43	1.17 [0.637 - 2.38]	10
<i>Tethya</i> sp.	10	1.15	0.82 [0.32 – 2.35]	5	0.67	0.69 [0.29 – 2.11]	5
	30	1.17	0.77 [0.32 – 2.35]	5	1.66	1.07 [0.44 – 3.27]	5
	Both	1.6	1.32	10	1.21	1.03	10

			[0.71 - 2.65]			[0.558 - 2.09]	
<i>Latrunculia fiordensis</i>	10	6.02	3.96	3	0.23	0.16	5
			[0.95 - 17.5]			[0.07 - 0.50]	
	30				0.25	0.16	5
						[0.06 - 0.48]	
	Both				0.28	0.228	10
						[0.126 - 0.477]	

Table 14. Pairwise tests estimating differences in isotopic niche size (SEA_C) between 10 m and 30 m at two sites in Doubtful Sound, Fiordland.

	Probability (%)	Significance
Inner Fiord site		
<i>Raspailia topsenti</i> (30 m < 10 m)	88.0	0.120
<i>Cymbastela tricalyciformis</i> (30 m < 10 m)	95.5	0.045
<i>Axinella cf. richardsoni</i> (30 m < 10 m)	50.0	0.500
<i>Tethya</i> sp. (30 m < 10 m)	50.4	0.496
Outer Fiord site		
<i>Cymbastela tricalyciformis</i> (30 m > 10 m)	50.4	0.496
<i>Axinella cf. richardsoni</i> (30 m > 10 m)	86.5	0.135
<i>Tethya</i> sp. (30 m > 10 m)	73.7	0.263
<i>Latrunculia fiordensis</i> (30 m > 10 m)	48.6	0.514

Table 15. Pairwise tests estimating differences in standard ellipse area (SEA_C) between the Inner Fiord and the Outer Fiord for four sponge species.

Groups	Probability (%)	Significance
<i>Raspailia topsenti</i> (Inner site < Outer site)	86.8	0.132
<i>Cymbastela tricalyciformis</i> (Inner site < Outer site)	84.6	0.154
<i>Axinella cf. richardsoni</i> (Inner site < Outer site)	99.0	0.010
<i>Tethya</i> sp. (Inner site < Outer site)	29.6	0.704

Table 16. PERMANOVA results for carbon sources collected from Doubtful Sound, Fiordland.

Factor	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Source	5	2643	528.6	226.06	0.0001	9941
Res	68	159.01	2.3383			
Total	73	2802				

PERMDISP on group factor 'Source', F: 0.27463 df1: 5 df2: 68, P(perm): 0.9387.

Table 17. PERMANOVA pairwise comparisons of carbon sources collected from Doubtful Sound, Fiordland.

Groups	t	P(perm)	Unique perms
<i>Codium</i> sp., <i>Ecklonia radiata</i>	1.9903	0.0435	9415
<i>Codium</i> sp., TOM	17.098	0.0001	9374
<i>Codium</i> sp., <i>Ulva</i> sp.	7.4667	0.0001	9440
<i>Codium</i> sp., Picoplankton	14.922	0.0001	9893
<i>Codium</i> sp., 2-200	7.2113	0.0001	9928
<i>Ecklonia radiata</i> , TOM	19.305	0.0001	9378
<i>Ecklonia radiata</i> , <i>Ulva</i> sp.	7.7994	0.0001	9411
<i>Ecklonia radiata</i> , Picoplankton	16.309	0.0001	9889
<i>Ecklonia radiata</i> , 2-200	8.0693	0.0001	9936
TOM, <i>Ulva</i> sp.	20.766	0.0001	9363
TOM, Picoplankton	12.805	0.0001	9910
TOM, 2-200	19.834	0.0001	9926
<i>Ulva</i> sp., Picoplankton	22.432	0.0001	9884
<i>Ulva</i> sp., 2-200	16.665	0.0001	9918
Picoplankton, 2-200	12.204	0.0001	9932

Table 18. Input parameters for dual isotope mixing models to estimate the proportional contribution of TOM, picoplankton, the 2-200 fraction of the water column and macroalgal carbon to sponge diets at two sites in Doubtful Sound, Fiordland.

Site		TOM Mean \pm SD	Picoplankton Mean \pm SD	2-200 Mean \pm SD	Macroalgae Mean \pm SD
Inner	$\delta^{13}\text{C}$	-30.15 ± 1.21	-30.72 ± 1.34	-25.35 ± 1.22	-16.4 ± 0.91
	$\delta^{15}\text{N}$	-2.77 ± 0.12	5.57 ± 1.53	7.12 ± 0.7	2.93 ± 1.26
Outer	$\delta^{13}\text{C}$	-30.15 ± 1.21	-30.68 ± 0.65	-24.10 ± 0.22	-20.62 ± 1.36
	$\delta^{15}\text{N}$	-2.77 ± 0.12	5.83 ± 1.09	7.01 ± 0.95	6.04 ± 0.94

Table 19. Percentage estimates of carbon source contributions (TOM, picoplankton 2-200 and Macroalgal OM) of four sponge species sampled at the Inner Fiord in Doubtful Sound, Fiordland.

	TOM Mean \pm SD [95% CI]	Picoplankton Mean \pm SD [95% CI]	2-200 Mean \pm SD [95% CI]	Macroalgae Mean \pm SD [95% CI]
<i>Raspailia</i>	2.9 ± 1.8	7.0 ± 4.8	70.1 ± 8.9	20.0 ± 5.3
<i>topsenti</i>	[0.6 – 7.5]	[1.1 – 18.7]	[48.7 – 84.4]	[9.5 – 30.8]
<i>Cymbastela</i>	4.6 ± 2.8	9.9 ± 7.3	47.7 ± 12.1	37.8 ± 10.2
<i>tricalyciformis</i>	[0.9 – 11.6]	[1.4 – 28.7]	[19.6 – 68.4]	[14.9 – 56.4]
<i>Axinella</i>	4.9 ± 4.3	11.5 ± 9.2	62.9 ± 19.9	20.7 ± 9.6
<i>richardsoni</i>	[0.6 – 16.4]	[1.0 – 34.5]	[18.4 – 92.7]	[0.03 – 39.6]
<i>Tethya</i> sp.	5.4 ± 2.7	13.9 ± 7.4	50.6 ± 10.4	30.1 ± 4.8
	[1.2 – 11.5]	[2.4 – 30.6]	[27.1 – 68.5]	[20.8 – 39.8]

Table 20. Percentage estimates of carbon source contributions (TOM, picoplankton 2-200 and Macroalgal OM) of four sponge species sampled at the Outer Fiord in Doubtful Sound, Fiordland.

	TOM Mean \pm SD [95% CI]	Picoplankton Mean \pm SD [95% CI]	2-200 Mean \pm SD [95% CI]	Macroalgae Mean \pm SD [95% CI]
<i>Raspailia</i>	6.0 \pm 3.5	10.5 \pm 6.7	36.1 \pm 19.1	47.5 \pm 15.8
<i>topsendi</i>	[1.1 – 14.4]	[1.6 – 26.0]	[4.2 – 76.2]	[12.9 – 74.4]
<i>Cymbastela</i>	12.4 \pm 4.3	4.9 \pm 3.1	8.4 \pm 6.0	74.4 \pm 15.8
<i>tricalyciformis</i>	[3.9 – 21.1]	[0.8 – 12.6]	[1.1 – 24.2]	[56.5 – 86.1]
<i>Axinella</i>	2.6 \pm 1.8	5.7 \pm 4.4	62.7 \pm 18.5	29.0 \pm 14.9
<i>richardsoni</i>	[0.5 – 7.5]	[0.6 – 17.8]	[17.3 – 91.7]	[4.1 – 62.6]
<i>Tethya</i> sp.	7.4 \pm 3.1	17.3 \pm 7.5	38.5 \pm 16.7	36.8 \pm 11.8
	[2.0 – 13.9]	[4.0 – 32.0]	[7.7 – 72.4]	[12.6 – 58.3]
<i>Latrunculia</i>	16.2 \pm 3.5	6.6 \pm 3.8	18.6 \pm 8.8	58.7 \pm 7.2
<i>fiordensis</i>	[9.1 – 22.9]	[1.3 – 15.4]	[3.6 – 36.7]	[44.2 – 72.3]