# Spatial variability of $\delta^{15}N$ and $\delta^{13}C$ in symbiotic corals

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**DECLARATION**

I hereby declare that the material presented in this thesis is my own original work unless otherwise stated. It has not been submitted in any form to another university or institution for any other higher degree.

I wish to acknowledge the following assistance in the research detailed in this report: Associate professor Jane Williamson, Dr. Troy Gaston, Dr. Vincent Raoult and Dr. Tim Smith for their assistance with the design of this project, sample collection, assisting with the methods, analyses and manuscript preparation.

All other research described in this report is my own original work.

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**ABSTRACT**

Primary production on coral reefs varies under changing conditions such as light and nutrient availability. Coral fragments and co-occurring macroalgae were collected along a depth gradient and at same depths around Hideaway Island, Vanuatu. The primary aim was to examine if there was a nutrient input around the island that could be detected in coral and macroalgae tissues using $\delta^{15}N$ signatures. The second aim was to examine the effect of depth over the $\delta^{15}N$ and $\delta^{13}C$ signatures of benthic primary producers to investigate their nutrient source and trophic dynamics. The $\delta^{15}N$ signatures did not show any trace of a nutrient input around the island. However, the chlorophyll *a* and *Symbiodinium* density from the coral *Montipora stellata* were the only indicators of a potential nutrient input around the island. Similarly, no effect of depth was found in the $\delta^{15}N$ of the macroalgae, epiphytes, and *Symbiodinium* from *Stylophora pistillata*. The only effect of depth was a decrease in *Symbiodinium* density and in the $\delta^{13}C$ values of macroalgae. We attribute these findings to *Symbiodinium* from *S. pistillata*, compensating for the decrease of nutrients as depth increases by decreasing the cell density but maintaining the chlorophyll *a* concentrations to satisfy the coral-host nutrient requirements.

**Key words**: bioindicators, corals, *Symbiodinium*, macroalgae, nitrogen, depth, nutrient recycling

## INTRODUCTION

Coral reefs are highly diverse and productive ecosystems that hold more than one-quarter of all known marine diversity from a range of phyla (Connell 1978). Scleractinian corals are the ecosystem engineers of tropical coral reefs due to their ability to precipitate calcium carbonate ($CaCO_3$) in their skeletons. These skeletal structures contribute to habitat complexity and, in part, control the availability of resources (Graham et al. 2007; Kennedy et al. 2013). These unique ecosystems are distributed mainly in the tropics and are surrounded by clear warm oligotrophic water (Burke et al. 2012) that is characteristically deficient in essential nutrients, such as nitrogen and phosphorus. Despite the low availability of nutrients, these ecosystems are highly productive (Odum and Odum 1955; Hatcher 1990; Gattuso et al. 1998). The high productivity of these diverse ecosystems is largely attributed to the recycling of nutrients between the reef biota (Muscatine and Porter 1977; Crossland et al. 1991). Nutrient recycling facilitates retention of these nutrients within the reef biota, reducing the loss of these nutrients back into the water column (Muscatine et al. 1994).

Coral reefs provide a wide range of ecosystem goods and services such as a source of food, income, recreational activities and coastal protection (Moberg and Folke 1999; Pratchett et al. 2014; Elliff and Kikuchi 2017). The ecological services that coral reefs provide to humans have been estimated at US$125 trillion/year (Costanza et al. 2014). Over 100 countries around the world have coasts with coral reefs and, their populations (tens of millions of people) depend directly on these ecosystems and thus rely heavily on their ecological "health" (Salvat 1992). Since the 19$^{th}$ century, coral reefs around the world have experienced declines in coral cover as a result of natural and anthropogenic disturbances (Pandolfi et al. 2003). Disturbances in coral reefs are a combination of global and local stressors that likely interact, such as climate change, changes in water quality, increased sedimentation, storms, disease, predation, overfishing and destructive fishing (Jackson et al. 2001; Hughes et al. 2003; Cooper et al. 2009). One of the principal threats to coral reefs is pollution generated by anthropogenic activities (Van Drecht et al. 2009). In the past 150 years, nutrient inputs have increased significantly in coastal areas and coral reefs (McCulloch et al. 2003). These nutrient loads are discharged into ground and surface water (Spalding et al. 2000; Fabricius 2005), adversely impacting these marine ecosystems (Richmond 1993). Nitrogen and phosphorus are the principal nutrients that cause eutrophication, and an increase in these nutrients into the clear oligotrophic environment can trigger algal blooms (Costa et al. 2000). The resulting overabundance of algae (phytoplankton, microphytobenthos, ephemeral macroalgae) leads to

turbid waters, reduces light penetration and ultimately reduces oxygen concentration (Cloern 2001; Porter 2001). These eutrophication events can adversely impact reef ecosystems by modifying trophic structures (Cloern 2001; Jupiter et al. 2008; Bell et al. 2014), altering biodiversity (Van Woesik et al. 1999), accelerating coral mortality (Kline et al. 2006) and reducing coral recruitment (Loya et al. 2004). Eutrophication is now considered a global threat to marine ecosystems and especially to coral reefs worldwide (Van Drecht et al. 2009).

**Coral-algal symbiosis**

Corals have developed the ability to thrive in nutrient-poor tropical waters through their symbiotic relationships, largely by co-evolving with unicellular dinoflagellate algae from the genus *Symbiodinium*, which live within the coral's endoderm (Muscatine et al. 1989b). While some scleractinian corals can survive without a symbiotic partner, the principal reef builder corals that dominate the reefs typically maintain this symbiotic relationship (Gattuso et al. 1993). The coral-*Symbiodinium* symbiosis has been defined as a mutualistic relationship that conveys nutritional benefits to both partners (Muscatine et al. 1994). *Symbiodinium* receives dissolved inorganic nitrogen (DIN) from the coral host and fixes inorganic carbon into organic molecules used for photosynthesis (Muscatine and Porter 1977; Heikoop et al. 1998). The photosynthetic products (glycerol, glucose, lipids, and amino acids) produced by the intracellular *Symbiodinium* are translocated to the coral host to satisfy their metabolic requirements and are used for light-enhanced calcification and growth (Muscatine et al. 1989b; Davy and Cook 2001; Muscatine et al. 2005). In addition, *Symbiodinium* use the excretory products from the coral such as ammonia, phosphate, and $CO_2$ (Trench 1979).

There has been much interest in the recycling and amount of nitrogen and carbon that is fixed and translocated by *Symbiodinium* to the coral host (Muscatine et al. 1989b; Tanaka et al. 2006; Seemann 2013; Ezzat et al. 2017). Carbon and nitrogen are essential for the photosynthesis of *Symbiodinium*, and for the translocation of organic molecules to the coral host. Previous research indicates that in shallow water and under high light conditions, photosynthesis rates are high (Reynaud et al. 2009). In contrast, under low light conditions, and as depth increases the photosynthetic production decreases (Gattuso et al. 1993; Anthony and Fabricius 2000). However, recent investigations suggest that *Symbiodinium* in some species have the ability to uncouple this relationship and increase its photosynthetic activity in low light conditions (Lesser et al. 2010; Seemann et al. 2012). Therefore, light plays a crucial role in the amount of carbon translocated from *Symbiodinium* to the coral host,

facilitating respiration and growth (Ezzat et al. 2017). This is important because the photosynthetically fixed carbon that is translocated from *Symbiodinium* to the coral host contributes to more than 70% of the daily carbon budget in shallow-water corals (Tanaka et al. 2006). Part of this carbon is deposited in the coral skeleton of scleractinian corals, which is composed of a two-phase composite of fibre-like crystals: aragonite, and calcite (McCulloch et al. 2012). Additionally, within the skeleton, the organic matrix (OM) is comprised of proteins and lipids, creating a chemical and physical environment for the deposition of the $CaCO_3$ skeleton (Muscatine et al. 2005). The OM is closely associated with the calcium carbonate structure and can reflect carbon and nitrogen sources of the symbiotic system between corals and *Symbiodinium* (Muscatine et al. 2005; Drake et al. 2013).

Furthermore, recent investigations have suggested that the symbiosis may be dynamic; depending on environmental conditions (Baker 2003) such as thermal stress (Grottoli et al. 2006; Ferrier-Pagès et al. 2010; Connolly et al. 2012), depth and light (Palardy et al. 2005; Palardy et al. 2008) and, nutrient availability (Tanaka et al. 2014b; Tanaka et al. 2017). It has also been suggested that the coral-algae symbiosis can even shift to a parasitic relationship if the conditions turn unfavourable (Wooldridge 2010; Lesser et al. 2013). In this context, scleractinian corals are sensitive to environmental changes, particularly to variations of nitrogen availability and changes in water quality (Lapointe 1997; Lesser 2013). Such environmental changes have affected their metabolism (Fabricius 2005), coral skeletal density and their growth patterns (Carricart-Ganivet et al. 2000), which are then reflected in changes to their soft tissue and skeletal structure (Wheeler and Björnsäter 1992). Such changes offer an opportunity to use corals as bioindicators to assess changes in water quality, trace nutrient inputs and nutrient recycling within the symbiotic system through the use of stable isotopes (Risk et al. 2009).

**Stable Isotopes**

The relative abundance of carbon and nitrogen stable isotopes can be used as a tool to investigate the sources of these elements in corals. Nitrogen, a naturally occurring nutrient available in all ecosystems, is essential for the functioning of all biological systems. In the ocean, biologically available nitrogen can be supplied to corals from nutrient-rich waters via biological fixation, atmospheric deposition and upwelling events (Ren et al. 2017). Carbon is present in the atmosphere and in the surface of the ocean as $CO_2$, which is used by photosynthetic organisms for respiration (Fry and Sherr 1989; Fry 2006). Nitrogen and

carbon each have two stable isotopes based on weight. $^{14}N$ and $^{12}C$ are considered the light isotopes, and $^{15}N$ and $^{13}C$ contain an extra neutron each, making them slightly heavier (Fry 2006).

The proportion of light to heavy isotopes in an organism indicates its isotopic composition (Fry 2006). Biological processes that rely on nitrogen and carbon assimilate the light isotopes ($^{12}C$ and $^{14}N$) from the heavy isotopes ($^{13}C$ and $^{14}N$). In the case of nitrogen, assimilation of $^{14}N$ over $^{15}N$ alters the proportion of each isotope compared to atmospheric $N_2$, resulting in an enrichment of the heavy isotope over the light isotope between each trophic level (Owens 1987). On the other hand, $^{12}C$ is excreted during respiration; usually, carbon stable isotope signatures of an animal are very similar to that in its diet (Wada 2009). The uses of carbon and nitrogen isotopic signatures are broad and varied. Carbon stable isotopes can be used as a tool to discriminate carbon fixation from an autotrophic or heterotrophic source, or various levels of heterotrophic sources (Fry and Sherr 1989; Seemann 2013), while nitrogen isotopes can discriminate from natural and anthropogenic sources (Owens 1987; Risk et al. 2009). Stable isotopes are measured using isotope ratio mass spectrometry (IRMS), and the values obtained are calculated in parts per mill (‰) with the following equation:

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

Recently $\delta^{15}N$ signatures have been used to trace nitrogen inputs, identify food sources and investigate nitrogen fluxes between trophic levels (Zanden Vander and Rasmussen 2001; McCutchan et al. 2003; Gaston and Suthers 2004; Michener and Kaufman 2007). Atmospheric nitrogen has a baseline $\delta^{15}N$ value of 0‰ therefore, nutrient sources can be tracked using $\delta^{15}N$ values because anthropogenic nutrients are usually depleted or enriched in $\delta^{15}N$, relative to naturally-occurring levels (Ward-Paige et al. 2005). In marine food webs, the stepwise enrichment of $^{15}N$ from each trophic level follows the same trend (Zanden Vander and Rasmussen 2001; Wada 2009). However, this enrichment rule is not always applicable to corals due to the dynamic symbiotic relationship they hold with *Symbiodinium* and the recycling of nutrients within this system (Muscatine et al. 1989b).

**Corals and macroalgae as bioindicators**

As with corals, nitrogen is a major growth-limiting nutrient for macroalgae (Lapointe 1997). Macroalgal tissue can provide accurate records of nitrogen assimilation (Umezawa et al. 2002) and can also be used to provide temporal information of biologically available nitrogen

in the environment (Costanzo et al. 2001). While similar in their use as bioindicators, corals and macroalgae differ in the way that they assimilate nitrogen. Macroalgae readily assimilate nutrients directly from the water column (Koop et al. 2001; Umezawa et al. 2002) or from heterotrophs (Williamson and Rees 1994), whilst uptake in hermatypic corals depends on nutrient recycling with their symbiotic algae and direct nutrient availability in the water column (Grottoli et al. 2006; Tanaka et al. 2010). Studies have used benthic macroalgae to identify nitrogen sources and nutrient inputs into marine ecosystems using stable isotopes (Barile and Lapointe 2005; Costanzo et al. 2005; Derse et al. 2007; Piñón-Gimate et al. 2017). Studies show that macroalgae reflect similar $\delta^{15}N$ values between different species and show decreasing trends in $\delta^{15}N$ values as distance increase from the main land-derived nitrogen source. This suggests that related macroalgae species inhabiting the same nutrient environment would reflect similar $\delta^{15}N$ values (Umezawa et al. 2002). Macroalgae are thus reliable proxies to investigate the nutrient availability in the water column and hence compare the isotopic signatures with other benthic organisms, such as symbiotic corals.

In addition to nutrient input, the $\delta^{15}N$ values of coral tissue and coral skeleton can be affected by environmental parameters such as light (Heikoop et al. 1998), terrestrial loads (Sammarco et al. 1999), eutrophication (Heikoop et al. 2000a; Hoegh-Guldberg et al. 2004), thermal stress bleaching events (Rodrigues and Grottoli 2006; Leggat et al. 2011), sewage-derived nitrogen (Baker et al. 2010), groundwater seepage (Erler et al. 2015) and upwelling events (Wang et al. 2016). Water depth, and associated changes in light, can also be an important factor when tracking $\delta^{15}N$ sources in corals and macroalgae (Risk et al. 2009). Few studies have investigated the relation of $\delta^{15}N$ values with depth on symbiotic corals. The first studies investigating the effect of depth and nutrient availability on the $\delta^{15}N$ signature of coral tissue and *Symbiodinium* found slightly higher $\delta^{15}N$ values in animal tissue than their corresponding zooxanthellae for the majority of the coral species tested (Muscatine et al. 1989b; Muscatine et al. 1994). However, they did not correlate this difference with depth, but rather with the hypothesis of $\delta^{15}N$ enrichment by an average of $2.6 \pm 2.1‰$ for marine invertebrates relative to its food source (Owens 1987). Their results also showed a tendency for $^{13}C$ and $^{15}N$ depletion as water depth increased, and these observations were consistent for both coral tissue and *Symbiodinium* in most of the coral species studied. Together, this suggests that corals from deeper waters are more heterotrophic than corals from shallow waters, and therefore have lower rates of photosynthesis. Conversely, other studies have found no correlation with $\delta^{15}N$ values and depth, but a decrease of $\delta^{13}C$ values in the host tissue and

symbionts with depth (Alamaru et al. 2009) suggesting that this relationship is not straightforward and need further research to untangle these issues. There has also been interest in investigating how depth affects the $\delta^{13}C$ values of different species of macroalgae. Some studies found more depleted $\delta^{13}C$ values at greater depths (Raven et al. 2002a; Marconi et al. 2011) suggesting that the observed depletion might be due to a limitation of photosynthesis by reduced light availability. However, this $\delta^{13}C$ depletion trend with increasing depth was not observed in other macroalgal species (Titlyanov et al. 2010; Marconi et al. 2011).

So far, it has been mentioned the uses and applicability of $\delta^{15}N$ signatures of coral tissue, its symbiotic algae and macroalgae. In addition to the latter, $\delta^{15}N$ signatures in the coral skeletal organic matrix provide another proxy to investigate the trophic dynamics in corals and its relation with the $\delta^{15}N$ signatures of the host tissue and symbiotic algae. Studies found a stepwise $\delta^{15}N$ enrichment in the coral tissue and organic matrix of non-symbiotic corals. Conversely, no $\delta^{15}N$ enrichment was found in symbiotic corals, attributing this to the effect of photosymbiosis and the contribution to the synthesis of the skeletal organic matrix from symbiotic algae (Muscatine et al. 2005).

Controlled experiments are important to prove and to further understand field observations. Studies have analysed the $\delta^{15}N$ signatures of coral tissue and *Symbiodinium* under controlled conditions, such as the effects of feeding and light (Reynaud et al. 2009), recycling and conservation of nutrients (Tanaka et al. 2006), the effect of high seawater temperature and nutrient enrichment (Tanaka et al. 2014b). Nevertheless, in situ observations are the first step of investigating the impact of environmental changes and anthropogenic disturbances. Johannes (1975) first described the negative ecological impacts of sewage pollution discharges in Kaneohe Bay, Hawaii, that caused 99% of coral mortality from anoxia and hydrogen sulphide toxicity. Other studies have shown that the influence of different nitrogen sources, such as fertilizer loads and sewage inputs, can increase or decrease $\delta^{15}N$ levels in corals (Heikoop et al. 2000a; Baker et al. 2010; Yamazaki et al. 2011; Baker et al. 2013; Wong et al. 2017). Anthropogenic nutrient inputs derived from wastewater including animal wastes, septic systems, and treated sewage can be traced because they are usually rich in $\delta^{15}N$ (Heikoop et al. 2000b). Studies that have used corals as bioindicators, have found higher $\delta^{15}N$ values that were directly correlated to anthropogenic nutrient inputs that led to eutrophication and consequently reef degradation (Marion et al. 2006; Baker et al. 2010;

Wong et al. 2017). Therefore, $\delta^{15}N$ values have demonstrated to be a powerful tool due to its ability to trace anthropogenic nutrient inputs (Heikoop et al. 2000b).

Coral reefs in countries that are currently being impacted by a combination of natural and anthropogenic disturbances offer an opportunity to study the impact of these disturbances at different temporal and spatial scales on the corals their corresponding symbiotic algae and surrounding macroalgae. The Pacific Island of Vanuatu is considered to be biodiverse marine habitat, but very little is known of the status of its coral reefs (Naviti and Aston 2000; Sulu 2007). The suspected input of anthropogenic-derived nutrients on many of its reefs makes Vanuatu an interesting location for assessing nutrient input on coral reef organisms. Here we examine spatial changes in nutrient recycling in two scleractinian corals, their symbiotic algae and associated macroalgae on a shallow coral lagoonal reef in Vanuatu using stable isotopes. We asked the following questions 1) Is it possible to detect nutrient inputs using coral, macroalgae and epiphytic algal tissues using stable isotopes? 2) Does depth influence any nutrient uptake detected in corals, macroalgae, and epiphytes? 3) Can the isotopic signatures of corals, macroalgae, and epiphytes be used to establish a baseline for $\delta^{15}N$ and $\delta^{13}C$ values of primary producers in this area?
Data obtained from this project will be useful for further studies, not only for coral reefs but also for other studies assessing trophic dynamics in similar habitats and locations.

## MATERIALS AND METHODS

### Study site

Sampled occurred in December 2017 on shallow fringing reef surrounding Hideaway Island (17° 41' 49.2324'' S, 168° 15' 49.0788'' E) located in Mele Bay , Efate, Vanuatu (Figure 1). As part of the coral triangle, this area is considered a hotspot for marine biodiversity (Burke et al. 2012). Vanuatu is an archipelago of volcanic origin that extends 1300 kilometres from the most northern to southern island, located in the Western Pacific (Preston 1996). Coral reefs in Vanuatu include exposed outer reefs, sheltered flats and lagoons, partially sheltered open embayments and sheltered embayments (Done and Navin 1990). The coral reef slopes typically harbour branching corals such as *Acropora, Montipora* and *Pocillopora* species, then dominance switches to a mix of massive and branching corals at 3-5 m depth. Coralline macroalgae also occur on the reef slopes and crests. Sheltered regions of the reef slope typically contain *Stylophora* spp. Open embayments are characterised by massive *Porites* and soft corals dominate the sheltered embayment (Done and Navin 1990).

Hideaway Island's fringing reef is in Mele Bay, 10 km north-east from the capital of Vanuatu, Port Vila. Mele Bay is among the most adversely impacted sites of Port Vila (Mosley and Aalbersberg 2003) as it receives severely polluted discharges from Tagabe River (Poustie and Deletic 2014). Waste management in Port Vila is limited and relies on septic systems that consistently floods, leading to water pollution with increased nutrient loads (Depledge 1994). In addition to the anthropogenic disturbances, Vanuatu's coral reefs, including those at Hideaway, are threatened by a range of episodic biotic and abiotic perturbations. In 2002 a bleaching event caused mass mortality in corals at Malapoa, Pango Point and Moso Island (Sulu 2007). The large coral-eating sea-star *Acanthaster planci*, commonly known as crown-of-thorns starfish, has also reached portions of reefs in Vanuatu, resulting in a 20-30% of live coral loss in the President Coolidge Reserve, Aore Island and Raddard area. Controlling this species has been a major focus of the country's fisheries department (Sulu 2007). The impact of *A. planci* on Hideaway Island, however, is unknown. In 2015 cyclone Pam caused severe damage in 80% of hard corals off Malapoa, Hideaway, Devils Point, and Efate.

**Sample collection**

The spatial variability of $\delta^{15}N$ signatures of benthic organisms such as corals and associated primary producers at Hideaway Island was examined. Branching *Montipora stellata* was chosen as the bioindicator species because it was consistently abundant at each site. Coral fragments of *M. stellata*, and co-occurring foliose macroalgae were assessed at ten sites in the shallow reef fringing the island (Figure 1). The purpose of collecting macroalgae and corals at different sites was to be able to detect inputs from all directions around the island. The ten sites were haphazardly chosen around the entire island and then categorized into zones according to their cardinal locations in relation to the island (western, eastern and southern) (Table 1). There were no sites directly north of the island because the fringing reef was absent in a northerly direction. We hypothesize that corals and macroalgae that are close to a nutrient input around Hideaway Island will have higher $\delta^{15}N$ values than the corals and macroalgae that are further away from the potential nutrient input.

Three replicates of small fragments (~ 5 cm tip of the branch) of live *M. stellata* were removed at 1-2 m depth from healthy colonies on snorkel at each of the 10 sites. The first macroalga closest to the sampled coral was also removed. As epiphytes were found attached to many of the macroalgae, these were also collected when visible. Samples were immediately placed in individual resealable bags and frozen at -20°C until processing at Macquarie University in Sydney, Australia.

The second sample collection consisted of collecting coral fragments (~ 5cm tip of the branch) and co-occurring macroalgae over a depth gradient that ranged from three to 26 m by SCUBA diving on the western zone of Hideaway Island. As few or no *M. stellata* were found under 3 m depth on the reef slopes, *Stylophora pistillata* which occurred across the depth gradient of the reef slope, was sampled. After collection, all coral and macroalgal samples were placed in individual resealable bags and frozen at -20°C until processing at Macquarie University. We hypothesize that as depth increases less nutrients will be available and therefore, the $\delta^{15}N$ of macroalgae will decrease while the $\delta^{15}N$ of corals will not change. This because S*ymbiodinium* compensates for the decrease in nutrients by increasing in cell density and chlorophyll *a* concentrations.

**Table. 1** Collection sites categorised by zones in relation to Hideaway Island: western, eastern and southern around Hideaway Island.

| Zone | Site | Longitude | Latitude |
|---|---|---|---|
| **Western** | 1 | 168.2628 | -17.6960 |
| **Western** | 2 | 168.2623 | -17.6962 |
| **Western** | 3 | 168.2629 | -17.6963 |
| **Western** | 4 | 168.2643 | -17.6964 |
| **Eastern** | 5 | 168.2621 | -17.6966 |
| **Eastern** | 6 | 168.2645 | -17.6970 |
| **Eastern** | 7 | 168.2641 | -17.6978 |
| **Southern** | 8 | 168.2623 | -17.6984 |
| **Southern** | 9 | 168.2627 | -17.6991 |
| **Southern** | 10 | 168.2635 | -17.6992 |

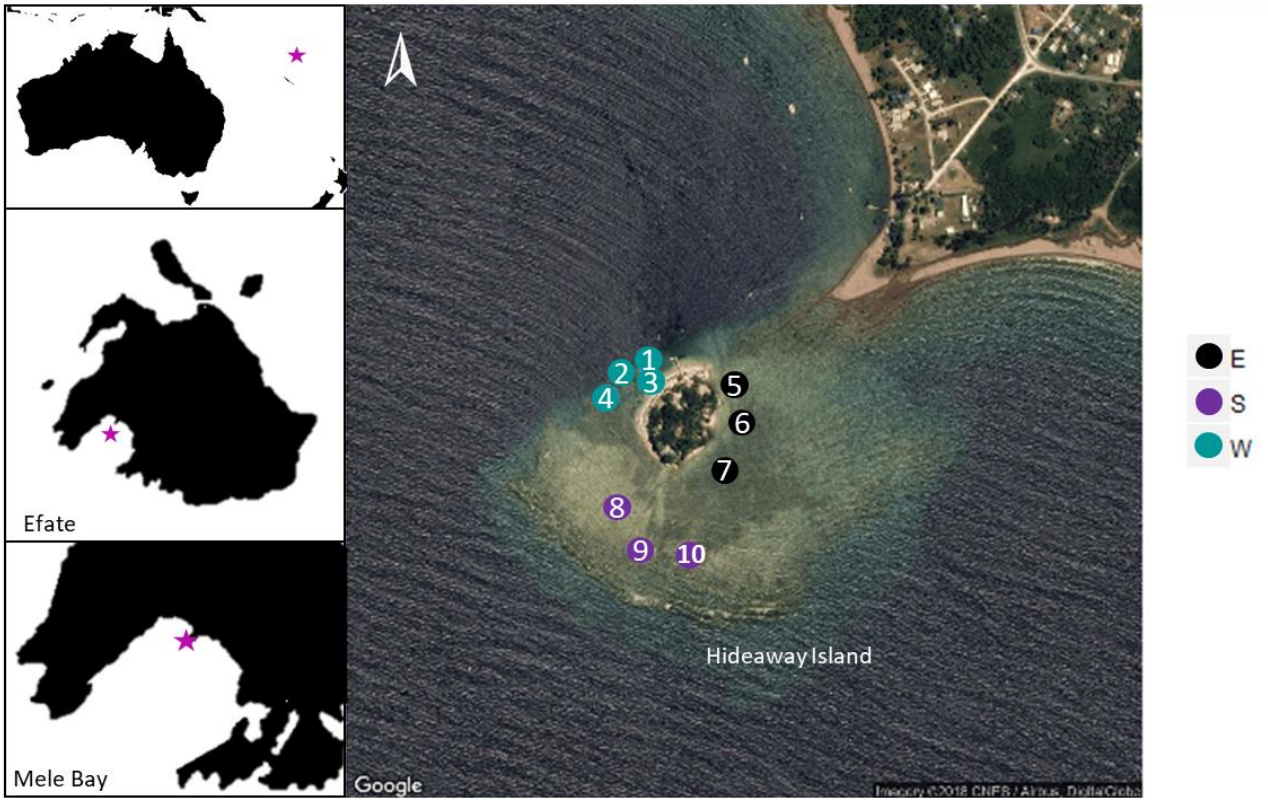


**Figure 1.** Location of Hideaway Island (17° 41' 49.2324'' S, 168° 15' 49.0788'' E), Vanuatu. Colours refer to the collection zones E (eastern), S (southern), W (western) around the island and numbers refer to the collection sites within each zone. Image courtesy of Google earth.

**Sample processing**

Coral tissue and *Symbiodinium*

Coral (all species) and symbiont tissue were removed from the calcium carbonate skeleton for each fragment using a Waterpik Ultra Water Flosser filled with RO (reverse osmosis) water. The resulting slurry for each replicate sample was placed in a 50 ml centrifuge tube and homogenised using a knife mill (Retsch GM200) set to speed 2 for 5 min. Three aliquots of 1 ml were separated from the resulting homogenized slurry to assess the *Symbiodinium* densities. Additionally, three aliquots of 1.5 ml were also separated from each slurry to perform measurements of chlorophyll *a* concentrations.

The remaining slurry samples containing host tissue and *Symbiodinium* were centrifuged (Eppendorf 5810 R) at 1700 RCF for 5 min to separate the host tissue from *Symbiodinium*. The supernatant containing the coral's host tissue was transferred to another 50 ml tube and to ensure all coral host tissue was removed, then the pellet containing *Symbiodinium* was resuspended with 5 ml RO water followed by centrifugation at 600 RCF for 5 min (Wong et al. 2017). The first and second supernatants containing the coral's host tissue were combined and filtered onto qualitative filter paper, Grade 1 (0.7 μm pore size, GF/F; Whatman) to collect the coral host tissue. The filters containing the host tissue and the *Symbiodinium* were oven dried at 60°C for 48 h. The dried filters were placed into individual (plastic 5 ml) vials for isotopic analysis. The *Symbiodinium* were ground to a fine powder using a mortar and pestle, then 1-2 mg was weighed into tin capsules for isotopic analysis.

*Symbiodinium* cell densities

To determine the density of *Symbiodinium* cells within the coral tissues, eight replicate counts were conducted from each 1ml aliquot collected above. 10 μl aliquots from the coral host tissue-*Symbiodinium* slurry were added to each chamber of a 0.1-mm-deep Improved Neubauer Haemocytometer and cells were counted immediately under a light microscope at 40x (Olympus BX51, Japan).

Chlorophyll *a* concentrations

The 1.5 ml aliquots of the homogenized coral host tissue-*Symbiodinium* slurry were centrifuged (Eppendorf 5810 R) at 600 RCF for 5 min in a 3 ml tube. The supernatant was discarded and the pellet containing the *Symbiodinium* cells used to extract the chlorophyll *a*. One ml of cold 100% acetone was added at each *Symbiodinium* sample. Samples were incubated for 24 h at 4°C (Grottoli et al. 2004). The absorbance of the *Symbiodinium* extract

was measured at 630, 660, and 750 nm using a spectrophotometer (SPECTROstar Nano, BMG Labtech Plate Reader). The following equation by Jeffrey and Humphrey (1975) for dinoflagellates was used estimate the chlorophyll *a* concentrations.

$$\text{Chlorophyll } a = 11.43\ E_{663} - 0.64\ E_{630}$$

**Surface area determination: Aluminium foil vs Photogrammetry**

Assessing the surface area of branching corals is difficult, often imprecise and, estimates vary depending on the technique used (Naumann et al. 2009). Two comparative methods were therefore used to determine the surface area of each coral fragment: the aluminium foil method and photogrammetry. The most common and simple method used when analysing nitrogen stable isotopes of coral fragments is the aluminium foil method developed by Marsh (1970). Photogrammetry is an image processing technique that allows the construction of 3 dimensional models from overlapping images surrounding the entire object (Raoult et al. 2017). Once a 3D coral has been produced, software can then reliably measure surface area. The aim was to assess the two methods and use the method that gave the most precise estimate of surface area for subsequent calculations of cell density and chlorophyll *a* concentration per area in this study.

Aluminium foil method (Marsh 1970)

The coral skeleton was wrapped with aluminium foil (Glad foil extra heavy duty) and moulded to fit the depressions and shape of the coral skeleton. All overlapping edges and V-shaped notches were cropped to ensure a good fit. Ten pieces of known surface area of the same aluminium foil were weighed to three decimal points using a balance to create a conversion factor from weight to surface area. The aluminium foil used for each coral fragment was then weighed and the surface area was converted using this factor.

Photogrammetry

Coral fragments were individually placed on a rotating table next to a 3 x 3 cm square scale. Overlapping photographs were taken while rotating the sample in a clockwise direction from above using a digital camera (Nikon D3100, 18-55mm lens, Tokyo, Japan) set on a tripod. The number of images taken varied from 80-150 depending on the size and complexity of the coral fragment. Digital models were constructed using a desktop computer with 32 GB of RAM under Windows 10 (version 1803). Images of each coral sample were compiled using Agisoft

Photoscan Professional (Version 1.2.5; Agisoft LLC, St Petersburg, Russia), which combines image correction, image alignment, mesh creation, texture rending, and auto calculates measurements of volume and surface area. Images were aligned using the high to medium accuracy setting, with a key point limit of 40000, and a tie point limit of 1000. Previous research on similar coral morphology show no difference in accuracy between the high and medium settings (Raoult et al. 2017). The software then determined the positions of the camera generating points into a three-dimensional space (Figure 2). This was followed by generating a dense point cloud using again the medium to high quality and an aggressive depth filtering. Once the dense point cloud was generated, the mesh was built from the overlapping images with the following settings; arbitrary surface type, dense cloud source data, high face count, and enabled (default) interpolation. Once the mesh was building, two markers were placed on the limits of the 3 x 3 square scale to create a scale bar. The model of the coral fragment was then manually trimmed from the rest of the mesh under high resolution, and any holes in the mesh were closed using the mesh tool. Cell densities and chlorophyll a concentrations were standardised to the volume and surface area of each coral fragment.

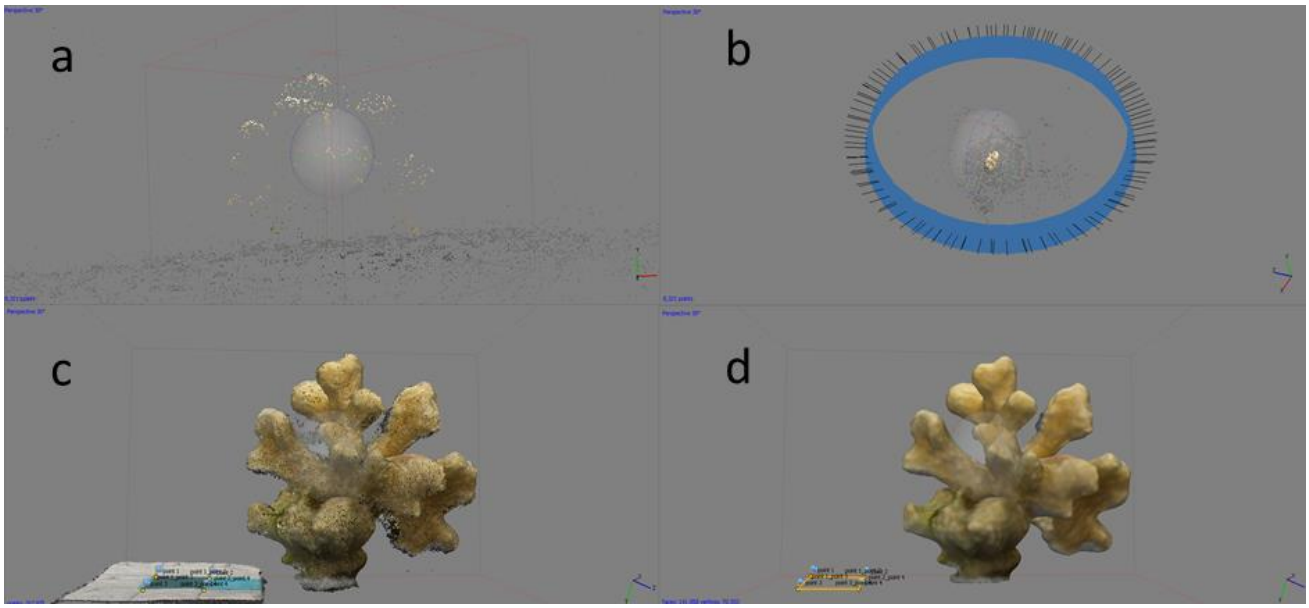


**Figure 2.** Photoscan workflow; a. aligned points from the coral images into a 3 dimensional space; b. alignment of pictures (in blue) and the creation of the dense point cloud; c. creation of the high polygon mesh of the coral fragment and the scale from the dense point cloud; d. cropped and completed 3D model of the coral fragment, including the markers on the scale.

**Extraction of the coral skeleton-bound organic matrix**

After removing the host tissue and *Symbiodinium* from the calcium carbonate skeleton, the skeletons were dried for 24 hours at 60°C and then ground into a fine powder using a mill (Fritsch pulverisette 2). To extract the coral skeleton-bound organic matrix, an adaptation of the method used by Muscatine et al. (2005) and the protocol developed by Wang et al. (2015) was followed as described below. For each sample, 5-10 mg of the coral skeleton was oxidatively cleaned to remove any possible contaminant of organic matter (coral tissue, *Symbiodinium*). Five to 10 mg of coral powder from each sample was placed in 15 ml centrifuge tubes. Ten ml of sodium hypochlorite (NaClO) (reagent grade, 10–15%) was then added to each sample and left for 24 hours. During this cleaning period, the tube was horizontally oriented and in constant vibration using a shaker (Infors Ht Multitron Pro). The NaClO reagent was then decanted and the sample rinsed three times with RO water then oven-dried at 60°C for 48 h. The dried sample was weighed and transferred into a 4 ml glass vial and decalcified using 4N HCl to release the aggregate organic matrix (i.e. soluble and insoluble). To oxidise to nitrate, 1 ml of freshly combined persulfate oxidizing reagent (1 g recrystallized low-N potassium persulfate and 2 g ACS grade NaOH in 100 mL deionized water) was added, and the sample was autoclaved (Astell autoclave) for 1.5 h. Once oxidized, 1 ml of RO water was added to the sample followed by centrifugation at 300 RCF for 10 min. The supernatant was transferred to a 4 ml glass vial and the pH tested using a probe (senssION +PH3) and adjusted to neutral using 4N HCl and 2N NaOH (Wang et al. 2015). When neutralised the samples were frozen at -80°C and lyophilised in a Christ freeze-dryer. Once lyophilised, all samples were powdered using a pestle and mortar and 8-10 mg was weighed into 8 mm x 5 mm pressed tin capsules (Oealabs) for isotopic analysis.

**Macroalgae and epiphytes**

Macroalgae were cleaned and separated from epiphytes following an adaptation of the mechanic technique described by Zimba and Hopson (1997). Here, each macroalga was placed in a (200 ml) screw-top jar with the macroalga completely covered with RO water or up to 50 ml of RO water. The jar was closed and manually shaken for 40 sec to remove the epiphytes from the macroalga. The macroalga was then removed from the jar and the epiphyte slurry. The surface of the macroalga was visually assessed and any remaining epiphytes were carefully removed using a scalpel and tweezers. After removal of epiphytes, the macroalga was rinsed with RO water and oven dried for 48 h at 60°C. Each epiphyte slurry was mixed

and filtered through a 100 μm mesh then oven-dried for 48 h at 60°C. All samples were then ground to a fine powder using a mortar and pestle. Of each sample 6-8 mg of was placed into a separate tin capsule for the nitrogen stable isotope analysis.

**Isotopic analysis**

All samples were analysed for nitrogen ($^{15}N$:$^{14}N$) stable isotopes using a Europa EA GSL elemental analyser coupled to a Hydra 2022 mass spectrometer (Sercon Ltd., UK) at Griffith University in Queensland, Australia. Precision is known to be within 0.10% for $\delta^{15}N$ ratios (Raoult et al. 2015). Ratios of $^{15}N$:$^{14}N$ ($\delta^{15}N$) were expressed as the relative difference between the sample and a standard of atmospheric nitrogen, in parts per thousand (‰).

**Statistical analysis**

All statistical analyses were performed using RStudio (Version 1.1.453, 2009-2018). To test the variability between Aluminium foil method and photogrammetry, and test whether these two methods were significantly different from each other, linear fixed effects models and ANOVAS were used. The area and method were fixed effects and the intercepts for sites (1-10), and coral replicates (A, B, C) were random effects.

A paired t-test was used to test for differences between the cell densities and the chlorophyll *a* concentrations of *Symbiodinium*. The cell densities and the chlorophyll *a* concentrations data were statistically analysed using Pearson's correlation, linear regression models and ANOVAS. A nested ANOVA with mixed effects models was used and a post-hoc Tukey multiple comparisons of means subsequently performed to test if the chlorophyll *a* concentrations and the cell densities of *Symbiodinium* were different between the three zones (eastern, western, and southern). Additionally, a one-way ANOVA followed by a Tukey HSD was run to test for significant differences in chlorophyll *a* concentrations and cell densities of *Symbiodinium* from the corals collected around the island regardless of the zone.

To test for differences in the $\delta^{15}N$ and $\delta^{13}C$ between macroalgae and epiphytes a paired t-test was used. The stable isotope data were analysed using Pearson's correlation, linear regression models and ANOVAS. Mean values are presented ± 1 SE unless otherwise specified.

## RESULTS

### Surface area determination: Aluminium foil vs Photogrammetry

There was no significant difference between the measurements of coral surface area calculated from aluminium foil or photogrammetry ($F_{8,47} = 9.44$, $p = 0.07$; Figure 3). Surface data derived from photogrammetry were subsequently used in this research because this method had a lower coefficient of variation than the aluminium foil method (Table 2). Therefore, the calculated area of each coral fragment determined by photogrammetry was used to standardize the chlorophyll *a* concentrations and the cell densities to surface area in $cm^2$ of all the coral fragments collected.

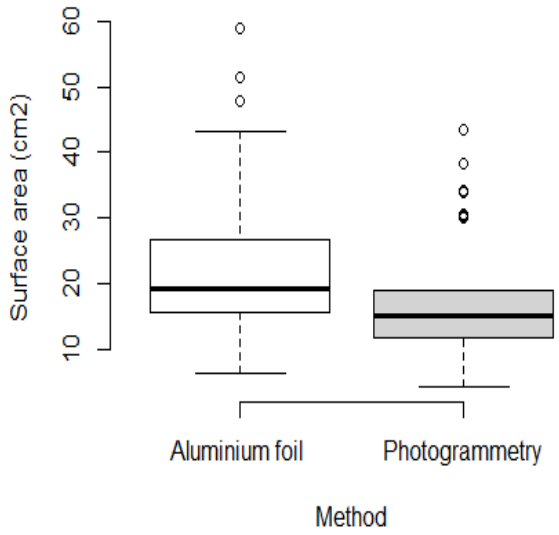


**Figure 3.** Measurements of the surface area calculated for the coral fragments using each method. Boxplots with any outliers shown as open circles.

**Table 2.** Summary table of the coefficient of variation, and standard deviation of the two methods used to calculate the surface area of the coral fragments (aluminium foil and photogrammetry).

| Method | Mean surface area ($cm^2$) ±S.E. | Standard deviation | Coefficient of variation (%) |
|---|---|---|---|
| **Aluminium foil** | 23.5 ± 2.5 | 14.0 | 59.6 |
| **Photogrammetry** | 18.0 ± 1.8 | 9.9 | 55.5 |

## Identifying a potential external nutrient input around Hideaway Island

### Spatial variability of the chlorophyll *a* and cell density of *Symbiodinium* of *Montipora stellata*

Cell densities of *Symbiodinium* were significantly different among the three zones (Table 4). Similar to patterns of chl *a* concentrations in *Symbiodinium*, the southern zone had significantly higher cell density than the western and eastern zones (Table 5). Cell densities in *Symbiodinium* in eastern and western zones did not differ significantly (Table 5), however, density of *Symbiodinium* from the three southern sites were significantly greater than densities from the four sites within the western zone (one-way ANOVA $F_{9,80} = 2.62$, $p < 0.01$; Figure 4a).

Corals in the southern zone had the highest chlorophyll *a* (chl *a*) concentration with a mean of 16.27 ± 1.67 µg/cm$^2$, while the western zone had the lowest chl *a* concentration (10.22 ± 0.92 µg/cm$^2$; Table 3; Figure 4b). *Symbiodinium* chl *a* concentrations among the three zones (southern, eastern, and western) were significantly different (Table 4). *Symbiodinium* in the western zone had significantly lower chl *a* concentrations than the southern zone (Table 3). The mean chl *a* of *Symbiodinium* from the eastern zone was not significantly different from the southern or western zones (Table 5). When analysing sites independently of zones, *Symbiodinium* in the three sites within the southern zone had significantly higher chl *a* concentrations than the four sites within the western zone (one-way ANOVA $F_{9,50} = 3.27$, $p < 0.01$; Figure 4b).

**Table 3.** Means ± S.E. of chlorophyll *a* and cell density of *Symbiodinium* of *Montipora stellata*, from the three zones around Hideaway Island.

| Zones | Chlorophyll *a* (µg/cm$^2$) | Cell density (x10$^5$ cells/cm$^2$) |
|---|---|---|
| **Eastern** | 11.88 ± 0.58 | 11.46 ± 0.94 |
| **Western** | 10.22 ± 0.92 | 10.70 ± 0.91 |
| **Southern** | 16.27 ± 1.67 | 17.150 ± 1.19 |

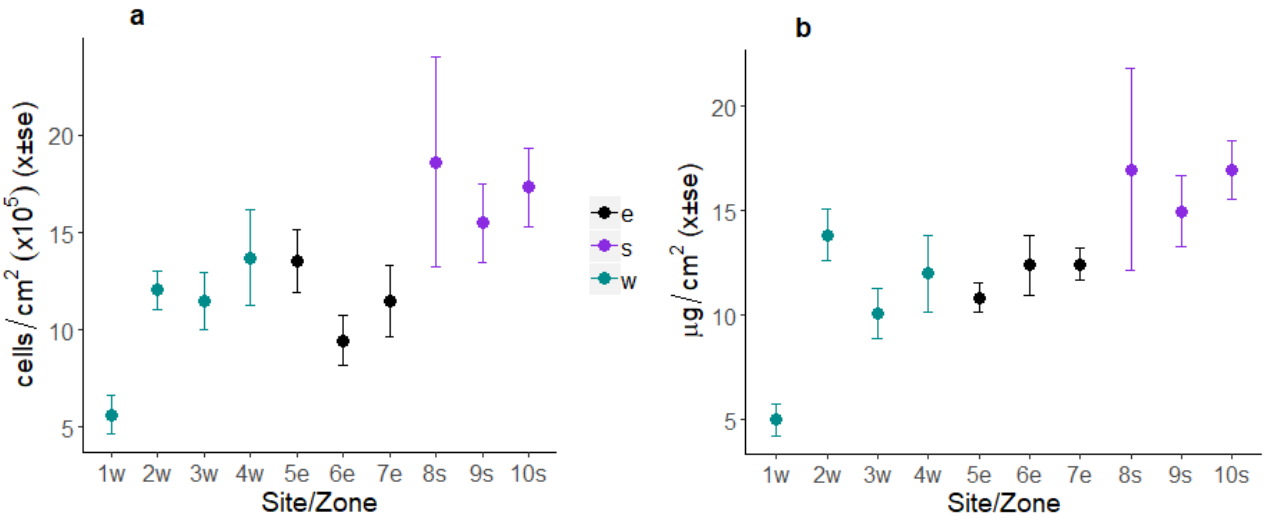


**Figure 4.** Mean ± S.E of **a.** cell density (cells cm $^{2}$ x10$^{5}$); **b.** chlorophyll *a* concentrations (µ cm $^{2}$) of *Symbiodinium* extracted from *Montipora stellata* at 10 sites around Hideaway Island. Letters (w, e, and s) correspond to the western, eastern and southern zones in relation to the island, respectively**.**

**Table 4.** Nested ANOVA with mixed effects models results describing the differences of chlorophyll *a* concentrations and cell densities of *Symbiodinium* from *Montipora stellata* among the three zones (western, eastern and southern). Values with * are significantly different at 0.05.

| Parameter | Measurement | df | F | p-value |
|---|---|---|---|---|
| **Zones** | Chlorophyll *a* | 2,7 | 4.72 | 0.05* |
| **Zones** | Cell density | 2,7 | 5.53 | 0.03* |

**Table 5.** Tukey post hoc test results describing the differences of chlorophyll *a* concentrations and cell densities of *Symbiodinium* in *Montipora stellata* among zones. Values with * are significantly different at 0.05.

| Zones | Chlorophyll *a* | Cell density |
|---|---|---|
| **Eastern-Southern** | p = 0.09 | p = 0.02* |
| **Eastern-Western** | p = 0.68 | p = 0.92 |
| **Southern-Western** | p < 0.01** | p < 0.01** |

**Spatial variability of $\delta^{15}N$ values around Hideaway Island**

There was no significant difference in $\delta^{15}N$ means of macroalgae between eastern, western and southern zones (Table 6,7). Significant differences in macroalgal $\delta^{15}N$ arose, however, when the 10 sites around the island were analysed regardless of zones (one-way ANOVA $F_{9,20} = 3.36$, $p = 0.01$). The $\delta^{15}N$ values of macroalgae from site 5 within the eastern zone was significantly higher than those at sites 2 ($p = 0.04$) and 4 ($p < 0.01$) within the western zone (Figure 5).

Overall, the $\delta^{15}N$ values of epiphytes were lower compared to those of the macroalgae. No significant differences were found in the $\delta^{15}N$ means of epiphytes between the three zones (Table 6, 7). Significant differences in epiphyte $\delta^{15}N$ occurred among the 10 sites when each site was independently analysed (one-way ANOVA $F_{9,20} = 3.98$, $p < 0.01$). Epiphytes from site 3 within the western zone had significantly higher $\delta^{15}N$ values than from sites 2 ($p = 0.024$) and 4 within the western zone ($p < 0.01$), and from site 6 in the eastern zone ($p = 0.053$). Moreover, epiphytes from site 4 within the western zone had significantly lower $\delta^{15}N$ values from site 10 in the southern zone ($p = 0.04$; Figure 5).

The $\delta^{15}N$ mean values of *Symbiodinium* were not significantly different between the three zones (Table 6,7), however, significant differences were observed at a site level (one-way ANOVA $F_{9,16} = 21.49$, $p < 0.01$). The $\delta^{15}N$ value of *Symbiodinium* from site 1 within the western zone was significantly higher than the rest of the nine the sites ($p < 0.01$; Figure 5).

**Table 6**. $\delta^{15}N$ means ± S.E of macroalgae, epiphytes, and *Symbiodinium* from the three zones around Hideaway Island.

| Zones | $\delta^{15}N$‰ macroalgae | $\delta^{15}N$ ‰ epiphytes | $\delta^{15}N$ ‰ *Symbiodinium* |
|---|---|---|---|
| **Eastern** | 6.42 ± 0.62 | 2.34 ± 0.19 | 4.14 ± 0.09 |
| **Western** | 5.14 ± 0.59 | 2.72 ± 0.35 | 4.39 ± 0.13 |
| **Southern** | 6.01 ± 0.39 | 3.37 ± 0.31 | 4.16 ± 0.06 |

**Table 7.** Nested ANOVA with mixed effects models results describing the differences on the $\delta^{15}N$ values of the macroalgae, epiphytes, and *Symbiodinium* of *Montipora stellata* between zones western, eastern and southern. Values with * are significantly different at 0.05.

| Parameter | $\delta^{15}N$ | df | F | p-value |
|---|---|---|---|---|
| **Zones** | macroalgae | 2,7 | 0.66 | 0.54 |
| **Zones** | epiphytes | 2,7 | 1.12 | 0.38 |
| **Zones** | *Symbiodinium* | 2,7 | 0.52 | 0.61 |

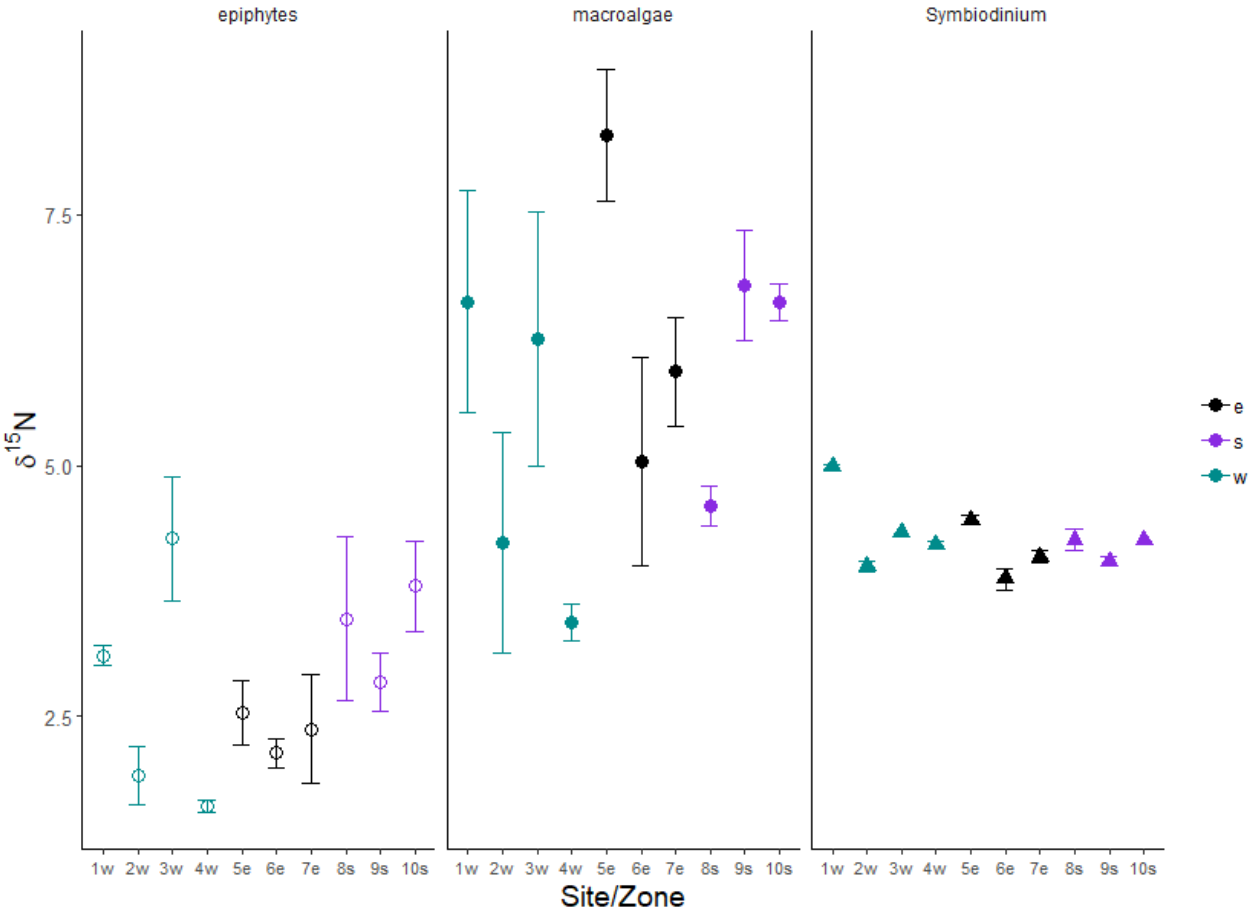


**Figure 5.** $\delta^{15}N$ mean ± S.E of macroalgae (●), epiphytes (○), and *Symbiodinium* (▲) of *Montipora digitata*, around Hideaway Island. Numbers refer to the site within each zone; letters correspond to the zone western, eastern and southern.

**Spatial variability of $\delta^{13}C$ values around Hideaway Island**

The mean $\delta^{13}C$ values of macroalgae collected at three different zones around Hideaway island at the same depth were not significantly different (Table 8, 9). There were no significant differences in the $\delta^{13}C$ values of the macroalgae when analysing sites independently regardless of the zone (one-way ANOVA, $F_{9,20} = 1.18$, $p = 0.36$) but the variability within a site was substantial (Figure 6; Table 8). No significant differences were found in the $\delta^{13}C$ values of the epiphytes among the three zones (Table 9). When analysing the $\delta^{13}C$ values of epiphytes from each site independently no significant differences were observed (one-way ANOVA $F_{9,20} = 1.96$, $p = 0.1$). Overall, the epiphytes had higher $\delta^{13}C$ values than the macroalgae.

Significant differences were detected between $\delta^{13}C$ values of *Symbiodinium* among the three zones around Hideaway Island were (Table 9). *Symbiodinium* from the western zone was significantly more enriched than *Symbiodinium* from the southern zone (Table 8, 10). However, the $\delta^{13}C$ values of *Symbiodinium* from the southern and eastern zones, nor the eastern and western zones did not differ significantly (Table 10). There were no significant differences in the $\delta^{13}C$ values of *Symbiodinium* among the ten sites (one-way ANOVA $F_{2,23} = 3.77$, $p = 0.27$).

**Table 8.** $\delta^{13}C$ means ± S.E of macroalgae, epiphytes, and *Symbiodinium* of *Montipora stellata* from the three zones around Hideaway Island.

| Zone | $\delta^{13}C$ ‰ macroalgae | $\delta^{13}C$‰ epiphytes | $\delta^{13}C$‰ *Symbiodinium* |
|---|---|---|---|
| **Eastern** | -9.92 ± 0.28 | -13.43 ± 0.70 | -13.99 ± 0.19 |
| **Western** | -9.11 ± 0.34 | -14.45 ± 0.68 | -13.82 ± 0.10 |
| **Southern** | -10.11 ± 0.21 | -13.71 ± 0.97 | -14.40 ± 0.12 |

**Table 9.** Nested ANOVA with mixed effects models results describing the differences on the $\delta^{13}C$ values of macroalgae, epiphytes, and *Symbiodinium* from *Montipora stellata* between zones western, eastern and southern. Values with * are significantly different.

| Parameter | $\delta^{13}C$ | df | F | p-value |
|---|---|---|---|---|
| **Zones** | macroalgae | 2,27 | 3.25 | 0.05 |
| **Zones** | epiphytes | 2,70 | 0.28 | 0.76 |
| **Zones** | *Symbiodinium* | 2,23 | 3.77 | 0.03* |

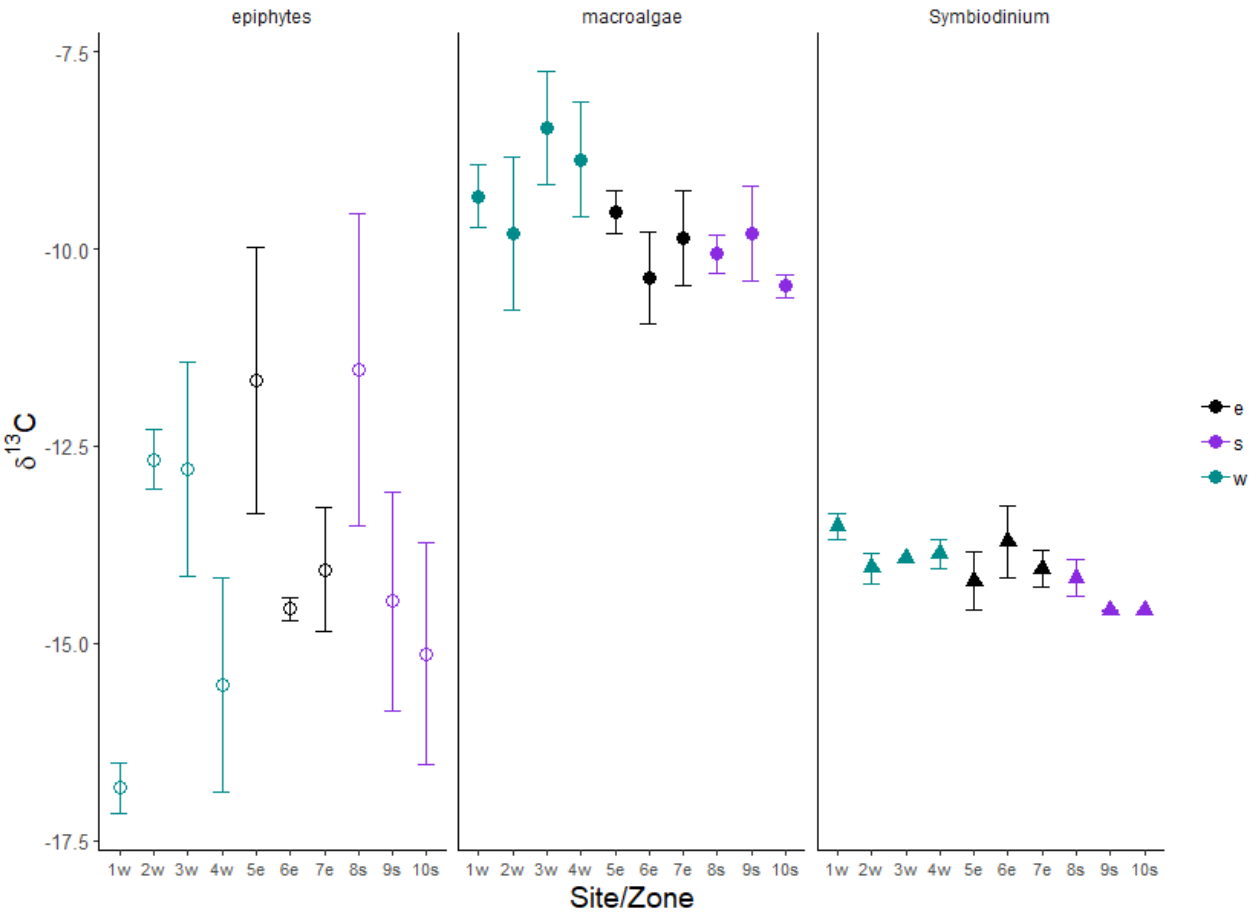


**Figure 6.** Variability of the mean ± S.E of $\delta^{13}C$ macroalgae (●), epiphytes (○), and *Symbiodinium* (▲) of *M. digitata* around Hideaway Island. Numbers refer to the site within each zone; letters correspond to the zone western, eastern and southern.

**Table 10.** Post hoc Tukey test Results describing the differences on the $\delta^{15}N$ of macroalgae, epiphytes and *Symbiodinium* of *Montipora stellata* among zones western, eastern and southern. Values with * are significant.

| Zone | $\delta^{13}C$ macroalgae | $\delta^{13}C$ epiphytes | $\delta^{13}C$ *Symbiodinium* |
|---|---|---|---|
| **Eastern-Southern** | p=0.90 | p=0.98 | p=0.14 |
| **Eastern-Western** | p=0.13 | p=0.75 | p=0.67 |
| **Southern-Western** | p=0.05 | p=0.86 | p= 0.01* |

**C:N ratios around Hideaway Island**

Mean C:N ratios of the macroalgae did not differ significantly between the southern, western and eastern zones (Table 11, 12), nor were significant differences found at site level (one-way ANOVA $F_{9,20} = 2.31$, $p = 0.05$).

Epiphyte and *Symbiodinium* C:N did not show significant differences between the three zones: eastern, southern and western (Table 11, 12) or at a site level (Epiphyte: one-way ANOVA $F_{9,20} = 2.26$, $p = 0.06$), (*Symbiodinium*: one-way ANOVA $F_{9,16} = 1.94$, $p = 0.12$). Overall, macroalgae and epiphytes collected around Hideaway island were highly variable in their C:N ratios while *Symbiodinium* had a narrower C:N range (Figure 7).

**Table 11.** C:N ratio means ± S.E of macroalgae, epiphytes, and *Symbiodinium* of *Montipora stellata* from the three zones around Hideaway Island.

| Zone | C:N ratios macroalgae | C:N ratios epiphytes | C:N ratios *Symbiodinium* |
|---|---|---|---|
| **Western** | 14.25 ± 0.67 | 17.18 ± 0.61 | 18.21 ± 0.15 |
| **Eastern** | 16.34 ± 0.50 | 15.77 ± 0.57 | 18.13 ± 0.35 |
| **Southern** | 16.12 ± 0.30 | 17.07 ± 0.71 | 18.63 ± 0.18 |

**Table 12.** Nested ANOVA with mixed effects models results describing the differences on the C:N ratios of macroalgae, epiphytes, and *Symbiodinium* of *Montipora stellata* among zones western, eastern and southern. Values with * are significantly different.

| Parameter | C:N ratio of | df | F | p-value |
|---|---|---|---|---|
| **Zones** | macroalgae | 2,7 | 2.40 | 0.16 |
| **Zones** | epiphytes | 2,7 | 0.52 | 0.61 |
| **Zones** | *Symbiodinium* | 2,7 | 1.30 | 0.33 |

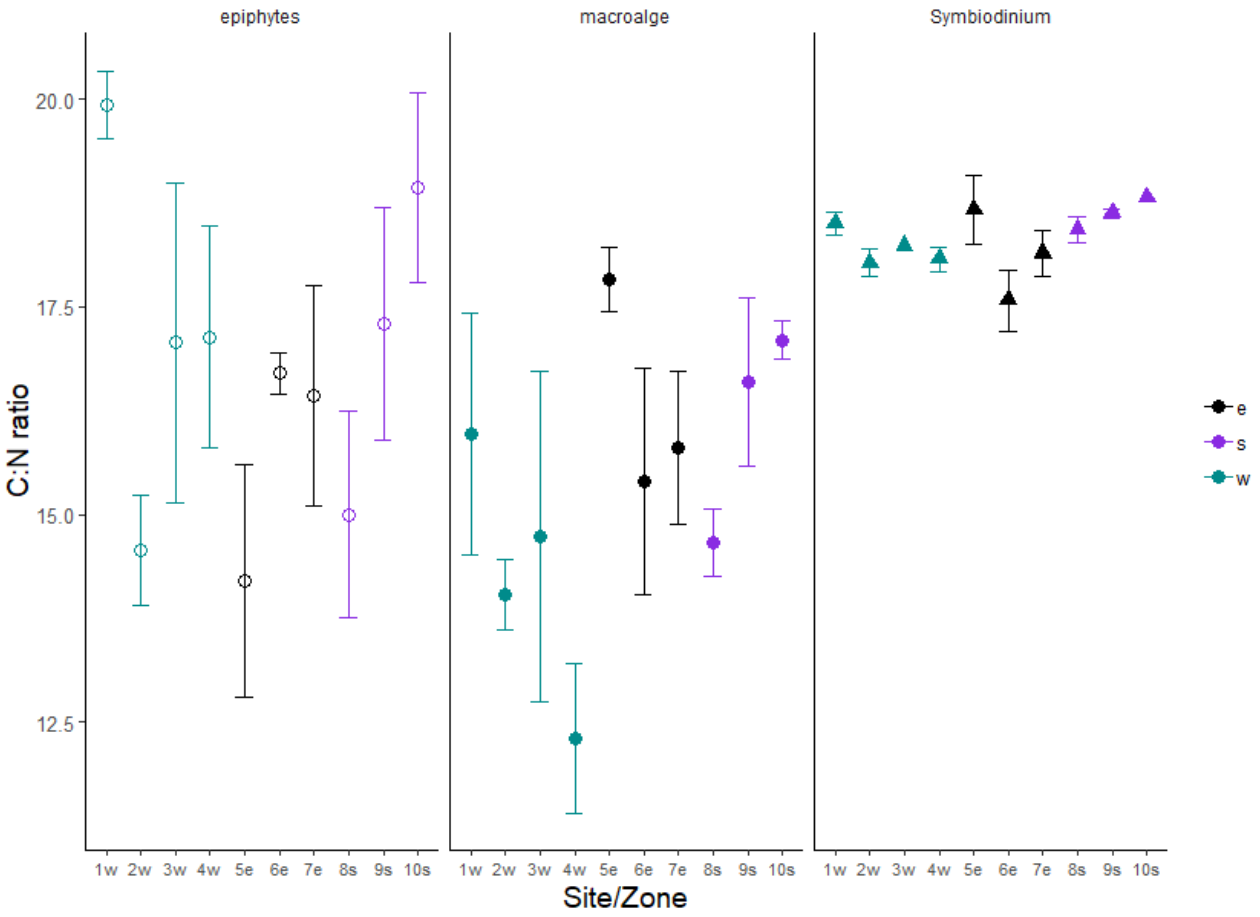


**Figure 7**. Variability of the mean ± S.E. C:N ratios of macroalgae (●), epiphytes (○), and *Symbiodinium* of *Montipora stellata* (▲) around Hideaway Island. Numbers refer to the sites within each zone (1-10); letters correspond to zones: western, eastern, and southern.

### Cell density and chl a of *Symbiodinium* of *Stylophora pistillata* relationship with depth

There was a significant correlation between the cell density and the chlorophyll *a* concentration of *Symbiodinium* with depth ($F_{1,37} = 14.64$, $p < 0.001$, $R^2 = 0.26$). Cell density decreased significantly as depth increased ($F_{1,37} = 10.38$, $p < 0.001$, $R^2=0.12$; Figure 8; a), even though the chl *a* did not decrease significantly with depth ($F_{1,37} = 2.06$, $p = 0.15$, $R^2 = 0.03$; Figure 8; b).

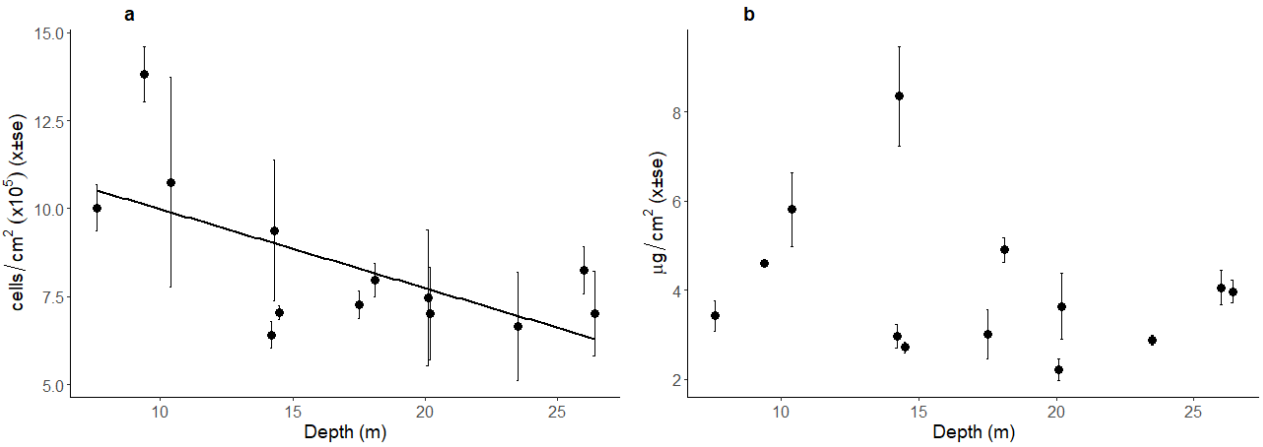


**Figure 8.** Variability of the mean ± S.E of **a.** cell density (cells/$cm^2$ x$10^5$), **b**. chlorophyll *a* concentration (µ/$cm^2$) of *Symbiodinium* extracted from *Stylophora pistillata* along a depth gradient.

### $\delta^{15}N$ relationship with depth

Macroalgal $\delta^{15}N$ values did not show any relationship with depth ($F_{1,15} = 0.67$, $p = 0.43$; Figure 9). The $\delta^{15}N$ values of the epiphytes were highly variable along the depth gradient (Figure 9) and no significant correlation between the $\delta^{15}N$ values of the epiphytes with depth was found ($F_{1,18} = 1.23$, $p = 0.28$). The results of the paired t-test revealed no significant differences between the mean $\delta^{15}N$ values of the macroalgae and epiphytes (paired t-test, $p = 0.61$).

$\delta^{15}N$ values of *Symbiodinium* of *S. pistillata* ranged from a minimal value of 3.9‰ at 23.5 m to a maximal value of 5.2‰ at 26 m depth (Figure 9). There was no significant relationship between the $\delta^{15}N$ values of *Symbiodinium* with depth ($F_{1,9}=1.59$, $p=0.23$). Interestingly, no significant influence of depth was found over the $\delta^{15}N$ values of macroalgae, epiphytes, and *Symbiodinium* (Table 13). Overall, the $\delta^{15}N$ values of *Symbiodinium* were higher than those of the macroalgae and epiphytes (Figure 9).

**Table 13.** Linear regression models showing differences on the $\delta^{15}N$ values of the macroalgae, epiphytes, and *Symbiodinium* of *Stylophora pistillata* with depth. Values with * are significant.

| Parameter | $\delta^{15}N$ | df | F | p-value |
|---|---|---|---|---|
| **Depth** | macroalgae | 1,15 | 0.67 | 0.43 |
| **Depth** | epiphytes | 1,18 | 1.23 | 0.28 |
| **Depth** | *Symbiodinium* | 1,11 | 1.80 | 0.20 |

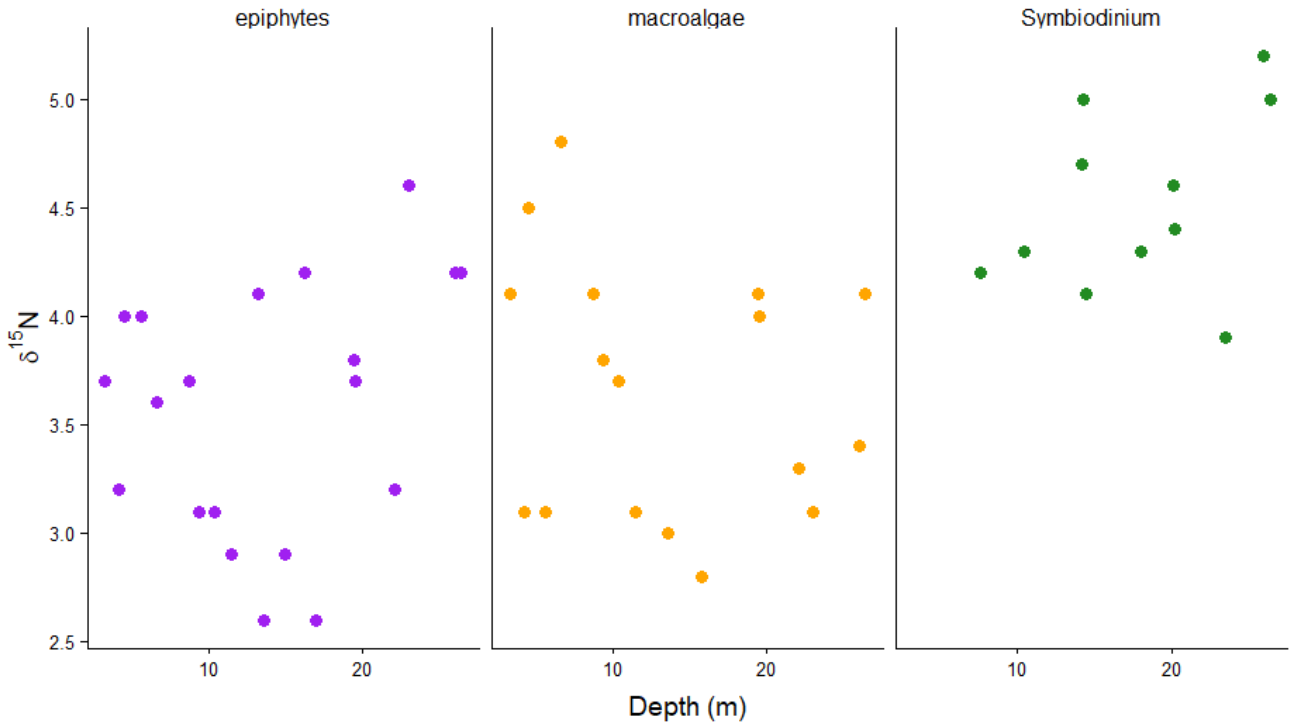


**Figure 9.** Variability of the $\delta^{15}N$ values of macroalgae, epiphytes, and *Symbiodinium* of *Stylophora pistillata*, along a depth gradient from 3 to 26 m depth.

### $\delta^{13}C$ relationship with a depth

The $\delta^{13}C$ values of the macroalgae were variable along the depth gradient. $\delta^{13}C$ values of macroalgae ranged from a minimum value of -20.3‰ at 19.4 m to a maximum value of -4.2‰ at 17 m depth (Figure 10). A significant depletion was observed below 20 m depth on the $\delta^{13}C$ values of the macroalgae ($F_{1,22} = 15.47$, $p < 0.001$, $R^2 = 0.39$).

The $\delta^{13}C$ values of the epiphytes were lower than the $\delta^{13}C$ values of the macroalgae, even so, both had high variability along the depth gradient. The $\delta^{13}C$ values of the epiphytes ranged

from a minimum value of -24.8‰ at 26.4m to a maximum value of -6.1‰ at 22.1 m depth (Figure 10). No influence of depth was found over the $\delta^{13}C$ values of the epiphytes ($F_{1,18}$=2.3, p=0.15 $R^2$=0.06). The paired t-test revealed there were significant differences in the $\delta^{13}C$ values between the macroalgae and epiphytes along the depth gradient (p<0.001).

Overall, the $\delta^{13}C$ values of *Symbiodinium* from *S. pistillata* were more depleted than the $\delta^{13}C$ values of macroalgae and epiphytes and had lower variability along the depth gradient compared to those of the macroalgae and epiphytes (Figure 10).

The $\delta^{13}C$ values of *Symbiodinium* ranged from a minimum value of -18‰ at 14.3 m to a maximum value of -15.2‰ at 10.4m depth (Figure 10). No significant differences were found in the $\delta^{13}C$ values of *Symbiodinium* as depth increased ($F_{1,11}$=1.81, p=0.21). There was a significant influence of depth on the $\delta^{13}C$ values of the macroalgae, nevertheless, depth had no significant influence over the $\delta^{13}C$ values of epiphytes and *Symbiodinium* (Table 14).

**Table 14.** Linear regression models showing differences on the $\delta^{13}C$ values of the macroalgae, epiphytes, and *Symbiodinium* of *Stylophora pistillata* with depth. Values with * are significant.

| Parameter | $\delta^{13}C$ | df | F | p-value |
|---|---|---|---|---|
| **Zones** | macroalgae | 1,22 | 15.47 | <0.001*** |
| **Zones** | epiphytes | 1,18 | 2.3 | 0.15 |
| **Zones** | *Symbiodinium* | 1,11 | 1.81 | 0.21 |

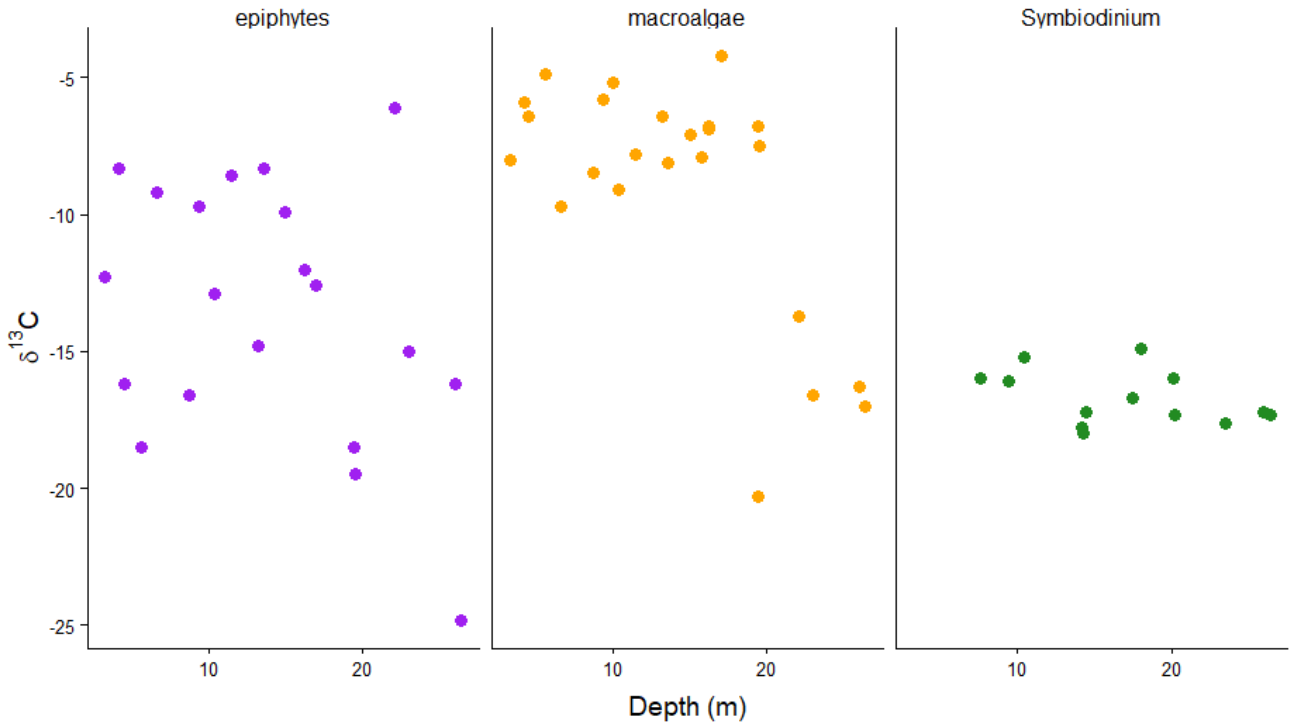


**Figure 10.** Variability of the $\delta^{13}C$ values of macroalgae, epiphytes and *Symbiodinium* from *Stylophora pistillata*, along a depth gradient from 3 to 26 m depth.

### C:N ratio relationship with depth

The carbon to nitrogen ratios (C:N) of the macroalgae ranged from a minimum value of 8 at 5.6 m to a maximum value of 24.4 at 19.4 m depth (Figure 11); while the C:N ratios of the epiphytes ranged from a minimum value of 10.9 at 13.6 m to a maximum value of 29 at 26.4 m depth (Figure 11). There was a significant increase of the C:N ratio of macroalgae and epiphytes as depth increases (Table 15). The C:N ratios of *Symbiodinium* ranged from a minimum value of 19.2 at 18 m and a maximal value of 22.5 at 14.2 m depth (Figure 11). The C:N ratios of *Symbiodinium* isolated from *S. pistillata* did not display a significant relationship with depth (Table 15).

**Table 15.** Linear regression models showing differences on the C:N ratios of the macroalgae, epiphytes, and *Symbiodinium* of *Stylophora pistillata* with depth. Values with * are significant.

| Parameter | C:N ratio | df | F | p-value |
|---|---|---|---|---|
| **Zones** | macroalgae | 1,15 | 19.55 | <0.001*** |
| **Zones** | epiphytes | 1,17 | 5.19 | 0.03* |
| **Zones** | *Symbiodinium* | 1,9 | 1.80 | 0.21 |

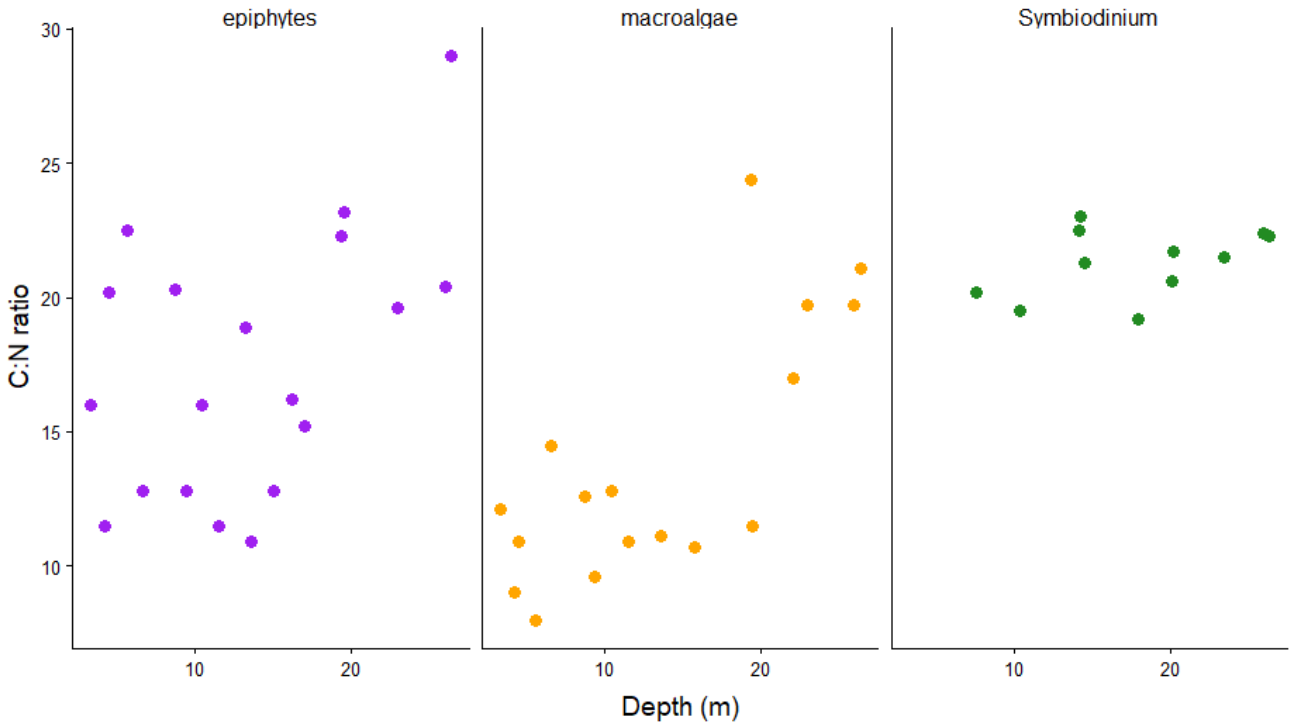


**Figure 11.** C:N ratios of macroalgae, epiphytes, and *Symbiodinium* from *Stylophora pistillata* along a bathymetric gradient from 3-26 m depth.

## DISCUSSION

Nitrogen and carbon stable isotopes of benthic primary producers were analysed to assess the spatial variability in order to detect a potential external nutrient input into the reef around Hideaway Island. Additionally, we examined the effects of depth on the $\delta^{15}N$ and $\delta^{13}C$ signatures of benthic primary producers to investigate their nutrient sources and trophic dynamics. This study is the first to analyse the spatial variability of *Symbiodinium* densities, chl *a* concentrations and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) of two symbiotic corals, macroalgae and epiphytes from a representative fringing reef at Hideaway Island, Vanuatu.

*Symbiodinium* densities and chl *a* concentrations of *Symbiodinium* were standardized to surface area ($cm^2$) using the measurements obtained from photogrammetry. The aluminium foil method developed by Marsh (1970) has been widely used because it keeps the coral skeleton intact without adding any chemicals, however, this method is ambiguous. In this study, we used photogrammetry, which is a novel, modern and emerging tool to calculate the surface area of the coral fragments collected. This approach provides results that are more precise, having less variability within the measurements. It has the added advantage of allowing easy calculations of volume and rugosity. Statistical analyses performed showed that the measured surface area using both methods were not significantly different, showing that both methods are valid when measuring the surface area of coral fragments.

### Identifying a potential external nutrient input around Hideaway Island

Cell densities and chl *a* concentrations of *Symbiodinium* extracted from *M. stellata* differed between zones around Hideaway Island. As *Symbiodinium,* density is positively correlated with nutrient availability (Stimson and Kinzie III 1991; Hoegh-Guldberg 1994; Muller-Parker et al. 1994), results from my research suggest that nutrient availability may also differ around the island. Corals collected in the southern zone of the island presented higher *Symbiodinium* densities and higher chl *a* concentrations. While *Symbiodinium* from corals collected on the western and eastern zones of the island had lower cell densities and lower chl *a* concentrations. It has been well documented in controlled experiments that *Symbiodinium* densities increase in nutrient-enriched environments (Tanaka et al. 2007; Tanaka et al. 2014a). The latter is supported by the argument that growth of *Symbiodinium* is limited by the availability of nutrients in the environment (Hall et al. 2018). Therefore, the high *Symbiodinium* densities and high chl *a* concentrations found in corals in the southern zone of

Hideaway Island appears to be driven by a higher level of nutrients available in this zone, compared to the western and eastern zones of the island.

As the southern zone of the island is sheltered by the reef crest, we hypothesise that a lower flow of water may occur in that zone, while the eastern and western zones of the island are more influenced by the currents that flow from the east. This could be the reason for the similarities in *Symbiodinium* densities and chl *a* concentrations between the western and eastern zones, and this circulation pattern may be responsible for the elevated *Symbiodinium* densities and chl *a* concentrations in corals found in the southern zone of Hideaway Island. The results presented here are only correlative, therefore we cannot be certain that any increases in *Symbiodinium* density and/or chl *a* concentration are related to higher level of nutrients in the southern zone of the island. Monitoring the flow of water in the zones around the island, along with manipulative experiments assessing relationships between these factors would help elucidate any causal relationships here.

Biotic interactions between benthic marine macroalgae and other organisms are quite broad (Hurd et al. 2014). Most of the macroalgae collected around Hideaway Island had epiphytes attached. Stereoscopic observations revealed that the epiphytes attached to the macroalgae were mainly composed of epiphytic algae. Differences were observed in the $\delta^{15}N$ of macroalgae and epiphytes collected in shallow waters. Macroalgae displayed more enriched $\delta^{15}N$ values than their attached epiphytes. This could be attributed to the indirect effects due to increased shading of macroalgae by epiphytes (Duarte 1995). We, therefore, hypothesise that the epiphytes are taking up the nitrogen before it gets to the macroalgae, reducing the amount of nitrogen available to the macroalgae. On the other hand, differences in $\delta^{15}N$ values between the two may also be due to the nutrient and light competition between macroalgae and their attached epiphytes (Worm and Sommer 2000).

Benthic marine macroalgae will differ in their isotopic composition depending on the species (Jaschinski et al. 2008; Zheng et al. 2015). While all effort was made to collect macroalgae that superficially looked similar, the macroalgae were only identified to morphospecies level. The high variability of $\delta^{15}N$ values found in macroalgae in this study, therefore, could be attributed to the collection of different species. Alternatively, differences in $\delta^{15}N$ values within macroalgae could be due to variations in age of the macroalgae collected or the amount of secondary metabolites produced. The age of a marine plant can impact the amount of triacylglycerols (TAG) produced, and less polar lipids are produced in older individuals (Alonso et al. 2000), and it seems reasonable to assume this to be equally true of macroalgae.

As nitrogen affects the fatty acid profile, this has implications for the amount of nitrogen produced. Equally production of secondary metabolites in response to small-scale external factors such as grazing may have also influenced the nitrogen content in the macroalgae. Grazing on individual plants can induce production of secondary metabolites in temperate macroalgae (Pavia and Brock 2000). A further explanation could be that because secondary metabolite production relies on nitrogen availability (Peckol et al. 1996), variations in the amount of secondary metabolites within individual plants in an area may also reflect variations in essential nutrients used to generate them, such as nitrogen. The magnitude of variation observed between samples in this study was surprising and suggests that future research using this method should discriminate between algal species and an increase in the replication of samples collected.

In contrast, *Symbiodinium* of *M. stellata* displayed low variability in $\delta^{15}N$ values compared to macroalgae and epiphytes. Observed differences in $\delta^{15}N$ values of the benthic organisms analysed could be attributed to the fact that macroalgae obtain nutrients directly from the water column (Umezawa et al. 2002), while *Symbiodinium* depends on nutrient recycling within the coral host in addition to nutrients directly from the environment (Grottoli et al. 2006). Moreover, $\delta^{15}N$ values of *Symbiodinium* (3.9 to 5‰) around Hideaway island were similar to those reported free of anthropogenic nitrogen sources (4.7‰) (Wong et al. 2017). Likewise, the range of $\delta^{15}N$ of macroalgae in this study was lower (2.8 to 8.8‰) than the values previously reported for macroalgae that rely on sewage as a nutrient source (Lapointe 1997; Costanzo et al. 2001). We can, therefore, hypothesise that the water around Hideaway Island is well-mixed and any potential nutrient input is well diluted, hence reducing a potential localized impact. Alternatively, it can be suggested that no anthropogenic nutrient source is substantially influencing the $\delta^{15}N$ values of benthic primary producers around Hideaway Island. Nutrient content in the waters around Hideaway Island need to be validated and compared to those at potential nutrient sources to further understand this.

As with nitrogen, $\delta^{13}C$ values in macroalgae can also be used to discriminate carbon sources. Differences in macroalgal $\delta^{13}C$ values vary with species: green algae usually are more $^{13}C$ enriched (-12%) than red algae (-18.3%) (Yamamuro et al. 1995; Wang and Yeh 2003). Therefore, we can hypothesise that any differences in $\delta^{13}C$ for macroalgae and epiphytes could be attributed to the collection of different species.

Many marine macroalgal species have a greater affinity for $HCO_3$- (bicarbonate) than $CO_2$, particularly when $CO_2$ is limited for photosynthesis (Yamamuro et al. 1995). The $\delta^{13}C$ values

of macroalgae had a narrower range and were more enriched (-7.6 to -11.1%) than the $\delta^{13}C$ values of epiphytic algae (-8.3 to -17.8%). Similar trends of macroalgae showing more enriched $\delta^{13}C$ values than epiphytes have been previously reported (Jaschinski et al. 2008; Zheng et al. 2015). The more positive $\delta^{13}C$ values of macroalgae suggest that macroalgae are relying on $HCO_3$- as a nutrient source, while the more negative $\delta^{13}C$ values of epiphytic algae suggest that their main carbon source is $CO_2$. This affinity of carbon sources may be the reason for the observed differences in $\delta^{13}C$ in the macroalgae and epiphytic algae. Another explanation for the observed $\delta^{13}C$ depletion of epiphytes may be related to associated bacteria derived from the macroalgae, which carbon sources are usually enriched in $\delta^{13}C$ (Zheng et al. 2015). Furthermore, the $\delta^{13}C$ values of macroalgae from shallow waters around Hideaway Island were more depleted than in previous studies (Moncreiff and Sullivan 2001; Kang et al. 2003; Zheng et al. 2015). The range in $\delta^{13}C$ values on epiphytic algae found in this study were very much within the range found in other studies (Raven et al. 2002b; Titlyanov et al. 2010). The depleted $\delta^{13}C$ values of macroalgae observed in this study compared to previous studies, which could be possibly because macroalgae are not entirely relying on $HCO_3$- but also in part on $CO_2$, which contributed to the observed depleted $\delta^{13}C$ values.

Carbon stable isotopes can be used as a tool to differentiate from an autotrophic or heterotrophic source (Seemann 2013). The $\delta^{13}C$ values of *Symbiodinium* of *M. stellata* in this study (-13.1 to -14.7‰) were in a range similar to those previously reported for several symbiotic corals by Muscatine et al. (1989a) (-10 to -13‰), Risk et al. (1994) (-10 to -16‰) and Swart et al. (2005)(-12 to -14‰). The previous studies associated this range $\delta^{13}C$ values to autotrophy in corals that were obtaining carbon sources from the symbiotic algae. The narrow range on the $\delta^{13}C$ values of *Symbiodinium* indicates that is mainly relying on carbon sources recycled from the coral host.

To summarise, the consistent $\delta^{15}N$ values analysed in macroalgae, epiphytes, and *Symbiodinium* of *M. stellata* in shallow waters around Hideaway Island did not show any obvious pattern or spatial variability that could be related to the zones around Hideaway Island. This suggests that no point source of nutrient input around the island was identified using macroalgae, epiphytes, and *Symbiodinium* of *M. stellata* as bioindicators in shallow water. The sample number and replication need to be increased to be able to identify any external nutrient inputs and the sampling of the benthic primary producers needs to be extended to a larger spatial scale including a sampling of any potential point source.

Results on stable isotopes in this research can be used as a baseline for future studies for benthic primary producers. The $\delta^{13}C$ and $\delta^{15}N$ values of the coral host tissue and skeleton were not included in this thesis, as these results are still pending. However, it would have been interesting to have these data to compare with data from *Symbiodinium*, macroalgae, and epiphytes.

**Depth effect on the nutrient source of benthic primary producers**

There was a significant influence of depth on cell density of *Symbiodinium* of *Stylophora pistillata* but not on chl *a* concentration. Below 15 m depth, similar trends were observed for both cell density and chl *a* concentrations. The density of *Symbiodinium* was higher at shallow waters, and as depth increased the cell density and chl *a* concentration decreased. However, after 15 m depth this decrease tapered off for both chl *a* and *Symbiodinium*, and a more linear trend was observed. Similar findings were reported for *Symbiodinium* of the same species as a result of photoacclimation (Borell et al. 2016). The stability of the cell density and the chl *a* concentration after 15 m depth observed in this study match the findings of previous studies where *S. pistillata* increases its photosynthetic activity to compensate for the decrease of light to satisfy the coral host nutritional budgets (Mass et al. 2007). Contrary to our results, other studies have reported an increase in the chl *a* concentration of *Symbiodinium* of *S. pistillata* as depth increases in a range of 5-30 m depth (Gattuso et al. 1993). These differences may be driven by the different genotypes that *S. pistillata* could host at different depths and in different parts of the world (Baker 2003). Alternatively, the low photosynthetic rates at 15 m depth compared to shallow water corals, attributed to lower irradiance levels at that depth (Ramsby et al. 2014).

The stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) for *Symbiodinium* presented here did not correlate with depth in Hideaway Island. The low variability and lack of enrichment with increasing depth, suggests that *Symbiodinium* is compensating for the changes in nutrient availability by increasing its photosynthetic rates and maintaining the cell density and chlorophyll *a* concentrations along the depth gradient (Mass et al. 2007). If this was not the case, we would have observed an enrichment in the $\delta^{15}N$ on *Symbiodinium* with increasing depth, which was not observed in this study.

There were no differences in trophic level or nitrogen sources along the depth gradient for *Symbiodinium* of *S. pistillata*, suggested by the isotopic $\delta^{15}N$ that ranged from 4.2‰ at 7.6 m to 5‰ at 26.4 m depth, however, this slight enrichment was not significant. We can also suggest that corals have different trophic adaptations as depth increases. This was previously

reported by Grottoli et al. (2006), who found that different species of corals will differ in their trophic plasticity depending on factors such as depth, light, available nutrients and bleaching. Moreover, the $\delta^{15}N$ values obtained for *Symbiodinium* were higher to those previously reported for *S. pistillata* symbionts but followed the same trend of no enrichment with increasing depth (Alamaru et al. 2009). This could be explained by different $\delta^{15}N$ values of inorganic nitrogen sources that are present at different locations and are reflected in *Symbiodinium*.

The $\delta^{13}C$ values of *Symbiodinium* presented in this study did not show a decrease with increasing depth. Our results concur with those documented by Einbinder et al. (2009), where no significant depletion in the $\delta^{13}C$ of *Symbiodinium* was observed above 30 m depth. Previous studies analysing the isotopic composition in a depth range of 1-60 m, including different coral species, have documented depletions in the $\delta^{13}C$ values of *Symbiodinium* and coral tissue with increasing depth (Muscatine et al. 1989a; Alamaru et al. 2009). However, the observed depletion trend in previous studies on the coral tissue and *Symbiodinium* was only significant from 15 m to 60 m depth.

A significant depletion of $\delta^{13}C$ in macroalgae below 20 m depth was observed, this depletion could be mainly attributed to a reduced rate of photosynthesis as a result of reduced light availability. None the less, the macroalgae $\delta^{13}C$ values analysed for this study showed a constant trend from 3-20 m depth. The finding of a reduced rate of photosynthesis below 20 m depth is supported by previous studies that reported constant photosynthetic rates above 15 m depth, followed by reduced photosynthetic rates below 15 m depth (Mass et al. 2007), which coincide with the results obtained in this study. Moreover, studies have suggested that macroalgae species with $\delta^{13}C$ values more positive than -10‰ are using $HCO_3^-$ as a carbon source, which has ~10‰ $\delta^{13}C$ value (Raven 1997). Therefore, it can be suggested that macroalgae inhabiting a depth range from 3-20 m with $\delta^{13}C$ values of -4.2‰ to -10‰ are using $HCO_3^-$ as a carbon source, while macroalgae in deeper waters (below 20 m depth) are using dissolved $CO_2$ which has more negative values than $HCO_3^-$ (Raven 1997). The $\delta^{13}C$ depletion found in macroalgae in this study suggests that at shallow depths, macroalgae have higher photosynthetic rates and a different carbon source compared to macroalgae from deeper waters. We found that the carbon source available above 20 m depth is $HCO_3^-$, and as depth increases the carbon source available changes to $CO_2$, as is suggested by the $\delta^{13}C$ data of macroalgae.

The $\delta^{13}C$ values obtained for *Symbiodinium* of *S. pistillata* along a depth range from 7.6 to 26 m suggest that there is a tight recycling of carbon sources between the coral host and the symbiont. *Symbiodinium* appears to be using $CO_2$ from the coral host for photosynthesis, and the coral host is using the photosynthetic products of *Symbiodinium* to satisfy its carbon budgets along the depth gradient. This can be supported by the different $\delta^{13}C$ values observed for macroalgae and *Symbiodinium*. Macroalgae displayed enriched $\delta^{13}C$ values (-5 to -10‰) at 3-20 m depth compared to those in *Symbiodinium* (-15 to -18‰), indicating that their carbon sources are different at these depths. While macroalgae are relying only on photosynthesis, the depleted and constant $\delta^{13}C$ values of *Symbiodinium* suggest that the carbon source might be partly from photosynthesis and partly from the coral host. These findings confirm the tight recycling of carbon sources between the coral host and the symbionts along the depth gradient and strengthens the recycling theory (Reynaud et al. 2009). An alternate explanation is that there is a high rate of carbon and nitrogen recycling between the coral host and *Symbiodinium* as depth increases which results in a lower fractionation (Einbinder et al. 2009). This supports our hypothesis of no enrichment in the isotopic composition of *Symbiodinium* due to the active nutrient recycling within the symbiotic system (Tanaka et al. 2006).

Moreover, the higher $\delta^{13}C$ values of macroalgae compared to those found in epiphytes (-6.1 to -24.8‰) might indicate that macroalgae are using $HCO_3$ as a source of carbon due to a lack of available $CO_2$. The depleted $\delta^{13}C$ values of epiphytes suggest they are relying on $CO_2$ as a carbon source. The differences in the $\delta^{13}C$ values of macroalgae and epiphytes suggest their preference for different sources of carbon.

Despite the proposed different carbon sources of macroalgae and epiphytes along the depth gradient and between organisms, depth had no significant effect on the $\delta^{14}N$ values of macroalgae and epiphytes. These results support data in previous studies, where there was no clear pattern in the $\delta^{15}N$ values that could correlate to depth, taxonomy of the species nor to climatic zones as seen for the $\delta^{13}C$ values (Marconi et al. 2011). However, there were significant similarities in the $\delta^{15}N$ values of epiphytes and macroalgae compared to the $\delta^{13}C$ values across the depth gradient, this might reflect the similar N sources utilized by both benthic organisms. Moreover, both macroalgae and epiphytes displayed a high variability along the depth gradient, but no pattern was observed. The high variability in the $\delta^{15}N$ values of the macroalgae and epiphytes along the depth gradient could be related to a combination of the following biological and environmental factors. These include 1) no significant decrease in

nitrogen as depth increases in the range of which these organisms where collected or 2) that the water in that depth range is well mixed, so no depletion in the $\delta^{15}N$ values was reflected in the macroalgae and epiphyte tissues.

**CONCLUSION**

We found mixed support for the presence of an external nutrient input at Hideaway Island, on one hand increased *Symbiodinium* densities could indicate an external input, whereas using $\delta^{15}N$ signatures of *Symbiodinium* and macroalgae we found no such evidence. Observed increases in *Symbiodinium* densities in the southern part of Hideaway Island suggest that there may be a potential external nutrient input that is triggering population growth in these cells. According to previous studies, we can confirm that *Symbiodinium* densities in situ are not entirely regulated by the coral host but may be limited by the level and variety of nutrients in the surrounding environment. Even when the chlorophyll *a* concentration and *Symbiodinium* density from the coral *Montipora stellata* were the only indicators of a potential external nutrient input in the southern zone of the island, there was no evidence on the $\delta^{15}N$ signatures of *Symbiodinium* or macroalgae that could indicate the presence of such nutrient input. The $\delta^{15}N$ data of benthic primary producers obtained in this study did not support our hypothesis of a potential external nutrient input at Hideaway Island. Future studies assessing this hypothesis should increase replication within each zone, as well as extend the collection of benthic primary producers to larger spatial scales in various conditions. It will also be interesting to see changes in the $\delta^{15}N$ and $\delta^{13}C$ signatures of benthic primary producers over different temporal scales, such as, before and after rainstorms, cyclones and at different seasons of the year.

Depth had no a significant effect on the $\delta^{15}N$ of macroalgae, epiphytes, and *Symbiodinium* of *S. pistillata*. The only effect of depth was a decrease in *Symbiodinium* density and in the $\delta^{13}C$ values of macroalgae. These results confirm our hypothesis of no $\delta^{15}N$ enrichment of *Symbiodinium* as depth increases due to the tight recycling between the coral host and the symbiont. However, as reported in previous studies, this enrichment was significant below 20 m depth, so further studies should consider this and increase the depth range at which corals are collected. It would also be interesting to assess the isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) of different co-occurring symbiotic coral species to see how *Symbiodinium* is behaving at different depths. Additionally to investigating the isotopic composition of *Symbiodinium*, further studies could include the host tissue and the coral skeleton $\delta^{15}N$ and $\delta^{13}C$ signatures to better understand the nutrient recycling between the symbiotic system as depth increases.

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