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Reef Building Corals Rely on Symbionts over a Large Depth Range
in an Internal Tidal Bore System, Florid Keys Reef Tract

by

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Environmental and Forest Biology
With Honors

May 2012

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Abstract

Corals living in shallow waters typically acquire their nutrients and energy from their photosynthetic symbiotic zooxanthellae, whereas deeper corals may rely to a lower extent on photosynthetic derived materials due to lower light levels. Whether these deeper corals feed to a greater extent is hotly debated within the community. Our study was based in the Florida Keys Reef Tract, which is unique because it is characterized by upwelling or tidal bores that periodically bring nutrient rich waters onto the Florida Shelf which can be used by benthic organisms. We separately measured the stable carbon and nitrogen isotope signatures of the coral host and symbiotic zooxanthellae of three species of reef building corals (*Porites astreoides, Montastraea cavernosa, and Montastraea faveolata*) along a depth gradient (3-35 m) in order to determine the feeding strategies of these corals as well as to assess whether corals were able to utilize upwelled water as a source of nutrients. The $\delta^{13}C$ and $\delta^{15}N$ values of the zooxanthellae and host tissue of all three species became progressively more depleted in ^{13}C and ^{15}N with depth, and $\delta^{15}N$ values were strikingly low. The trend in the $\delta^{15}N$ values with depth suggest that feeding is less important at depth. Further, we found a strong correlation between the $\delta^{13}C$ values of the host and their zooxanthellae at all depths, suggesting that even as photosynthetic rates decrease with depth, hosts continue to acquire most of their carbon from their symbionts and do not rely to any greater extent on feeding heterotrophically. Finally we suggest that the strikingly low $\delta^{15}N$ values for deep water corals are due to the utilization of upwelled water as a source of nutrients.

Table of Contents

List of Figures

List of Tables

Glossary

Endosymbiotic – a symbiotic relationship between two organisms where one organism lives within the other organism

Fractionation – variation in the equilibrium distribution of isotopes during incorporation into an organism

Internal tidal bores – a phenomenon in which the leading edge of an incoming tide forms an internal wave

Irradiance – the amount of light that is able to penetrate into the water column

RubisCO – an enzyme used in the Calvin cycle to catalyze the first major step in carbon fixation

Scleractinian – an order of corals which build calcium carbonate skeletons, often referred to as “stony corals”

Semidiurnal – occurring twice a day

Thermocline – the depth in the water column where the change in temperature is the steepest, usually forming to separate water masses that cannot mix

Trophic – referring to the strategy an organism uses to obtain nutrition

Zooxanthellae – a dinoflagellate that lives within the tissue of corals and provides the corals energy through photosynthesis

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Introduction

Scleractinian corals can satisfy their nutritional requirements by a combination of heterotrophic assimilation (active feeding) and translocation from autotrophic endosymbiotic dinoflagellates (zooxanthellae) (Muscatine 1990; Reynaud et al. 2002; Houlbrèque and Ferrier-Pagès 2009; Alamaru et al. 2009). They grow throughout a large depth range that extends down to 150 m, or the lower boundary for photosynthetic primary production (Alamaru et al. 2009). In shallow waters where irradiance is high, up to 95% of compounds produced by zooxanthellae in photosynthesis can be translocated to the coral host (Muscatine et al. 1990; Maier et al. 2010) but little is known about how corals obtain energy in deeper waters where irradiance is significantly lower (Leichter et al. 2008). Several studies have been done to try to shed light on which trophic strategy Scleractinian corals use to obtain energy in deeper waters. From the results of these studies two leading hypotheses have emerged which are currently being hotly debated. It has been well established that photosynthetic rate decreases with decreasing irradiance (Lesser et al. 2010) so one hypothesis is that translocation of carbon must also decrease with depth and corals must feed heterotrophically to compensate for energy (Muscatine et al. 1989; Lesser et al. 2010). The second hypothesis is that corals are still able to obtain most of their energy from their zooxanthellae as Scleractinian corals only grow to the lower boundary for photosynthetic primary production (Alamaru et al. 2009; Einbinder et al. 2009; Maier et al. 2010).

The stable carbon and nitrogen isotope compositions of an organism are commonly used to study the feeding behavior of an organism and have been used to study the feeding behavior of Scleractinian corals over a range of depths. The stable

carbon isotope signature of an organism is similar to the carbon signature of its food source (Sulzman 2007). The stable nitrogen signature is used as an indicator of feeding strategy because it increases ~+3.5‰ with respect to the food source for each higher trophic level on which the organism is feeding (Heikoop et al. 1998), and can also identify the source of nutrients used by photosynthetic organisms. All studies which have looked at the stable carbon isotope signature of Scleractinian corals found that the carbon signature of both coral tissue and zooxanthellae decreases with depth (Muscatine et al. 1998; Mass et al. 2007; Alamaru et al. 2009; Einbinder et al. 2009; Maier et al. 2010; Lesser et al. 2010), however not all researchers agree on the feeding behavior indicated by this trend. Muscatine et al. (1989) and Lesser et al. (2010) found that while both coral tissue and zooxanthellae carbon signatures decreased with depth, the difference between the carbon signatures of the two organisms increased with depth. They suggested that this showed that corals were becoming less dependent on their zooxanthellae as a source of carbon with increasing depth. In contrast, Alamaru et al. (2009) found that the difference between the carbon isotope signatures of coral tissue and zooxanthellae did not correlate with depth.

The stable nitrogen isotope signature of Scleractinian corals over a depth range do not show a consistent trend with depth. Some studies reported a decreasing nitrogen isotope signature with increasing depth (Muscatine et al. 1994; Heikoop et al. 1998; Maier et al. 2010; Baker et al. 2011), while others found that depth had no effect on nitrogen isotope signature (Alamaru et al. 2009; Lesser et al. 2010). Lesser et al. (2010) was among those that found that depth had no effect on nitrogen isotope signature but suggested that since the nitrogen values did not increase over the depth range, there was

no evidence that corals were feeding heterotrophically to any greater extent. Maier et al. (2010) found that nitrogen signature in corals decreased until 30 m and then remained constant for the rest of the depth range (47 m). He suggested that constant nitrogen isotope values over a depth range were due to nitrogen limitation within that depth range. Baker et al. (2011) found that the nitrogen signature of Gorgonian corals decreased with increasing depth and then in a later laboratory experiment found that nitrogen fractionation in these corals was negatively correlated with light intensity suggesting that a decreasing nitrogen isotope signature with increasing depth is related to light availability.

Our study was based in the Florida Keys Reef Tract, which is unique because it is characterized by upwelling or tidal bores that periodically bring nutrient rich waters onto the Florida Shelf which can be used by benthic organisms. Evidence of these tidal bores was found by Leichter et al. (1996), who discovered that the water temperature at 35 m on Conch Reef, located 8 km off of Key Largo, Florida, decreased at 12 hour intervals each day. The temperature decrease was coupled with an increased salinity that matched the salinity of subthermocline water (Leichter et al. 1996). Similar patterns in temperature and salinity were found at 40 m and 21 m, but not at 7 m (Leichter et al. 1996). The mechanism behind these tidal bores is the semidiurnal tide which pulls on the thermocline as it comes in creating a wave that breaks twice a day on the lower part of the reef (around 40 to 20 m) bathing it in subthermocline water. Concentrations of nitrate below this thermocline are 10-40 times the concentration of nitrate at the surface (Leichter et al. 2003) so Scleractinian corals on the deeper part of this reef experience high concentrations of nutrients twice a day (Leichter et al. 2006).

The objective of this study was to determine the feeding strategies of three Scleractinian corals, *Porites astreoides, Montastraea faveolata,* and *Montastraea cavernosa*, over a depth range of 3-35 m in the Florida Keys Reef Tract using stable carbon and nitrogen isotope signatures for coral tissue, zooxanthellae, and local zooplankton. These methods were also used to assess whether corals are able to utilize upwelled water from internal tidal bores as a source of nutrients to sustain growth in the deeper part of this system.

Methods

Sample collection:

Tissue samples of three coral species (*Montastraea faveolata, Porites astreoides, and Montastraea cavernosa*) were collected from reef sites in the Upper and Middle Florida Keys (Fig. 1) ranging in depth (3-35m; Table 1). Coral tissue were removed from the skeleton using the air-brush technique (Szmant et al. 1990), the resulting slurry homogenized (Tissue Tearor; 15 s) and separated into a zooxanthellae and animal fraction (Teece et al. 2011). Zooplankton were collected by horizontally towing nets (50 μm) immediately above coral heads. All samples were frozen, and lyophilized at -55^{o}C prior to analysis.

Stable isotope analyses

The weight percentage of organic carbon and nitrogen, and the $\delta^{13}C$ and $\delta^{15}N$ values of zooplankton, zooxanthellae, and coral host were measured at the Environmental Science Stable Isotope Laboratory (EaSSIL) at the State University of New York College

of Environmental Science and Forestry (SUNY-ESF) using a Costech elemental analyzer linked via a ThermoFinnigan Conflo III interface to a Finnigan MAT Delta XL Plus stable isotope mass spectrometer (EA-IRMS). All samples were treated with dilute HCl to remove inorganic carbon, which can affect overall $\delta^{13}C$ values (Teece and Fogel 2004). Samples were analyzed in triplicate and accuracy and precision of the stable isotope measurements (expressed in the standard per mil notation) was verified using National Institutes of Standards and Technology RM8573 ($\delta^{13}C$ ≠ -26.4 ± 0.1‰ [*n*=38], ($\delta^{15}N$ = -4.5 ± 0.3‰ [*n*=38]), and RM8574 ($\delta^{13}C$ = +37.6 ± 0.2‰ [*n*=38], ($\delta^{15}N$ = +47.6 ± 0.3‰ [*n*=38]). Daily precision of the instrument was verified by repeated analyses of internal laboratory standards including acetanilide ($\delta^{13}C$ =-30.1±0.1‰, $\delta^{15}N$ =-0.2±0.3‰ [*n*=16]), valine ($\delta^{13}C$ = -10.9±0.1‰, $\delta^{15}N$ = -6.6±0.3‰ [*n*=5]), and daphnia ($\delta^{13}C$ = -24.8±0.1‰, $\delta^{15}N$ = +17.2±0.5‰ [*n*=3]), during the sample runs.

Statistical analyses

Correlations between $\delta^{13}C$ and $\delta^{15}N$ values with depth were assessed using a Pearson correlation (Mintab Release 16).

Results

$\delta^{13}C$ values vs. depth

The $\delta^{13}C$ values of for each of the three species versus depth for coral tissue ranged from -12.8‰ to -19.5‰ (Figure 2a), and the $\delta^{13}C$ signature for the zooxanthellae ranged from -12.2‰ to -19.0‰ (Figure 2b). The $\delta^{13}C$ values for both the coral tissue and zooxanthellae were more variable at shallower depths than in deep water. The $\delta^{13}C$

values of coral tissue for all species ranged from -12.9‰ to -19.2‰ at 5 m and from -15.5‰ to -19.5‰ at 35 m, and the $\delta^{13}C$ values of zooxanthellae ranged from -12.9‰ to -19.0‰ at 5 m and from -17.0‰ to -19.0‰ at 35 m. The $\delta^{13}C$ values for *Porites astreoides* ranged from -12.9‰ to -19.3‰ for the coral tissue and -11.3‰ to -19.0‰ for the zooxanthellae, and $\delta^{13}C$ values of *Montastraea faveolata* ranged from -13.2‰ to -17.9‰ for the coral tissue and from -12.8‰ to -19.0‰ for the zooxanthellae. *Montastraea cavernosa* was only collected below 23m, and the $\delta^{13}C$ values of coral tissue ranged from -15.1‰ to -18.2‰ and -13.3‰ to -17.6‰ for the zooxanthellae. The $\delta^{13}C$ values of animal hosts showed significant correlations between decreasing $\delta^{13}C$ values and increasing depth for *M. faveolata* (Pearson correlation r = -0.630, p=0.000), and *P. astreoides* (Pearson correlation r = -0.367, p=0.028).

$\delta^{15}N$ values vs. depth

The $\delta^{15}N$ values for each of the three species versus depth for coral tissue ranged from -19.0‰ to +19.0‰ (Figure 3a), and the $\delta^{15}N$ values for the zooxanthellae ranged from -10.0‰ to +4.0‰ (Figure 3b). The $\delta^{15}N$ values for both the coral tissue and the zooxanthellae were more variable at shallower depths than in deep water. For the coral tissue, the $\delta^{15}N$ values ranged from -19.0‰ to +19.0‰ at 5 m and from -19.0‰ to -8.0‰ at 35 m. The $\delta^{15}N$ values of zooxanthellae ranged from -10.0‰ to +4.0‰ at 5 m and from -9.5‰ to -3.0‰ at 35 m. The $\delta^{15}N$ values for *P. astreoides* ranged from 1.1‰ to -19.1‰ for coral tissue and from +2.9‰ to -7‰ for zooxanthellae, and $\delta^{15}N$ values for *M. faveolata* ranged from +2.1‰ to -18.2‰ for coral tissue and from +1.7‰ to -9.9‰ for

zooxanthellae. The $\delta^{15}N$ values for *Montastraea cavernosa* ranged from -2.9‰ to -15.6‰ for coral tissue and from 0.1‰ to -9.8‰ for zooxanthellae.

Variation in $\delta^{13}C$ and $\delta^{15}N$ values of zooplankton

The $\delta^{13}C$ values of zooplankton from inshore sites (Coral Gardens, Tavernier Rocks; -14.7‰ to -16.9‰) were depleted relative to those from deeper offshore sites (Shallow Conch, Deep Conch, Deep Fore; -18.7‰ to -19.0‰). Except for zooplankton from Deep Conch (+6.7‰), the $\delta^{15}N$ values of zooplankton spanned a small range (+2.7‰ to +4.3‰).

Discussion

Variation of $\delta^{13}C$ vs. depth

In general, the $\delta^{13}C$ values for the coral tissue and zooxanthellae of all three species of corals decreased with depth (Figure 2a; Figure 2b). This trend is consistent with previous studies for several species of corals in the Caribbean including *Montastraea cavernosa* (Muscatine et al. 1989; Lesser et al. 2010) , *Madracis mirabilis, Acropora cervicornis, Acropora palmata, Porites astreoides, Montastraea annularis, Eusmilia fastigiata, Dendrogyra cylindrus* (Muscatine et al. 1989) , *Madracis aurentenra,* and *Madracis formosa* (Maier et al. 2010). Similar trends were also observed with S*tylophora pistillata* (Mass et al. 2007; Einbinder et al. 2009; Alamaru et al. 2009) and *Favia favus* in the Gulf of Aqaba (Alamaru et al. 2009). The $\delta^{13}C$ values for the coral tissue and zooxanthellae of all three species of corals were more variable in shallow waters than at depth. Such high variability in the $\delta^{13}C$ values in coral hosts and

zooxanthellae in shallow water corals were also observed in *M. faveolata* from the Florida Keys (Swart et al. 2005), and in several species from Banda, Australia, Jepara, Jamaica and Zanzibar (Heikoop et al. 2000). The variability was tentatively attributed to changes in light availability determined by latitude, season, turbidity and cloudiness (Heikoop et al. 2000). Our samples were collected between April and June and although we observed no correlation between dates and the $\delta^{13}C$ values, a variety of factors are probably responsible for this variation including, but not limited to, light availability, temperature fluctuations and food availability. In our study, the $\delta^{13}C$ values of coral hosts and zooxanthellae were less variable at lower depths, which may reflect lower variability in light and food availability.

Zooxanthellae obtain carbon dioxide (CO_2) for photosynthesis from two main sources: CO_2 from metabolism of the coral host, and an internal pool of bicarbonate (HCO_3^-) in the host tissue which forms when metabolic CO_2 reacts with cell water (Figure A.1; Muscatine et al. 1989; Furla et al. 2002). There is also some HCO_3^- within the internal pool which comes from seawater HCO_3^- diffusing into the host tissue (Figure 4; Muscatine et al. 1989). Fractionation of carbon isotopes by zooxanthellae is controlled by the uptake and intracellular diffusion of HCO_3^-, as well as the enzyme RubisCO (ribulose 1,5-biphosphate carboxylase/oxygenase) which participates in the fixation of CO_2 into organic molecules during photosynthesis (Muscatine et al. 1989). Under normal light conditions, RubisCO discriminates against ^{13}C so the resulting carbon biomass is depleted in ^{13}C relative to the carbon source.

In shallow waters, where light is abundant and rates of photosynthesis are high, zooxanthellae discriminate against ^{13}C leading to typical $\delta^{13}C$ values of -12 to -15‰. The

carbon supply/demand theory of Swart et al. (2005) states that under the high light conditions of shallow environments, RubisCO will not discriminate against ^{13}C as strongly to keep up with high photosynthetic rates (Swart et al. 2005; Einbinder et al. 2009). With increasing depth and concurrent lower light levels, photosynthetic rates decrease, and RubisCO will discriminate more against ^{13}C during carbon fixation, leading to depleted $\delta^{13}C$ values in zooxanthellae associated with deeper coral colonies. We observed a strong correlation between decreasing $\delta^{13}C$ values of zooxanthellae with increasing depth which adds support to this theory.

Variation of $\delta^{15}N$ vs. depth

The $\delta^{15}N$ values of the host tissue and zooxanthellae of all three species of corals decreased with depth. This trend is consistent with previous studies for several species of Scleractinian corals including *M. mirabilis, A. cervicornis, A. agaracities, A. palmata, P. astreoides, M. annularis, M. cavernosa, E. fastigiata*, and *D. cylindrus* (Muscatine & Kaplan 1994; Heikoop et al. 1998), and *P. lobata* in Zanzibar (Heikoop et al. 1998). It was also observed in Gorgonian octocorals, *Pseudopterogorgia americana* and *Gorgonia ventalina*, in the Florida Keys and Mexico (Baker et al. 2011). The $\delta^{15}N$ values of the coral host and zooxanthellae showed greater variability in shallow waters than at lower depths for all three species of corals, similar to that observed in several coral species (Heikoop et al. 2000). Heikoop et al. (1998) suggested that light is the primary factor controlling the $\delta^{15}N$ signature of coral tissues due to the strong relationship found between the $\delta^{15}N$ signature of corals and light attenuation. Baker et al. (2011) also suggested that light was the primary factor based on observations in laboratory

experiments. The higher variability in $\delta^{15}N$ values of corals and symbionts in shallow waters in our study may be due to variations in light availability at shallower depths. The mechanism suggested for the increase in fractionation of ^{15}N with decreased light is similar to the carbon supply/demand theory. In areas with high light availability, photosynthetic rates are high and in order to keep up with the demands of a high photosynthetic rate zooxanthellae will take up any available nitrogen isotopes (Heikoop et al. 1998; Baker et al. 2001). However, in deeper waters where there is less light available, photosynthetic rates are slower, and growth rates are slower (Lesser et al. 2010). Under these conditions corals can discriminate against the heavier ^{15}N isotope without affecting growth rate or metabolic needs leading to depleted $\delta^{15}N$ values of zooxanthellae (Heikoop et al. 1998; Baker et al. 2001). Similarly, pelagic algae fractionate to a greater extent during assimilation of inorganic nitrogen at low growth rates (Wada and Hattori 1978).

Are corals feeding more at depth?

Previous studies which support that Scleractinian corals feed more heterotrophically at lower depths found that the $\delta^{13}C$ values of the coral were similar to the $\delta^{13}C$ values of particulate matter at lower depths (Muscatine et al. 1989; Lesser et al. 2010). This reasoning supports the general rule that the carbon signature of an organism is similar to the carbon signature of its food source (Sulzman 2007; Gannes et al. 1998). In the Florida Keys, the $\delta^{13}C$ values of zooplankton range from -17‰ to -19‰ (Table 2) and particulate organic matter (POM) averages -20‰ (Swart et al. 2005). These values are similar to the values we found for the coral animal tissue at depth, which were -16‰

to -20‰ (Figure 2a), and so could suggest that the corals are feeding heterotrophically to obtain their carbon for biomass production.

However, if corals were feeding heterotrophically, we would expect the $\delta^{15}N$ values for the host tissue to be ~+3.5‰ greater than that of the zooplankton (or POM) on which they are feeding. Heterotrophs are typically enriched in ^{15}N relative to their diet (Minagawa and Wada 1984), and the $\delta^{15}N$ values for zooplankton in the Florida Keys ranged from +0.2‰ to +4.7‰ (Table 2). Therefore, if our corals were feeding more on zooplankton at depth we would expect the $\delta^{15}N$ values of the host tissue to approach ~+3.7‰ to +8.5‰. In contrast, coral animal tissue at depth was highly depleted in ^{15}N (-19‰ to -20‰; Figure 3b), and showed the same decreasing trend with increasing depth as the $\delta^{15}N$ values of the zooxanthellae. Therefore, the main source of N for deeper water corals at our sites in the Florida Keys Reef Tract was not from increased feeding, rather a continued reliance on their symbiotic zooxanthellae even at lower depths.

Is it photosynthesis?

The $\delta^{13}C$ values for the coral tissue were similar to the $\delta^{13}C$ values of the zooxanthellae at depth (-17 to -19‰; Figure 2b). We found a strong relationship between the coral tissue and zooxanthellae $\delta^{13}C$ values at all depths for all three of our coral species (Figure 5). We also found a strong relationship when we made the same plot using data from previous studies (Figure A.2; Pearson correlation r = 0.850, P=0.000). This plot includes data from the studies which found that the difference in the $\delta^{13}C$ values between the coral host and zooxanthellae increased with depth. Since all of these studies were done in different locations with many species of corals these plots imply that this

relationship is universal and supports the carbon supply/demand theory (Swart et al. 2005) that the decreasing $\delta^{13}C$ values of corals growing in deeper water is the result of increased fractionation due to lower light levels and decreased rates of photosynthesis by zooxanthellae.

Further support of the importance of photosynthetically derived nutrition to the coral host is our observation of the decrease in $\delta^{15}N$ values of both symbiont and host with increasing depth, which is in total contrast to results which would suggest that corals were obtaining more of their nutrition from feeding heterotrophically at lower depths. Our data support the suggestions of Heikoop et al. (1998) and Baker et al. (2011) that the $\delta^{15}N$ signature is primarily dependent on light availability. That the $\delta^{15}N$ signatures of both the coral tissue and zooxanthellae reflect this pattern is evidence that the coral and zooxanthallae are still strongly dependent on light at all depths. Based on our data we conclude that coral colonies at lower depths (down to 35 m), do not use heterotrophic feeding to obtain significant quantities of carbon and nitrogen compared with colonies growing in shallow water. We suggest that light and photosynthesis by the symbionts are the most important factors in providing carbon and nitrogen for corals, and that Scleractinian corals continue to obtain energy from their symbionts even at 35 m. These symbionts may be adapted to lower light conditions, as these symbionts still produce sufficient energy reserves from photosynthesis, and continue to translocate materials to the coral host to ensure growth, albeit slower, at depth.

Source of nitrogen for deep water corals?

While decreasing trends for $\delta^{15}N$ values with increasing depth were consistent with other studies, our $\delta^{15}N$ values of coral tissue and zooxanthellae in deeper waters (-12 to -19‰) were strikingly lower than any other study (~-3‰; Muscatine et al. 1994; Heikoop et al. 1998; Alamaru et al. 2009; Lesser et al. 2010; Maeir et al. 2010; Baker et al. 2011). The $\delta^{15}N$ signature can be dependent on two factors, either the $\delta^{15}N$ value of the source of nitrogen or fractionation of nitrogen isotopes during nitrogen assimilation and uptake. In order to determine which of these factors is affecting the nitrogen signature of our corals and zooxanthellae we must examine both the isotopic composition of the source and the fractionation during uptake of nitrogen.

The main source of nitrogen for zooxanthellae and corals is nitrate, which is found in high concentrations at lower depths on the Florida Keys Reef Tract (FRT; Leichter et al. 2003) Ammonium can sometimes be an important nitrogen source on coral reefs but in the FRT concentrations of ammonium are low at all depths (0-0.6 µmol L^{-1} (Leichter et al. 2007). The high concentrations of nitrate at depth can be attributed to internal bores, which are breaking internal waves (Leichter et al. 1996). In the Florida Keys Reef Tract internal bores are caused by semidiurnal tidal forcing which generates waves along the thermocline that break on the deep part of the reef (Leichter et al. 1996), making them a high frequency source of upwelling for the Florida Keys (Leichter et al. 1996). These internal bores bring high concentrations of nitrate (1.0 to 4.0 µM), which is 10-40 times greater than background concentrations in the Florida Keys (0.1 to 0.2 µM) (Leichter et al. 2003) onto deeper reefs, and the amount of nitrate being delivered to the reef twice a day by internal bores could equal or exceed the amount of nitrate necessary

for a reef in a given day (Leichter et al. 2003). The $\delta^{15}N$ value of deep water nitrate that is upwelled onto the reef tract (+5.3‰) is similar to that of nitrate found in shallow reef waters. Therefore, the differences in $\delta^{15}N$ values of corals with increasing depths are not solely related to the isotopic composition of the source nitrate, rather the higher concentrations of nitrate at depth, and/or fractionation during uptake of this nitrogen source leads to the depleted $\delta^{15}N$ values of corals at lower depths.

There are many factors which can affect the process of fractionation including concentration of the N source, and also the rate of uptake. Wada and Hattori (1978) demonstrated that greater fractionation of nitrogen isotopes occurred during nitrate assimilation in the marine diatom *Phaeodactylum tricornutum* under higher concentrations of nitrate. Since the concentration of nitrate at depth in the FRT is considerably higher (10 to 40 times greater; Leichter et al. 2003) than in shallow waters, we would expect greater fractionation of nitrogen during uptake in our deeper coral colonies which is what we observed. Growth rate is another factor that can affect fractionation. For the same diatom species, Wada and Hattori (1978) found that isotope fractionation was inversely related to growth rate, meaning that higher fractionation occurred at slower growth rates. At lower depths, many coral species grow at slower rates (Lirman and Fong 2007) and the depleted $\delta^{13}C$ values of our corals at lower depths reflect lower photosynthetic rates which in turn suggest lower growth rates of corals in deeper waters. Therefore, both the lower growth rates indicated by depleted $\delta^{13}C$ values and the higher concentrations of nitrate at depth lead to higher fractionation during nitrogen uptake which is reflected in the highly depleted $\delta^{15}N$ values of our deep water corals.

Conclusion

Our study shows that Scleractinian corals growing in shallow to deep waters (35 m) rely on their symbionts for energy and nutrition even when growth rates in deeper waters may be low. Heterotrophic feeding by coral colonies does not increase with increasing depth as previously suggested, rather the symbiotic zooxanthellae provide the majority of C and N needed by the coral host over this depth range. We also suggest that corals in the Florida Keys Reef Tract directly utilizes deep water nitrate that is periodically upwelled onto deeper reefs as a major source of their nitrogen.

Table 1 Locations of corals collected for this study.

Code	Site name	Depth (m)	Latitude	Longitude
608CGARD	Coral Gardens	3.2	24° 50.236'N	80° 43.695'W
TAV	Tavernier Rocks	4.5	24° 56.339'N	80° 33.763'W
608 Marker 44	Marker 44	5.2	24° 47.809'N	80° 46.989'W
608 EETURT	East Turtle	5.2	24° 43.903'N	80° 54.037'W
608 TRANSP 11	11' Mound	5.6	24° 43.415'N	80° 51.639'W
SHALC	Shallow Conch	6.1	24° 56.782'N	80° 30.124'W
DEEPC	Deep Conch	18.7	24° 56.793'N	80° 27.412'W
810DC2	Deep Pickles	23.2	24° 58.915'N	80° 24.732'W
810DC1	Deep Conch Deep	30.0	24° 56.792'N	80° 27.412'W
210Deep	Molasses Deep	35.7	25° 45.440'N	80° 21.157'W

Table 2 Stable carbon and nitrogen isotope values, and atomic C/N ratios of zooplankton from reefs in the Florida Reef Tract (±SD).

Date	Site	# samples analyzed	$\delta^{15}N$	$\delta^{13}C$	At C/N
608	Coral Garden	3	+3.0 (0.9)	-15.0 (0.8)	4.9 (0.4)
608	Coral Garden Night	6	+2.4 (0.8)	-14.7 (0.3)	6.0 (0.3)
409	Tavernier Rocks	10	+2.6 (0.5)	-16.8 (1.5)	7.1 (1.6)
608	Marker 44	3	+1.3 (1.6)	-16.8 (1.1)	4.4 (0.4)
608	East Turtle	3	+3.1 (0.7)	-16.9 (0.9)	4.9 (0.6)
608	11’ Mound	6	+3.4 (0.6)	-17.6 (0.1)	5.2 (0.0)
409	Shallow Conch	12	+4.3 (2.0)	-18.9 (0.5)	5.9 (0.7)
509	Deep Conch	9	+6.7 (0.9)	-18.7 (0.3)	5.8 (0.3)
409	Deep Fore N	10	+2.7 (0.8)	-19.0 (0.5)	4.9 (0.3)

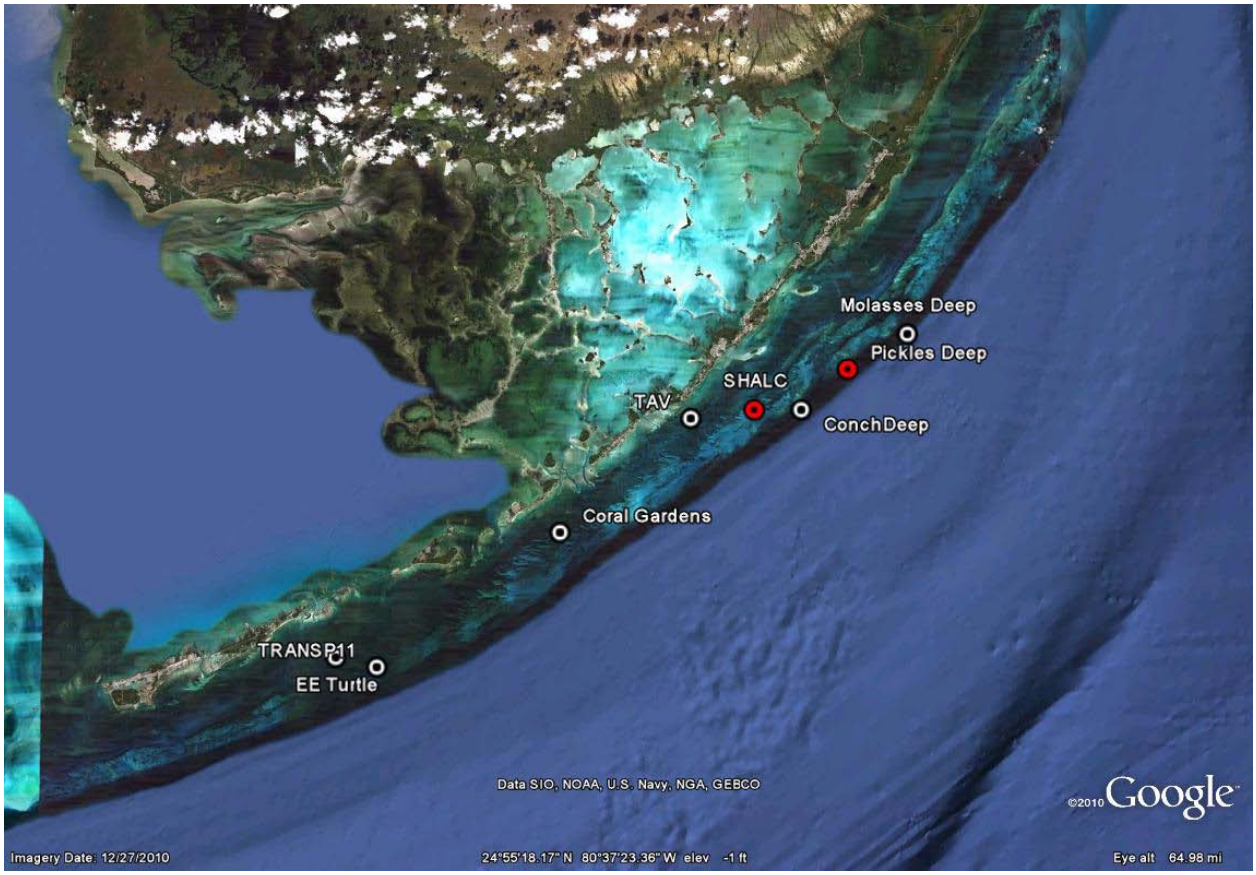

Fig. 1 Location of collection sites in the Florida Keys reef tract.

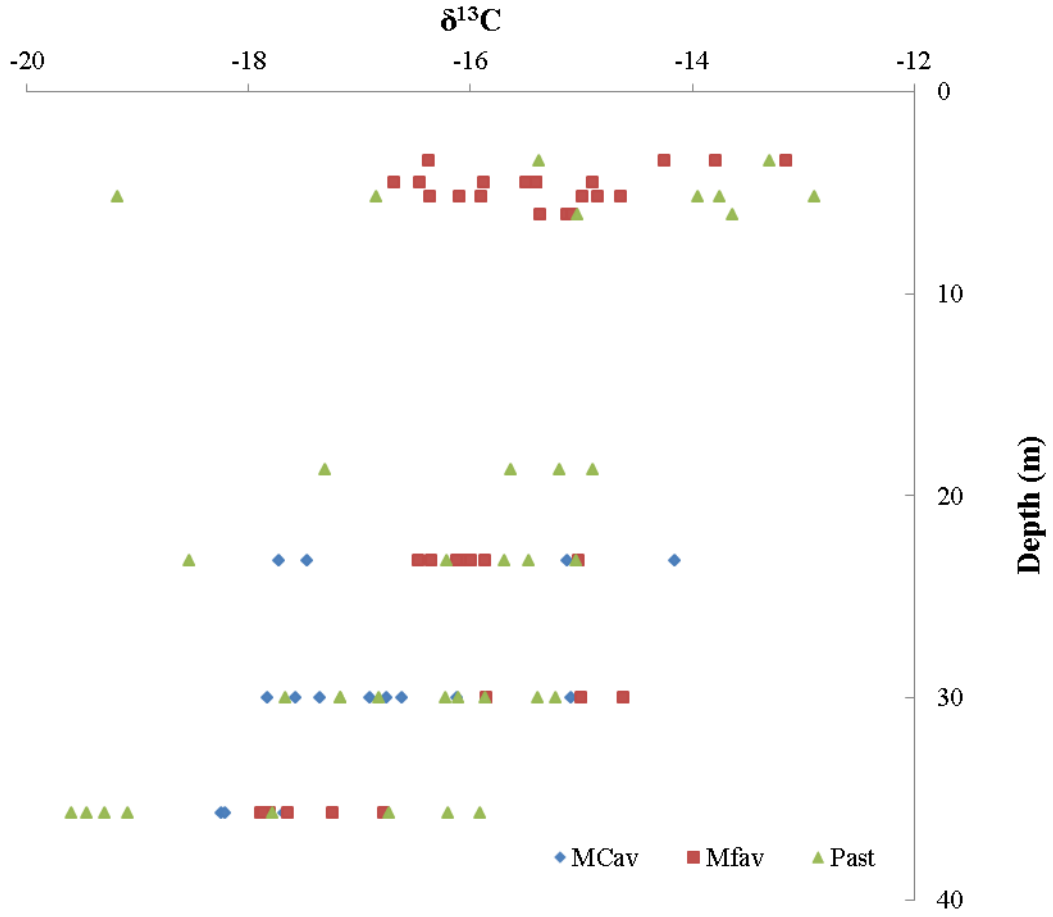

Figure 2a $\delta^{13}C$ values for coral tissue versus depth.

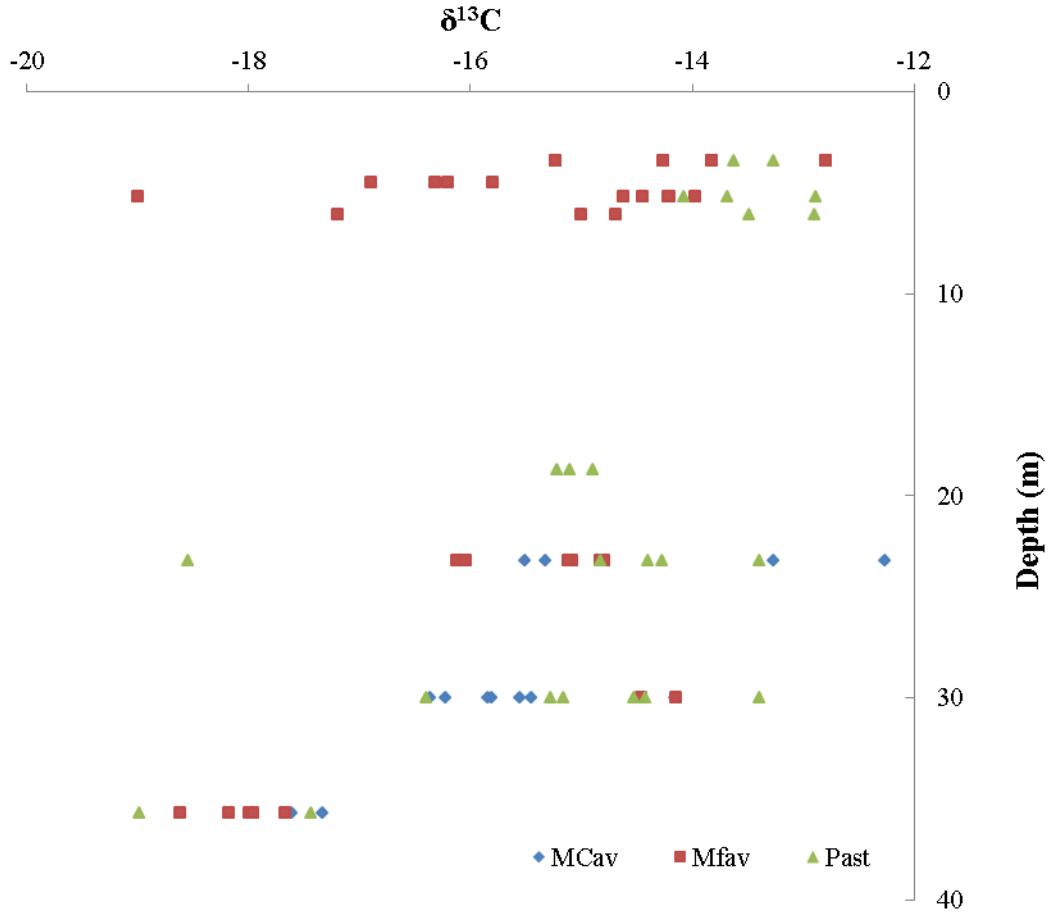

Figure 2b $\delta^{13}C$ values for zooxanthellae versus depth.

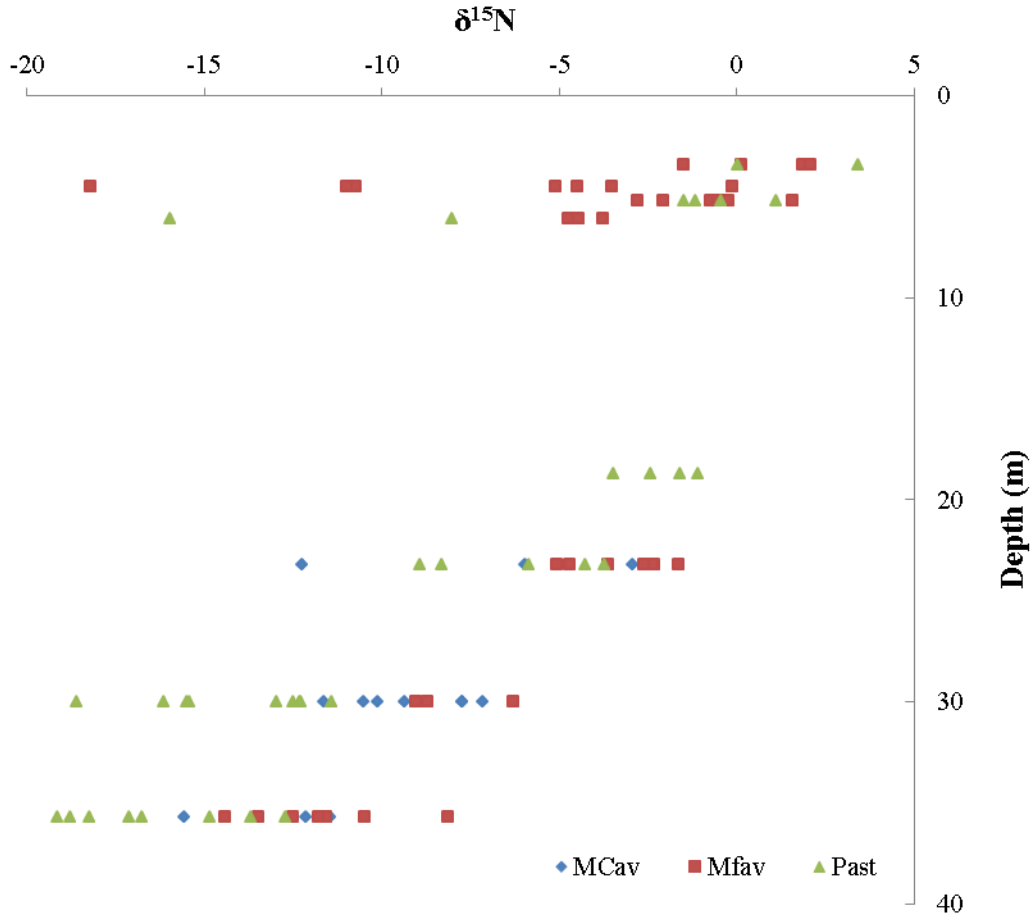

Figure 3a $\delta^{15}N$ values for coral tissue versus depth.

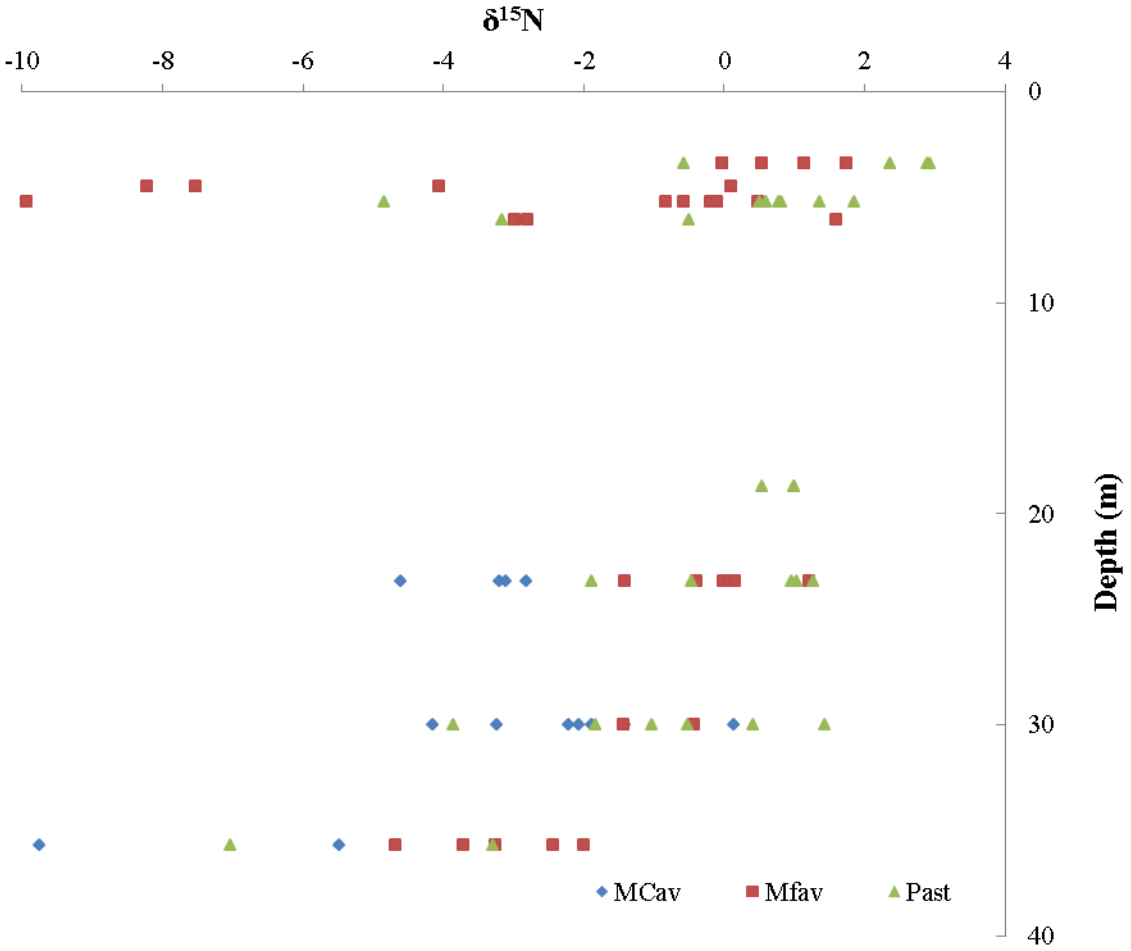

Figure 3b $\delta^{15}N$ values for zooxanthellae versus depth.

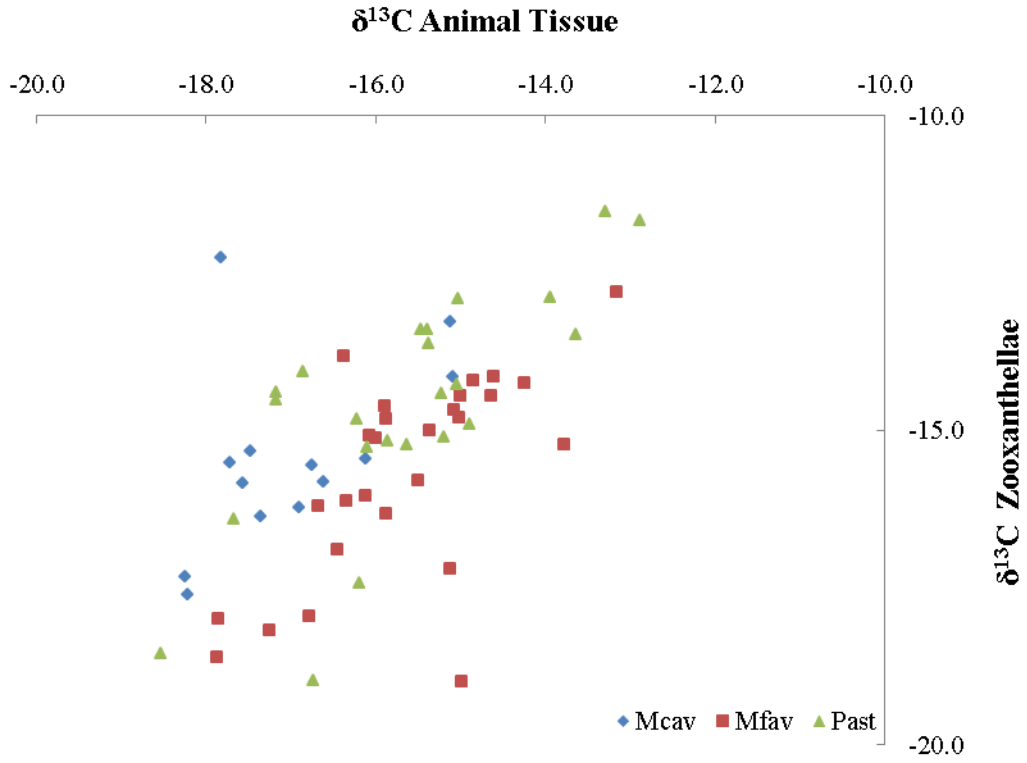

Figure 4 $\delta^{13}C$ values for coral tissue versus $\delta^{13}C$ for zooxanthellae.

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Appendices

A. 1. Carbon uptake and fractionation by zooxanthellae and coral

There are two main sources of carbon dioxide ($CO_{2)}$ which zooxanthellae can use for photosynthesis. One of these sources is the metabolic CO_2 formed from respiration in the animal tissue (Muscatine et al. 1989). The second source is an internal pool of bicarbonate (HCO_3^-) within in the oral endoderm of the animal tissue (Figure A.1; Muscatine et al. 1989). This internal bicarbonate pool itself originates from two processes: either by diffusion of seawater bicarbonate into the oral endoderm, or by metabolic CO_2 from animal respiration reacting with water molecules in the oral endoderm. Corals may actively uptake seawater bicarbonate using anion exchangers (Furla et al. 2000). Muscatine et al. (1989) determined that at high rates of photosynthesis approximately 60% of CO_2 used for photosynthesis is metabolic and 40% is from the internal bicarbonate pool.

Fractionation of stable isotopes occurs because lighter isotopes have weaker chemical bonds than heavier isotopes (Maier et al. 2010). This causes lighter carbon isotopes to be used more quickly in chemical reactions. For zooxanthellae this is thought to occur during the primary carboxylation reaction in C3 photosynthesis, which is catalyzed by the enzyme ribulose bisphosphate carboxylase (RUBISCO). To explain the changes that can take place in zooxanthellae fractionation over a depth range Muscatine et al. (1989) developed the “diffusion-depletion” hypothesis, also referred to as the “carbon supply/demand” hypothesis (Swart et al. 2005). This hypothesis states that in shallow, well lit waters where the rate of zooxanthellae photosynthesis is greater than the rate of animal tissue respiration, all metabolic CO_2 from respiration will be used for

photosynthesis. In order to maintain photosynthesis at high rates when metabolic CO_2 has been depleted, zooxanthellae must turn to the internal bicarbonate pool as a source of CO_2, and this pool must be replenished by diffusion of seawater bicarbonate into the oral endoderm. When the demand for CO_2 for photosynthesis is so high that all CO_2 is used RUBISCO will not discriminate against ^{13}C and fractionation will decrease (Muscatine et al. 1989; Swart et al. 2005).

A. 2. Nitrogen uptake and fractionation by zooxanthellae and coral

The main source of nitrogen for zooxanthellae is dissolved inorganic nitrogen (DIN) in the form of nitrate and/or ammonium. Nitrate and ammonium can enter the coral by diffusion or by active uptake from seawater (Muscatine et al. 1994). Zooxanthellae are also able to use ammonium waste from coral catabolism (Muscatine et al. 1994). Nitrogen may also be supplemented with particulate organic nitrogen (PON) which can be consumed by the coral heterotrophically (Maier et al. 2010).

It has been demonstrated that fractionation of ammonium and nitrate does not occur during uptake but does occur during assimilation in diatoms (Wada & Hattori 1978). It was suggested that this is also true in zooxanthellae (Muscatine et al. 1994). More fractionation occurs at depth, where light is limited and nitrogen is sufficient, than in shallow waters where light is high and nitrogen is limited (Muscatine et al. 1994; Heikoop et al. 1998; Maier et al. 2010; Baker et al. 2011). The hypothesis to explain the fractionation of nitrogen over a depth is known as the "depletion-diffusion" hypothesis, which is similar to the supply/demand hypothesis used to explain changes in the fractionation of carbon over a depth range. This hypothesis states that in high light

conditions all available ammonium and nitrate will be used for photosynthesis leading to passive diffusion of seawater DIN into the coral (Cook et al. 1988; Heikoop et al. 1998). Under these circumstances heavier ^{15}N isotope will not be discriminated against. However, under light limited conditions where rates of photosynthesis are lower but sufficient ammonium and nitrate are available, the lighter ^{14}N isotope will be preferentially used leading to higher fractionation (Baker et al. 2011).

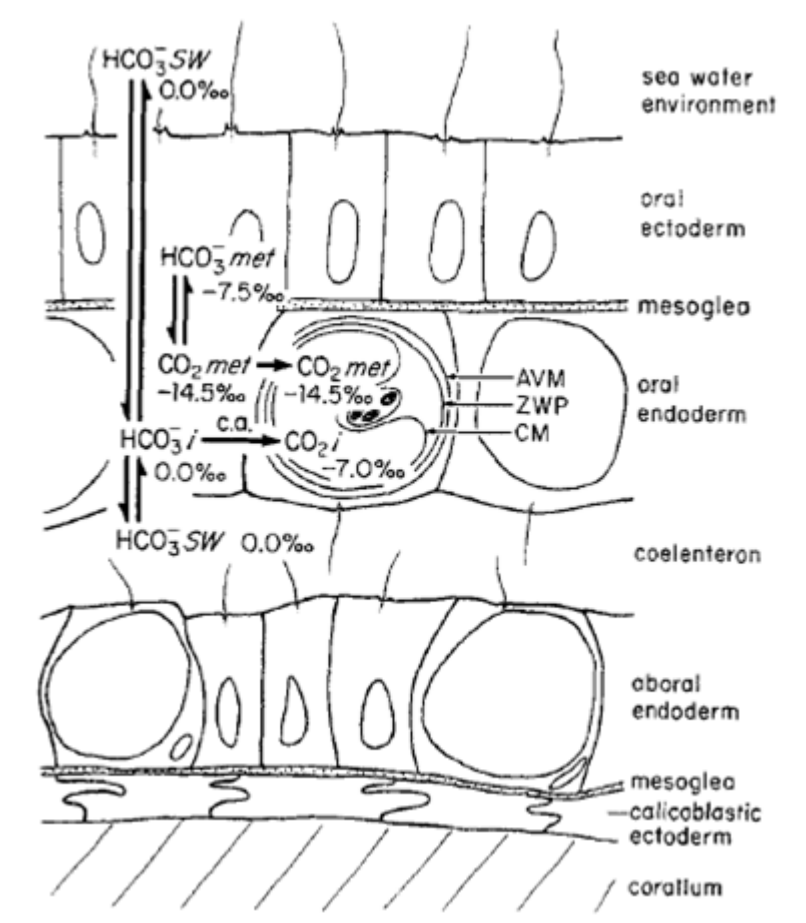

Figure A.1 Model of carbon fixation and translocation in coral-zooxanthellae symbiosis (taken from Muscatine et al. 1989).

Table A.1: The $\delta^{13}C$ and $\delta^{15}N$ values of zooxanthellae (zoox), animal host (host), total coral holobiont and zooplankton (zoopl) from published studies used for our comparison of isotopic compositions of corals from around the world

Depth	$\delta^{13}C$ host	$\delta^{13}C$ zoox	Species	Location	$\delta^{13}C$ zoop	$\delta^{15}N$ host	$\delta^{15}N$ zoox	$\delta^{15}N$ holobiont	Ref.
1.0	-13.1	-12.5	*A. agaricites*	Jamaica	-18.4	3.0	1.6		5,6
10.0	-15.6	-13.9	*A. agaricites*	Jamaica	-18.4		1.9		5,6
30.0	-15.5	-14.5	*A. agaricites*	Jamaica	-18.4	1.5	1.9		5,6
50.0	-17.9	-15.6	*A. agaricites*	Jamaica	-18.4	1.5	0.3		5,6
1.0			*A. cervicornis*	Jamaica	-18.4	4.1	1.8		6
10.0	-15.3	-14.1	*A. cervicornis*	Jamaica	-18.4	1.9	1.7		5,6
30.0	-16.0	-16.0	*A. cervicornis*	Jamaica	-18.4	1.6	0.2		5,6
1.0	-15.1	-14.8	*A. palmata*	Jamaica	-18.4		1.8		5,6
10.0	-15.2	-14.7	*A. palmata*	Jamaica	-18.4	2.1	1.5		5,6
10.0	-15.5	-14.9	*D. cylindrus*	Jamaica	-18.4	2.2	2.4		5,6
1.0			*E. fastigiata*	Jamaica	-18.4	3.5	3.5		6
10.0	-15.4	-15.1	*E. fastigiata*	Jamaica	-18.4	2.5	2.2		5,6
30.0	-19.5	-19.2	*E. fastigiata*	Jamaica	-18.4	2.8	0.9		5,6
5.0	-12.8	-12.8	*F. favus*	Gulf of Aqaba	-21.0	2.1	1.1		1
10.0	-12.0	-12.0	*F. favus*	Gulf of Aqaba	-21.0	2.5	1.3		1
20.0	-12.2	-12.2	*F. favus*	Gulf of Aqaba	-21.0	2.7	1.1		1
25.0	-13.5	-13.0	*F. favus*	Gulf of Aqaba	-21.0	2.4	0.7		1
45.0	-17.0	-17.0	*F. favus*	Gulf of Aqaba	-21.0	2.5	1.2		1
60.0	-19.2	-19.0	*F. favus*	Gulf of Aqaba	-21.0	2.4	1.0		1
1.0	-11.9	-9.4	*M. annularis*	Jamaica	-18.4	3.3	3.0		5,6
10.0	-13.6	-13.9	*M. annularis*	Jamaica	-18.4	2.4	1.8		5,6
30.0	-19.6	-18.3	*M. annularis*	Jamaica	-18.4	0.2	2.4		5,6
50.0	-19.3	-15.6	*M. annularis*	Jamaica	-18.4	1.9	-0.2		5,6
5.0	-17.1	-16.7	*M. aurentenra*	Curacao				5.1	3
5.0	-17.4	-17.3	*M. aurentenra*	Curacao				4.5	3
10.0	-17.8	-17.1	*M. aurentenra*	Curacao				4.7	3
10.0	-17.7	-17.5	*M. aurentenra*	Curacao				4.3	3
20.0	-18.5	-18.0	*M. aurentenra*	Curacao				4.9	3
20.0	-19.2	-19.6	*M. aurentenra*	Curacao				4.4	3
30.0	-19.1	-19.0	*M. carambi*	Curacao				3.4	3
30.0	-20.0	-20.0	*M. carambi*	Curacao				3.3	3
1.0	-10.8	-11.3	*M. cavernosa*	Jamaica	-18.4	3.0	1.0		5,6
5.0	-12.6	-12.8	*M. cavernosa*	Bahamas	-19.9	2.7	2.0		4
10.0	-14.6	-14.4	*M. cavernosa*	Bahamas	-19.9	1.8	2.2		4
10.0	-13.5	-14.0	*M. cavernosa*	Jamaica	-18.4	1.1	0.4		5,6
15.0	-15.4	-15.8	*M. cavernosa*	Bahamas	-19.9	2.6	2.1		4
20.0	-13.8	-14.8	*M. cavernosa*	Bahamas	-19.9	3.7	2.0		4

25.0	-14.8	-15.2	*M. cavernosa*	Bahamas	-19.9	2.3	1.5			4
30.0	-16.8	-14.7	*M. cavernosa*	Jamaica	-18.4	1.2	-2.2		5,6	
45.0	-15.0	-15.2	*M. cavernosa*	Bahamas	-19.9	2.8	2.5			4
50.0	-22.4	-14.3	*M. cavernosa*	Jamaica	-18.4	3.4	-1.7		5,6	
60.0	-16.0	-15.8	*M. cavernosa*	Bahamas	-19.9	3.2	1.7			4
90.0	-19.2	-17.2	*M. cavernosa*	Bahamas	-19.9					4
40.0	-19.6	-19.1	*M. formosa*	Curacao				3.3		3
40.0	-20.2	-20.1	*M. formosa*	Curacao				2.2		3
47.0	-19.2	-19.2	*M. formosa*	Curacao				2.9		3
47.0	-20.2	-20.2	*M. formosa*	Curacao				1.6		3
1.0	-19.0	-18.4	*M. mirabilis*	Jamaica	-18.4	3.9	3.5		5,6	
10.0	-17.7	-16.8	*M. mirabilis*	Jamaica	-18.4	3.1	3.3		5,6	
30.0	-19.6	-16.4	*M. mirabilis*	Jamaica	-18.4	1.8	2.6		5,6	
1.0	-11.8	-10.2	*P. astreoides*	Jamaica	-18.4	2.8	3.0		5,6	
10.0			*P. astreoides*	Jamaica	-18.4	2.1	2.3			6
30.0	-17.9	-14.6	*P. astreoides*	Jamaica	-18.4	1.7	2.0		5,6	
1.0	-14.0	-13.2	*S. pistillata*	Red Sea	-18.4				5,6	
2.0	-13.5	-13.5	*S. pistillata*	Gulf of Aqaba	-21.0	1.8	0.5			1
5.0	-14.1	-13.5	*S. pistillata*	Gulf of Aqaba	-21.0	1.9	-0.2			1
5.0	-16.0	-14.0	*S. pistillata*	Gulf of Aqaba	-20.0					2
10.0	-14.1	-13.3	*S. pistillata*	Gulf of Aqaba	-21.0	1.5	0.2			1
10.0	-17.0	-15.0	*S. pistillata*	Gulf of Aqaba	-20.0					2
10.0	-15.4	-13.3	*S. pistillata*	Red Sea	-18.4					5
15.0	-14.0	-13.5	*S. pistillata*	Gulf of Aqaba	-21.0	1.1	-0.1			1
20.0	-16.2	-16.0	*S. pistillata*	Gulf of Aqaba	-21.0	1.0	-0.8			1
25.0	-16.3	-16.2	*S. pistillata*	Gulf of Aqaba	-21.0	1.5	-1.0			1
30.0	-18.2	-18.0	*S. pistillata*	Gulf of Aqaba	-21.0	1.3	-0.5			1
30.0	-18.5	-15.0	*S. pistillata*	Gulf of Aqaba	-20.0					2
30.0	-18.6	-15.7	*S. pistillata*	Red Sea	-18.4					5
45.0	-19.2	-19.2	*S. pistillata*	Gulf of Aqaba	-21.0	1.0	-1.0			1
50.0	-21.8	-17.8	*S. pistillata*	Gulf of Aqaba	-20.0					2
60.0	-21.5	-20.0	*S. pistillata*	Gulf of Aqaba	-21.0	0.9	-0.1			1
65.0	-22.5	-18.5	*S. pistillata*	Gulf of Aqaba	-20.0					2

[1] Alamaru et al. 2009; [2] Einbinder et al. 2009; [3]Maier et al. 2010; [4]Lesser et al. 2010; [5] Mucatine et al. 1989; [6] Muscatine et al. 1994.

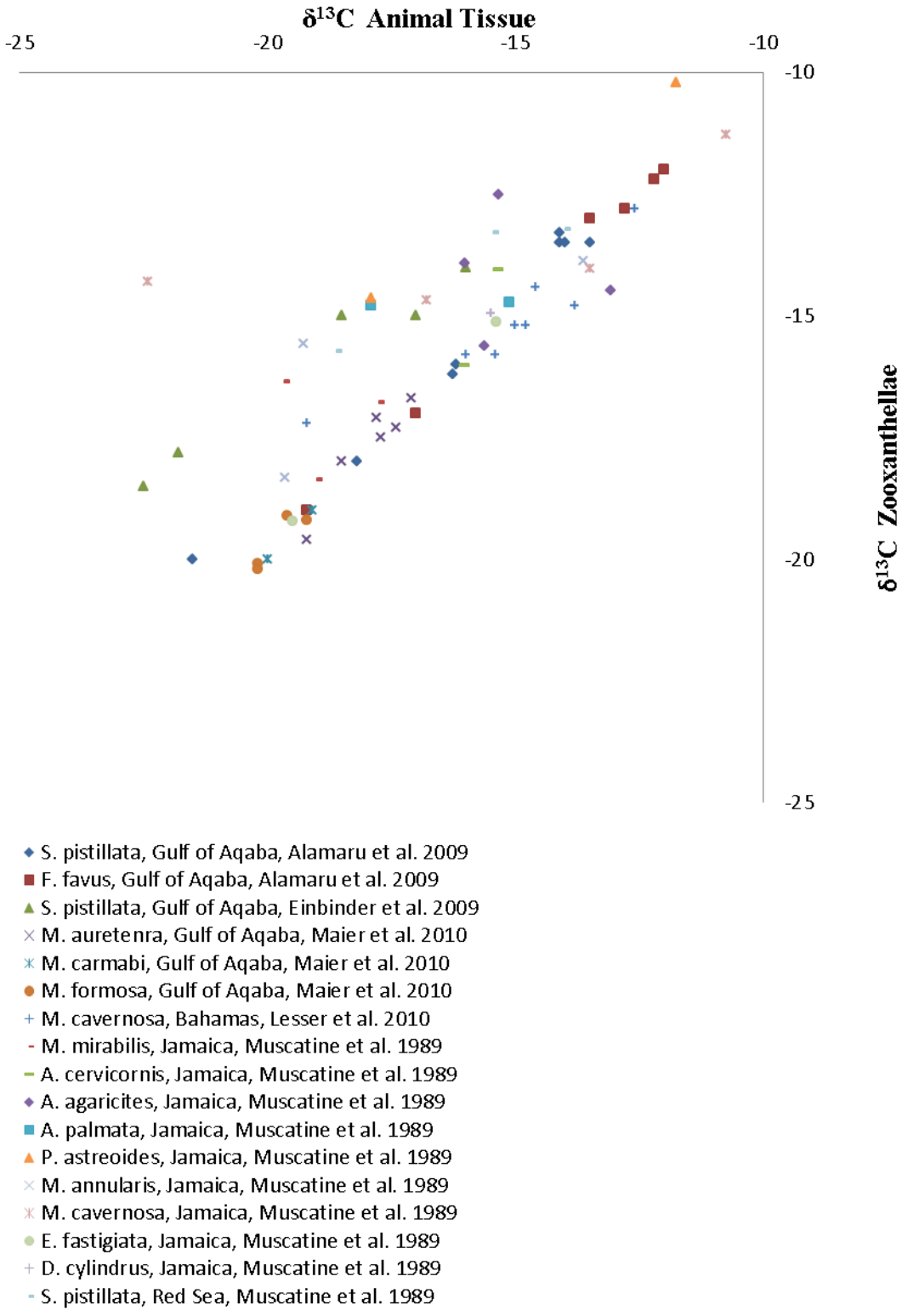

Figure A.2 $\delta^{13}C$ values of coral tissue versus $\delta^{13}C$ values of zooxanthellae (data from studies in Fig A.1)

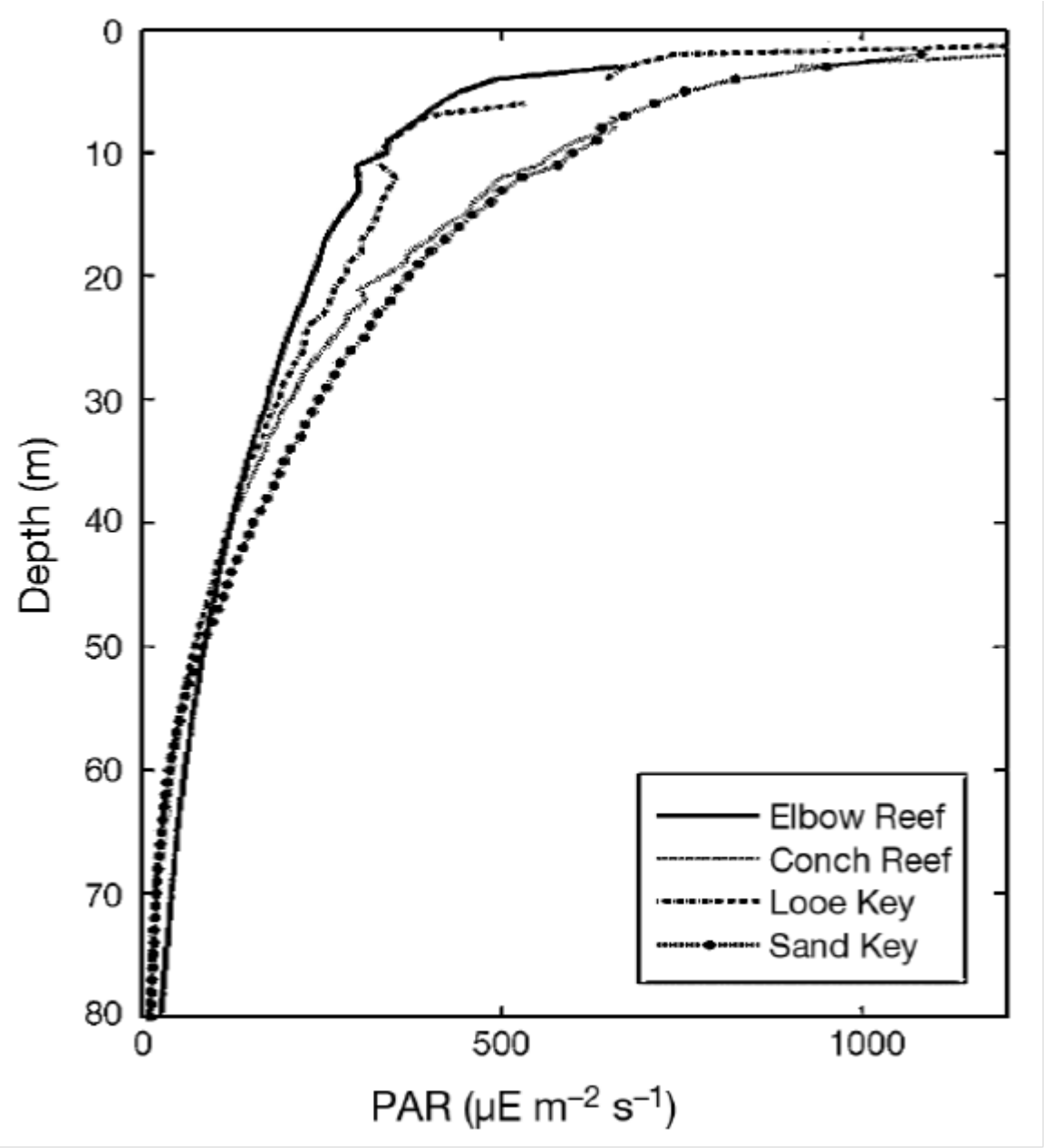

Figure A.3 Photon irradiance (PAR, μmol photons m^{-2} s^{-1}) as a function of depth measured in 2002 and 2003 in the offshore waters of the Florida Reef Tract (taken from Leichter et al. 2008).

Table 1. Calculated mean percent of surface irradiance at depth for water column with light extinction coefficient $-0.06\ m^{-1}$

Depth (m)	Percent surface irradiance
0	100.0
10	54.9
20	30.1
30	16.5
40	9.1
50	5.0
60	2.7
70	1.5
80	0.8
90	0.5
100	0.2

Figure A.4 Calculated mean percent of surface irradiance at depth in waters offshore of the Florida Reef Tract (taken from Leichter et al. 2008).