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Carbon and nitrogen utilization in two species of Red Sea corals along a depth gradient: Insights from stable isotope analysis of total organic material and lipids

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Abstract

We examined the utilization of carbon and nitrogen in two common Red Sea coral species (*Stylophora pistillata* and *Favia favus*), differing in colony morphology and polyp size, along a depth gradient down to 60 m. We describe the changes in C/N ratios and in the stable isotope composition of carbon and nitrogen of coral's tissue and algal symbionts. We also measured the carbon isotopic composition of the lipid fraction extracted from both coral tissue and algal symbionts in order to reveal the changes in the carbon source utilized by the host coral for lipid synthesis.

The results show that for both species, $\delta^{13}C$ decreases by 7–8‰ in animal tissue, algal symbionts and in the lipid fractions as depth increases. However, in contrast to previous reports, the difference between $\delta^{13}C$ values of coral tissue and algal symbionts does not increase with depth. $\delta^{15}N$ values of coral tissue and algal symbionts in both species do not correlate with depth suggesting that the heterotrophic capacity of these corals does not increase with depth. $\delta^{13}C$ values of tissue lipids were depleted by an average of ~3.5‰ compared to $\delta^{13}C$ of the entire tissue at all depths. $\delta^{13}C$ values of algal lipids were depleted by an average of ~2‰ compared to $\delta^{13}C$ of the entire zooxanthellae at all depths, indicating high efficiency of carbon recycling between the two symbiotic partners along the entire gradient. The depletion of lipids is attributed to the fractionation mechanism during lipid synthesis. In addition, for both species, $\delta^{13}C$ values of algal lipids were enriched compared with $\delta^{13}C$ of tissue lipids. In *S. pistillata*, the difference between $\delta^{13}C$ values of tissue lipids and algal lipids increased linearly with depth, indicating a change in the sources of carbon utilized by the coral for lipid synthesis below 20 m from an autotrophic to a heterotrophic source. However, in *F. favus*, this average difference was ~4 times larger compared to shallow *S. pistillata* and was constant along the entire depth gradient, suggesting that *F. favus* uses heterotrophically-acquired carbon for lipid synthesis regardless of depth. Overall, *F. favus* exhibited enriched $\delta^{13}C$ and $\delta^{15}N$ values compared to *S. pistillata* along the entire gradient. We attribute these differences to both morphological differences (i.e. colony morphology, tissue thickness and polyp size) between the two species and to a higher heterotrophy/autotrophy ratio in *F. favus* at all depths. The C/N ratio in *S. pistillata* tissue decreased with increasing water depth whereas in *F. favus* it remained constant. This reflects a higher heterotrophic capacity in the large polyped *F. favus*, at all depths.

1. INTRODUCTION

Coral reefs extend down to 150 m, a depth which represents the lower boundary for photosynthetic primary production, the driving force behind hermatypic corals

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(Veron, 2000; Stambler and Dubinsky, 2005). The physical properties of the environment change gradually along such a bathymetric gradient (e.g. temperature, light intensity and spectrum, currents etc.), indicating a high degree of physiological and trophic diversity (Anthony and Fabricius, 2000).

Zooxanthellate corals are mixotrophic organisms which derive their energy from both photosynthates translocated from their symbiotic algae, commonly referred as zooxanthellae, and from a variety of external food sources (Goreau et al., 1971; Sebens et al., 1996; Ferrier-Pages et al., 1998). The two main extrinsic sources of carbon utilized by corals are dissolved inorganic carbon, which in the Gulf of Aqaba has a mean $\delta^{13}C$ of 0.45‰ (Mizrachi, 2008), and zooplankton, with a mean $\delta^{13}C$ of −21‰ (Lorian, 1991; Rosenfeld, 2004). Zooxanthellae inside the coral tissues utilize bicarbonate, rather than $CO_{2(aq)}$, as the primary source for photosynthesis (Goiran et al., 1996; Allemand et al., 1998; Furla et al., 2000).

In shallow water, up to 95% of the carbon fixed by the zooxanthellae during photosynthesis is translocated to the coral tissue, potentially satisfying its energetic needs for respiration and growth (Muscatine et al., 1984; Muscatine, 1990). Any excess energy is stored in the coral tissues as lipids (Patton et al., 1977; Patton and Burris, 1983) which represent the major energy reserves and can form up to 40% of the coral dry biomass (Ben-David Zaslow and Benayahu, 1999; Yamashiro et al., 1999). These reserves can then be utilized by the coral during energy deficiency periods (Yamashiro et al., 2005). It was postulated that coral lipids are mainly derived from carbon which was photosynthetically fixed by the symbiotic zooxanthellae (Patton et al., 1977; Oku et al., 2003). However, Grottoli et al. (2006) demonstrated that some coral species can maintain their lipid reservoirs through heterotrophic means during periods of bleaching as opposed to others which consume their lipids during this period.

Photosynthesis rates decrease significantly with depth, as light intensity decreases exponentially (Gattuso et al., 1993; Mass et al., 2007), forcing the coral host to acclimate in order to compensate for this reduction. The acclimation of corals to changes in light intensity involves a variety of phenotypic responses of both the symbiotic zooxanthellae and the coral host. However, photosynthesis is nonetheless lower in low-light intensities despite the fact that zooxanthellae in deeper water are well adapted to low-light intensities due to higher photosynthetic efficiency (Kaiser et al., 1993; Lesser et al., 2000; Titlyanov et al., 2001; Mass et al., 2007). This then possibly results in lower translocation of photosynthetically-fixed carbon to the coral host. The reduced contribution of photosynthetically-fixed carbon from the algal symbionts in deeper water may be partially compensated by increasing the carbon input from extrinsic sources. The ability of a coral species to change its autotrophic/heterotrophic ratio may give it an advantage by enabling it to extend its bathymetric distribution and also increase its resilience to bleaching events as demonstrated by Grottoli et al. (2006).

Relatively few studies examined the changes in the feeding rates of corals as a consequence of reduced photosynthetic performance due to natural conditions such as changes along bathymetric gradients (Grottoli, 2002; Palardy et al., 2005, 2008) or during bleaching (Grottoli et al., 2006).

Here, changes in the autotrophic/heterotrophic ratio were studied in two common coral species (*Stylophora pistillata* and *Favia favus*) differing in colony morphology and polyp size along a significant depth gradient down to 60 m. We analyzed the natural variability of carbon and nitrogen isotopes in zooxanthellae and host tissues and the natural abundance of carbon isotopes in the lipid fraction extracted from both coral tissue and zooxanthellae in order to define the carbon source utilized for lipid synthesis.

2. MATERIALS AND METHODS

2.1. Site description

The study site is located at the northern tip of the Gulf of Aqaba, Red Sea (29°30.05′N, 34°55′E). The average winter and summer temperatures range between 21 and 27 °C (Paldor and Anati, 1979). During summer the water column is stratified, leading to large differences in temperature along the bathymetric gradient (e.g. from 27 °C at the surface to 21 °C at 150 m). During winter the water column is well mixed down to 150 m, reaching temperatures around 21–22 °C (Genin et al., 1995). In the clear water of the Gulf, the penetration of solar irradiance in seawater is high, with a diffuse attenuation coefficient (K_d) of 0.0726 m^{-1} (Kuguru et al., 2007).

We chose two of the most common coral species in the Gulf of Aqaba as model organisms. *Stylophora pistillata* (Esper, 1797) which forms branching colonies with small (*ca.* 1 mm) polyps and *Favia favus* (Forskal, 1775) which forms massive colonies with large polyps (*ca.* 10 mm) (Veron, 2000). Both species are found along a relatively large bathymetric gradient down to at least 65 m and exhibit morphological changes along the gradient (Fig. 1).

2.2. Corals collection and processing

Stylophora pistillata nubbins and *F. favus* fragments were sampled during September 2007 along a depth gradient from 1 m down to 60 m depth using SCUBA (technical diving using TRIMIX gas for deep dives). The corals were frozen at −80 °C immediately after sampling for further analysis. Coral tissue was removed from the skeleton using an Air-brush system (PAASCHE) with 0.2 μm filtered sea water (FSW). The resulting slurry containing both coral tissue and algal symbionts was homogenized and the separation between host tissue and algal symbionts was done according to Muscatine et al. (1989). To remove all carbonates from the sample, algal pellets were then decalcified by adding 0.5 ml of HCl (1 N) until the slurry had stopped bubbling. The zooxanthellae were transferred to Eppendorf tubes and the coral tissue was concentrated and filtered on a pre-combusted GF/F filter (0.7 μm pore size) (Muscatine et al., 1989; Rosenfeld, 2004). All samples were washed with double-distilled water (DDW) in order to wash out the salts, and dried at 60 °C for further isotopic analysis.

Fig. 1. Shallow 3 m (left) and deep 60 m (right) colonies of *Favia favus* (A and B) and *Stylophora pistillata* (C and D).

2.3. Lipid extraction and preparation for isotopic analysis

Lipids were extracted from both coral tissue and algal symbionts according to Bligh and Dyer (1959). Briefly, to each 1 ml of homogenate, 3.75 ml of a pre-mix chloroform/methanol (1/2 v:v) were added. The samples were vortexed for 10 min and 1.25 ml of chloroform were added, followed by another minute of vortex and an addition of 1.25 ml DDW. The samples were then centrifuged at $155\times g$ for 5 min in order to separate the chloroform phase from the methanol phase. Following separation, the lower phase containing the lipids was carefully collected and transferred to glass vials. The chloroform was evaporated under N_2 atmosphere and the residual lipids were analyzed for carbon isotopic composition.

2.4. Stable isotope analyses

All samples including entire coral tissue (i.e. bulk tissue), algal symbionts (i.e. bulk zooxanthellae) and lipid fractions extracted from both, were analyzed using an Elemental Analyzer (Carlo Erba 1110) connected in-line to a Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS). For the bulk zooxanthellae (B_z), 200–300 μg of organic matter were used to measure C and N isotopes. Filters containing bulk coral tissue (B_t) were subsampled by cutting out a portion (1/16–1/2 according to the concentration of organic matter estimated from the filter's color). Lipid samples (L_z for zooxanthellae lipids and L_t for tissue lipids) were re-dissolved in 100–250 μl chloroform and 12 μl samples were transferred into tin cups. Clean tin cups filled with chloroform were used as control. The cups were heated at 60 °C for 40 min until full evaporation of the chloroform, and the remaining lipid samples were analyzed for carbon isotopes. The C/N ratios were calculated from the % carbon and nitrogen in each sample, calibrated by using Acetanilid.

All carbon measurements are reported in permil (‰) units relative to international Vienna-Peedee Belemnite Limestone standards (V-PDB). The nitrogen measurements are reported relative to atmospheric nitrogen standards. Internal standards were used to evaluate the analytical error. The precision of the measurements for $\delta^{13}C$ is 0.05‰ and for $\delta^{15}N$ is 0.2‰. All samples were run in duplicates except for lipids, which were run in triplicates and the average δ values are reported.

2.5. Statistical analyses

Normality and homogeneity of variances assumptions were verified using the Kolmogorov–Smirnov test and Cochran test, respectively. We used the homogeneity of slopes model in order to test whether depth has the same effect on both coral species. In cases that homogeneity of variances was achieved and no interaction was found between the continuous variable and the categorical variable, ANCOVA was applied. In order to test for possible differences between coral tissue, zooxanthellae and lipid fraction isotopic composition, a paired *t*-test was used when analyz-

ing dependent samples. Unless otherwise specified, mean values are presented ± SE.

3. RESULTS

3.1. $\delta^{13}C$ variability along the depth gradient

Bulk coral tissue revealed large variation in $\delta^{13}C$ values of both *S. pistillata* and *F. favus* along the depth gradient, ranging from a maximal value of −13.51‰ in shallow water to a minimal value of −20.54‰ in deep water and from −11.89‰ to −19.1‰, respectively (Fig. 2A and C). The significant depletion is evident in both species only below 15 m (Fig. 2A and C). $\delta^{13}C$ values of both coral species changed with depth at a similar rate (i.e. no difference between slopes, ANCOVA $p < 0.001$; $\beta = -0.128‰\ m^{-1}$; $r^2_{Stylophora} = 0.922$, $r^2_{Favia} = 0.861$). However, values of *S. pistillata* tissue were depleted by *ca.* 2.43‰ compared with *F. favus* along the entire gradient (Intercepts = −13.246, −10.815‰, respectively) (Fig. 2A and C). The difference between $\delta^{13}C$ values of coral tissue and algal symbionts was significant in *S. pistillata* (paired *t*-test, $p < 0.001$) but not in *F. favus* (paired *t*-test, $p > 0.05$).

3.2. $\delta^{13}C$ of the lipid fractions

Depth had a significant negative effect on $\delta^{13}C$ values of lipids extracted from both animal tissue and zooxanthellae in both coral species (Table 1). $\delta^{13}C$ of tissue lipids in *S. pistillata* ranged between a maximal value of −16.16‰ in the shallow to a minimal value of −24.09‰ at 60 m depth, while $\delta^{13}C$ of zooxanthellae lipids ranged between a maximal value of −15.54‰ and a minimal value of −22.04‰ along this depth range (Fig. 3A). $\delta^{13}C$ values of tissue lipids in *F. favus* ranged between a maximal value of −15.84‰ in the shallow to a minimal value of −22.55‰ at 60 m depth, while $\delta^{13}C$ of zooxanthellae lipids ranged between a maximal value of −13.44‰ and a minimal value of −21.23‰ along this depth range (Fig. 3B). $\delta^{13}C$ of the lipid fractions were significantly depleted compared with both whole tissue and zooxanthellae in both species (paired *t*-test, $p < 0.001$) (Fig. 3A and B). Lipids extracted from the tissue of *S. pistillata* were depleted by 3.63‰ ± 1.51 compared with bulk tissue, while lipids extracted from the algal symbionts were depleted by 2.82‰ ± 0.33 compared with bulk zooxanthellae. In *F. favus*, lipids extracted from the tissue were depleted by 3.43‰ ± 0.37 compared with bulk tissue, while

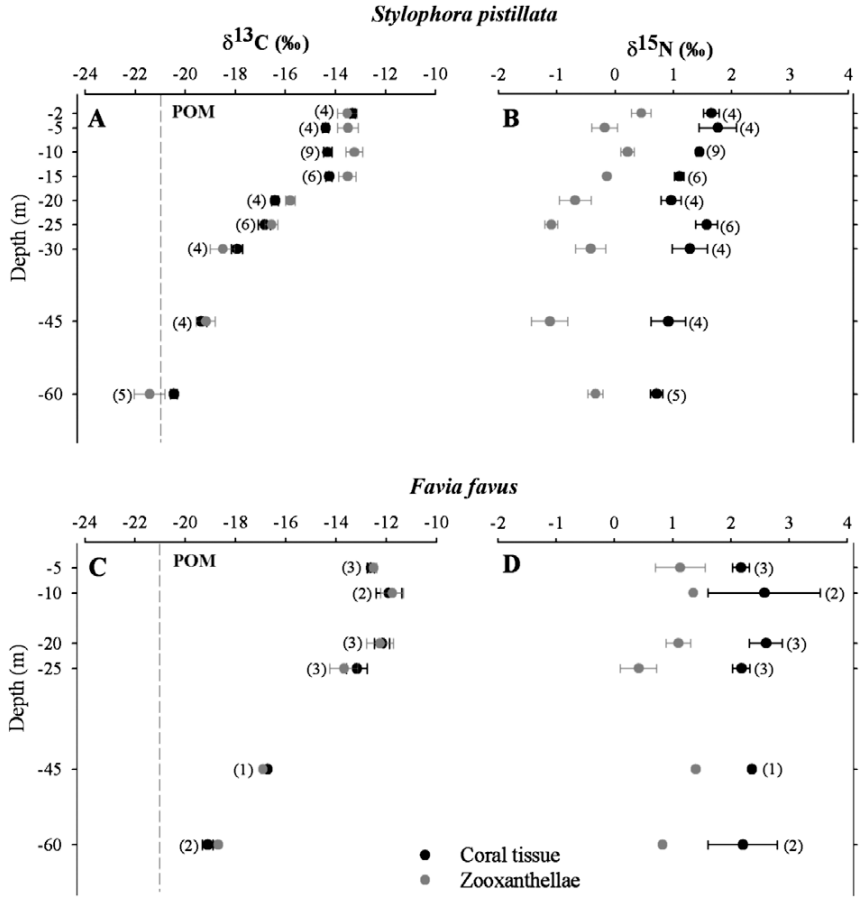

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ of *Stylophora pistillata* (A and B) and *Favia favus* (C and D) tissues (black) and zooxanthellae (grey) along a depth gradient from 2 to 60 m depth (average ± SE). Number of corals analyzed from each depth is indicated in parentheses. Dashed line represents the average isotopic value for particulate organic matter (POM).

Table 1
Regression coefficients for *Stylophora pistillata* and *Favia favus* ($\delta^{13}C$ of lipid fraction *vs.* depth).

	Stylophora pistillata		*Favia favus*	
	$\delta^{13}C_{Lt}$	$\delta^{13}C_{Lz}$	$\delta^{13}C_{Lt}$	$\delta^{13}C_{Lz}$
r^2	0.84	0.72	0.76	0.82
p Value	$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Slope (‰ $m^{-1)}$	−0.13	−0.08	−0.11	−0.13
Intercept (‰)	−17.43	−17.14	−15.93	−12.99

lipids extracted from the algal symbionts were depleted by 1.19‰ ± 0.25 compared with the zooxanthellae. In both species the lipids isolated from the zooxanthellae were enriched compared with the tissue lipids (paired *t*-test, $p < 0.005$) (Fig 3A and B). The difference between $\delta^{13}C_{Lz} - \delta^{13}C_{Lt}$ in *S. pistillata* significantly increased with depth ($r^2 = 0.44$, $p < 0.05$, slope of 0.04‰ m^{-1} and intercept of 0.29‰) (Fig. 4). In shallow water down to 20 m the difference was relatively small (0.49‰ ± 0.1), while from 30 m down it increased significantly to a value 2.48‰ ± 0.39. In *F. favus*, the average difference between lipid fraction of tissue and algal symbionts was ~4 times larger (2.23 ± 0.3 SD) compared with shallow *S. pistillata* and almost constant along the entire depth range (Fig. 3).

3.3. $\delta^{15}N$ variability along the depth gradient

Both coral species revealed a lower variability in $\delta^{15}N_{Bt}$ and $\delta^{15}N_{Bz}$ values along the depth gradient compared with $\delta^{13}C$ values (Fig. 2B and D). $\delta^{15}N_{Bt}$ *S. pistillata* was slightly affected by depth ($r^2 = 0.267$, $p < 0.001$, $\beta_{Stylophora\ t} = -0.014$), exhibiting values ranging from a maximum of 1.75‰ at 5 m to a minimum of 0.71‰ at 60 m (Fig. 2B). The $\delta^{15}N_{Bz}$ of *S. pistillata* were also negatively effected by water depth ($r^2 = 0.325$, $p < 0.001$, $\beta_{Stylophora\ z} = -0.023$) and exhibited values ranging from 0.8‰ at 2 m to −1.73‰ at 45 m.

In *F. favus* $\delta^{15}N_{Bt}$ values ranged from 3.54‰ to 1.61‰ while $\delta^{15}N_{Bz}$ presented lower values ranging from 2.04‰ to −0.2‰ (Fig. 2D). Neither tissue nor zooxanthellae values correlated with depth ($r^2 = 0.005$, $p > 0.05$, $\beta_{Favia\ t} = -0.002$ and $r^2 = 0.032$, $p > 0.05$, $\beta_{Favia\ z} = -0.006$, respectively). As seen for the $\delta^{13}C$ values, *S. pistillata* was depleted in ^{15}N along the entire gradient compared with *F. favus* (Fig. 2B and D).

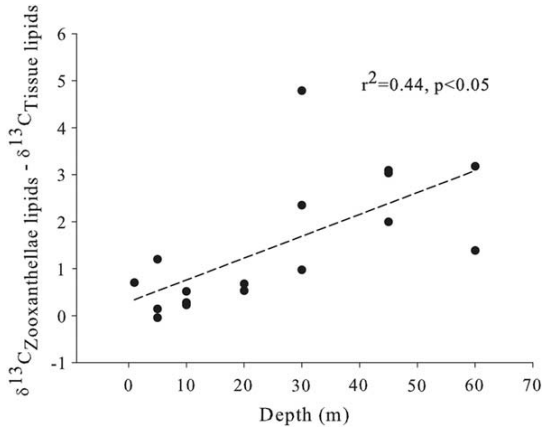

Fig. 4. Relationship between the differences in $\delta^{13}C$ values of lipid fractions in the coral tissue and zooxanthellae versus depth for *Stylophora pistillata*.

3.4. C/N ratio along the depth gradient

The carbon to nitrogen ratios (C/N) of *S. pistillata* tissue decreased significantly with depth ($r^2 = 0.862$, $p < 0.001$, $\beta_{Stylophora\ t} = -0.050$) from a maximal value of 8.49 at 2 m to a minimal value of 5.3 at 60 m depth (Fig. 5A). However, the C/N ratios of zooxanthellae isolated from *S. pistillata* were not affected by depth ($r^2 = 0.006$, $p > 0.05$, $\beta_{Stylophora\ z} = 0.003$) and had an average value of 7.18 ± 0.17 along the entire depth gradient (Fig. 5A). For *F. favus* the C/N ratios of both symbiotic zooxanthellae and coral tissue were not affected by depth ($r^2 = 0.192$, $p > 0.05$, $\beta_{Favia\ z} = -0.017$ and $r^2 = 0.002$, $p > 0.05$, $\beta_{Favia\ t} =$

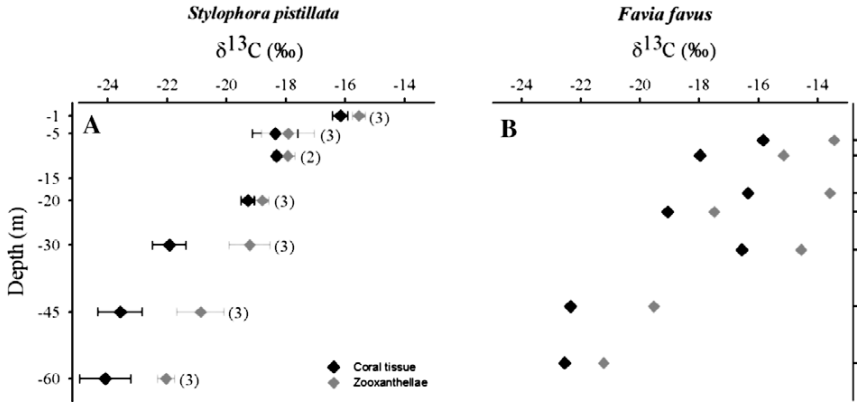

Fig. 3. $\delta^{13}C$ of the lipid fraction extracted from the coral tissue (black) and from the zooxanthellae (grey) of *Stylophora pistillata* and *Favia favus* (average ± SE). Number of corals analyzed from each depth is indicated in parentheses. For *Favia favus* only one coral was analyzed from each depth.

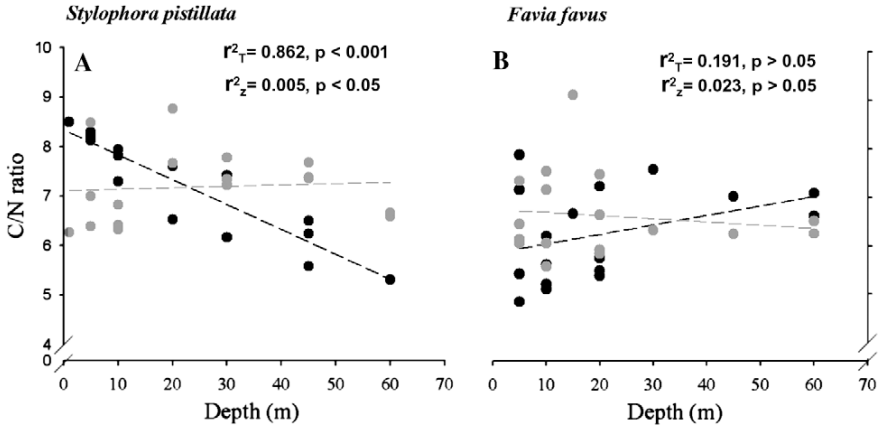

Fig. 5. C/N ratio of *Stylophora pistillata* and *Favia favus* tissue (black) and symbiotic algae (grey) along a bathymetric gradient 2–60 m depth. Dashed lines represent linear regression.

0.002, respectively) and had average values of 6.62 ± 0.19 and 6.24 ± 0.23, respectively (Fig. 5B).

4. DISCUSSION

4.1. Heterotrophy vs. autotrophy

Overall, $\delta^{13}C$ values of both coral species, including coral tissue, zooxanthellae (Fig. 2A and C) and lipid fractions (Fig. 3A and B), decrease with increasing depth and approach the $\delta^{13}C$ value of particulate organic matter (POM) at a depth of 60 m (i.e. *ca.* $-21‰$, Lorian, 1991). The observed trends in the bulk samples of these coral species from the Gulf of Aqaba generally agree with earlier findings for Caribbean corals (Land et al., 1975; Muscatine et al., 1989) and coral larvae in the Gulf of Aqaba (Alamaru et al., 2009). However, the depletion trend in the Gulf of Aqaba was noticed only between 15 and 60 m. At the upper most 15 m there is no significant change in the isotopic composition of carbon in the bulk tissue and zooxanthellae (Fig. 2A and C). This observation is also supported by measurements of photosynthesis rates which are almost constant in the upper 15 m (Winters et al., 2006; Mass et al., 2007). We suggest that the significant depletion from 15 m to 60 m is mainly due to a reduction in the rates of photosynthesis in the symbiotic zooxanthellae. Up until now, it was postulated that shallow water corals are autotrophic and that the $\delta^{13}C$ values of coral tissue and zooxanthellae are relatively similar. Thus, it was suggested (Land et al., 1975; Muscatine et al., 1989; Swart et al., 2005b), that with increasing depth, the difference between the $\delta^{13}C$ values of coral tissue and algal symbionts increases due to the higher heterotrophy of deep corals. However, our data indicates that the difference between $\delta^{13}C_{Bt}$ and $\delta^{13}C_{Bz}$ does not correlate with depth in the Gulf of Aqaba. This result may indicate that either there is no increase in heterotrophy with increasing depth and/or the increase in heterotrophy with depth is masked by a fast recycling of carbon between the animal host and the zooxanthellae (Einbinder et al., 2009). Bulk tissue and zooxanthellae $\delta^{15}N$ values enable to distinguish between the two hypotheses. A stepwise "trophic" enrichment in $\delta^{15}N$ values is expected as the heterotrophic capacity of corals increases. We did not observe a significant enrichment along the entire gradient which supports the hypothesis that heterotrophic rates do not increase significantly with depth (Fig. 2B and D). This later finding may imply that corals do not shift from almost exclusive autotrophy in shallow water to heterotrophy in the deep reef, in contrast to previous suggestions. The differences in the species morphology along the depth gradient also support this conclusion (Fig. 1). Coral colonies become more flat and the distance between polyps increases with increasing depth, resulting in a lower number of polyps per cm^2 (Nir, 2006; Einbinder et al., 2009). This adaptation maximizes the amount of light available to colonies rather than increasing their heterotrophic capabilities in deep water because less oral openings are found per cm^2. Furthermore, if the trophic effect (i.e. increased heterotrophy in deep water) was the correct explanation, it might have been expected that the difference between $\delta^{15}N$ values of coral tissue and algal symbionts will increase with depth, as a result of higher dependency of corals on zooplankton for their nutrition and higher fractionation by the algae due to lower photosynthesis rates (Muscatine et al., 1989; Muscatine and Kaplan, 1994; Swart et al., 2005a). Our results do not show such correlation between the differences in $\delta^{15}N$ values and depth. In addition, we did not find a decrease in the $\delta^{15}N$ values of zooxanthellae of both coral species with depth, indicating a tight recycling of nitrogen compounds between the coral host and its algal symbionts (Tanaka et al., 2006). This recycling possibly occurs via translocation of organic nitrogen through compounds with low C/N ratios from zooxanthellae to the coral host.

The $\delta^{15}N$ values obtained for both coral species are low in comparison with the values reported for diverse zooxanthellate coral species around the world (Muscatine et al., 2005). A possible explanation for these differences may be the higher rates of nitrogen fixation in these two Red Sea coral species (Shashar et al., 1994a,b) as $\delta^{15}N$ values of fixed N_2 approach $0‰$ as reported by Lesser et al. (2007)

for the Caribbean coral *Montastrea cavernosa* which is known to contain symbiotic nitrogen fixing bacteria in its tissues (Lesser et al., 2004).

We report here, for the first time, the $\delta^{13}C$ values of lipids extracted from both coral tissue and algal symbionts along the entire depth gradient. This compound specific approach revealed that $\delta^{13}C$ values of lipids extracted from the animal tissue and algal symbionts in both coral species decrease with depth (Fig. 3A and B and Table 1). The fact that the lipid fraction is significantly depleted in ^{13}C compared with bulk organic material is attributed to the carbon fractionation mechanism during the formation of acetyl-coenzyme A, the precursor for fatty acids biosynthesis (DeNiro and Epstein, 1977; Melzer and Schmidt, 1987; Swart et al., 2005b). The depletion of the lipid fractions along the depth gradient is correlated with the depletion of bulk tissue and zooxanthellae (Figs. 2 and 3). This correlation may be due to the depletion of the source material available for lipid synthesis, or to a depth dependent decrease in the rate of lipid synthesis. So far, no clear correlation has been reported between total lipid content of coral tissues and depth (Harland et al., 1993). However, it is reasonable to assume that deeper corals contain lower amounts of lipid reservoirs, due to the suggestion that lipid synthesis in corals is light dependent (Patton et al., 1977; Crossland et al., 1980; Oku et al., 2003). We suggest that lower rates of lipid synthesis in deep water may enhance the metabolic fractionation effect during lipid synthesis leading to even more depleted values compared to the source (Fig. 3A and B). Another possible explanation is that in deep water the source of carbon for lipid synthesis is mainly from heterotrophy, since the translocation of photosynthetic carbon is not sufficient to maintain the required lipid pool. Szmant et al. (1990) and Swart et al. (2005b) suggest that during periods characterized by low photosynthetic rates (i.e. autumn and winter) corals respire more lipids and a ^{13}C enrichment in coral tissues is expected. Correspondingly, since the photosynthetic rates of deep corals are relatively low (Mass et al., 2007) this suggestion may be applied also to deep corals. However, our results contradict this hypothesis as coral tissues become depleted with increasing depth (Fig. 2A and C).

The carbon to nitrogen ratios of *S. pistillata* tissue clearly decrease with increasing depth as opposed to *F. favus*, in which this ratio remains constant along the depth gradient (Fig. 5A and B). This decrease may reflect a reduction in the amounts of stored lipids in the coral tissue as C/N ratio is considered a good proxy for an organism's condition since it reflects the ratio of lipids and carbohydrates to proteins (Bodin et al., 2007). Furthermore, a strong correlation between % lipids and C/N ratio is reported for a range of marine and terrestrial animals (Bodin et al., 2007; Post et al., 2007). It may be that this small-polyped coral species (*S. pistillata*) is not able to compensate for the reduction in photosynthates translocated from its zooxanthellae by heterotrophic means. Depth had no significant effect on the C/N ratios of algal symbionts in both corals as opposed to the reduction seen in coral tissue (Fig. 5A and B). Our direct measurements of C/N ratios do not agree with the trends deduced from nitrogen uptake experiments by Muscatine et al. (1984) and Falkowski et al. (1984). They suggested that the C/N ratios of zooxanthellae in high-light environments will be three times higher than in shaded environments. Another possibility that should not be ruled out is that the decrease in % carbon as a result of lower photosynthesis rates is accompanied by a decline in % nitrogen.

Overall our results suggest that heterotrophy does not increase significantly with depth. The depletion in $\delta^{13}C$ values is presumably due to lower rates of photosynthesis which in-turn enhances the fractionation during carbon fixation as demonstrated by Swart et al. (2005b). The carbon source utilized for lipid synthesis is affected both by depth and by the coral species.

4.2. Comparison between coral species

Significant differences were found between the two corals species in both $\delta^{13}C$ and $\delta^{15}N$ values at all depths (Figs. 2 and 3). *Favia favus* exhibited enriched $\delta^{13}C$ and $\delta^{15}N$ values compared with *S. pistillata* along the entire depth gradient (Figs. 2 and 3). This result may be attributed to the morphological differences between the two genera. *Favia favus* is a "fleshy" (i.e. thick tissue) genus that forms massive colonies thus having a low surface to volume ratio (S/V) (Fig. 1A), while *S. pistillata* is characterized as a "skinny" (i.e. thin tissue) genus that forms branching colonies with high S/V ratio (Fig. 1C) (Veron, 2000; Loya et al., 2001). The differences at all parameters between the two species can be attributed to; (1) diffusion limitations of carbon species which reduce the fractionation and/or (2) differences in feeding rates between these corals. The complex structure of the coral colony may attenuate the diffusion by the thickness of the tissue, thereby lowering the discrimination against the heavier isotope (Mason et al., 1967). Hence, the branching *S. pistillata* which has a high S/V and a lower diffusion resistance exhibits more depleted ^{13}C values compared with *F. favus*. Also, the lack of differences between $\delta^{13}C$ values of coral tissue and zooxanthellae in *F. favus* may also be a consequence of higher diffusion limitations in this species that in-turn lead to greater recycling of carbon between the host and its algal symbionts. The fact that *F. favus* tissue displays enriched $\delta^{15}N$ values compared with *S. pistillata* supports our hypothesis that this species is characterized by higher feeding rates regardless of depth, in accordance with the use of S/V as an indicator to the heterotrophic capacity of corals (Palardy et al., 2008).

The sources of carbon utilized by the corals for lipid synthesis differ between the two species. In *S. pistillata* the difference between $\delta^{13}C$ values of zooxanthellae lipids and tissue lipids increases by almost four-fold with depth (Fig. 4A). This difference indicates that below 15 m lipid synthesis in coral tissue is primarily based upon heterotrophically-acquired carbon, which is ^{13}C depleted compared with autotrophically-acquired carbon. This conclusion is also supported by the fact that in shallow water lipids are directly translocated from the algal symbionts to the coral host (Patton et al., 1977; Patton and Burris, 1983; Treignier et al., 2008). However, in deep water translocation is limited to water soluble products of photosynthesis. Moreover, this

increasing difference from a depth of 20 m agrees with the sudden depletion in ^{13}C seen in bulk samples from a depth of *ca.* 20 m (Fig. 2A and C). In *F. favus* the difference between algal lipids and tissue lipids is ~4 times larger compared with shallow *S. pistillata* and does not change with increasing depth, suggesting that the carbon source for lipid synthesis originates from heterotrophy along the entire gradient and also that the rate of heterotrophy is not depth dependent. These findings, together with the fact that *F. favus* exhibited enriched $\delta^{15}N$ values compared with *S. pistillata* (Fig. 2B and D), indicate that *F. favus* is characterized by higher heterotrophic/autotrophic ratios compared with *S. pistillata*.

The contribution of zooxanthellae photosynthates to the overall coral carbon budget decreases significantly with decreasing light levels. The same reduction occurs during bleaching events, when the symbiotic zooxanthellae are expelled from the coral tissue. Therefore, the physiological state of deep corals may be comparable to that of bleached ones. The mechanisms enabling deep Faviid corals to cope with extreme light conditions by exploiting extrinsic nutritional resources may be the same as the mechanisms which enable Faviids to survive bleaching events (Loya et al., 2001; Winters et al., 2006). *Stylophora pistillata*, on the other hand, is known to be sensitive to bleaching and usually dies following a bleaching event (Loya et al., 2001; Winters et al., 2006). This may emphasize the importance, from an energetic point of view, of the symbiotic algae and the autotrophic carbon translocated to the host coral.

5. CONCLUSIONS

The tight coupling between $\delta^{13}C$ values of coral tissue and algal symbionts (both total material and lipid fractions) along a large bathymetric gradient, down to 60 m, suggests a fast recycling of carbon between the coral host and its algal symbionts. For both corals, an isotopic depletion in $\delta^{13}C$ values starts only at depths greater then 15 m. Lipids isolated from both coral tissue and zooxanthellae were found to be significantly depleted compared with the bulk material, in accordance with existing data for other taxa. A decrease in C/N ratio in *S. pistillata* tissue but not in its zooxanthellae with increasing depth may result from a decrease in the lipid reservoirs in the coral tissue. In *F. favus*, however, the C/N ratio does not change with depth suggesting maintenance of stable lipid reservoirs. The carbon source used by the small-polyped coral, *S. pistillata*, for lipid synthesis changes along the bathymetric gradient from an autotrophic source in the upper 20 m to a heterotrophic source bellow 30 m. In contrast, the large polyped coral, *Favis favus*, uses heterotrophic carbon for lipid synthesis regardless of depth.

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