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Stable isotopes reveal limitations in C and N assimilation in the Caribbean reef corals *Madracis auretenra*, *M. carmabi* and *M. formosa*

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ABSTRACT: We used tissue $\delta^{15}N$ and $\delta^{13}C$ to elucidate nitrogen and carbon utilization in 3 branching coral species of the genus *Madracis*. Coral branches were sampled between 5 and 47 m water depth, and tissue was sub-sampled from the tips and sides of branches. Photosynthetically active radiation (PAR) ranged between 1 and 71% of surface PAR. There was a good correlation between $\delta^{15}N$ and $\delta^{13}C$, and these had similar ranges of 5.6 and 5.2‰, respectively, suggesting that light-driven photosynthesis is a strong factor in isotope fractionation. However, at shallow depths, $\delta^{13}C$ decreased more strongly while $\delta^{15}N$ remained constant, while at deeper depths $\delta^{15}N$ decreased while $\delta^{13}C$ remained constant. Comparisons of $\delta^{13}C$ in polyps and zooxanthellae revealed that prey capture at the tips of branches was more pronounced than at the corresponding sideward-facing positions and that corals at deeper depths did not increase their food uptake to compensate for a decrease in photosynthetic carbon assimilation. This indicates that foraging in branching *Madracis* depends on current regimes and prey encounter rather than selective food capture. The systematic analysis of tissue $\delta^{15}N$ along a steep depth gradient revealed that the shallow-water coral *M. auretenra* was nitrogen limited throughout its entire depth range.

KEY WORDS: Stable isotopes · Nitrogen limitation · Carbon · Heterotrophy · Scleractinian coral

INTRODUCTION

Tropical scleractinian reef corals are extremely well adapted to oligotrophic conditions through their harbouring of endosymbiotic dinoflagellates (zooxanthellae) of the genus *Symbiodinium*. The algal symbionts contribute significantly to the energy demand of their host and translocate up to 95% of photosynthetic products to the coral animal (Muscatine 1990). In shallow-water corals, this mechanism is considered sufficient to meet the daily carbon demand for animal respiration and growth (Davies 1984, McCloskey & Muscatine 1984). In deeper depths, zooxanthellae adapt to lower light by increasing their relative photosynthetic efficiency (Kaiser et al. 1993, Lesser et al. 2000, Titlyanov et al. 2001). Less photosynthetically fixed carbon is supplied to the animal part of the coral, but the reduced contribution of photosynthetic carbon at increasing water depth can partially be compensated by increasing the allochthonous nutrient supply (McCloskey & Muscatine 1984, Ferrier-Pagès et al. 1998, Titlyanov et al. 2000).

We used tissue $\delta^{15}N$ and $\delta^{13}C$ to investigate how 3 branching coral species of the genus *Madracis* at reefs of the Caribbean island Curaçao (Netherlands Antilles) cope with decreasing light regimes. On the reefs of Curaçao, each of the 3 species shows a strict zonation pattern and one species successively replaces the other at increasing water depth (Vermeij & Bak 2003). *Madracis auretenra* (Locke, Weil and Coates 2007),

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also described as *Madracis mirabilis* sensu Wells, 1973, is abundant at shallow depths between 2 and 25 m, *M. carmabi* Vermeij et al., 2003 at intermediate depths of 20 to 40 m, and *M. formosa* Wells, 1973 is confined to depths between 30 and 60 m. Photoadaptation has its limits, and absolute photosynthetic rates are lower in deep corals than in shallow ones. We therefore investigate, by means of tissue $\delta^{13}C$ and $\delta^{15}N$ analyses, whether deep corals indeed compensate for the inevitable energy loss by increasing their heterotrophic food supply. Tissue $\delta^{15}N$ and $\delta^{13}C$ are good indicators for trophic relationships and metabolic functioning (DeNiro & Epstein 1978, DeNiro & Epstein 1981, Gannes et al. 1998, Alamaru et al. 2009a,b). The separate analysis of $\delta^{13}C$ of endosymbionts and the host organism can indicate the shared portion of metabolic energy (Muscatine et al. 1989). Also, relatively low values of $\delta^{15}N$ in primary producers can hint at nitrogen fixation (Yamamuro et al. 1995, France et al. 1998). The application of $\delta^{15}N$ analysis has considerably increased our knowledge of food webs. With each trophic level, heterotrophic organisms are enriched in $\delta^{15}N$ by an average of 3.4 ± 1.1‰ (Minagawa & Wada 1984). We systematically sampled tissue $\delta^{13}C$ and $\delta^{15}N$ over a steep depth and light gradient to elucidate patterns of carbon and nitrogen assimilation, heterotrophic feeding and resource partitioning between zooxanthellae and cnidarian hosts of branching *Madracis* spp. We further propose that combining and extending existing models of C and N isotope fractionation can reveal a coral's 'strategy' of investing in either enhanced tissue or skeletal growth.

MATERIALS AND METHODS

Study site, sampling and sample treatment

A total of 42 colonies of *Madracis* spp. were subsampled in June 2002 on the leeward coast of Curaçao, Netherlands Antilles (12° 12′ N, 68° 56′ W). Pairs of tissue samples were taken from the upward facing tip (0°) and the sideward-facing (90°) position of a branch from each colony (see Table 1). Coral tissue was removed with a water-pik, using about 200 ml of filtered seawater (Millipore, pore size 0.22 µm). The tissue slurry was passed through a 20 µm mesh gauze to remove skeleton parts and other debris. Comparisons of $\delta^{13}C$ analyses with acidified and non-acidified aliquots proved that the 20 µm net was sufficient to remove all carbonate (data not shown), and acid-treatment prior to stable isotope analyses was consequently omitted. Half of the slurry was filtered on precombusted Whatman GF/F glass-fibre filters using low pressure. The remaining part was homogenised (Ultra-Turrax® T50) for 5 to 10 min to release zooxanthellae from the animal tissue. The homogenate was repeatedly centrifuged (2000 × *g*) and washed to separate the zooxanthellae from animal tissue (Muscatine et al. 1989). Random microscopic inspections revealed high separation efficiency for the 2 types of material. The supernatant containing animal tissue was filtered on GF/F filters. The pellet containing zooxanthellae was re-suspended in filtered seawater and also filtered on GF/F filters. The GF/F filters were dried for at least 24 h in an oven at 60°C and stored for later analyses of tissue stable isotopes.

$\delta^{15}N$ and $\delta^{13}C$ of particulate organic matter (POM) in seawater from intermediate depths (about 25 m) was filtered onto a GF/F filter and processed in a similar manner to the coral samples. To measure $\delta^{13}C$ of dissolved inorganic carbon (DIC) in seawater we took water samples (30 ml each) at 4 depths (5, 15, 25, 40 m) between April 16 and June 26, 2001 at at least biweekly intervals (N = 9 at each depth). Directly after sampling, $HgCl_2$ was injected, and bottles were sealed (air-tight) and stored at 4°C until analysis of $\delta^{13}C$.

Photosynthetically active radiation

At the study site, photosynthetically active radiation (PAR) with a wavelength of 400 to 700 nm was determined during 16 days in May 2001 using a LI-192SA underwater quantum sensor (LI-COR) connected to a LI-1000 data logger (LI-COR). PAR of upward-facing, horizontal positions (0°) was determined at intervals of 1 m to a maximum depth of 40 m. For PAR of the sideward-facing, vertical position (90°), we repeatedly measured at 4 distinct depths (10, 15, 25 and 35 m) and used the average PAR relative to the upward-facing position (see Table 1).

Isotopic analyses

Isotopic data are presented as $\delta^{15}N$ and $\delta^{13}C$ in ‰ notation with δX (‰) = $[(R_{sample} \times R_{standard}) - 1] \times 1000$, where X = ^{15}N or ^{13}C, and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. The $\delta^{15}N$ and $\delta^{13}C$ of coral holobiont and POM were analysed in parallel and in triplicate with a Finnigan MAT Delta Plus mass spectrometer after being combusted at 1050°C in a NC 2500 Elemental Analyser (CE Instruments). $\delta^{15}N$ is reported relative to air N_2. We used pure N_2 (99.996 %) as a laboratory working standard gas and IAEA standards N-1 and N-2. $\delta^{13}C$ is reported relative to Vienna PDB (VPDB). Sucrose and polyethylene foil (PEF) were used as standards for $\delta^{13}C$. The analytical precision for both $\delta^{15}N$ and $\delta^{13}C$ based on repeated measurements of a labora-

tory sediment standard (WST2) was <0.26‰ ± 1 SD (N = 143). Samples of separate cnidarian host and zooxanthellae fractions were analysed for $\delta^{13}C$ with benzoic acid as the $\delta^{13}C$ standard. The analytical precision for $\delta^{13}C$ was <0.15‰ ± 1 SD (N = 27) based on repeated measurements of a laboratory standard (acetanalide). Sampled material was insufficient to also analyse $\delta^{15}N$ of separate cnidarian host and zooxanthellae.

Statistical analyses

Statistical analyses were conducted using the Windows software package Statistica 7. For each coral colony, the tip and side positions were sampled separately thus resulting in a pair of samples from the tip and side for a single branch. Also, the $\delta^{13}C$ of polyp and zooxanthellae was derived from corresponding samples. For corresponding sample pairs, we used the paired *t*-test for the statistical comparison of the bulk $\delta^{15}N$ and $\delta^{13}C$ of the tip and side position or $\delta^{13}C$ of polyp and zooxanthellae. We also tested the mean differences of sample pairs for the 6 depths against zero. This provided information on whether or not the differences between paired samples are consistently (either negatively or positively) offset from each other throughout sampling depths.

To test the combined effects of depth and colony position, a 2-way ANOVA was conducted for each data set with bulk $\delta^{15}N$, bulk $\delta^{13}C$, $\delta^{13}C$ of polyp and $\delta^{13}C$ of zooxanthellae. For all tests, the chosen significance level was $p < 0.05$.

RESULTS

Light, seawater DIC and POM at the study site

Dependent on depth and colony position, PAR varied between 1 and 71% of surface PAR (Table 1). The average mid-day surface PAR was 817 µE m^{-2} s^{-1}. Changes in insulation and cloud cover between sampling days accounted for a highly variable PAR with an average coefficient of variance (CV) of 34.7% ± 0.75 SE. This variability was independent of depth or colony position ($r^2 = 0.033$, $p = 0.265$, $n = 40$). Exponential regression functions for PAR at upward (PAR[0°]) and sideward (PAR[90°]) positions as a function of depth were $PAR(0°) = 817.1\ \mu E\ m^{-2}\ s^{-1} \times e^{-0.068 \times depth}$ ($r = 0.985$, $p \ll 0.001$) and $PAR(90°) = 93.7\ \mu E\ m^{-2}\ s^{-1} \times e^{-0.052 \times depth}$ ($r = 0.918$, $p = 0.041$). This means that the attenuation coefficient k' for the upward and sideward positions was −0.068 and −0.052, respectively. Stable isotope signatures of POM sampled from the intermediate depth ranged between 3.9 and 4.8‰ and 20.1 and 20.3‰ for $\delta^{15}N$ and $\delta^{13}C$ (N = 3, each), respectively. Dissolved inorganic carbon in seawater at this site was on average 1.32‰ ± 0.02 (N = 36) and was independent of water depth or season (Maier 2004).

Holobiont tissue $\delta^{13}C$ and $\delta^{15}N$

The stable isotope values from the coral holobiont ranged from 0.4 to 6.0‰ for $\delta^{15}N$ and from −22.1 to

Table 1. *Madracis auretenra*, *M. carmabi*, *M. formosa*. Sampling regimes, light characteristics and tissue isotope mean and SE values. Samples taken from the tip of a colony were horizontal (angle of 0°) and the side positions were vertical (90°). Depth is the sampling depth and the numbers in brackets give the theoretical depth for side positions at which same light intensity would occur relative to tip position. $\delta^{15}N$: holobiont $\delta^{15}N$; H-$\delta^{13}C$: holobiont $\delta^{13}C$; P-$\delta^{13}C$: host polyp $\delta^{13}C$; Z-$\delta^{13}C$: zooxanthellae $\delta^{13}C$; N: number of colonies investigated

Species	N	Depth (m)	Branch position	PAR µE m^{-2} s^{-1}	PAR (%)	$\delta^{15}N$ (‰)	H-$\delta^{13}C$ (‰)	P-$\delta^{13}C$ (‰)	Z-$\delta^{13}C$ (‰)
M. auretenra	6	5	Tip	581.0	71.1	5.1 ± 0.1	–17.1 ± 0.1	–17.1 ± 0.1	–16.7 ± 0.2
	6	5 (35)	Side	72.4	8.9	4.5 ± 0.2	–17.7 ± 0.2	–17.4 ± 0.1	–17.3 ± 0.1
	8	10	Tip	413.1	50.6	4.7 ± 0.2	–17.7 ± 0.2	–17.8 ± 0.1	–17.1 ± 0.2
	8	10 (39)	Side	55.9	6.8	4.3 ± 0.3	–18.0 ± 0.3	–17.7 ± 0.2	–17.5 ± 0.2
	7	20	Tip	208.9	25.6	4.9 ± 0.1	–18.5 ± 0.3	–18.5 ± 0.2	–18.0 ± 0.2
	7	20 (47)	Side	33.3	4.1	4.4 ± 0.2	–19.5 ± 0.4	–19.2 ± 0.3	–19.6 ± 0.3
M. carmabi	6	30	Tip	105.6	12.9	3.4 ± 0.3	–19.6 ± 0.3	–19.1 ± 0.2	–19.0 ± 0.2
	6	30 (55)	Side	19.9	2.4	3.0 ± 0.4	–20.2 ± 0.2	–20.0 ± 0.3	–20.0 ± 0.3
M. formosa	10	40	Tip	53.4	6.5	3.3 ± 0.1	–19.7 ± 0.2	–19.6 ± 0.2	–19.1 ± 0.2
	10	40 (62)	Side	11.8	1.4	2.2 ± 0.2	–20.8 ± 0.3	–20.2 ± 0.3	–20.1 ± 0.3
	5	47	Tip	33.1	4.1	2.9 ± 0.2	–20.1 ± 0.4	–19.7 ± 0.3	–19.2 ± 0.3
	5	47 (67)	Side	8.2	1.0	1.6 ± 0.4	–20.4 ± 0.2	–20.0 ± 0.2	–20.2 ± 0.2
All samples	94	–	–	–	–	3.7 ± 0.1	–19.1 ± 0.2	–18.9 ± 0.2	–1.7 ± 0.2

–16.9‰ for $\delta^{13}C$. The $\delta^{15}N$ and $\delta^{13}C$ values had an almost similar isotopic range with 5.6‰ and 5.2‰, respectively. Furthermore, $\delta^{15}N$ and $\delta^{13}C$ were significantly and positively correlated to each other, ($R = 0.760$, $p \ll 0.001$, $N = 82$) and each decreased significantly with water depth ($R = -0.782$ for $\delta^{15}N$ and $R = -0.816$ for $\delta^{13}C$; with each $N = 84$ and $p << 0.001$). Despite the good overall correlation between $\delta^{15}N$ and $\delta^{13}C$, correlations were weaker if corals from shallow (5 to 20 m, $R = 0.327$, $p = 0.033$, $N = 43$) or deep (30 to 47 m, $R = 0.520$, $p = 0.001$, $N = 39$) were considered separately (Fig. 1).

$\delta^{13}C$ in zooxanthellae and cnidarian host

The average $\delta^{13}C$ values of polyp tissue and zooxanthellae were –18.9‰ ± 0.1 and –18.6‰ ± 0.2, respectively. The difference between polyp and zooxanthellae averaged 0.24‰ and was highly significant (paired *t*-test, $t = 5.08$, $p << 0.001$, $N = 83$). Paired *t*-tests between zooxanthellae and cnidarian $\delta^{13}C$ at respective depths and colony positions revealed significant differences at all depths except at 30 m for the colony tips, whereas at side position zooxanthellae and host tissue at 10 and 20 m depth were significantly different (Fig. 2). At the colony tips, $\delta^{13}C$ values of zooxanthellae were consistently heavier than corresponding values of the polyp tissue. This difference was consistent and significant at tips (test of means against zero, $p = 0.002$, $N = 6$), while there was no consistent difference between zooxanthellae and host $\delta^{13}C$ at side positions (test of means against zero, $p = 0.721$, $N = 6$). The difference between zooxanthellae and polyp $\delta^{13}C$ was more pronounced for $\delta^{13}C$ of tips of the shallow water coral *Madracis auretenra* with a significant mean difference of 0.58‰ ± 0.06 ($t = 10.5$, $p \ll 0.001$, $N = 20$) than for the tip position of the deeper colonies with a difference of 0.37‰ ± 0.09 ($t = 3.9$, $p < 0.001$, $N = 21$). However, the differences between zooxanthellae and polyp $\delta^{13}C$ at the tip of a colony showed no significant depth effect (1-way ANOVA, $F(5,35) = 1.815$, $p = 0.135$).

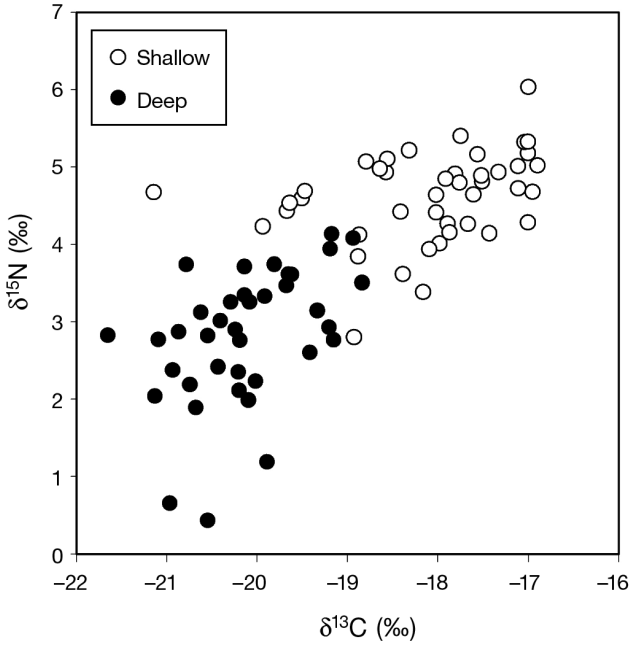

Fig. 1. *Madracis* spp. Correlation between $\delta^{15}N$ and $\delta^{13}C$ in holobiont tissue with $\delta^{15}N = 16.7 + 0.69 \times \delta^{13}C$ ($r = 0.769$, $p < 0.0001$, $N = 84$). (○) shallow (5 to 20 m), (●) deeper (30 to 47 m) water corals

Effect of depth and colony position

Depth and colony position had a significant effect on stable isotope fractionation (2-way ANOVA, always $p \ll 0.001$, $N = 42$). Paired sample *t*-tests of tissue isotopes from tip and side position at distinct depths were also performed. At each depth, the $\delta^{13}C$ values of zooxanthellae were significantly higher in tip than in side positions ($p < 0.05$), whereas $\delta^{13}C$ of cnidarian host tissue was only significantly different at 20, 30 and 40 m (Fig. 2). For holobiont tissue, the paired *t*-test revealed significant differences between colony positions for $\delta^{13}C$ at 5, 20, 30 and 40 m and for $\delta^{15}N$ at 20, 40 and 47 m (Fig. 3). Generally, the tissue stable isotope signals were heavier at the tip (0°) than at the side (90°) of a colony. The mean differences between tip and side were significant for $\delta^{15}N$ and $\delta^{13}C$ of holobiont and zooxanthellae (test of means against zero, $p < 0.01$, $N = 6$), but not for $\delta^{13}C$ of polyp host (test of means against zero, $p = 0.07$, $N = 6$).

DISCUSSION

Stable isotope models

In general, tissue stable isotopes were more depleted at deeper water depths regardless of tissue type or colony position, a pattern which generally substantiates earlier findings on photosynthetically driven isotope fractionation for both $\delta^{15}N$ (Muscatine & Kaplan 1994, Heikoop et al. 1998) and $\delta^{13}C$ (Land et al. 1975, Muscatine et al. 1989). Our data further indicate dissolved inorganic nitrogen (DIN) limitation for shallow water corals and a consistently more pronounced heterotrophic signature in the tips of branches compared to the sides. Contrary to earlier studies (McCloskey & Muscatine 1984, Ferrier-Pagès et al. 1998, Titlyanov et al. 2000), our results do not show that lower photosyn-

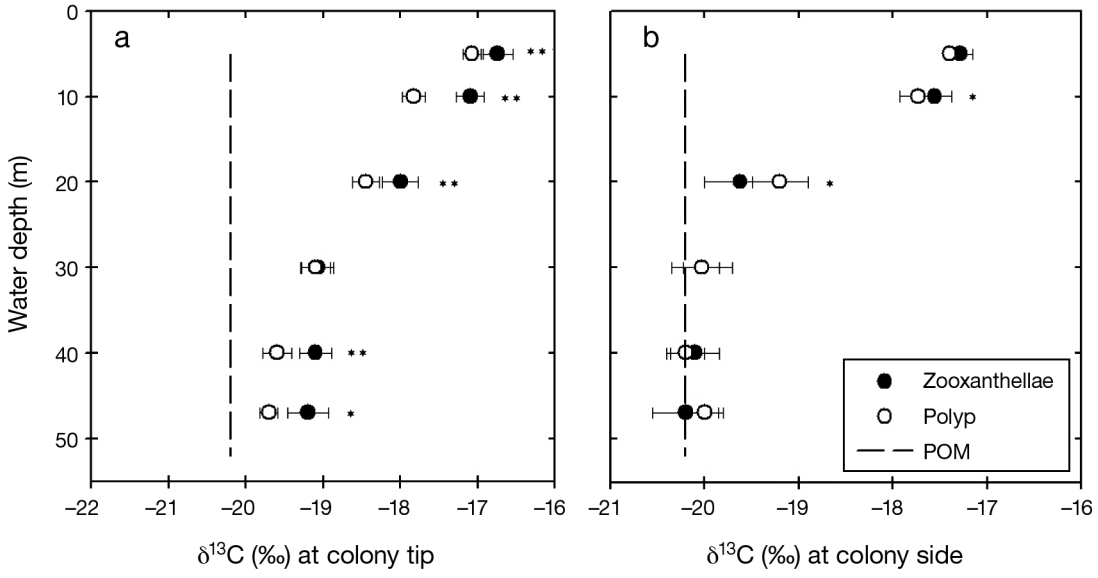

Fig. 2. *Madracis* spp. $\delta^{13}C$ of zooxanthellae and host polyp for (a) tip and (b) side of colony positions versus water depth. Error bars are SE of mean values. Vertical dashed line gives estimate for $\delta^{13}C$ of POM. $^{*}p < 0.05$ and $^{**}p < 0.005$ (paired *t*-test for tips and side of colony positions)

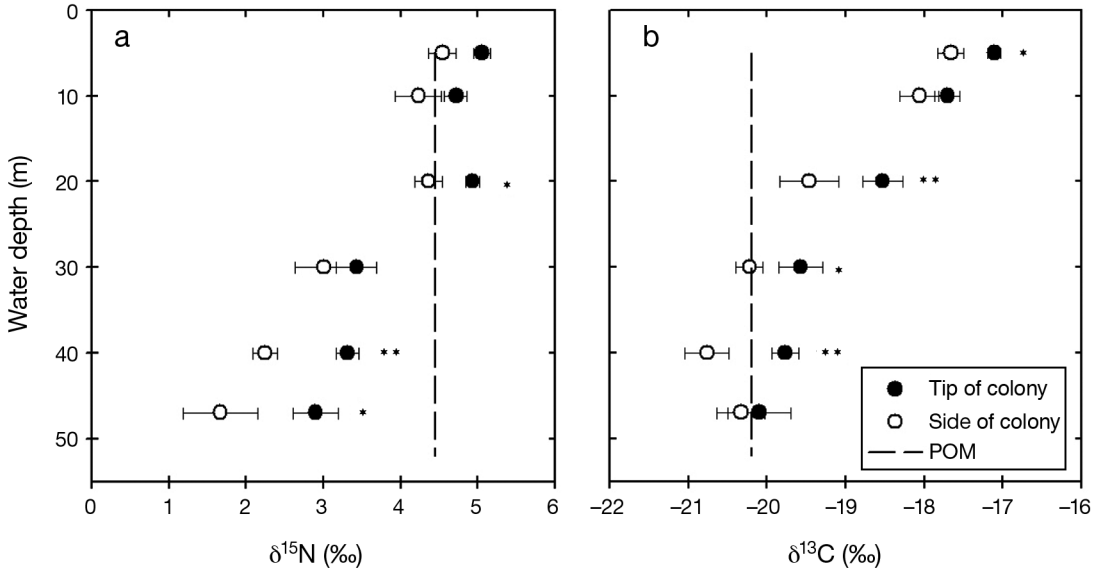

Fig. 3. *Madracis* spp. (a) Mean $\delta^{15}N$ and (b) $\delta^{13}C$ of holobiont tissue at the side and tip of branches versus water depth. Error bars are SE of mean values. Vertical dashed line gives estimate for $\delta^{15}N$ and $\delta^{13}C$ of POM. $^{*}p < 0.05$ and $^{**}p < 0.005$ (paired *t*-test for tips and side of colony positions)

thetic performance at deeper depths is compensated for by an increase in heterotrophy.

We combined several models on carbon acquisition (Furla et al. 2000a), carbon isotope fractionation (Muscatine et al. 1989, Reynaud et al. 2002), nitrogen flux and isotope fractionation (Heikoop et al. 1998) to elucidate physiological constraints in the branching coral *Madracis* spp. (Fig. 4). Stable isotope fractionation occurs during chemical reactions where lighter isotopes usually form weaker chemical bonds and react faster than the heavier isotopes. The isotopic signature in biological systems depends on the isotope ratio of source molecules, enzymes involved in reactions, reaction kinetics and physical constraints that control the replenishment of source molecules at the site of (bio-) chemical reactions (e.g. Urey 1947, Owens 1988, Laj-

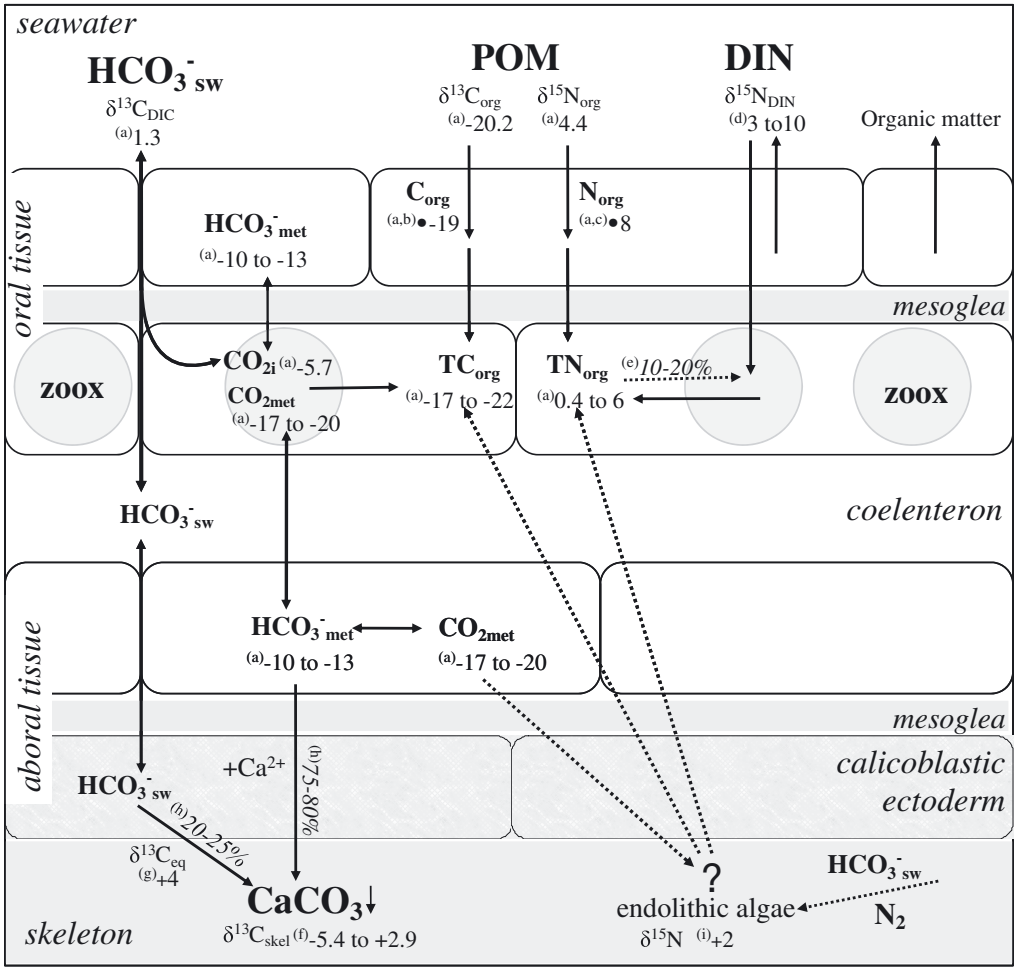

Fig. 4. *Madracis* spp. Model of pathways and fluxes of carbon and nitrogen contributing to stable isotope fractionation and composition of total organic carbon and nitrogen (TC_{org} and TN_{org}) as measured in the tissues of branching *Madracis* spp. The model was combined and modified from models for carbon acquisition (Furla et al. 2000a), carbon isotope fractionation (Muscatine et al. 1989) and nitrogen flux and isotope fractionation (Heikoop et al. 1998). Numbers with letters in superscript are estimates for $\delta^{15}N$ or $\delta^{13}C$ (‰). The values were approximated from various sources as follows: (a) this study with average $\delta^{13}C$ seawater DIC ($\delta^{13}C_{DIC}$), seawater bicarbonate ($HCO_{3\,SW}^-$), metabolic CO_2 (CO_{2met}), which is similar to $\delta^{13}C$ of animal tissue (Muscatine et al. 1989), while metabolic bicarbonate ($HCO_{3\,met}^-$) is enriched by ~7‰ relative to CO_{2met} and vice versa. The same fractionation factor of 7‰ was applied between $\delta^{13}C_{DIC}$ and inorganic CO_2 ($CO_{2\,i}$), which is taken up by zooxanthellae (zoox). (b) $\delta^{13}C$ of organic carbon (C_{org}) is about 1‰ enriched to prey (DeNiro & Epstein 1978). In our study, we assume that POM is the major allochthonous food source. (c) Enrichment of organic $\delta^{15}N$ ($\delta^{15}N_{org}$) by heterotrophic feeding (N_{org}) by an average 3.4‰ (Minagawa & Wada 1984). (d) Likely range of $\delta^{15}N$ of seawater DIN ($\delta^{15}N_{DIN}$) (Heikoop et al. 1998). (e) Maximum of 20% of assimilated prey nitrogen has been shown to appear in endosymbiotic zooxanthellae (Piniak et al. 2003). (f) Range in skeletal $\delta^{13}C$ ($\delta^{13}C_{skel}$) for branching *Madracis* spp. with a depth range similar to colonies of this study (Maier et al. 2003). (g) Equilibrium ^{13}C fractionation ($\delta^{13}C$) between aragonite and bicarbonate (Romanek et al. 1992). (h) Suggested contribution to skeletal carbon of 75 to 80% of metabolic carbon and 20 to 25% of seawater bicarbonate (Erez 1978, Furla et al. 2000a). (i) Nitrogen fixers, such as endolithic algae, have an $\delta^{15}N$ value of ~2‰ (France et al. 1998). A transfer of photoassimilates from endolithic algae to coral tissue (dashed arrows) may be of significance for coral metabolism in extreme situations such as in very low light environments or in bleached corals (Schlichter et al. 1995, Fine & Loya 2002). The '?' indicates that it is uncertain if endolithic algae utilize CO_2 respired by corals. Dotted line indicates optional contributions/pathways

tha & Michener 1994). A reef-building coral consists of several micro-environments with an oral and aboral layer of an ecto- and endoderm, which is separated by the mesoglea and the coelenteron. The gastroderm contains photosynthetic algal symbionts and the aboral ectodermal layer forms the calicoblast, where the aragonite exoskeleton is excreted. The oral side is exposed to surrounding seawater, while at the aboral side the calicoblast layer is shielded from ambient seawater by the skeleton (Fig. 4). Consequently, tissue isotope frac-

tionation depends on reactions taking place within these micro-environments, the replenishment with source C and N, as well as the translocation of C and N from and to respective sites for biochemical reactions.

At the study site, the $\delta^{13}C$ of external seawater DIC averaged 1.3‰ and temperature was 26.5°C (299.5 K) (Maier et al. 2003). The cellular CO_2, which is derived from ambient seawater DIC, would thus have a $\delta^{13}C$ of −7.45‰ according to the equation $\delta^{13}C_{CO2} = \delta^{13}C_{DIC} + 23.644 \times 9701.5/T_K$ (Rau et al. 1996). The $\delta^{13}C$ of metabolic CO_2 has an expected isotopic range between −17‰ for shallow and −20‰ for deep corals, which actually corresponds to our findings (Table 1). It has also been hypothesized that the internal DIC pool actually increases in size as a consequence of a light-dependent CO_2 concentrating mechanism to avoid limitation in CO_2 supply to photosynthesis (Weis et al. 1989, Al-Moghrabi et al. 1996, De Beer et al. 2000, Furla et al. 2000a).

Diffusion constraints

The maxima of shallow *Madracis auretenra* zooxanthellae $\delta^{13}C$ were around −16‰. These values are relatively low compared to other zooxanthellate coral species, which mostly range between −12 and −14‰ at the same depths (Muscatine et al. 1989, Swart et al. 2005, Alamaru et al. 2009a), but similar values have been previously reported for *M. auretenra* (Muscatine et al. 1989). Lower $\delta^{13}C$ values can hint at lower photosynthesis and/or at faster replenishment of internal DIC from ambient seawater DIC due to reduced diffusion distances. The genus *Madracis* indeed has several characteristics that could account for reduced diffusion distances and thus allow for faster exchange rates between internal and seawater DIC: (1) The tissue of *M. auretenra* is removed very easily by water-picking indicating that it is relatively superficially bound to the skeleton and does not extend deeply into the skeleton. Other corals often have either big corallites, as found in *Montastraea*, or a porous skeleton as in *Porites*, and in both cases the polyp tissue reaches several millimetres deep into the skeleton. This can increase diffusion distances or create stagnant regions on a small scale, and thus slow down DIC exchange between seawater and calicoblast layer. (2) The genus *Madracis* hardly produces any mucus (C. Maier pers. obs.) that may decelerate diffusion of DIC between ambient seawater and coral tissue. This means that a relatively thin tissue layer and the lack of mucus production, as observed in the branching *Madracis* species investigated here, would allow for rapid replenishment of DIC from ambient seawater into the coral tissue, and this would favour stronger isotopic discrimination. This could be an explanation for the relatively low isotope values observed in *Madracis* spp. (Muscatine et al. 1989, this study).

For the calcification of carbon, 75 to 80% is supposedly supplied by metabolic (respired) DIC, while the remaining 20 to 25% originate from external seawater DIC (Erez 1978, Furla et al. 2000b). If indeed the lighter metabolic DIC is used for calcification, the internal carbon pool becomes heavier, and light-enhanced calcification further augments the enrichment of tissue $\delta^{13}C$, specifically in rapidly calcifying corals (McConnaughey 1989a,b, McConnaughey & Whelan 1997). This very likely explains the higher tissue $\delta^{13}C$ of the shallow-water coral *Madracis auretenra* in relation to its deeper congeners.

N-limitation and saturation levels

Similar fractionation regimes and diffusion-limited N-supply can influence the nitrogen isotope fractionation. The 'depletion-diffusion' and 'host assimilation' models for zooxanthellate corals are thoroughly discussed in Heikoop et al. (1998). DIN is taken up from the external seawater into the cytoplasm of the host, and from this internal DIN pool, nitrogen is taken up by the zooxanthellae, assimilated into organic nitrogen and finally translocated back to the host (Fig. 4). Also, nitrogen from host metabolism is released to the internal DIN pool.

The $\delta^{15}N$ values of shallow-water *Madracis auretenra* remained constant at their maxima between 5 and 20 m water depth, while $\delta^{13}C$ remained fairly constant at minimum values for deep corals between 30 and 47 m (Fig. 2). These constant maxima or minima at either shallow or deep depths indicate a saturation state for the processes involved in the respective isotope fractionation. The constant $\delta^{15}N$ maxima between 5 and 20 m water depth show that *M. auretenra* is actually DIN limited almost throughout its entire depth range at the reefs of Curaçao. This consequently suggests that *M. auretenra* has a high photosynthetic rate that causes DIN limitation even at a depth of 20 m. At the same time, tissue $\delta^{13}C$ of *M. auretenra* decreased from 5 to 20 m, signifying that more carbon is fixed by photosynthesis and/or calcification at shallow depths. An increase in photosynthetic carbon fixation at shallow depths accompanied by a decrease in nitrogen fixation as a consequence of very high photosynthesis has been suggested before, but not to the extent of DIN limitation (Muscatine & Kaplan 1994). However, it has been suggested that shallow water corals under high light can become nitrogen-restricted (Rees 1991, Jokiel et al. 1994, Muscatine & Kaplan 1994) and the data presented in our study provide the first record of DIN limitation actually being indicated by tissue $\delta^{15}N$.

Heterotrophy and resource partitioning

The $\delta^{13}C$ and $\delta^{15}N$ values for POM are bordering corresponding minimum and maximum plateaus of tissue isotopes from deep and shallow colonies, respectively (Figs. 2 & 3). Both $\delta^{13}C$ and $\delta^{15}N$ were lower at the side than at the upward-facing tips of colony branches (Fig. 3). Also, at the colony tip there was a consistent and significant offset in $\delta^{13}C$ between zooxanthellae and cnidarian host tissue, which was lacking for side positions (Fig. 2). The lack in clear differences between zooxanthellae and cnidarian $\delta^{13}C$ at the side indicates an almost complete recycling of carbon between zooxanthellae and the animal host. This suggests that prey is captured only sporadically at the side position of a branch, which is actually contrary to our initial idea. We expected a more pronounced heterotrophic isotope signature at the side position to compensate for lower light intensity and consequently for less photosynthetic energy being available to the polyps.

Because photosynthetic DIN assimilation was limited and a saturation plateau for isotope fractionation was reached in shallow *Madracis auretenra,* it is possible to estimate heterotrophic nitrogen uptake from the observed difference in $\delta^{15}N$ at tip and side positions. As indicated by similar polyp and zooxanthellae $\delta^{13}C$, food capture is negligible at side positions. We can also assume that photosynthetic DIN assimilation rates at the tip and side positions were similar because they were saturated at both positions. As a consequence, the observed difference between $\delta^{15}N$ at the tip and side has to be attributed to heterotrophic feeding. If POM is the main heterotrophic food source and an average enrichment factor of 3.4‰ is assumed (Minagawa & Wada 1984), the average difference of 0.5‰ between the tip and side suggests that about 15% of the nitrogen was derived from POM. This is a conservative estimate because it is based on the assumption that no particulate organic nitrogen is taken up at the side position via prey capture. In any case, this estimate is well within the range of earlier estimates on uptake of organic matter (Anthony 1999).

We further suggest that the depth for saturation states for processes involved in stable isotope fractionation is indicated by a change in direction of the isotope curves: A change occurs for $\delta^{15}N$ between 20 and 30 m water depth and for $\delta^{13}C$ between 30 and 40 m (Fig. 3). This indicates that seawater DIN supply would be sufficient at, or below, this depth to meet levels of the actual photosynthetic capacity of the *Madracis* species investigated. The $\delta^{13}C$ values of POM — as an allochthonous food source — are similar to $\delta^{13}C$ values expected for enzymatically driven photosynthetic isotope fractionation by RubisCO, form II, known for dinoflagellates (Leggat et al. 1999, Robinson et al. 2003). This ambiguity limits our ability to further interpret the trophic state of deep-water specimens by means of stable isotopes. However, differences in $\delta^{13}C$ between zooxanthellae and polyp remained constant with depth, which is in contrast to previous observations (Muscatine et al. 1989). This suggests that over the entire depth range from 5 to 47 m the branching *Madracis* species investigated do not necessarily increase prey capture and, thus, may not compensate for any inevitable reduction in photosynthetic carbon assimilation. Our findings that heterotrophy in branching *Madracis* appears to be the result of food availability as indicated by $\delta^{15}N$ at the tip and side positions also support this theory. Food availability is controlled by current regimes around and between colony branches. It has been shown that prey encounter and prey capture depend on flow speed and coral colony morphology (Sebens et al. 1997) as well as on the concentration of prey items (Anthony 1999). At the outer boundaries of branching colonies, i.e. at the outward-facing tip, faster flow regimes favour particle encounter and the possibility to capture prey. The sideward-facing positions are farther 'inside' of a branching colony, and water flow can reach a stagnant phase reducing particle encounter and thus, prey encounter (Kaandorp et al. 2003).

Growth strategy of *Madracis auretenra*

The shallow water species *Madracis auretenra* has a remarkable growth strategy and skeletal growth is very fast at the tip of a branch. Skeletal growth rates approximate 0.5% d^{-1} (Bak 1977) and a mean linear skeletal extension of 16 mm yr^{-1} (Nagelkerken et al. 2000). Often only upper parts of branches are covered with live tissue while lower parts are void of tissue. Bare skeleton allows burrowing organisms to penetrate and destabilise the skeleton. This generates skeletal breaking points that facilitate reproduction by fragmentation (Highsmith 1982). On the shallow reefs of Curaçao, *M. auretenra* indeed forms large meadows (Bak 1977, van Duyl 1985) that are likely a result of spreading by colony fragmentation. Such a life strategy, with fast skeletal growth at branch tips and reproduction by fragmentation, is actually a good solution if tissue growth is nitrogen-limited. This seems to be the case for *M. auretenra,* for which tissue $\delta^{15}N$ indicates nitrogen-limitation, while at the same time excess carbon still facilitates rapid calcification rates under high-light conditions. This may explain why *M. auretenra* is not abundant below a certain depth, where photosynthetic carbon assimilation might be light-restricted, and a resultant reduction in calcification rates may not be able to sustain a life strategy that is based on fast skeletal growth and reproduction by fragmentation.

CONCLUSIONS

By the combined and systematic comparison throughout a large depth and light range of the $\delta^{15}N$ and $\delta^{13}C$ signals of branching *Madracis* species we showed a very distinct pattern with respect to carbon and nitrogen assimilation and resource partitioning between coral polyp and algal symbionts. We showed DIN limitation for shallow water *M. auretenra* while DIN and DIC assimilation of corals at intermediate depths seemed neither carbon nor nitrogen restricted. Stable isotope signals revealed that food capture was more pronounced at colony tips than at side positions throughout all depths. This indicates that in the branching species of *Madracis,* heterotrophy is a function of food availability. Ultimately, we conjecture that tissue $\delta^{15}N$ and $\delta^{13}C$ can indicate a species' potential life strategy with respect to either investing in tissue growth and maintenance, or investing in faster skeletal growth at the expense of individual, older coral polyps.

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