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Resource partitioning by reef corals as determined from stable isotope composition*

I. $\delta^{13}C$ of zooxanthellae and animal tissue vs depth

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Abstract

The pattern of resource partitioning vs depth by corals collected in February 1983 from Jamaica and the Red Sea was determined from their stable carbon isotope composition. Observations were made on isolated zooxanthellae and corresponding algae-free animal tissue from eight species at four depths over a 50 m bathymetric range. Zooxanthellae $\delta^{13}C$ was high in shallow water and became lower as depth increased. This trend correlated significantly with the annual integrated photosynthetic rate. The trend is interpreted according to a "depletion-diffusion" hypothesis; in shallow water, at high rates of photosynthesis, metabolic CO_2 is nearly depleted and the supply of CO_2 from seawater bicarbonate is limited by diffusion. Since most of the available CO_2 is fixed, isotope fractionation is minimal. In deeper water, at lower rates of photosynthesis, metabolic CO_2 is ample, and isotope fractionation is greater. Animal tissue $\delta^{13}C$ was slightly lower than corresponding zooxanthellae values in shallow water. As depth increased the difference between zooxanthellae and animal tissue $\delta^{13}C$ increased and the latter approached the $\delta^{13}C$ of oceanic particulate organic carbon. These data suggest that carbon is translocated at all depths and that deep-water corals draw significantly on allocthonous sources of carbon.

Introduction

Symbiotic dinoflagellates (=zooxanthellae) are important primary producers on coral reefs. There is much interest in the amount of carbon fixed by zooxanthellae in individual reef corals, and in the amount and nature of the carbon translocated to the coral animal host (Muscatine and Porter 1977, Muscatine 1980, Rinkevich and Loya 1983, Black and Burris 1983, Falkowski et al. 1984, see also Trench et al. 1981, Battey and Patton 1984, 1986).

Daily and annual budgets of photosynthetically-fixed carbon have been established for a variety of corals (Davies 1984, Falkowski et al. 1984, Muscatine et al. 1984, McCloskey and Muscatine 1984, Porter et al. 1984, Porter 1985, Edmunds and Davies 1986). The data indicate that in shallow water, photosynthetic rates are high, and translocated carbon can easily meet the animal carbon demand for respiration and growth (Muscatine et al. 1985). In contrast, in deep water, photosynthetic rates are low, and much less translocated carbon is available for animal respiration and growth. We predict that in deep water there is substantial input of carbon from other sources (Falkowski et al. 1984, McCloskey and Muscatine 1984, Porter 1985). Since direct observation of feeding at depth is impractical, we attempted to evaluate this prediction by analyzing the stable carbon isotope composition ($\delta^{13}C$) of coral animal tissue and zooxanthellae as a function of depth (i.e., decreasing irradiance).

An animal's stable carbon isotope composition is similar to that of its diet (De Niro and Epstein 1978, Haines and Montague 1979, other references in Rau et al. 1983), and provides an index of carbon assimilation over the long term which is difficult to obtain by standard short-term physiological measurements. Thus, as noted by Goreau (1977 a, b), the stable carbon isotope composition should indicate if coral animal tissue derives more carbon from allocthonous sources (e.g. zooplankton, dissolved organic carbon) than from zooxanthellae photosynthesis and translocation, particularly if these alternative sources have distinctive $\delta^{13}C$ values.

In the vicinity of coral reefs of Jamaica the $\delta^{13}C$ for demersal zooplankton, on which many corals feed (Porter 1974, Alldredge and King 1977), ranges from −17.1 to −19.8‰ (Land et al. 1975). The $\delta^{13}C$ for carbon translocated by coral zooxanthellae is unknown, but it would be a function of the primary carboxylation mechanism in photosynthesis. Goreau (1977 a) modeled carbon isotope fractionation in corals and described the system as "a partially closed one in which most of the inorganic carbon is *ultimate-*

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ly consumed." Fixation of nearly all available CO_2 would result in limited fractionation, and so the zooxanthellae would be enriched in ^{13}C. From data of Land et al. (1975) Goreau assigned a $\delta^{13}C$ value of $-13.5‰$ for the zooxanthellae internal organic carbon pool available for translocation. He also predicted that the $\delta^{13}C$ of zooxanthellae and animal tissue would be similar as a result of internal exchange of carbon.

Previous studies have measured $\delta^{13}C$ from coral skeletal carbonate (Land et al. 1975, Weber et al. 1976, Erez 1977, Weil et al. 1981, Cummings and McCarty 1982), and zooxanthellae sterols and terpenes (Kokke et al. 1984), but none have yet measured $\delta^{13}C$ of zooxanthellae and animal tissue organic fractions separately. Land et al. (1975, 1977, see also Black and Bender 1976) approximated this type of analysis by measuring the $\delta^{13}C$ of total coral tissue (i.e., animal tissue plus algae) and isolated algae from three species of Jamaican corals to 60 m. In both fractions $\delta^{13}C$ was high in shallow water (-12 to $-13‰$, 1 to 6 m) but lower in deep water (-16 to $-18‰$, 30 to 60 m). The high zooxanthellae $\delta^{13}C$ was interpreted as the result of less isotopic fractionation at high photosynthetic rates. The similarity of $\delta^{13}C$ of both fractions was interpreted as the result of translocation of carbon from algae to animal tissue. The tendency of the total tissue $\delta^{13}C$ to approach that of demersal zooplankton (-17 to $-18‰$) as depth increased was not addressed.

In the present study, we measured the stable carbon isotope composition of isolated zooxanthellae and the corresponding algae-free animal tissue and attempted to determine the extent of intraspecific resource partitioning by ten corals from Jamaica and one from the Red Sea over a 30 to 50 m bathymetric range. The Jamaican corals included presumed "autotrophic" species, adapted for photosynthesis, with branching skeletons, small polyps, and high surface to volume ratios, and presumed "heterotrophic" species, adapted for holozoic feeding, with massive skeletons, large polyps, and low surface to volume ratios (Porter 1976). We also measured the photosynthetic rate of each species and computed the annual integrated photosynthetic rate to determine how this parameter correlated with zooxanthellae $\delta^{13}C$. The results confirm and extend those of Land et al. (1975) and are consistent with the model of Goreau (1977a). The data show that $\delta^{13}C$ for zooxanthellae from shallow-water corals is relatively high, and correlates significantly with photosynthetic rate. The $\delta^{13}C$ of coral animal tissue and zooxanthellae are similar. As depth increases, all $\delta^{13}C$ values become lower, and the coral animal $\delta^{13}C$ approaches that of the environmental particulate organic carbon pool.

Materials and methods

Selection of corals; in situ measurement of diel oxygen flux

Study sites were established using SCUBA in February 1983 at 10, 30, and 50 m on the fore-reef, and at 1 and 10 m on the back-reef, opposite the Discovery Bay Marine Laboratory (DBML), Jamaica, West Indies. Ten species of corals (nine zooxanthellate, one azooxanthellate) listed in Table 1, were selected for analysis. Specimens were dislodged from the substrate or chiseled from massive corals (150 to 200 cm^2). Each zooxanthellate specimen was subdivided into three pieces and each piece was placed in one of three chambers fitted to a self-contained underwater respirometer (Porter 1980, Porter et al. 1984). Diel oxygen flux in each chamber was measured in situ at each collection site. From diel oxygen flux data, annual gross photosynthesis was calculated as described by Porter (1985). Coral pieces were placed in plastic bags filled with seawater and taken to DBML within 30 min. Some samples were placed in the running seawater aquaria in the laboratory and processed within 24 h. Others were frozen at $-20\,°C$ until they could be processed, usually within 48 h. Freezing had no adverse effect on separation of zooxanthellae and animal tissue (Streamer et al. 1986). *Stylophora pistillata* was collected using SCUBA in February 1984 from the fore-reef adjacent to the H. Steinitz Marine Biological Laboratory, Eilat, Israel. Samples were processed on the same day.

Separation of zooxanthellae and animal tissue

Two coral specimens in each set were processed for biomass parameters for a companion study. The third specimen was processed for stable carbon isotope composition. Tissue was removed from fresh or thawed corals with a Water-Pik, using 30 to 50 ml Millipore-filtered seawater recycled through the apparatus to keep volumes small. Tissue was removed completely from each skeleton to ensure sample homogeneity. The homogenate was passed through several layers of surgical gauze to remove fragments of skeleton and then centrifuged (I.E.C. Model HN-S) in 10 ml conical glass centrifuge tubes at $2\,000 \times g$ for 3 to 4 min to pellet most of the zooxanthellae. The turbid supernatant was decanted and centrifuged at $4\,000 \times g$ for 4 min to pellet residual zooxanthellae. After this treatment, the supernatant, often containing a surface layer of lipid droplets, was decanted and thoroughly mixed in a hand-held tissue homogenizer, and a sample was deposited on a Reeve-Angel (934AH) precombusted glass fiber filter using suction, until the filter was loaded. The filter was inspected for zooxanthellae with a compound microscope at $100\times$. If algae were detected, the original suspension was centrifuged at higher speed to pellet any remaining zooxanthellae and sampled again. The process was repeated until zooxanthellae could not be detected on the filter. The filter was dried at 50 °C. The pellet of zooxanthellae was resuspended and washed 3 to 5 times with filtered seawater at a centrifugation speed sufficient to just pellet the algae and leave residual animal tissue in suspension. Final pellets were inspected for animal tissue and when only minimally contaminated with identifiable animal constituents (e.g. nematocysts) they were dried at 50 °C overnight in the centrifuge tubes. The dry filters and pellets were stored in glassine envelopes until analyzed.

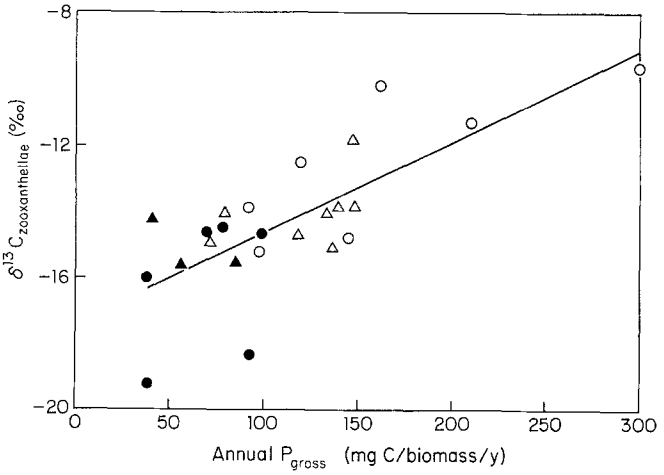

Fig. 1. $\delta^{13}C$ of zooxanthellae (from Table 1) plotted against annual integrated photosynthetic rate (mg carbon biomass^{-1} yr^{-1}) for each species of Jamaican coral (except *Madracis mirabilis*) at all depths. (○: 1 m; △: 10 m; ●: 30 m; ▲: 50 m) ($\delta^{13}C = -17.4 + P_{gross}$ 0.02; $r = 0.75$; $p > 0.001$). Photosynthetic rate was normalized per unit area of coral surface. This provided the least variance of any normalizing biomass unit

Analytical techniques

The $\delta^{13}C$ was determined as follows: Filters containing animal tissue, and dried algal pellets were treated overnight with 0.001 N H_3PO_4 to remove CO_2 from carbonate contaminants. Samples were then freeze-dried and combusted as described by Minagawa et al. (1984), using an apparatus with small inner, and large outer combustion tubes. Samples on filters were analyzed using the large outer tube. Mass spectrometry was performed at the University of California, Los Angeles (UCLA) on a Varian MAT 250 instrument. The $^{13}C/^{12}C$ of the samples are reported as $\delta^{13}C$, in units per ml (‰), where:

$$\delta^{13}C\ (‰) = [(R_{sample}(R_{PDB\ standard}) - 1) \times 1\,000$$

and

$$R = {}^{13}C/{}^{12}C$$

The analytical precision of these measurements is ca. 0.2‰.

Table 1. $\delta^{13}C$ for algae and animal tissue from zooxanthellate corals from Jamaica and Eilat, and for tissue from an azooxanthellate coral from Jamaica, over a 50 m bathymetric range. Jamaican species listed approximately in order of decreasing surface area: volume and increasing polyp diameter (cf. Porter 1976)

Zooxanthellate corals	Depth (m) $\delta^{13}C$			
	1	10	30	50
Jamaica				
Madracis mirabilis				
algae	−18.37	−16.79	−16.35	*
animal	−18.98	−17.74	−19.64	*
Acropora cervicornis				
algae	−13.89	−14.05	−16.02	*
animal	**	−15.34	−16.02	*
Agaricia agaricites				
algae	−12.52	−13.91	−14.47	−15.61
animal	−13.08	−15.63	−15.49	−17.90
Acropora palmata				
algae	−14.80	−14.72	*	*
animal	−15.11	−15.19	*	*
Porites astreoides				
algae	−10.21	−11.82	−14.64	*
animal	−11.76	**	−17.91	*
Montastraea annularis				
algae	− 9.63	−13.87	−18.33	−15.58
animal	−11.90	−13.63	−19.64	−19.27
Montastraea cavernosa				
algae	−11.28	−14.04	−14.67	−14.29
animal	−10.75	−13.49	−16.79	−22.42
Eusmilia fastigiata				
algae	−15.22	−15.12	−19.21	*
animal	**	−15.39	−19.48	*
Dendrogyra cylindrus				
algae	*	−14.94	*	*
animal	*	−15.50	*	*
Red Sea, Israel				
Stylophora pistillata				
algae	−13.22	−13.29	−15.74	
animal	−13.97	−15.43	−18.59	
Azooxanthellate coral				
Tubastraea coccinea	−20.27			

* Not found at depth
** Samples lost

Results

$\delta^{13}C$ of zooxanthellae

Table 1 shows that $\delta^{13}C$ for zooxanthellae ranges from −9.63 to −19.21‰. Except for zooxanthellae in *Madracis mirabilis*, the $\delta^{13}C$ is relatively high in shallow water and becomes lower as depth increases.

To determine the effect of photosynthetic rate on zooxanthellae $\delta^{13}C$, we plotted zooxanthellae $\delta^{13}C$ against the annual integrated rate of gross photosynthesis by each species at each depth. As Fig. 1 shows, there is a significant correlation between zooxanthellae $\delta^{13}C$ and photosynthetic rate. As photosynthetic rate increases, isotopic discrimination decreases.

To evaluate the possibility that ^{13}C depletion might be influenced by a depth-dependent factor other than irradiance, we analyzed the regression of gross photosynthetic rate vs zooxanthellae $\delta^{13}C$ in the species from each depth. Table 2 shows that there is a significant positive correlation between $\delta^{13}C$ and rate of photosynthesis at 1 m. The higher the rate, the more reduced is the isotopic discrimination. However, the correlation is not significant at 10, 30 and 50 m, probably due to small sample sizes. Similarly, when gross photosynthesis is plotted against $\delta^{13}C$ for each species at each depth, there is a trend toward lower $\delta^{13}C$ with depth,

Table 2. Linear regression data for $\delta^{13}C$ of zooxanthellae vs annual integrated gross photosynthesis (mg carbon biomass^{-1} yr^{-1}) at each depth. s: significant correlation; ns: not significant ($\delta^{13}C = a\,P_{gross} + b$)

Species	Depth (m)	$\delta^{13}C$	P_{gross}	a	b	r	$p<0.05$
Acropora palmata	1	−14.81	146.55				
Acropora cervicornis		−13.89	93.02				
Agaricia agaricites		−12.52	120.48				
Porites astreoides		−10.21	162.69				
Montastraea cavernosa		−11.28	211.56				
Eusmilia fastigiata		−15.22	98.58				
Montastraea annularis		− 9.63	301.14				
				−16.32	0.02	0.78	s
Acropora palmata	10	−14.72	119.51				
Acropora cervicornis		−14.05	80.47				
Agaricia agaricites		−13.91	140.87				
Porites astreoides		−11.82	148.78				
Montastraea annularis		−13.87	149.97				
Montastraea cavernosa		−14.04	134.71				
Dendrogyra cylindrus		−15.12	137.73				
Eusmilia fastigiata		−14.94	72.71				
				−15.91	0.01	0.46	ns
Acropora cervicornis	30	−16.02	38.76				
Agaricia agaricites		−14.47	79.32				
Porites astreoides		−14.64	70.63				
Montastraea annularis		−18.33	93.92				
Montastraea cavernosa		−14.67	100.10				
Eusmilia fastigiata		−19.21	39.57				
				−18.21	0.02	0.36	ns
Agaricia agaricites	50	−15.61	58.86				
Montastraea annularis		−15.58	86.32				
Montastraea cavernosa		−14.29	41.88				
				−13.62	−0.02	0.75	ns

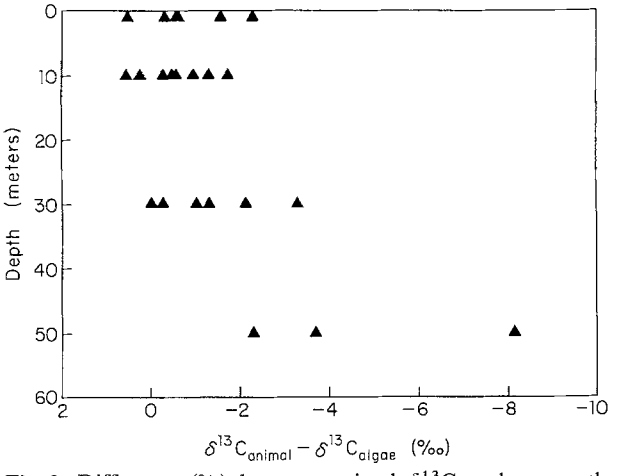

Fig. 2. Difference (‰) between animal $\delta^{13}C$ and zooxanthellae $\delta^{13}C$ plotted against depth

but the trend is statistically significant only for *Acropora cervicornis* ($p<0.05$; $r=0.99$). Therefore, although isotopic discrimination seems to increase as irradiance diminishes, the possible influence of other depth-dependent variables is not ruled out.

$\delta^{13}C$ of animal tissue

Table 1 also shows that the animal tissue $\delta^{13}C$ ranges from −10.75 to −22.42‰, and is almost always slightly lower than the zooxanthellae $\delta^{13}C$ (exceptions: *Montastraea annularis* 10 m, *M. cavernosa* 1 and 10 m).

In shallow water (1 to 10 m), the mean difference between animal and algal $\delta^{13}C$ is small, less than 1.0‰ (Fig. 2). We interpret the similarity between zooxanthellae and animal $\delta^{13}C$ as the result, in part, of translocation of fixed carbon from algae to animal. From 10 to 50 m the differences increase with depth (Fig. 2). The mean differences at 30 and 50 m are 1.6 and 4.7‰, respectively. We interpret this disproportionate depletion of ^{13}C in animal tissue with depth as the result of acquisition of carbon by the animal tissue not only from translocation but also from other ^{13}C-depleted sources as well. This interpretation is supported by the observation that the $\delta^{13}C$ value of *Tubastrea coccinea*, an azooxanthellate coral, is −20.27‰ (Table 1). This value is representative of coral animal tissue which has acquired carbon solely by holozoic feeding or uptake of dissolved organic carbon (DOC).

Discussion

$\delta^{13}C$ of zooxanthellae

Except for *Madracis mirabilis*, the $\delta^{13}C$ for zooxanthellae is relatively high in shallow water and becomes lower as depth increases. To explain these values and their change with depth we must first consider the $\delta^{13}C$ of the source CO_2 and

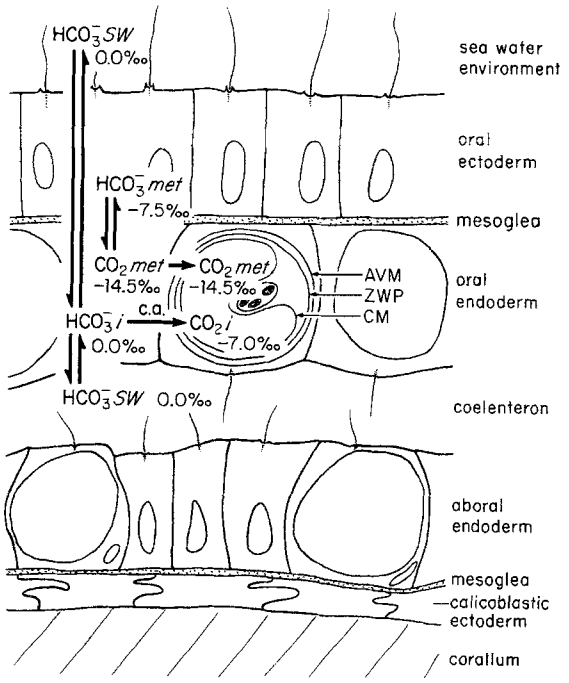

Fig. 3. Schematic diagram of section through reef coral tissue and skeleton (between polyps) showing morphology in relation to sources and fluxes of inorganic carbon. AV: animal vacuolar membrane; ZWP: zooxanthella cell wall and plasma membrane; CM: chloroplast membrane; N: nucleus; c.a: carbonic anhydrase

then the mechanisms by which the source CO_2 is fractionated.

Sources of CO_2 for zooxanthellae photosynthesis

The principal sources of CO_2 for zooxanthellae photosynthesis in corals are animal metabolism (CO_2met) and the internal animal tissue pool of bicarbonate (HCO_3^-i). With reference to Fig. 3, the internal bicarbonate pool is derived in part from the reaction of CO_2met with cell water to form HCO_3^-met, and in part from inward diffusion of seawater bicarbonate (HCO_3^-sw). Seawater CO_2(aq.) is negligible as a direct source due to its low concentration at pH 8.2 and its high resistance to diffusion in aqueous media (Benedict 1978).

The $\delta^{13}C$ of CO_2met is assumed to be similar to that of the animal tissue from which it is derived. In shallow water corals (1 to 10 m), this value ranges from −10.75 to −18.98‰ (mean = −14.5‰) (Table 1). The CO_2met may be fixed directly or may equilibrate with HCO_3^-met, as it must at night. Since the magnitude of equilibrium fractionation between CO_2(aq.) and HCO_3^-sw at 28 °C is ca. Δ7.0‰ (Deuser and Degens 1967, Deuser et al. 1968, see also Mook et al. 1974), we assume that CO_2met and HCO_3^-met will behave similarly, resulting in no net change in the $\delta^{13}C$ for the CO_2met pool.

The $\delta^{13}C$ for HCO_3^-i is assumed to be close to 0.0‰, the mean $\delta^{13}C$ for oceanic (Atlantic) bicarbonate (Deuser et al. 1968, Deuser and Hunt 1970). The $\delta^{13}C$ of CO_2i resulting from equilibrium fractionation with HCO_3^-i will be −7.00‰.

Using the model equations of Goreau (1977a, p. 302), and the data in Table 1, we calculate that, at high rates of photosynthesis, ca. 60% of the CO_2 is from metabolic CO_2 and 40% from the seawater bicarbonate pool. The $\delta^{13}C$ of the internal CO_2 pool, calculated from [(CO_2met × 0.6) + (CO_2i × 0.4)], should then range from −9.25 to −14.18‰, with a mean of −11.71‰.

Fractionation mechanisms

The major carbon isotope fractionation mechanisms in marine microalgae (i.e., phytoplankton) are the uptake and intracellular diffusion of bicarbonate and/or CO_2 and the primary carboxylation reaction in C_3 photosynthesis, catalyzed by ribulose bisphosphate carboxylase (RUBPCase). The $\Delta^{13}C$ of RUBPCase is ca. 27‰ (O'Leary 1981). Marine microalgae are thought not to exhibit C_4 photosynthesis since they lack the requisite anatomy, and there is little evidence for decarboxylation and reassimilation of CO_2 via RUBPCase (Benedict 1978, Appleby et al. 1980, Morris 1980, Descolas-Gros and Fontugne 1985, Kerby and Raven 1985). They do, however, exhibit "C_4 metabolism" (Morris 1980) so that carbon isotopes could still be fractionated by a major β-carboxylating enzyme, phosphoenolpyruvate carboxylase (PEPCase) ($\Delta^{13}C = 2.37$‰, Benedict 1978). Little is known of carbon isotope fractionation by other β-carboxylating enzymes, including phosphoenolpyruvate carboxykinase (PEPCK), and pyruvate carboxylase (PC) (Beardall et al. 1976, Appleby et al. 1980, Morris 1980, Descolas-Gros and Fontugne 1985, Kerby and Raven 1985). The β-carboxylation replenishes carbon anaplerotically to supplement the TCA cycle (Morris 1980) and accounts for, at most, ca. 10% of the total CO_2 fixed (Appleby et al. 1980, Descolas-Gros and Fontugne 1985).

The high zooxanthellae $\delta^{13}C$ could be the result of a low ratio of RUBPCase:PEPCase activity (Descolas-Gros and Fontugne 1985). This ratio varies in microalgae, including zooxanthellae, with environmental conditions and the physiological state of the cells. Beardall et al. 1976, Ting 1976, Schmitz and Kremer 1977, Morris 1980, Hofmann and Kremer 1981, Trench and Fisher 1983, Descolas-Gros and Fontugne 1985, Tytler and Trench 1986). In fact, free-living dinoflagellates in culture and in field samples (Descolas-Gros and Fontugne 1985), and cultured zooxanthellae from the Hawaiian coral *Montipora verrucosa* (Tytler and Trench 1986), exhibit high PEPCase activity. However, the high activity in the former is correlated with the onset of a stationary growth phase and in the latter with low growth irradiance. Neither condition is typical for tropical shallow water corals (Muscatine et al. 1984). Moreover, as light intensity decreases with depth, zooxanthellae $\delta^{13}C$ becomes lower rather than higher (Table 1). Finally, if we assume that bicarbonate is the substrate fixed by PEPCase (Cooper and Wood 1971, Kerby and Raven 1985), and that β-carboxylation is the sole pathway of carbon fixation, then the $\delta^{13}C$ of the zooxanthellae at any depth would be significantly lower than any corresponding values in Table 1. This is

because the $\delta^{13}C$ of HCO_3^-i and HCO_3^-met is always lower than that of CO_2i and CO_2met (e.g. compare $\delta^{13}C$ zooxanthellae minus 7.0‰ due to equilibrium fractionation plus −2.4‰ due to kinetic fractionation by PEPCase). Therefore, it is unlikely that the high zooxanthellae $\delta^{13}C$ is due to a low ratio of RUBPCase : PEPCase activity.

Alternatively the high zooxanthellae $\delta^{13}C$ could be due to the well-established phenomenon that when C_3 plants, including microalgae, fix all available CO_2, the stable carbon isotope discrimination by RUBPCase is substantially reduced, and the $\delta^{13}C$ of the fixed carbon approaches that of the source carbon. Degens et al. 1968, Deuser et al. 1968, see also reviews of Troughton 1979, Benedict et al. 1980, O'Leary 1980, Smith and Walker 1980).

Based on this phenomenon and the model of Goreau (1977a), we offer the following hypothesis. In shallow water, when photosynthesis is greater than respiration, CO_2met is totally consumed, and the algae must draw on the CO_2i from the internal bicarbonate pool. In turn, the bicarbonate must be replaced from the seawater bicarbonate reservoir (cf. Goreau 1977a). If replacement of internal bicarbonate is limited, isotopic fractionation will be reduced. Since seawater bicarbonate must move inward through a diffusion-resistant unstirred surface layer of seawater (Walker and Smith 1980), animal plasma membrane, cytosol, animal vacuolar membrane, perialgal envelope, plasmalemma, and chloroplast envelope, its supply could be diffusion-limited. Diffusion discrimination of unknown magnitude could accompany inward movement. Internal CO_2 depletion could also be exacerbated by the high cell densities of zooxanthellae, as noted by Cummings and McCarty (1982, see also Pardue et al. 1976). Therefore, if, at the site of photosynthesis, the algae fix most or all of the available CO_2met + CO_2i, fractionation by RUBPCase would be minimal and the zooxanthellae $\delta^{13}C$ wuld approach the value for the internal pool of CO_2. The lowest calculated value −9.25‰ is virtually identical with the lowest observed value in Table 1 (−9.63‰, *Montastraea annularis* zooxanthellae 1 m).

Replacement of CO_2 from the internal bicarbonate pool by simple equilibration might not provide enough CO_2 for high photosynthetic rates. However, carbonic anhydrase (CA) would effect immediate and rapid adjustment of the chemical and isotopic equilibrium between internal bicarbonate and CO_2 (Goreau 1977a, O'Leary 1980, Kerby and Raven 1985). In zooxanthellate corals, both the algae and the animal tissue exhibit CA activity (Graham and Smillie 1976, Isa and Yamazato 1984, Weis et al. 1988). The extent of isotopic discrimination by CA is unknown.

This "depletion-diffusion" hypothesis (D'Elia et al. 1983) does not imply that zooxanthellae photosynthesis is CO_2-limited as we have no evidence for this. It holds only that at high rates of photosynthesis all or nearly all of the CO_2 in the internal pool is fixed. Whereas Burris et al. (1983) have argued that coral zooxanthellae are not CO_2-limited, Streamer et al. (1986) suggest that a lag period in fixation of ^{14}C-bicarbonate by *Acropora scandens* might be due to rate-limiting delivery of CO_2. Dennison and Barnes (1987) observed a significant increase in rates of photosynthesis and calcification in the coral *Acropora scandens* when surrounding water was stirred. However, when photosynthesis was near compensation, stirring had no effect, suggesting that at high rates, photosynthesis was limited by the diffusion of substrate.

Our hypothesis is supported by data in Fig. 1, which demonstrate directly that carbon isotope fractionation is progressively reduced as annual integrated gross photosynthetic rate increases. This relation between light intensity (i.e., photosynthetic rate) and $\delta^{13}C$ has been observed in other marine algae (Degens et al. 1968, Deuser 1970, Benedict et al. 1980, Wefer and Killingley 1986). In an analogous case, $\delta^{13}C$ of the symbiotic chemoautotrophic bacteria and trophosome tissue of the hydrothermal vent vestimentiferan *Riftia pachyptila* is ca. −11.0‰. This high value is thought to be the result of diminished supply of CO_2 at the site of chemosynthesis and concomitant fixation of a ^{13}C-enriched CO_2 pool (Rau 1981).

From 10 to 50 m, the $\delta^{13}C$ ranges from −15 to −19‰ and the algae become progressively depleted in ^{13}C with depth. According to our hypothesis, the increase in isotopic discrimination is due to the decreased photosynthetic rate and to the increase in the proportion of fixed CO_2met.

Within-depth differences in $\delta^{13}C$ might also be influenced by the length of the path of diffusion of bicarbonate through coral tissue. Corals with more protein per cm^2, might offer greater resistance to diffusion of bicarbonate than "skinny" corals with less protein cm^2. A test of this hypothesis reveals a significant correlation between protein per cm^2 and $\delta^{13}C$ in corals at 1 m. This might explain why, for example, in shallow water, the zooxanthellae in the fleshy *Montastraea* spp. and the non-branching, perforate *Porites astreoides* are isotopically heavier than the perforate but branching *Acropora* spp. and the imperforate, branching *Madracis* sp. Since *Madracis* sp. has a high surface : volume ratio (Porter 1976) it is probably less diffusion-resistant to bicarbonate, which could explain why its initial $\delta^{13}C$ value is relatively low in shallow water.

The lowest $\delta^{13}C$ values yet observed for zooxanthellae are −24.1 and −23‰, from the scyphozoan medusa *Mastigias* sp. (Muscatine et al. unpublished data) and from the clam *Tridacna maxima* (Black and Bender 1976), respectively. The zooxanthellae are present in relatively low densities in the mantle of *T. maxima* (Trench et al. 1981) and in the fluid-bathed mesoglea beneath a single layer of epithelial cells in *Mastigias* sp. Diffusion of bicarbonate in *Mastigias* sp. mesoglea could be enhanced by circulation of fluid as the bell contracts. These features provide a basis for increased kinetic fractionation at modest photosynthetic rates and fixation of a greater proportion of ^{13}C-depleted metabolic CO_2.

The effect of respiration of translocated carbon, photorespiration, "back translocation" (i.e., algal heterotrophy, Steen 1986), and calcification on $\delta^{13}C$ of zooxanthellae is unknown. However, if one molecule of calcium carbonate is precipitated for each molecule of CO_2 fixed in photosynthesis (Goreau 1961), then half the inorganic carbon

pool is consumed by calcification during the day, further decreasing the immediate availability of CO_2, but only at light-limited rates of photosynthesis.

Finally, the effects of temperature on bicarbonate-CO_2 equilibrium fractionation (Sackett et al. 1965, Mook et al. 1974) cannot explain the decrease in $\delta^{13}C$ vs depth. According to Deuser et al. (1968) $\delta^{13}C$ changes by 0.109‰ $°C^{-1}$, but over the 50 m bathymetric range in Discovery Bay, the temperature change is less than 0.3 °C (Land et al. 1975).

$\delta^{13}C$ of coral animal tissue

In shallow water, the similarity between $\delta^{13}C$ of algae and animal tissue is probably due to translocation, since more than 90% of the carbon photosynthetically-fixed by zooxanthellae is translocated to the animal tissue, potentially satisfying its carbon demand for respiration and growth (Davies 1984, Muscatine et al. 1984). This interpretation does not deny that shallow water corals can feed on particulate organic carbon (Lewis 1976, 1977).

Since translocation represents movement of carbon from a producer trophic level to a consumer level, and since fractionation per trophic level is customarily on the order of $\Delta 1.0$‰ (De Niro and Epstein 1978, Haines and Montague 1979, Rau and Anderson 1981, Rau et al. 1983), it is not surprising that the animal tissue $\delta^{13}C$ is similar to, but consistently lower than that of the algae.

As depth increases, animal tissue $\delta^{13}C$ becomes even lower (Fig. 2). In five of the seven species whose range extends at least to 30 m, it decreases to a greater extent than algal $\delta^{13}C$, and reaches the lowest value of −22.43‰ in *Montastraea cavernosa* at 50 m (Table 1). In this case the difference between algae and animal tissue is −8.13‰. This difference strongly suggests that animal tissue at depth incorporates carbon from sources other than zooxanthellae, especially since the absolute amount of translocated carbon diminishes with depth due to reduced rates of photosynthesis. This conclusion is consistent with the observation that *M. cavernosa* has large polyps and a low surface to volume ratio typical of a "heterotrophic" coral. By comparison with the azooxanthellate coral *Tubastrea coccinea,* a $\delta^{13}C$ of at least −20.27‰ could result from feeding on allocthonous sources such as demersal zooplankton whose $\delta^{13}C$ is −18.9 to −19.8‰ (Land et al. 1977). Where the $\delta^{13}C$ of animal tissue at depth is even lower, perhaps other allocthonous sources of carbon are assimilated. Oceanic POC/DOC ranges from −18.4 to −24.4‰ (Williams and Gordon 1970, Rau et al. 1982).

Another explanation may be that corals at depth produce and store more substrates depleted in ^{13}C such as lipid. Although there is little data on total lipid vs depth for Jamaican corals, there is apparently no systematic change in total fatty acid composition with depth (Meyers et al. 1978). In fact, some deep water corals (25 to 30 m) from Florida and Grand Cayman contain large amounts of polyunsaturated fatty acids, especially docohexanoic acid. This fatty acid is also found in high concentration in oceanic copepods. Its abundance in corals is also interpreted as evidence for a dietary constituent from allocthonous sources (Meyers 1979).

In conclusion, the $\delta^{13}C$ of reef corals provides modest support for the prediction that as photosynthesis and translocation diminish with increasing depth, corals draw more on allocthonous sources of carbon.

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