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REPORT

Baseline shifts in coral skeletal oxygen isotopic composition: a signature of symbiont shuffling?

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Abstract Decades-long records of the stable isotopic composition of coral skeletal cores were analyzed from four sites on the Mesoamerican Reef. Two of the sites exhibited baseline shifts in oxygen isotopic composition after known coral bleaching events. Changes in pH at the calcification site caused by a change in the associated symbiont community are invoked to explain the observed shift in the isotopic composition. To test the hypothesis that changes in symbiont clade could affect skeletal chemistry, additional coral samples were collected from Belize for paired *Symbiodinium* identification and skeletal stable isotopic analysis. We found some evidence that skeletal stable isotopic composition may be affected by symbiont clade and suggest this is an important topic for future investigation. If different *Symbiodinium* clades leave consistent signatures in skeletal geochemical composition, the signature will provide a method to quantify past symbiont shuffling events, important for understanding how corals are likely to respond to climate change.

Keywords *Symbiodinium* · Skeletal geochemistry · Coral biomineralization · Coral calcification · Isotopic records

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Introduction

Massive scleractinian corals provide unique insight into past tropical climate because they grow continuously and record changes in water chemistry and temperature in their calcium carbonate skeletons (Gagan et al. 2000). Coral annual growth bands provide clear chronologies and offer data with a time resolution of approximately 1 month. Accordingly, corals have been exploited extensively for paleoclimatic studies, particularly for reconstructing historic records of ocean temperature and precipitation (Dunbar et al. 1994; Charles et al. 1997; Gagan et al. 1998; Draschba et al. 2000; Tudhope et al. 2001; Cobb et al. 2003; McGregor and Gagan 2004). These paleoclimate inferences are typically derived from analysis of stable isotope and minor elemental ratios, which are partitioned into skeleton based on ambient seawater temperature and composition. For precise climate reconstructions, it is essential to understand the range of variables that affect skeletal proxies, including those associated with coral physiology.

Here we present two datasets that point to a previously unidentified but potentially determining variable: the clade of symbiotic dinoflagellate, *Symbiodinium,* present in the

coral tissue. First, we introduce the geochemical measures used here as well as models of coral calcification, specifically with respect to the ways that stable carbon and oxygen isotopes vary with changes in the calcification medium. Coral biomineralization is an active arena of investigation, and we review the current understanding to put our findings in context. We then discuss physiological differences between *Symbiodinium* clades and how symbiont physiology might affect coral geochemistry. We then use this information to interpret our results from four long-term records of coral skeletal stable isotopic composition that exhibited unusual baseline shifts in $\delta^{18}O$, and an exploratory analysis of paired measurements of symbiont clade and skeletal isotopic composition.

Coral skeletal geochemistry

Isotopes contain different numbers of neutrons and vary in atomic mass, but behave chemically similar with equivalent protons and electrons. Here we measured ratios of stable oxygen ($^{18}O/^{16}O$) and carbon ($^{13}C/^{12}C$) isotopes, expressed in permil (‰) notation versus the Pee Dee Belemnite (PDB) standard:

$$\delta^{18}O = \left[\frac{^{18}O/^{16}O_{sample} - ^{18}O/^{16}O_{standard}}{^{18}O/^{16}O_{standard}}\right] * 1,000\ ‰$$

The $\delta^{18}O$ of the coral skeleton is largely controlled by water temperature as well as the $\delta^{18}O$ of the seawater, which varies with salinity (McConnaughey 1989; Leder et al. 1996; Fairbanks et al. 1997). Ions with equivalent charges can substitute for calcium in the coral aragonite. For instance, the coral skeletal Sr/Ca ratio is controlled primarily by water temperature (Beck et al. 1992). Empirical relationships between instrumental water temperature records and coral $\delta^{18}O$ and Sr/Ca are therefore used to reconstruct water temperature using corals (e.g., Quinn et al. 1998; Cobb et al. 2003; Goodkin et al. 2005). Paired measurements of both $\delta^{18}O$ and Sr/Ca can also be used to isolate changes in the $\delta^{18}O$ of seawater, a property that essentially mirrors local changes in the precipitation–evaporation balance (McCulloch et al. 1994; Ren et al. 2003).

Coral calcification models

In addition to temperature and salinity, the so-called vital effects related to the growth and metabolic rate of the coral also affect coral chemical signature (McConnaughey 2003). The origin of these "vital effects" has been debated since the earliest investigations of reef carbonates (Weiner and Dove 2003). Calcification occurs in a fluid- or hydrogel-filled space between the coral tissue and skeleton (Cohen and McConnaughey 2003; Allemand et al. 2011; Fig. 1). The products necessary for calcification, Ca^{2+} and HCO_3^-, enter the calcifying space either by active transport through the tissue via Ca-ATPase and carbonic anhydrase (CA), respectively, from CO_2 passively diffusing from the tissues and then being transformed via hydration or by hydroxylation, possibly with the aid of CA activity, and/or from seawater or dissolved ions directly entering the space through paracellular "leakage" or vacuolization (Furla et al. 2000; Adkins et al. 2003; Al-Horani et al. 2003; Cohen and McConnaughey 2003; Colombo-Pallotta et al. 2010; Allemand et al. 2011; Tambutté et al. 2011; Gagnon et al. 2012). The pH of the calcifying medium is increased relative to ambient seawater, which promotes calcification, probably mostly due to Ca-ATPase pumping protons from the calcifying space into the cells in exchange for Ca^{2+} and/or the activity of CA (Adkins et al. 2003; Al-Horani et al. 2003; Colombo-Pallotta et al. 2010; Allemand et al. 2011; Tambutté et al. 2011; Venn et al. 2011; McCulloch et al. 2012).

The specific mechanisms of isotopic fractionation during coral biomineralization are still not fully understood, but several models have been proposed. The original "kinetic" model proposes that the rate of calcification controls the $\delta^{13}C$ and $\delta^{18}O$ via kinetic fractionation at the rate-limiting step where CO_2 is either hydrated ($CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$) or hydroxylated ($CO_2 + OH^- \rightarrow HCO_3^-$) before $CaCO_3$ precipitation, leading to differences in both $\delta^{13}C$ and $\delta^{18}O$ (McConnaughey 2003). Kinetic controls on isotopic fractionation should cause $\delta^{13}C$ and $\delta^{18}O$ to covary with changes in calcification rates, as has been observed in some symbiotic and asymbiotic corals (McConnaughey 1989;

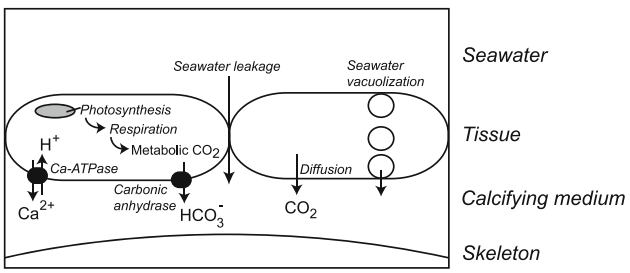

Fig. 1 Model showing different methods for calcification products to enter the calcifying medium between the coral tissue and skeleton, based on references in text. Ca^{2+} is either actively pumped across the tissue layer via Ca-ATPase or enters directly from seawater by leakage or vacuolization. After being produced by respiration of O_2 from symbiont photosynthesis in the coral tissue, CO_2 enters the calcifying space via diffusion or active pumping of HCO_3^- by CA across the tissue layer; these species can also enter directly as DIC in seawater via leakage between cell junctions or vacuolization

Allison et al. 1996; Adkins et al. 2003). However, metabolic effects in symbiotic corals can change the skeletal $\delta^{13}C$ without necessarily affecting the $\delta^{18}O$; for instance, increased photosynthesis removes isotopically light carbon from the calcification pool, and respiration adds heavy carbon to the pool (McConnaughey 1989; Swart et al. 1996; Hartmann et al. 2010). Heterotrophy can also add isotopically light carbon to the pool (Grottoli and Wellington 1999). Other research suggests that instead of kinetic controls, the $\delta^{13}C$ is controlled by the balance of isotopically light metabolic CO_2 that diffuses across the calicoblastic membrane into the calcifying space relative to the leak rate of seawater containing heavier dissolved inorganic carbon (DIC) that directly enters the calcifying space (Adkins et al. 2003; Rollion-Bard et al. 2003; Allison et al. 2010).

So far, no controls on $\delta^{18}O$ independent of changes in $\delta^{13}C$ have been clearly identified. However, there is increasing evidence that $\delta^{18}O$ may be influenced by the pH of the calcifying medium (Adkins et al. 2003; Rollion-Bard et al. 2003; Allison et al. 2010). Changes in pH of the calcifying medium could affect $\delta^{18}O$ by (i) changing the speciation of dissolved carbon in the calcifying space, with higher pH leading to species with lighter $\delta^{18}O$ (i.e., CO_3^{2-} is the most isotopically depleted species; Adkins et al. 2003), (ii) changing the balance of hydration versus hydroxylation of CO_2 (Rollion-Bard et al. 2003; Allison et al. 2010), or (iii) as with $\delta^{13}C$, simply increasing the amount of metabolic CO_2 versus seawater DIC involved in calcification, where the former is lighter in $\delta^{18}O$ (Allison et al. 2010) (Fig. 2).

While changes in $\delta^{13}C$ associated with changes in photosynthetic rates have been explored (Grottoli 2000; Risk et al. 2003; Hartmann et al. 2010), the ways in which $\delta^{18}O$ may be affected by photosynthesis-induced changes in calcification site dynamics remain largely unexplored. The ratio of metabolic CO_2 and seawater DIC in the calcifying space could be changed by an increase in the metabolic $[CO_2]$ gradient between the tissues and the calcifying fluid, as would be expected from increased production of O_2 by photosynthesis and subsequent respiration to CO_2 in the tissues (Furla et al. 2000; Allemand et al. 2011). Additionally, Ca-ATPase activity appears to increase with increased metabolic activity (Al-Horani et al. 2003), which could raise the pH in the calcifying fluid (Rollion-Bard et al. 2003). Both of these mechanisms would lead to the diffusion of more metabolic CO_2 into the calcifying space (Allemand et al. 2011), increasing the metabolic CO_2/seawater CO_2 ratio and decreasing the skeletal $\delta^{18}O$. An increase in pH due to enhanced Ca-ATPase activity would also decrease $\delta^{18}O$ through the other mechanisms described earlier: (i) changing the speciation of dissolved carbon toward species with lighter $\delta^{18}O$ or (ii) shifting the balance toward hydroxylation instead of hydration of CO_2. In addition to Ca-ATPase, CA appears to play a critical role in coral calcification, either pumping DIC in or removing carbonic acid to reduce pH in the calcifying medium; CA may also change activity with photosynthetic rate (Colombo-Pallotta et al. 2010; Allemand et al. 2011; Tambutté et al. 2011). Thus, with higher photosynthetic rates and increased metabolic activity, pH

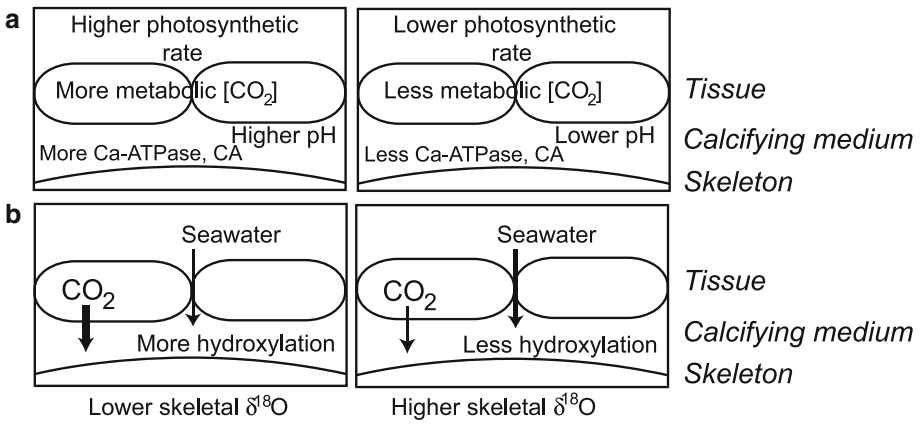

Fig. 2 Possible mechanisms causing lower skeletal $\delta^{18}O$ with increased photosynthetic rate. In (**a**) greater photosynthesis would cause an increase in tissue CO_2 concentrations, increasing the gradient of CO_2 between the tissues and the calcifying fluid. Greater photosynthesis would also potentially increase Ca-ATPase activity, and therefore pH in the calcifying fluid as well as CA activity; these mechanisms would all cause an increase in metabolic CO_2 transport or diffusion from the tissues into the calcifying space. In (**b**) the width of the arrows represents the relative contributions of CO_2 to calcification from either metabolic CO_2 from the tissues or seawater leaking into the calcifying space. The relative amount of hydroxylation versus hydrolyzation of CO_2 between the two scenarios is noted, and these differences might occur if pH were affected as in (**a**). Lower skeletal $\delta^{18}O$ in the greater photosynthetic rate scenario could result from either increased pH at the calcifying site, an increase in the relative amount of metabolic versus seawater CO_2 used in calcification, or increased hydroxylation of CO_2 in the calcifying space

in the calcifying medium would be increased, and a relatively larger amount of metabolic CO_2 and/or DIC would contribute to calcification, thus reducing skeletal $\delta^{18}O$ (Fig. 2; Furla et al. 2000; Adkins et al. 2003; Al-Horani et al. 2003; Rollion-Bard et al. 2003; Allison et al. 2010; Colombo-Pallotta et al. 2010; Allemand et al. 2011; Tambutté et al. 2011).

Physiological differences associated with different symbiont clades

There are currently eight recognized clades of *Symbiodinium*: A–H (Coffroth and Santos 2005). Some coral colonies harbor several different *Symbiodinium* clades. The coral species studied here, *Montastraea faveolata*, displays a zonation of *Symbiodinium* clades in different areas of the coral heads, probably due to variation in light exposure (Rowan et al. 1997). In addition, a study of *Symbiodinium* variability between/within the *Montastraea annularis* species complex (including *M. faveolata*, *Montastraea franksi*, and *M. annularis*) from Belize and Panama found that zooxanthellae clade associations were correlated with local conditions (Garren et al. 2006). Some *Symbiodinium* clades are more susceptible to heat stress, and corals harboring these clades are correspondingly less resistant to temperature-induced bleaching (during which the symbionts are expelled or killed, Brown 1997) (Berkelmans and Van Oppen 2006).

Most studies investigating differences in physiology between *Symbiodinium* clades have focused on their response to heat and light stresses to investigate the roles of these stressors in coral bleaching. To that end, studies have investigated the photophysiology of *Symbiodinium* either alone in culture, in culture with host factor (coral animal amino acids; Gates et al. 1995), or *in hospite*. However, because most corals selectively associate with certain *Symbiodinium* clades (Goulet 2006), comparative studies of symbiont physiology within or isolated from a single host are less common. Although *Montastraea* spp. have been found to associate with clades A, B, C, and D *Symbiodinium* (Toller et al. 2001; Garren et al. 2006), comparative studies of photosynthetic rates have not yet been completed on different clades in this host. Importantly, the host appears to affect *Symbiodinium* physiological performance, so gleaning information on comparative performance between different clades from the literature is not straightforward. The relative photosynthetic rates of *Symbiodinium* can differ depending on the species they are hosted within, sometimes showing opposite results [for instance, clade C1 > clade D in *Acropora millepora* (Cantin et al. 2009), but clade C = clade D in *Pocillopora damicornis* (Rowan 2004); clade A + C > clade C alone in *Acropora vallida* (Ulstrup et al. 2007), but clade A > clade C in *Acropora cytherea* (Stat et al. 2008)]. Other physiological differences may also occur; for example, all clades produce mycosporine-like amino acids when cultured with host factor, but only clade A does so in culture alone (Banaszak et al. 2000).

Physiological differences between *Symbiodinium* clades could in principle affect the resulting chemistry in the coral's calcification space. A recent study by McCulloch et al. (2012) investigated the ability of corals to raise the pH in the calcifying space despite a decrease in ambient seawater pH associated with climate change. Those authors calculated that <1 % of the free energy supplied on average by photosynthesis would be required to increase pH in the calcifying fluid by an additional 0.5 pH units compared to ambient seawater (i.e., to counteract ocean acidification impacts). Considering that an increase in pH by 0.5 units could result in $\delta^{18}O$ depletion by approximately 2 ‰ (Rollion-Bard et al. 2003), larger than the annual range of millimeter-scale $\delta^{18}O$ in most coral records (Charles et al. 1997; Tudhope et al. 2001; Cobb et al. 2003), it becomes apparent that small differences in photosynthetic rates could strongly influence the measured coral skeletal $\delta^{18}O$.

Here we explore four records of coral skeletal stable isotopic composition. Each is >70 years long, and two exhibit unusual baseline shifts in $\delta^{18}O$. We then examine changes in the dominant *Symbiodinium* clade as a potential driver for this baseline shift.

Methods

Coral core collection

Coral cores were collected from *M. faveolata*, the dominant reef builder on the deep fore reef in Mesoamerica (McField 2000) from four sites on the Mesoamerican Reef: Turneffe Atoll (January 2007), the Sapodilla Cayes (January 2006), Utila (July 2006), and Cayos Cochinos (July 2006) (Table 1; Fig. 3). Cores were drilled vertically to capture the maximum growth axis using a pneumatic drill with a 5-cm-diameter core barrel. After collection, the tissue was removed with a Waterpik™, and the cores were rinsed in freshwater and air-dried.

Coral core isotopic analyses

At the Scripps Institution of Oceanography, an 8-mm-thick slab was removed from each coral core using a carbide-tipped double-bladed table saw lubricated with water. The slabs were X-rayed to reveal annual density bands that were used both for growth rate analysis (Carilli et al. 2009a) and to guide isotopic sampling. Samples of

Table 1 Coral core collection site locations, with dive site name or nearby caye, and coordinates

Site	Dive site name	Coordinates
Turneffe Atoll	Harry Jones	17°18′25″N, 87°48′04″W
Sapodilla	Frank's Caye, NE buoy	16°07′45″N, 88°14′59″W
Utila	Diamond Caye	16°03′52″N, 86°57′30″W
Cayos Cochinos	Pelican Point, Peli 2	15°58′41″N, 86°29′06″W

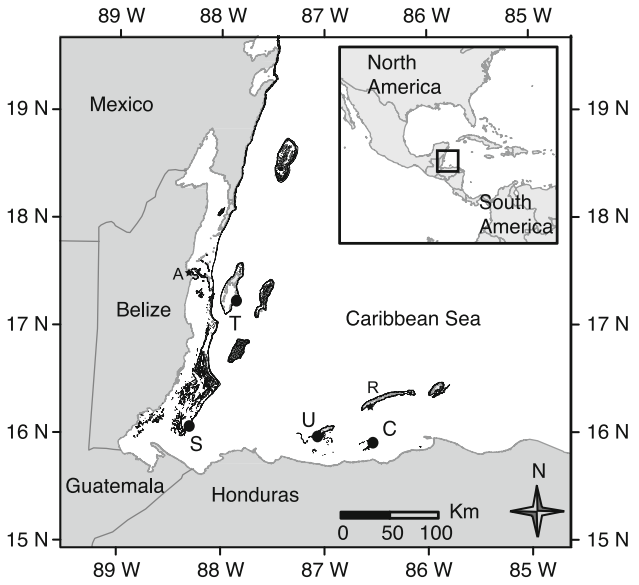

Fig. 3 Map of Mesoamerican Reef, in *black*, and surrounding countries in *gray*. Coral samples were collected from Turneffe Atoll (*T*), the Sapodilla Cayes (*S*), Utila (*U*), and Cayos Cochinos (*C*). Also denoted with *stars* are weather stations at the Belize International Airport (*A*), the Roatan airport (*R*). Another weather station is located at Hunting Caye in the Sapodilla Cayes (*S*)

~200–300 µg of skeletal powder were drilled along corallite walls using a small drill press and a drill bit ~0.5 mm in diameter. Samples were spaced 1 mm apart along the majority of all cores, but 0.5 mm apart near tops of the cores from the Sapodilla Cayes, Utila, and Cayos Cochinos. Each sample was reacted in a common phosphoric acid bath at 90 °C for 11 min and was then analyzed using a Finnigan MAT 252 stable isotope ratio mass spectrometer. Each set of 40 unknowns included seven interspersed standards of ground *Porites* sp. coral to quantify instrumental precision, which was ±0.11 ‰ for $\delta^{13}C$ and ±0.066 ‰ for $\delta^{18}O$ (1σ, $n = 446$). Isotopic ratios are expressed in permil (‰) notation versus the PDB standard for both oxygen and carbon isotopes (Fig. 4).

Coral core statistical analyses

We compared our isotopic records with sea surface temperature (SST) data products as well as records of various climatic indices. Due to inherent seasonality, isotopic and SST records are non-normal. We first calculated annual mean values for the oxygen and carbon isotope records as well as annual maximum and minimum values for the oxygen isotope records (Fig. 4). We also calculated annual negative oxygen isotopic anomalies (where more negative values correspond to warmer and/or wetter conditions) and annual heat stress anomalies from the ERV2SST SST dataset (Fig. 5; Smith and Reynolds 2004). We calculated annual oxygen isotopic anomalies over the length of each record starting from October of 1 year through September of the following year to correspond with the coral's growth year (delineated by changes in skeletal density). Each month's isotopic anomaly was subtracted from the long-term minimum, set at −4.26 for all sites (corresponding to the long-term average). Any values that exceeded this threshold were then summed for the total negative oxygen isotopic anomaly in each coral year (Fig. 5). We also constructed records of "degree-heating-months" (DHM) from the ERV2SST global SST dataset for two different grid cells: the 2 × 2° boxes centered on 18°N, 88°W to represent Turneffe and Sapodilla and 18°N, 86°W to represent Cayos Cochinos and Utila. This method calculates DHM as the annual sum (again from October to September) of the difference between the average monthly SSTs that exceeded the long-term maximum monthly mean (Lough 2000).

We calculated Pearson correlation coefficients in *R* between annual mean $\delta^{18}O$ and $\delta^{13}C$ and annual mean SST from the ERV2SST and HadISST (Rayner et al. 2003) datasets, as well as annual index values from the following climate indices: Atlantic Meridional Oscillation (AMO; Enfield et al. 2001), North Atlantic Oscillation (NAO; Hurrell 1995), Tropical North Atlantic Index (TNAI; Penland and Matrosova 1998), Caribbean Index (CAR; Penland and Matrosova 1998), and the Multivariate El Niño Southern Oscillation Index (MEI; Wolter and Timlin 1998).

Bleaching occurred on the Mesoamerican Reef in 1995, and again more strongly in 1998 (McField 1999, 2000). The oxygen isotopic anomalies, annual mean, minimum, and maximum values were checked for normality using Shapiro–Wilkes tests in *R*; the majority of the data were non-normal, and permutation tests in *R* were used to test for significant differences between the pre- and post-bleaching data. The pre- and post-bleaching cutoff date was 1995 for Cayos Cochinos and 1998 for Turneffe, Sapodilla, and Utila; this was based on the apparent shift in $\delta^{18}O$ in the Cayos Cochinos and Turneffe datasets.

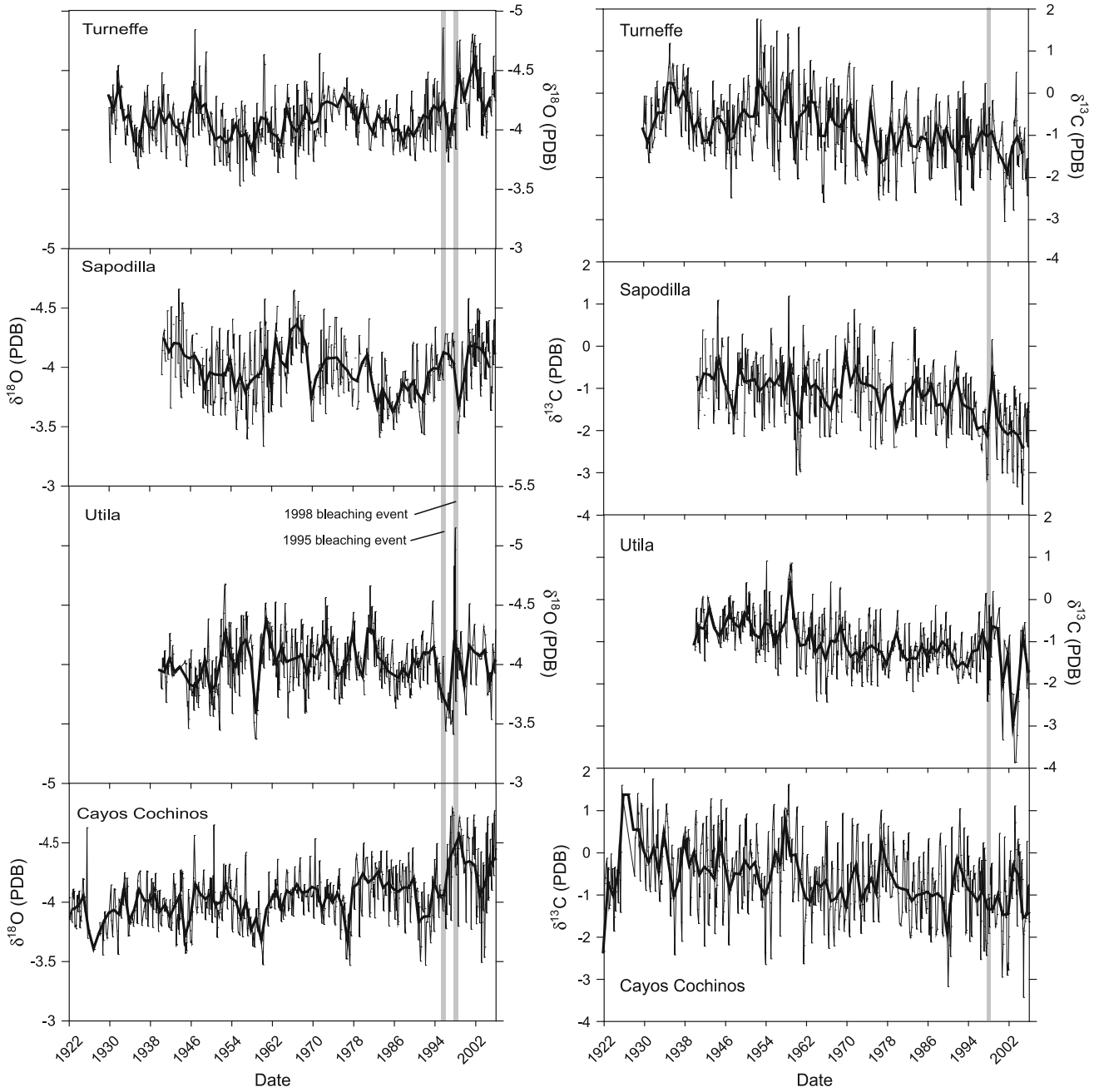

Fig. 4 Long-term approximately monthly oxygen and carbon isotope records from each of the four coral cores. *Bold line* denotes annual mean. Note reversed *y* axis in oxygen isotope plots

Because $\delta^{18}O$ and $\delta^{13}C$ were correlated in our datasets (Fig. 6; with Pearson correlation coefficients on the raw data ranging between 0.45 and 0.61), we also used an ANCOVA to test for significant differences in $\delta^{18}O$ before and after bleaching while controlling for $\delta^{13}C$. However, because the data were non-normal, even upon transformation, we first binned the $\delta^{18}O$ data by 0.5 ‰ increments of $\delta^{13}C$ (Fig. 7). This resulted in average $\delta^{18}O$ values across the range of $\delta^{13}C$ in each dataset that were normally distributed. We fit linear regressions to the pre- and post-bleaching binned data to visualize the differences between the binned datasets (Fig. 7).

Coral core Sr/Ca analyses

As an independent check of the $\delta^{18}O$ data, we analyzed Sr/Ca ratios on a subset of splits of the material, retained in acid-washed polypropylene tubes, from the coral core isotopic analyses across the 1998 bleaching event for three of the cores. Unfortunately, relevant samples from the

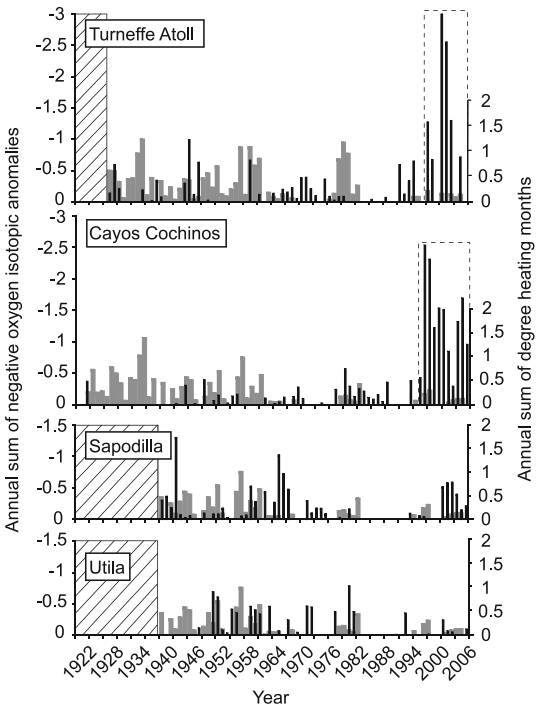

Fig. 5 Annual oxygen isotopic anomalies from each of the four coral cores (*black bars*) and annual DHM (*gray bars*) for each of the four sites (see Fig. 3 for locations). *Hashed boxes on left* delineate periods without isotope data. *Dashed boxes on right* delineate shift in oxygen isotopic baseline after bleaching in 1998 for Turneffe Atoll and 1995 for Cayos Cochinos

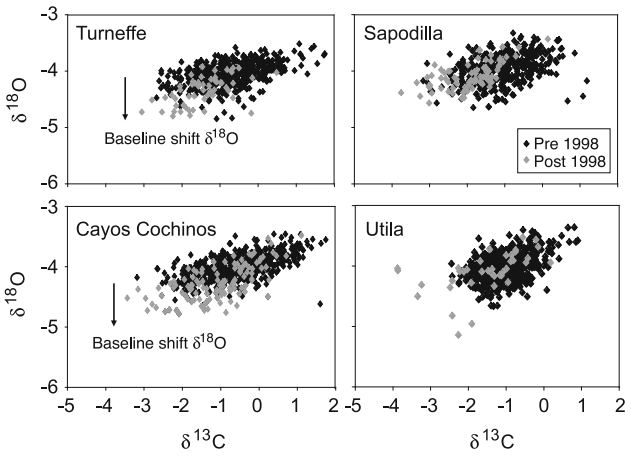

Fig. 6 $\delta^{13}C$ versus $\delta^{13}O$ plots of all data from coral cores. Data from before bleaching (1995 at Cayos Cochinos, 1998 at other sites) are *black*, and data from after bleaching are *gray*

Cayos Cochinos core were lost by an external laboratory prior to this analysis. Samples from the Sapodilla and Utila cores were analyzed at the Australian Nuclear Science and Technology Organization (with an instrumental precision of 0.01 mmol mol^{-1} [1 SD]), while samples from the Turneffe core were analyzed at Woods Hole Oceanographic Institution (with an instrumental precision of 0.02 mmol mol^{-1} [1 SD]). Samples were analyzed using inductively coupled plasma-atomic emission spectrometry (Schrag 1999; de Villiers et al. 2002; Goodkin et al. 2005). For the Turneffe core, Sr/Ca was analyzed on individual splits that were diluted with the same volume of acid, leading to a range of Ca^{2+} concentrations. To account for different matrix effects associated with different sample concentrations, a large range of standards was also analyzed, encompassing the range of sample concentrations, and the data were corrected for matrix effects after analysis (Schrag 1999). For the Sapodilla and Utila cores, Sr/Ca ratios were also analyzed on individual splits for approximately half of the samples, while the other half were combined (i.e., two or more biweekly or monthly samples) due to small sample sizes. For these samples, 0.3–1.0 mg of coral powder was weighed out, and they were diluted with acid so that all samples were at 40 ppm [Ca^{2+}], negating the need for matrix effect corrections. Time series of Sr/Ca were non-normal based on Shapiro–Wilkes tests in *R*, and permutation tests in *R* were used to test for significant differences in Sr/Ca before and after the bleaching event. We used approximately 8 years worth of data on either side of the bleaching event for each of these cores. The Sr/Ca record from the Turneffe core was wiggle-matched to HadISST to fine-tune the timescale in Analyseries 2.0; the resulting correlation coefficient between Turneffe Sr/Ca and SST was 0.49 (Electronic Supplemental Material, ESM Fig. S4).

Coral plug sample collections

To examine whether *Symbiodinium* clade affected the coral skeletal isotopic signature in *M. faveolata*, cylindrical plugs (1 cm in diameter and ~1 cm deep) of live coral tissue and the underlying skeleton were collected from Turneffe Atoll, Belize (Fig. 2; Table 1). Samples were collected with a metal punch from the tops of 20 coral heads on a single day in April 2010 at the same approximate depth (Table 2), from within a roughly 30 m radius. The samples were wrapped in aluminum foil and frozen immediately.

Plug sample *Symbiodinium* clade analyses

At Scripps Institution of Oceanography, frozen coral fragments were thawed on ice and then airbrushed using 2 mL of sterile seawater to remove the tissue from the skeleton. DNA was extracted from 250 µL of tissue slurry using the UltraClean™ Soil Kit (MoBio). The small-subunit ribosomal *RNA* gene (SSUrDNA) was amplified from each DNA sample using primers ss5 and ss3z (Rowan and Powers 1991) with the following PCR cycling series: a 94 °C denaturing step for 5 min, followed by 30 cycles of amplification (3-min denaturation at 94 °C; 1-min

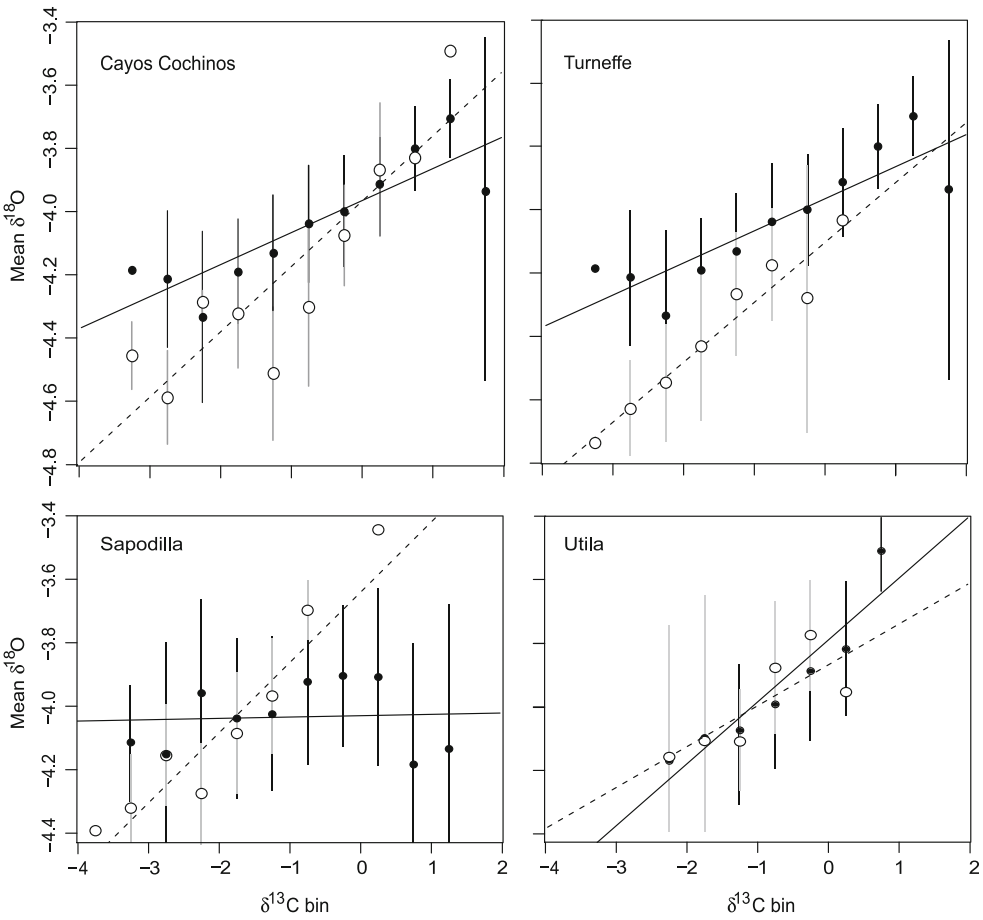

Fig. 7 Mean $\delta^{18}O$ of each 0.5 ‰-$\delta^{13}C$ bin for each coral core isotopic record before bleaching (*filled circles*) and after bleaching (*open circles*). The standard deviation at each point is shown by a *vertical line* (*black lines* for pre-bleaching data, *gray lines* for post-bleaching data). Where no standard deviation is visible, only one datapoint fell in that $\delta^{13}C$ bin. Linear regressions on the mean data are also shown: *Solid lines* are regressions on pre-bleaching data, and *broken lines* are regressions on post-bleaching data

annealing starting at 65 °C for the first cycle and reduced 0.5 °C per cycle to 50 °C; 3-min extension at 72 °C), and a final extension of 10 min at 72 °C before a 4 °C hold. The PCR product was digested separately with two restriction enzymes, *TaqI* and *DpnII* (New England Biolabs), to identify *Symbiodinium* lineages A, B, C, and D following the restriction fragment length polymorphism (RFLP) protocol in Rowan and Powers (1991). While subcladal genetic variability likely exists in these corals, significant physiological differences have been observed between groups at the coarser clade level (Ulstrup et al. 2008; Oliver and Palumbi 2011), and we therefore retain the clade grouping here.

Plug sample isotopic analyses

The $\delta^{18}O$ and $\delta^{13}C$ of each skeletal sample were analyzed using several thin slices of the coral skeleton corresponding to four different time horizons: the top ~0.5 mm of septa, bottom ~0.5 mm of septa, top ~0.5 mm of coenosteum, and a subsample of homogenized material from the top ~4 months of coral growth (Fig. 8). Assuming an average annual growth rate of 8 mm, each ~0.5 mm sample should represent approximately 3 weeks of growth. The sampling was carried out using a clean razor blade. The samples were analyzed for stable isotopic composition using the methods described above for the coral cores.

Plug sample statistical analyses

Because $\delta^{18}O$ and $\delta^{13}C$ were correlated in our plug sample data (Pearson's correlation coefficient = 0.80), and different coral samples had inherently different $\delta^{13}C$ ranges (Fig. 9), we could not simply compare $\delta^{18}O$ between corals with different *Symbiodinium* clades. Instead, to control for changes in $\delta^{13}C$, we fit linear regressions to the data from each plug sample and then evaluated those linear regressions to calculate the $\delta^{18}O$ at the mean $\delta^{13}C$ of the entire dataset of −0.69 ‰ (Fig. 10). The resulting data were normally distributed, and we performed *t* tests to compare groups of calculated mean $\delta^{18}O$ values from plug samples with different *Symbiodinium* clades.

Results

The strongest correlations between annual mean isotopic data and climatic indices were as follows: Cayos

Table 2 Live coral punch sample details

Coral colony #	Depth of colony top	*Symbiodinium* clade
1	4	B
2	4	A
3	4.5	A
4	5.3	A
5	4.6	B/D
6	3.9	A
7	2.9	B/A
8	4.2	B/D
9	3.3	B/A
10	3.9	A
11	3.9	–
12	3.2	–
13	3.6	A
14	4.1	A
15	4.5	A
16	5.1	B/A
17	4.2	B/D
18	4.2	A
19	4.2	–
20	3.8	A

Colonies with multiple *Symbiodinium* clades have a / between clades; those with no results are marked with –

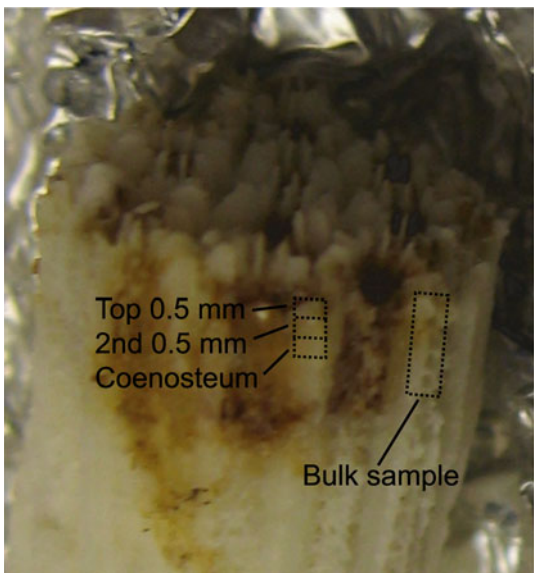

Fig. 8 Example of live-collected coral sample analyzed for *Symbiodinium* clade and skeletal isotopic signature. *Boxes* show approximate locations of sampling for isotopic analyses on individual live-collected coral plugs

Cochinos, $\delta^{18}O$ and TNAI ($R = -0.34$); Turneffe Atoll, $\delta^{13}C$ and MEI ($R = -0.35$); and Sapodilla Cayes, $\delta^{13}C$ and CAR ($R = -0.43$) and TNAI ($R = -0.45$). We did not find strong correlations between annual mean or annual anomalies of $\delta^{18}O$ and SST or other climatic indices from our four core records; all other correlation coefficients were $R < 0.3$. Annual average isotopic records also had correlation coefficients <0.3 between cores. However, similar trends were observed in $\delta^{13}C$ in all cores toward more negative values with time (0.13–0.16 ‰ per decade).

In two of our coral core isotopic records, we found a significant baseline shift in $\delta^{18}O$ anomalies without an equivalent change in $\delta^{13}C$ after known bleaching events, which occurred in 1995 at Cayos Cochinos and 1998 at Turneffe Atoll (Figs. 4, 5, 6, 7). There were significant differences in annual $\delta^{18}O$ anomalies, means, minima, and maxima at Turneffe Atoll, and in annual $\delta^{18}O$ anomalies, means, and minima at Cayos Cochinos ($p < 0.05$ based on permutation tests of pre- and post-bleaching data in *R*). In contrast, none of these measures were significantly different between pre- and post-bleaching data from our Sapodilla Cayes and Utila cores. In addition, our ANCOVA on the binned $\delta^{18}O$ versus $\delta^{13}C$ data indicated that for a given $\delta^{13}C$ value, $\delta^{18}O$ was lighter (more negative) in the post-bleaching data at Turneffe Atoll and Cayos Cochinos, but not at Sapodilla Cayes or Utila. Notably, all of the cores had skeletal growth anomalies associated with the 1998 bleaching event (as well as the 1995 bleaching event in the Cayos Cochinos core), suggesting that all of the sampled corals bleached during those events (ESM Fig. S1; Carilli et al. 2009a). The mean difference between the pre- and post-bleaching annual mean $\delta^{18}O$ in the Turneffe Atoll and Cayos Cochinos cores was 0.3 ‰. We found no significant differences in Sr/Ca between pre- and post-bleaching event data ($p = 0.76$, 0.34, and 0.89, based on permutation tests on data from the Sapodilla Cayes, Utila, and Turneffe Atoll, respectively).

Symbiodinium clades were successfully identified in 17 of the 20 plug samples. Ten samples contained only clade A symbionts, while the other colonies contained B and A (B/A; 3 corals), only B (1 coral), or B and D (B/D; 3 corals). Colony water depth ranged between 2.9 and 5.3 m and was not significantly correlated with either $\delta^{18}O$ or $\delta^{13}C$ ($r = 0.05$, $p = 0.70$; $r = 0.09$, $p = 0.43$, respectively, using Pearson's correlation coefficient in *R*, Table 2). There was only one coral with clade B *Symbiodinium* only, so isotopic data from this coral could not be statistically compared with the others. That coral had the lowest calculated $\delta^{18}O$ at the mean $\delta^{13}C$ (−3.56 ‰) based on a linear regression of all $\delta^{18}O$ versus $\delta^{13}C$ data from that plug sample compared to the coral samples hosting other *Symbiodinium* groups (A mean −3.35 ‰, B/A mean −3.47 ‰, B/D mean −3.53 ‰; Fig. 10). The differences in the means between the other groups were not significantly different based on *t* tests in *R*.

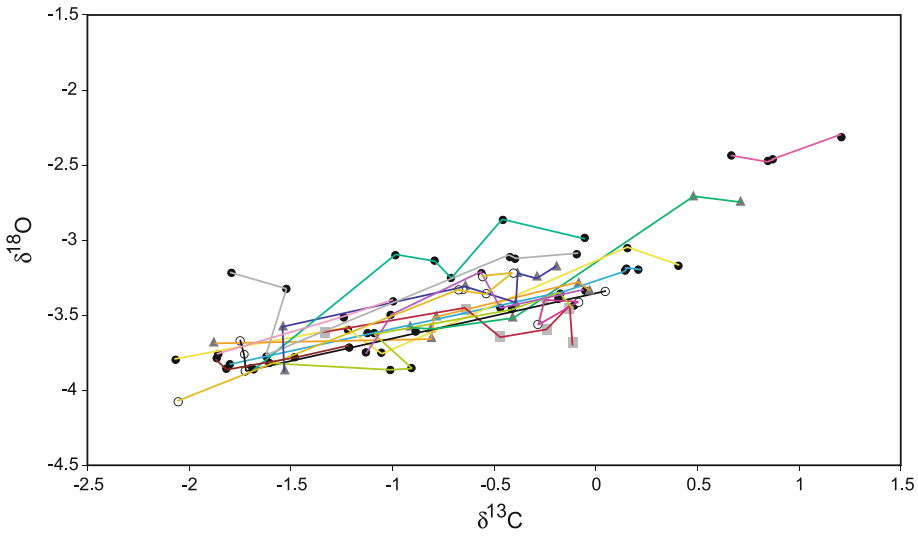

Fig. 9 Isotopic data from coral plug samples. *Different colored lines* are provided to visually identify samples from the same plug sample. Symbols identify *Symbiodinium* clade: *Filled circles* are from clade A corals, *open circles* are from clade B/D corals, *gray triangles* are from B/A corals, and *gray squares* are from clade B coral

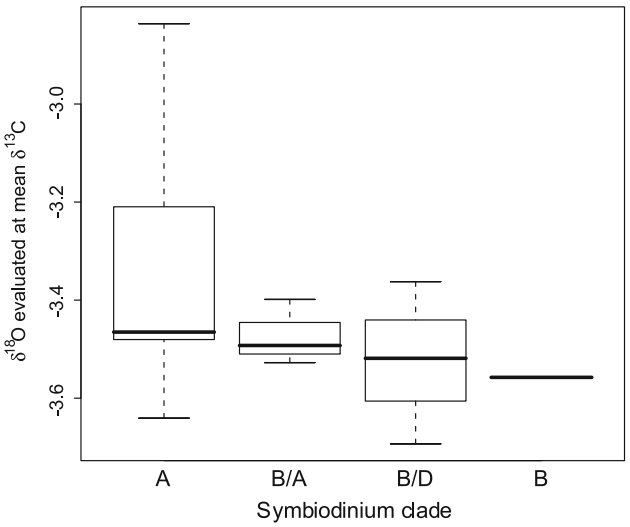

Fig. 10 Boxplot of δ^{18}O evaluated at the mean δ^{13}C of −0.69 ‰ from linear regressions on δ^{18}O and δ^{13}C from each plug sample, grouped by *Symbiodinium* clade

Discussion

Our multi-decadal δ^{18}O and δ^{13}C records from the core samples do not clearly reflect regional climatic phenomena; the records are dissimilar to one another and are not well correlated on either a monthly or annual average scale with SST reconstructions or climate indices (Fig. 5). Our Turneffe Atoll δ^{18}O record is broadly similar to a record published in Gischler and Oschmann (2005) from a lagoonal coral from Turneffe Atoll (correlation coefficient on annual averages, $R = 0.33$). Their lagoonal coral δ^{18}O record also did not correlate well with SST or climatic indices, but they did find significant correlations from other cores collected from the middle of the Belizean section of the Mesoamerican Reef (Gischler and Oschmann 2005).

We suspect that the unclear relationships between our isotopic records and climatic data stem from a combination of differences in the timing of peak rainfall and SST across the region, which do not covary and may cause conflicting δ^{18}O changes (ESM Fig. S2); impacts on seawater δ^{18}O from land-based runoff or lagoonal influence (Carilli et al. 2009b); or, as we discuss further below, changes in the dominant zooxanthellae clade. The long-term depletion in δ^{13}C is consistent with the Seuss effect, wherein isotopically lighter CO_2 is liberated into the atmosphere from burning fossil fuels (Keeling 1979). The magnitude of this trend is similar to those reported in Swart et al. (2009), and references therein, on corals and sclerosponges from the wider Caribbean basin.

However, the shift in δ^{18}O after bleaching in two of our records cannot be satisfactorily explained by changes in SST, seawater δ^{18}O composition/salinity, or gross skeletal growth rates. Skeletal extension, density, and calcification rates (Carilli et al. 2009a) were compared between cores across the bleaching event (ESM Fig. S3). Extension and calcification were reduced below pre-bleaching rates in these cores after bleaching in 1998 at both Cayos Cochinos and Utila, but they recovered and even exceeded the pre-bleaching rates at Turneffe Atoll and Sapodilla. These patterns cannot explain the observed shifts in δ^{18}O at Cayos Cochinos and Turneffe Atoll, as those cores had opposite skeletal growth rate changes after bleaching. Other studies have also found significantly different, unexplained isotopic ratios from neighboring coral colonies, even after growth rate corrections have been applied (Linsley et al. 1999; Felis et al. 2003). If interpreted solely as indicative of a change in water temperature, a 0.3 ‰ shift in δ^{18}O would correspond to a ~1.5 °C difference in mean temperature before and after the bleaching event, but this is not reflected in SST reconstructions (Fig. 5).

An SST change is also not supported by our Sr/Ca analyses, which were not significantly different before and after the bleaching event. A change in average salinity of ~20 psu would be required to cause this magnitude (0.3 ‰) $\delta^{18}O$ shift in seawater composition. This is unlikely, considering that the salinity observed at Cayos Cochinos varied by a maximum of ~4 psu over 2 years of in situ data collection (USGS SBE Seabird 16-plus instrument).

No established mechanisms are available to explain the observed change in $\delta^{18}O$ in our cores, so we hypothesized that the shift could be the geochemical signature of a change in dominant *Symbiodinium* clade in the coral tissues. A switch or shuffling of the dominant symbiont clade after bleaching to a more heat-tolerant variety has been suggested as an adaptive response to increasing heat stress (Buddemeier and Fautin 1993). While several studies have found that the symbiont association sampled from the same location on *M. faveolata* heads is often stable over the long term, even after bleaching (Toller et al. 2001; Thornhill et al. 2006, 2009), some corals did temporarily change before reversion to the original clade (Thornhill et al. 2006, 2009). Also, Ulstrup et al. (2008) found that *Symbiodinium* clade changed after severe experimental bleaching, and Berkelmans and van Oppen (2006) found that some corals changed from clade C to D dominance when transplanted to a warmer location.

The various clades of *Symbiodinium* differ physiologically in their response to heat and light stress and photosynthetic rate and efficiency (Iglesias-Prieto and Trench 1994; Kinzie et al. 2001; Savage et al. 2002; Rowan 2004; Goulet et al. 2005; Warner et al. 2006; Loram et al. 2007; Ulstrup et al. 2007; Abrego et al. 2008; Stat et al. 2008; Cantin et al. 2009; Hennige et al. 2009; van Oppen et al. 2009). For instance, clade A appears adapted to high light conditions; it was the only *Symbiodinium* clade able to produce photoprotective mycosporine-like amino acids in culture (Banaszak et al. 2000), and it was also the only clade that increased the use of alternative photosynthetic pathways, another photoprotective mechanism, in high light conditions (Reynolds et al. 2008). However, these photoprotective mechanisms are also likely to make clade A less photosynthetically efficient overall (LaJeunesse 2002), and this may lead to differences in the skeletal isotopic composition in corals hosting clade A compared with other clades of *Symbiodinium*. Similarly, there are likely other physiological differences between clades that could affect skeletal chemistry.

Thus, different clades may have varying effects on the isotopic chemistry of the coral skeleton. For instance, corals hosting *Symbiodinium* that photosynthesize at higher rates may incorporate more metabolic CO_2 into their skeletons and could increase the pH in the calcifying space in a number of ways, both of which could lead to more depleted $\delta^{18}O$ signatures in the coral skeleton (Figs. 1, 2; Furla et al. 2000; Adkins et al. 2003; Al-Horani et al. 2003; McConnaughey 2003; Rollion-Bard et al. 2003; Allison et al. 2010; Colombo-Pallotta et al. 2010; Venn et al. 2011; McCulloch et al. 2012). Indeed, Spero et al. (1997) observed an analogous change in the intercept of the $\delta^{13}C$/$\delta^{18}O$ relationship in symbiont-bearing foraminifera raised under high light or dark conditions; at any given $\delta^{13}C$ value, foraminifera raised with high light had significantly lower $\delta^{18}O$ than those raised in the dark. While foraminiferal biomineralization may be different from that of corals, these results are consistent with variations in $\delta^{13}C$ and $\delta^{18}O$ with symbiont photosynthetic rates.

Taken together, the observed shift in $\delta^{18}O$ after bleaching in our coral cores, the calcification mechanisms reported in the literature, and the isotopic signatures in our plug samples implicate symbiont physiology as a factor in skeletal isotopic fractionation that cannot be ignored. The coral plug samples we collected to test this hypothesis were dominated by clade A *Symbiodinium*, reducing our power to statistically separate differences in isotopic signatures between corals hosting different symbiont clades. However, these data serve as an important motivator for further investigation using archived collections, field samples, or material used in experimental aquaria, to further elucidate the impacts of symbiont clade on coral skeletal chemistry. This effect offers a potential mechanism with which coral skeletons could provide a historical record of the incidence and frequency of symbiont clade shuffling.

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