October 2005

Pre-treatment effects on coral skeletal delta^{13}C and delta^{18}O

Andrea G. Grottoli
University of Pennsylvania

Lisa J. Rodrigues
University of Pennsylvania

Kathryn A. Matthews
University of Pennsylvania

James E. Palardy
University of Pennsylvania

Olivia T. Gibb
University of Pennsylvania

Pre-treatment effects on coral skeletal delta^{13}C and delta^{18}O

Abstract

Pre-treatments are often used to remove organic "contaminant" material prior to isotopic analyses of coral skeletal samples. Here we conducted three experiments to test the pre-treatment effect of water, 30% hydrogen peroxide (H_2O_2), and household bleach [5.25% sodium hypochlorite ($NaClO_3$) and 0.15% sodium hydroxide ($NaOH$)], on the stable isotopic composition of coral skeletal samples. First, using a mass balance approach we calculated the expected change in skeletal delta^{13}C due to the removal of all organic carbon. The model showed that (1) the removal of organic carbon (which has a low delta^{13}C value relative to skeletal delta^{13}C) from the skeletal sample should theoretically result in a higher delta^{13}C value of the remaining organic-carbon-free carbonate, and that (2) only at the highest concentrations of skeletal organic carbon within the tissue layer of corals is the contribution of the organic carbon to the overall delta^{13}C skeletal value potentially large enough to be detectable by mass spectrometry. We then conducted two sets of experiments to test the model where we pre-treated a large number of skeletal samples from five species of corals with water, H_2O_2, bleach, or no pre-treatment for 24 h. Skeletal delta^{13}C generally decreased significantly with water, bleach, and H_2O_2 pre-treatments which is contrary to the model-predicted increase in delta^{13}C following such pre-treatments. Thus, organic carbon within the skeleton is not a net source of contamination to delta^{13}C analyses. Skeletal delta^{18}O decreased the most with water and bleach pre-treatments. In addition, the effect of H_2O_2 or bleach pre-treatments on either delta^{13}C or delta^{18}O was not consistent among species or locations. The direction of change in delta^{13}C and delta^{18}O with pre-treatments was no different for skeletal samples taken within or below the tissue layer. Based on our results, we suggest that pre-treatment is not necessary and recommend that pre-treatment not be performed on coral skeletal samples prior to stable isotope analysis to avoid any pre-treatment-induced variability that could significantly compromise inter-colony and inter-species comparisons.

Keywords
Coral; Aragonite, Pre-treatment, Method

Pre-treatment effects on coral skeletal δ^{13}C and δ^{18}O

Andrea G. Grottoli, Lisa J. Rodrigues, Kathryn A. Matthews, James E. Palardy and Olivia T. Gibb

Department of Earth and Environmental Science, University of Pennsylvania, 240 South 33rd Street, Philadelphia, PA 19104, United States

Abstract

Pre-treatments are often used to remove organic "contaminant" material prior to isotopic analyses of coral skeletal samples. Here we conducted three experiments to test the pre-treatment effect of water, 30% hydrogen peroxide (H_2O_2), and household bleach [5.25% sodium hypochlorite ($NaClO_3$) and 0.15% sodium hydroxide ($NaOH$)], on the stable isotopic composition of coral skeletal samples. First, using a mass balance approach we calculated the expected change in skeletal δ^{13}C due to the removal of all organic carbon. The model showed that (1) the removal of organic carbon (which has a low δ^{13}C value relative to skeletal δ^{13}C) from the skeletal sample should theoretically result in a *higher* δ^{13}C value of the remaining organic-carbon-free carbonate, and that (2) only at the highest concentrations of skeletal organic carbon within the tissue layer of corals is the contribution of the organic carbon to the overall δ^{13}C skeletal value potentially large enough to be detectable by mass spectrometry. We then conducted two sets of experiments to test the model where we pre-treated a large number of skeletal samples from five species of corals with water, H_2O_2, bleach, or no pre-treatment for 24 h. Skeletal δ^{13}C generally decreased significantly with water, bleach, and H_2O_2 pre-treatments which is *contrary* to the model predicted increase in δ^{13}C following such pre-treatments. Thus, organic carbon within the skeleton is not a net source of contamination to δ^{13}C analyses. Skeletal δ^{18}O decreased the most with water and bleach pre-treatments. In addition, the effect of H_2O_2 or bleach pre-treatments on either δ^{13}C or δ^{18}O was not consistent among species or locations. The direction of change in δ^{13}C and δ^{18}O with pre-treatments was no different for skeletal samples taken within or below the tissue layer. Based on our results, we suggest that pre-treatment is not necessary and recommend that pre-treatment not be performed on coral skeletal samples prior to stable isotope analysis to avoid any pre-treatment-induced variability that could significantly compromise inter-colony and inter-species comparisons.

1. Introduction

Mounding coral species used for paleoclimatic and paleoceanographic reconstruction deposit their aragonitic skeleton below the basal epidermis in discrete annual bands, can grow for several hundred years, and have high annual growth rates that allow for monthly or better sampling resolution. The stable carbon [δ^{13}C = permil deviation of the ratio of ^{13}C / ^{12}C relative to the Vienna Peedee Belemnite (V-PDB) Limestone standard] and oxygen [δ^{18}O = permil deviation of the ratio of ^{18}O / ^{16}O relative to V-PDB] isotopic composition of coral skeletal records have been used over the past few decades for

paleoclimatic/paleoceanographic studies and for coral physiological studies. Both isotopes can be very powerful tools provided that the methods of skeletal sample preparation are scientifically valid and consistent among publications. Several pre-treatment methods have been applied to coral skeletal material prior to stable isotopic analyses over the past few decades (Table 1) with mixed results regarding the effects of the pre-treatment on the skeletal isotopic values.

Table 1. Published papers using various coral skeletal pre-treatment methods

Pre-treatment	References
None reported and/or briefly washed or sonicated in water	Cardinal et al., 2001, Cobb et al., 2001, Cobb et al., 2003a, Cobb et al., 2003b, Cohen and Hart, 1997, Cole et al., 1993, Cole et al., 2000, Cole and Fairbanks, 1990, Emiliani et al., 1978, Fairbanks and Dodge, 1979, Felis et al., 1998, Felis et al., 2000, Felis et al., 2003, Gagan and Chivas, 1995, Gagan et al., 1994, Gagan et al., 1996, Grottoli, 1999, Grottoli, 2002, Grottoli and Wellington, 1999, Guilderson and Schrag, 1999, Guzman and Tudhope, 1998, Hemming et al., 1998, Hughen et al., 1999, Kefu et al., 2002, Klein et al., 1992, Klein et al., 1993, Klein et al., 1997, Kuhnert et al., 1999, Kuhnert et al., 2002, Leder et al., 1991, Linsley et al., 1999, Linsley et al., 2000, Maier et al., 2003, McCulloch et al., 1994, Morimoto et al., 2002, Patzold, 1984, Patzold et al., 1991, Suzuki et al., 2000, Suzuki et al., 2003, Swart et al., 1996a, Swart et al., 1996b, Swart et al., 1996c, Swart et al., 1998, Swart et al., 1999, Watanabe et al., 2002, Watanabe et al., 2003, Wellington et al., 1996 and Winters et al., 1991
Bleach (NaOH or NaClO$_3$) 5% for 1–24 h	Allison et al., 1996, Carriquiry et al., 1988, Carriquiry et al., 1994, Guzman and Tudhope, 1998, Land et al., 1975, Land et al., 1977, Leder et al., 1996, Tudhope et al., 1995, Tudhope et al., 1996 and Tudhope et al., 1997
Hydrogen peroxide (H$_2$O$_2$) 5–35% for 1–36 h	Allison et al., 1996, Bessat et al., 1997, Boiseau et al., 1998, Boiseau et al., 1999, Boiseau and Juillet-Leclerc, 1997, Carriquiry, 1994, Heikoop et al., 2000, Heiss, 1996, Reynaud et al., 2002, Reynaud-Vaganay et al., 1999, Reynaud-Vaganay et al., 2001 and Weil et al., 1981
Roasting at 200, 375 or 475 °C for 30–60 min or oxygen plasma roasting at 400 °C for 20 min.	Aharon, 1991, Chakraborty and Ramesh, 1993, Chakraborty and Ramesh, 1997, Chakraborty and Ramesh, 1998, Druffel, 1985, Druffel, 1997, Druffel and Griffin, 1993, Druffel and Griffin, 1995, Druffel and Griffin, 1999, Druffel et al., 2001, Dunbar et al., 1994, Epstein et al., 1953, Goreau, 1977, Linsley et al., 1994, Nozaki et al., 1978, Porter et al., 1989, Quinn et al., 1993, Quinn et al., 1996a, Quinn et al., 1996b, Quinn et al., 1998 and Wellington and Dunbar, 1995
Bleach and/or H$_2$O$_2$ and roasting	Dunbar and Wellington, 1981, Dunbar et al., 1990, McConnaughey, 1989, Weber et al., 1976 and Weber and Woodhead, 1972

Pre-treatments have been applied for the purpose of removing organic "contaminants" from the skeletal material prior to isotopic analysis. The most common pre-treatment techniques are vacuum roasting, bleaching with hydrogen peroxide (H$_2$O$_2$), and bleaching with sodium hypochlorite (NaClO) combined with sodium hydroxide (NaOH) (household bleach). Previous studies have shown that vacuum roasting results in an unpredictable and thus uncorrectable isotopic shift in coral skeletal δ^{18}O (e.g.: Dunbar and Wellington, 1981, Gaffey et al., 1991 and Land et al., 1975), with the conclusion that vacuum roasting pre-treatment should no longer be practiced. However, pre-treatment bleaching with H$_2$O$_2$ or household bleach continues to be practiced by some researchers (particularly for removing organic "contaminants" from the skeletal material within the tissue layer) while other researchers have opted not to pre-treat coral skeletal material at all (Table 1).

The logic for not pre-treating skeletal samples is threefold. First, of the total carbon, the organic carbon content (by mass) of coral skeletal material ranges from 0.02% to 0.5% below the tissue layer and up to 0.7% within the tissue layer (Boiseau and Juillet-Leclerc,

1997 and Ingalls et al., 2003). This is a very small fraction of the total skeletal carbon mass. Second, when the coral material is acidified for isotopic analysis only some of the organic material reacts with acid. Thus the total amount of organic carbon-derived CO_2 produced from the reaction is small. Third, many researchers (but not all, see Boiseau and Juillet-Leclerc, 1997) have found that the variation in both $\delta^{18}O$ and $\delta^{13}C$ composition of pre-treated coral skeletal material was greater than in non-treated corals and that shifts in the skeletal isotopic composition after pre-treatment with either bleach or hydrogen peroxide were not always consistent (Cole and Fairbanks, 1990 and McConnaughey, 1989; Mucciarone unpublished).

A study by Boiseau and Juillet-Leclerc (1997) recommended that H_2O_2 be used to remove organic "contaminants" prior to coral $\delta^{18}O$ skeletal isotopic analysis. They presented data from a single *Porites lutea* coral core showing that the H_2O_2 pre-treatment did not isotopically exchange with skeletal oxygen and that the overall mean and variation in $\delta^{18}O$ of the H_2O_2 pre-treated skeletal samples was the same as for the non pre-treated samples. However, a closer examination of the data reveals that while the overall average is the same between the pre-treated and non pre-treated samples, the direction and magnitude of the shift in the skeletal isotopic composition after pre treatment was not always consistent: $\delta^{18}O$ increased by an average of 0.11‰ ± 0.11 [1 standard deviation (std)] in six of the 24 samples and decreased by 0.18‰ ± 0.14 (1 std) in the remaining 18 samples, while $\delta^{13}C$ increased by 0.39‰ ± 0.20 (1 std) in eight of the 24 samples and decreased by 0.25‰ ± 0.12 (1 std) in the remaining 16 samples. These changes in $\delta^{13}C$ and $\delta^{18}O$ are large and easily detectable by stable isotope ratio mass spectrometry. If a single coral core is analyzed at 1 mm resolution (as is typically done for coral-based paleoclimate reconstruction), pre-treatment could shift the $\delta^{13}C$ and $\delta^{18}O$ of any given sample significantly and in an unpredictable and non-correctable manner. A 0.18‰ shift in $\delta^{18}O$ can indicate as much as a ∼ 1 °C seawater temperature shift in some geographical regions. Another study by Watanabe et al. (2001) found no difference in the $\delta^{13}C$ and $\delta^{18}O$ values of a single *Montastraea* coral that was pre-treated for 15 min with either H_2O_2 + NaOH or with weak nitric acid. In both the Watanabe et al. (2001) and the Boiseau and Juillet-Leclerc (1997) papers, all of the analyses were performed on a single coral core. Thus the researchers were unable to evaluate if the skeletal material of all coral individuals and species have the same isotopic response to pre-treatments.

Despite the amount of published literature on the topic, the question still remains: is the removal of organic "contaminants" from coral skeletal samples necessary or valid? If organic molecules do not readily break down when exposed to acids and are in very low concentrations in the skeleton (even in the tissue layer), can the small amount of added CO_2 from the organic material in the skeleton truly contaminate the sample? In other words, is the volume of organically-derived CO_2 large enough to cause a detectable shift in the isotopic value and therefore be of realistic concern? None of the previous studies contained a sufficient sample size or diversity of coral species to fully evaluate the effects of H_2O_2 or bleach pre-treatments on coral skeletal $\delta^{13}C$ and $\delta^{18}O$ values. Small sample size limits the statistical power in the data and thus the strength of the conclusions. Since coral skeletal structure varies between species, the surface contact area may differ between species resulting in variable or contradictory results. In addition, the previous

studies often did not include adequate controls in their experiments thus complicating the interpretation of the results.

Here, the effects of H_2O_2 and bleach pre-treatments on coral skeletal isotope values were assessed with a theoretical model and two analytical experiments. Relevant controls, multiple species, and replication were implemented in the design. The overarching goal was to determine if pre-treatments, as performed in the literature, are necessary prior to isotopic analysis of coral skeletal material. A simple mass balance equation to calculate the theoretically expected change in skeletal $\delta^{13}C$ due to the removal of organics during pre-treatment was constructed. Based on this model, it was hypothesized that pre-treatment of corals to remove organic "contaminants" would result in a detectable increase in the skeletal $\delta^{13}C$ of samples within the tissue layer, but have no analytically detectable effect on the skeletal $\delta^{13}C$ of samples below the tissue layer. This hypothesis was tested using two experiments. In the first experiment, the effect of pre-treatments on repeated analyses of five homogeneous samples extracted from below the tissue layer was assessed. This experiment evaluated if pre-treatment effects on $\delta^{13}C$ and $\delta^{18}O$ are reproducible, and therefore correctable, within a single homogenous sample. This experiment was repeated on five coral species. The second experiment was designed to determine two things: (1) if pre-treatment effects on $\delta^{13}C$ and $\delta^{18}O$ are reproducible, and therefore correctable, among coral fragments of the same species and among different species, and (2) if pre-treatment produced a larger shift in the $\delta^{13}C$ and $\delta^{18}O$ of skeletal material sampled both within and below the tissue layer and thus address whether organic-rich skeletal samples need to be pre-treated to remove organic "contaminants" prior to isotopic analysis.

2. Methods

2.1. Mass balance model

A simple mass balance equation was solved to calculate the theoretical isotopic contribution of the organic carbon fraction to the overall skeletal $\delta^{13}C$ value and the expected change in coral skeletal isotopic composition due to pre-treatments. Within the tissue layer, organic carbon accounts for 0.55–0.70% of the total skeletal carbon in *Porites lutea* coral from Tahiti (Boiseau and Juillet-Leclerc, 1997). Below the tissue layer, organic carbon accounts for 0.11–0.56%, 0.02–0.4%, and 0.04–0.11% of the total skeletal carbon in *P. lutea* from Tahiti (Boiseau and Juillet-Leclerc, 1997), in *P. lutea* from the Red Sea, and in *Montastraea annularis* from Florida, respectively (Ingalls et al., 2003). These published values represent a range of organic carbon content in corals and were used to set the maximum and minimum organic carbon content values in the model. Assuming that during acidification all of the organic carbon is oxidized to produce CO_2, we calculated the expected change in skeletal $\delta^{13}C$ due to the removal of all organic matter based on the mass balance equation:

$$\delta^{13}C_{wus} = \delta^{13}C_{org} \ (x) + \delta^{13}C_{carb} \ (1-x) \qquad (1)$$

where $\delta^{13}C_{wus}$ is the $\delta^{13}C$ value of the *w*hole *u*ntreated *s*keleton and is the sum of the $\delta^{13}C$ of the *org*anic tissue ($\delta^{13}C_{org}$) and *carb*onate skeletal components ($\delta^{13}C_{carb}$). x is the proportion of organic carbon in the skeleton, and at most ranges from 0.0002 to 0.007 (equivalent to 0.02–0.70%) (Boiseau and Juillet-Leclerc, 1997 and Ingalls et al., 2003). While organic carbon molecules do not readily react with acids, the model was constructed with the assumption that 100% of the organic material is acidified to yield the maximum theoretical contribution of organic carbon to the isotopic value of the coral skeleton. The $\delta^{13}C_{carb}$ was calculated for three species of corals for which both $\delta^{13}C_{wus}$ and $\delta^{13}C_{org}$ values were available: *Montipora verrucosa*, *Porites compressa*, and *Montastraea annularis* (Table 2). Bulk coral tissue samples were removed from the tops of coral fragments with a water pik and acidified to remove any potential skeletal particles prior to isotopic analysis of the tissue fraction to yield $\delta^{13}C_{org}$ values. Whole untreated skeletal samples were removed by gently shaving the top skeletal portion of the coral fragment with a diamond-tipped drill bit and the $\delta^{13}C$ of the resulting skeletal powder was analyzed by mass spectrometry. Since none of these corals were pre-treated prior to analysis, the reported skeletal $\delta^{13}C$ values are in fact $\delta^{13}C_{wus}$ values. Additional details on the analytical methods for both the skeletal and tissue fractions are given elsewhere (Grottoli et al., 2004 and Muscatine et al., 1989). For each species, $\delta^{13}C_{carb}$ was solved for using the mass balance equation above for values of x ranging from 0.02% to 0.70%. The difference between $\delta^{13}C_{wus}$ and $\delta^{13}C_{carb}$ represents the maximum predicted contribution of the organic fraction to the $\delta^{13}C_{wus}$ value.

Table 2. Description of coral data used in the model shown in Fig. 1

Species	Morphology	Depth (m)	Location	Average $\delta^{13}C_{wus}$	Average $\delta^{13}C_{org}$	Source
Montipora verrucosa	Branching/plating	1.2	Hawaii	− 2	− 14.5	Grottoli et al. (this study)
Porites compressa	Branching	1.2	Hawaii	− 3	− 14	Grottoli et al. (this study)
Porites compressa	Branching	5	Hawaii	− 4	− 15.5	Grottoli et al. (this study)
Porites compressa	Branching	8.3	Hawaii	− 5	− 16	Grottoli et al. (this study)
Montastraea annularis	Mounding	10	Jamaica	− 2.2	− 13.6	Land et al. (1975), Muscatine et al. (1989), Fairbanks and Dodge (1979)

$\delta^{13}C_{wus} = \delta^{13}C$ of whole untreated skeleton, $\delta^{13}C_{org} = \delta^{13}C$ of organic tissue fraction.

2.2. Experiment #1

The effect of pre-treatments on repeated analyses of a homogeneous sample extracted from below the tissue layer was evaluated. This experiment was conducted on five coral species. This experiment evaluated if pre-treatment had an effect on the $\delta^{13}C$ and $\delta^{18}O$ values, if those effects were reproducible (and therefore correctable) within a single homogenous sample, and if those results were consistent among species. A bulk 160 mg sample of skeletal material from below the tissue layer of one coral fragment of each species was used. None of the samples had any visible evidence of secondary calcification, intrusion of boring organisms or other possible sources of exogenous carbonate or organic contamination. The bulk sample was cleaned with high pressure deionized water for one minute, dried at 60 °C for at least 3 days, ground to a fine powder, and divided into twenty 8-mg subsamples: five subsamples were independently not pre-treated with anything, five were pre-treated with Milli-Q filtered water, five with H_2O_2, and five with bleach for 24 h. After pre-treatment, all subsamples were centrifuged at 6000 rpm for 10 min and their respective pre-treatment liquids were aspirated. The samples were then rinsed ten times with deionized water by centrifugation and pipetting techniques and then dried overnight. An 80 μg aliquot of each sample was acidified with 100% ortho-phosphoric acid in an automated Kiel carbonate device, and the $\delta^{13}C$ and $\delta^{18}O$ values of the resulting CO_2 measured in a Finnigan MAT 252 triple collecting mass spectrometer. All isotope values were reported as the per mil deviation relative to V-PDB. Repeated analyses of an internal carbonate standard ($n = 98$) yields $\delta^{13}C$ and $\delta^{18}O$ values of $3.00 \pm 0.03‰$ (± 1 std) and $-1.66 \pm 0.05‰$ (± 1 std), respectively. At least 20% of all measurements were made in duplicate. This was repeated for five species of coral (collection site and date given in brackets): *Porites compressa* (Hawaii, 12 September 2003), *Porites lobata* (Hawaii, 12 September 2003), *Pavona clavus* (Panamá, 11 July 2003), *Pavona gigantea* (Panamá, 11 July 2003), and *Montipora verrucosa* (Hawaii, 12 September 2003). By homogenizing each bulk sample prior to experimental manipulation, grain size and the exposed surface area per volume was relatively consistent among species with otherwise very different skeletal structure and surface to volume ratios.

A two-way fully factorial Model I ANOVA was performed on the $\delta^{13}C$ and $\delta^{18}O$ data where treatment and species were fixed effects. A posteriori Tukey tests were used to determine which treatments and species significantly differed from each other. Statistical computations were done using JMP 5.1 General Linear Model (GLM) programs (SAS Institute Inc., Cary, NC). Null hypotheses were rejected when the probability level was ≤ 0.05. In addition, the change in $\delta^{13}C$ and $\delta^{18}O$ ($\Delta\delta^{13}C$ and $\Delta\delta^{18}O$, respectively) was computed as the difference between the mean of the non pre-treated and the mean of each pre-treated samples for each species. The isotopic values of all five subsamples for each pre-treatment and species were used to calculate the means and standard deviation of the means shown in Fig. 2.

2.3. Experiment #2

The second experiment evaluated if pre-treatment had an effect on the skeletal $\delta^{13}C$ and $\delta^{18}O$ among genotypically different fragments of the same species, among species, and

among geographic locations. This experiment was also designed to assess if the higher organic content of the skeleton within the tissue layer produced a larger shift in the skeletal $\delta^{13}C$ and $\delta^{18}O$ after pre-treatment. Based on the model above, it was hypothesized that the pre-treatment of corals to remove organic "contaminants" would result in a detectable increase in the skeletal $\delta^{13}C$ of samples within the tissue layer (WTL), but have no analytically detectable effect on the skeletal $\delta^{13}C$ of samples below the tissue layer (BTL). The hypothesis was tested on corals from Hawaii (H) and Panamá (P) collected at 2 and 5 m depths. In total there were eight coral data sets: *M. verrucosa* (H-BTL-2 m), *P. compressa* (H-BTL-2 m), *P. compressa* (H-WTL-2 m), *P. lobata* (H-WTL-2 m), *P. lobata* (P-WTL-2 m), *P. clavus* (P-BTL-2 m), *P. clavus* (P-BTL-5 m), *P. clavus* (P-WTL-5 m). The term "coral data sets" refers to these eight groups throughout the remainder of the text unless otherwise stated. In all cases, between 7 and 10 genetically distinct coral fragments (genotypes) within each coral data set were grown in situ for several months, stained with Alizarin Red at two time intervals to establish specific reference dates within the growing skeleton, and allowed to grow out for at least 3 months beyond the last stain line prior to collection. Once collected, the corals were brought to the University of Pennsylvania, cut along their major axis of growth to reveal the stain lines, cleaned with high pressure deionized water, and dried at 60 °C for at least 3 days. Only samples that were free of any visible evidence of secondary calcification, intrusion of boring organisms or other possible sources of exogenous carbonate or organic contamination were used in the experiment.

For *P. compressa*, *P. lobata*, and *M. verrucosa* from Hawaii, skeletal material was removed between the two stain lines below the tissue layer (3 October 2002 to 12 January 2003) with a Dremmel tool fitted with a diamond-tipped dental bit. For *P. compressa*, skeletal samples were also extracted within the tissue layer between the last stain line and the growing edge of the coral (12 January 2003 to 12 September 2003). This represents 9 months of growth and an average of approximately 1.3 cm of linear growth. Since the tissue penetrates the skeleton for approximately the top 1 cm of the skeleton, the sampled section was 77% within the tissue layer. For *P. clavus* from Panamá at both depths, skeletal samples below the tissue layer were extracted between the two stain lines set on 6 February and 15 April, 2003. Skeletal samples within the tissue layer were extracted from both *P. lobata* and *P. clavus* between the last stain line and the growing edge of each coral fragment (15 April to 11 July 2003) representing 3 months of growth all within the tissue layer. In all cases, at least 32 mg of material was extracted for each sample, ground to a fine powder, and divided into four 8-mg subsamples: one subsample was not pre-treated with anything, one was pre-treated with water, one with H_2O_2, and one with bleach for 24 h according to the methods outlined in Experiment #1. $\delta^{13}C$ and $\delta^{18}O$ analyses of each sample were also conducted according to methods already described above. Sampling between known time frames established by the stain lines and between a stain line and the growing edge of the coral fragment ensured that the same time interval was sampled within each coral data set and that seasonal changes in $\delta^{13}C$ and $\delta^{18}O$ over time did not interfere with the interpretation of the treatment effects within each coral data set. In addition, by homogenizing each 32 mg sample prior to experimental manipulation, grain size and the exposed surface area were relatively

consistent among genotypes within a species and among species with otherwise very different skeletal structure and surface to volume ratios.

A partially nested ANOVA was performed on the δ^{13}C and δ^{18}O data where coral data set and treatment were fixed effects and genotype was a random effect. Data set and treatment were fully crossed and tested over the genotype nested within the coral data set random effect. A posteriori Tukey tests were used to determine which treatments and coral data sets significantly differed from each other. A partially nested ANOVA was also performed on the δ^{13}C and δ^{18}O data where section (within the tissue layer or below the tissue layer) and treatment were fixed effects and genotype was a random effect. Section and treatment were fully crossed and tested over the genotype that was nested within the section random effect. A posteriori Tukey tests were used to determine which treatments and sections differed from each other. Statistical computations were done using JMP 5.1 GLM programs. Null hypotheses were rejected when the probability level was ≤ 0.05. In addition, the change in δ^{13}C and δ^{18}O ($\Delta\delta^{13}$C and $\Delta\delta^{18}$O, respectively) was computed as the difference between the non pre-treated and each pre-treated subsample for each fragment. Within each data set, the isotopic value of each fragment within each pre-treatment was used to calculate the mean and standard deviation of the mean shown in Fig. 3 and Fig. 4.

3. Results

3.1. Model

The model revealed three findings. First, since δ^{13}C$_{org}$ is isotopically very depleted relative to the skeleton (Table 2), removal of organic "contaminant" from a skeletal sample by any pre-treatment method should result in a heavier coral δ^{13}C$_{carb}$ value compared to the original untreated δ^{13}C$_{wus}$ (Fig. 1). Second, as the proportion of organic carbon in the skeleton increases, the δ^{13}C$_{carb}$ value increases. However, it is only at the highest concentrations of organic material in the skeleton, such as is found within the tissue layer of corals, and assuming that all of the organic material is 100% acidified, that the predicted change in skeletal δ^{13}C is analytically detectable. At best, mass spectrometers have analytical errors of $\pm 0.03\%o$ for δ^{13}C. Thus, δ^{13}C values that differ by less than $0.03\%o$ are within error of each other and are not analytically distinguishable from each other. Third, there are no analytically detectable differences among all three species, or across depth for *P. compressa*.
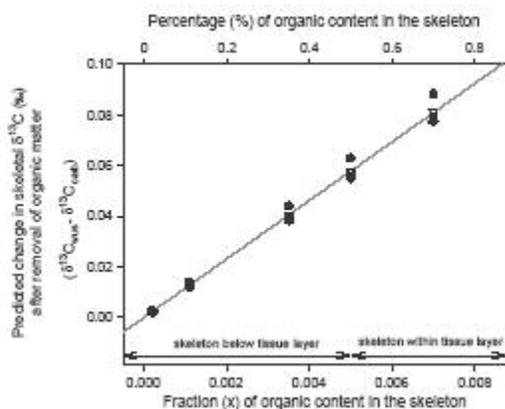
Fig. 1. Model of the predicted change in skeletal $\delta^{13}C$ after the removal of all organic matter "contaminants" for a range of organic content (x in Eq. (1)) ranging from 0.0002 to 0.007 (equivalent 0.02–0.7%) of the total carbon and assuming that all of the organic carbon in the skeleton completely acidifies and converts to CO_2. Regression is significant (Predicted change in $\delta^{13}C = 11.55$ (x) + 0.00005, $p < 0.001$, $r^2 = 0.99$). *Porites compressa* at 1.5 and 8 m overlap in all cases, ● = *Montipora verrucosa*, □ = *Montastraea annularis*, ▲ = *Porites compressa* 1.5 m depth, ▽ = *Porites compressa* 5 m depth, ♦ = *Porites compressa* 8.3 m depth.

Based on the model, it is hypothesized that pre-treatment of corals to remove isotopically depleted organic "contaminants" will result in an analytically detectable increase in the skeletal $\delta^{13}C_{carb}$ of samples within the tissue layer, but have no detectable effect on the skeletal $\delta^{13}C_{carb}$ of samples below the tissue layer. The model does not set forth any explicit hypotheses for skeletal $\delta^{18}O$ and it is not the goal of this paper to explore such a model at this time.

3.2. Experiment #1

One hundred samples were analyzed for $\delta^{13}C$ and $\delta^{18}O$. One $\delta^{13}C$ value was lost during analysis. The plot of the residual versus the predicted $\delta^{13}C$ and $\delta^{18}O$ values indicated that both data sets were normally distributed.

$\delta^{13}C$ showed significant pre-treatment, species, and interaction effects (Table 3) (Fig. 2a, b, c, respectively). An a posteriori Tukey test showed that $\delta^{13}C$ did not significantly differ between non pre-treated and water pre-treated corals, but was significantly lower in H_2O_2 and bleach pre-treated samples compared to non pre-treated samples by an average of 0.05‰ and 0.2‰, respectively (Fig. 2a). This is opposite of what was expected based on the model. Additional a posteriori Tukey tests showed that all species significantly differed from each other (Fig. 2b). The very small error bars about each $\delta^{13}C$ average in Fig. 2b show that the change in $\delta^{13}C$ due to pre-treatment are very reproducible. The pre-treatment by species interaction was also significant, indicating that the magnitude and direction of change in $\delta^{13}C$ due to pre-treatments were not the same for all species. This effect is best illustrated by examining the changes in $\delta^{13}C$ relative to non pre-treated samples ($\Delta\delta^{13}C$) in Fig. 2c.

Table 3. Two-way fully factorial Model I ANOVA of $\delta^{13}C$ ($n = 99$) and $\delta^{18}O$ ($n = 100$) bulk skeletal samples from Experiment #1

Model	df	F	p-value	Model r^2
$\delta^{13}C$	19	1359.01	< 0.0001	0.99
Treatment	3	46.05	< 0.0001	
Species	4	6403	< 0.0001	
Treatment * species	12	5.62	< 0.0001	
$\delta^{18}O$	19	241.74	< 0.0001	0.98
Treatment	3	6.84	0.0004	
Species	4	1138.53	< 0.0001	
Treatment * species	12	1.536	0.1286	

df = degrees of freedom, *p*-values ≤ 0.05 are significant.
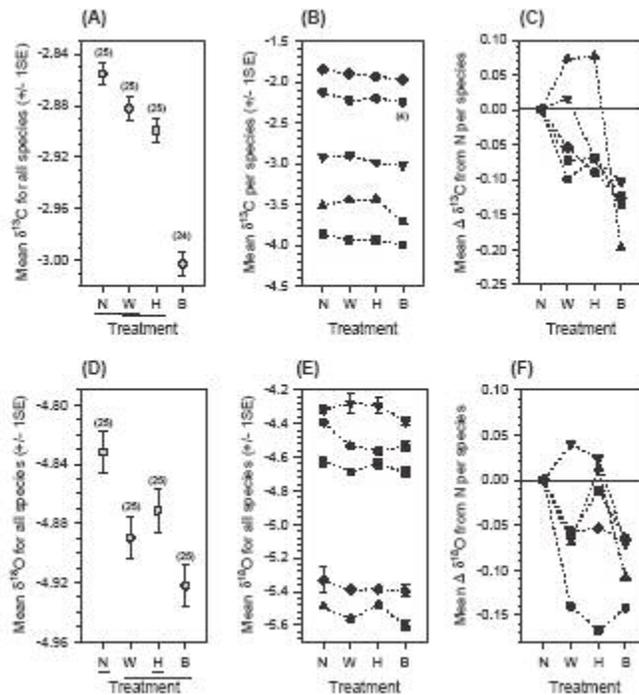

Fig. 2. Experiment #1, Pre-treatment of bulk skeletal samples below the tissue layer from five species of coral: *Pavona clavus* (♦), *Porites lobata* (●), *Montipora verrucosa* (▼), *Pavona gigantea* (▲), and *Porites compressa* (▪). Pre-treatments were: N = no pre-treatment, W = water pre-treatment, H = H$_2$O$_2$ pre-treatment, B = bleach pre-treatment. (A) and (D) Overall mean $\delta^{13}C$ and $\delta^{18}O$ for all species, respectively.

Within each panel, treatment labels along the *x*-axis that share a line indicate that the means do not significantly different from each other. (B) and (E) Mean $\delta^{13}C$ and $\delta^{18}O$ for each treatment within each species, respectively. In (B), error bars are always smaller than the symbols. (C) and (F) Mean change in $\delta^{13}C$ ($\Delta\delta^{13}C$) and in $\delta^{18}O$ ($\Delta\delta^{18}O$) from no pre-treatment for each species, respectively. SE = standard error of the mean, the sample size of each mean is 5 except where indicated. Statistics given in Table 3.

$\delta^{18}O$ showed significant pre-treatment and species effects, and no significant interaction effects (Table 3) (Fig. 2d, e, and f, respectively). A posteriori Tukey tests showed that $\delta^{18}O$ did not significantly differ between non pre-treated and H_2O_2 pre-treated samples but was significantly lower in the water and bleach pre-treatments samples by an average of 0.06‰ and 0.09‰ compared to the non pre-treated samples, respectively (Fig. 2d). Additional a posteriori Tukey tests showed that all species significantly differed from each other (Fig. 2e). The very small error bars about each mean $\delta^{18}O$ in Fig. 2e show that the changes in $\delta^{18}O$ due to pre-treatments were very reproducible. The absence of a significant pre-treatment by species interaction indicates that the magnitude and direction of change in $\delta^{18}O$ due to pre-treatments were statistically the same for all species. However, some interesting patterns were observed in Fig. 2f and will be addressed in the Discussion section.

3.3. Experiment #2

Between 7 and 10 individual corals from the following eight sets of corals were used in this experiment: *M. verrucosa* (H-BTL-2 m), *P. compressa* (H-BTL-2 m), *P. compressa* (H-WTL-2 m), *P. lobata* (H-WTL-2 m), *P. lobata* (P-WTL-2 m), *P. clavus* (P-BTL-2 m), *P. clavus* (P-BTL-5 m), *P. clavus* (P-WTL-5 m). In total, two hundred and ninety-five samples were analyzed for $\delta^{13}C$ and $\delta^{18}O$. The plot of the residual versus the predicted $\delta^{13}C$ and $\delta^{18}O$ values indicated that both $\delta^{13}C$ and $\delta^{18}O$ data were normally distributed.

$\delta^{13}C$ significantly differed among treatments and coral data sets (Table 4). A posteriori Tukey tests showed that $\delta^{13}C$ significantly differed among all treatments where no treatment > water > H_2O_2 > bleach (Fig. 3a). This pattern was observed in all species except one, *Montipora verrucosa* (Fig. 3b, c). On average, $\delta^{13}C$ values of water, H_2O_2, and bleach pre-treatment samples were 0.06‰, 0.08‰, and 0.12‰ lower than the non pre-treated coral samples, respectively (Fig. 3a). These results are opposite of what was expected based on the model. Additional Tukey tests showed that all coral data sets significantly differed from each other (Fig. 3b). Although the absolute $\delta^{13}C$ values are offset among coral data sets, changes in $\delta^{13}C$ due to pre-treatments are statistically comparable among coral data sets with the methods used here. The significant pre-treatment by coral data set interaction effect indicates that not all coral data sets responded to all pre-treatments in the same way (Table 4). This is best visualized by examining the intersecting lines in the change in $\delta^{13}C$ ($\Delta\delta^{13}C$) shown in Fig. 3c. Finally, the significant nested genotype effect indicates that $\delta^{13}C$ values significantly differed among individual coral fragments (genotypes) within coral data sets (Table 4).

Table 4. Partially nested ANOVA of $\delta^{13}C$ ($n = 295$) and $\delta^{18}O$ ($n = 295$) from Experiment #2

Model	df	F	p-value	Model r^2
$\delta^{13}C$	97	1394.5	< 0.0001	0.99
Set	7	23.34	< 0.0001	
Treat	3	115.46	< 0.0001	
Set * treat	21	5.07	< 0.0001	
Geno (set)	66	510.79	< 0.0001	
$\delta^{18}O$	97	422.11	< 0.0001	0.99
Set	7	133.2	< 0.0001	
Treat	3	44.09	< 0.0001	
Set * treat	21	1.62	0.048	
Geno (set)	66	40.88	< 0.0001	

Coral data sets are as listed in the methods section. Fixed effects tests are over the genotype nested within coral data sets [geno(set)] random effect. df = degrees of freedom, p-values ≤ 0.05 are significant.

The $\delta^{18}O$ also significantly differed among treatments and coral data sets (Table 4). A posteriori Tukey tests showed that no treatment > H_2O_2 > water = bleach (Fig. 3d). On average, H_2O_2, water, and bleach pre-treated samples were lower than non-pretreated samples by an average of 0.04, 0.06, and 0.09‰, respectively. However within each data set, the effect of pre-treatment on $\delta^{18}O$ varied significantly. The significant pre-treatment by coral data set interaction effect indicates that not all coral data sets responded to all pre-treatments in the same way (Table 4). This is best visualized by examining the intersecting lines in the change in $\delta^{18}O$ ($\Delta\delta^{18}O$) shown in Fig. 3f. Additional Tukey tests showed that all coral data sets significantly differed from each other (Fig. 3e). Finally, the significant nested genotype effect indicates that $\delta^{18}O$ values significantly differed among individual coral fragments (genotypes) within coral data sets (Table 4).
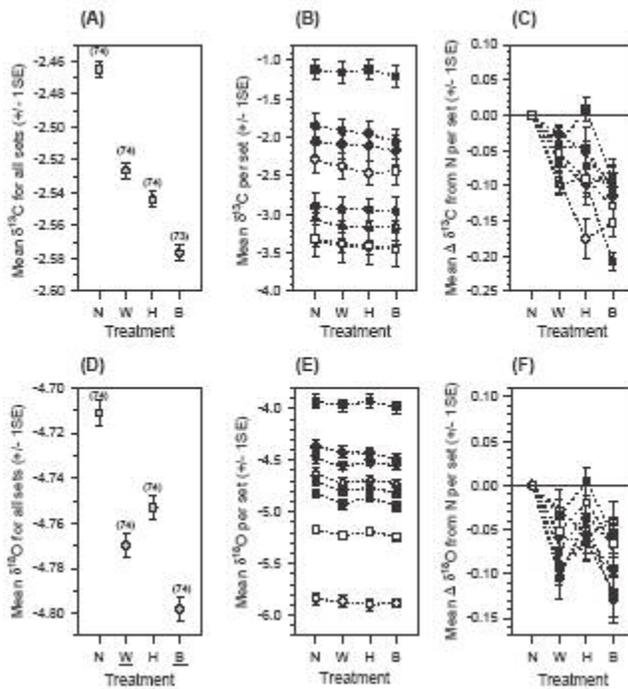
Fig. 3. Experiment #2, Pre-treatment of skeletal samples below the tissue layer (BTL, black symbols) and within the tissue layer (WTL, white symbols) for four species of corals from Hawaii (H) and Panama (P) collected at 2 or 5 m depth for a total of eight data sets: *M. verrucosa* (■) (H-BTL-2 m), *P. compressa* (▼) (H-BTL-2 m), *P. compressa* (▢) (H-WTL-2 m), *P. lobata* (♦) (H-BTL-2 m), *P. lobata* (▢) (P-WTL-2 m), *P. clavus* (.) (P-BTL-2 m), *P. clavus* (●) (P-BTL-5 m), *P. clavus* (○) (P-WTL-5 m). (A) and (D) Overall mean δ^{13}C and δ^{18}O for all data sets, respectively. Within each panel, treatment labels on the *x*-axis that share a line indicate that the means do not significantly different from each other. (B) and (E) Mean δ^{13}C and δ^{18}O for each treatment within each coral data set, respectively. ▼ and ▢ often overlap in (B) such that ▼ is not always visible. (C) and (F) Mean change in δ^{13}C ($\Delta\delta^{13}$C) and in δ^{18}O ($\Delta\delta^{18}$O) from no pre-treatment within each coral data set, respectively. SE = standard error of each mean, sample size of each mean ranges from 6 to 10 except where indicated in brackets. Pre-treatments as defined in Fig. 2. Statistics given in Table 4.

Closer examination of the effect of treatments on different sections of the coral skeleton (within the tissue layer versus below the tissue layer) revealed that the shape of the response curves appears similar for both δ^{13}C and δ^{18}O (Fig. 4a versus b, and c versus d). Statistical tests showed significant treatment and section effects for both δ^{13}C and δ^{18}O (Table 5). More importantly, the section by treatment interaction effect was significant for δ^{13}C and was not significant for δ^{18}O. This means that the magnitude and/or direction of the change in δ^{13}C due to pre-treatments statistically differed for skeleton within the tissue layer compared to skeleton below the tissue layer. δ^{18}O responded to pre-treatments in the same way within and below the tissue layer.
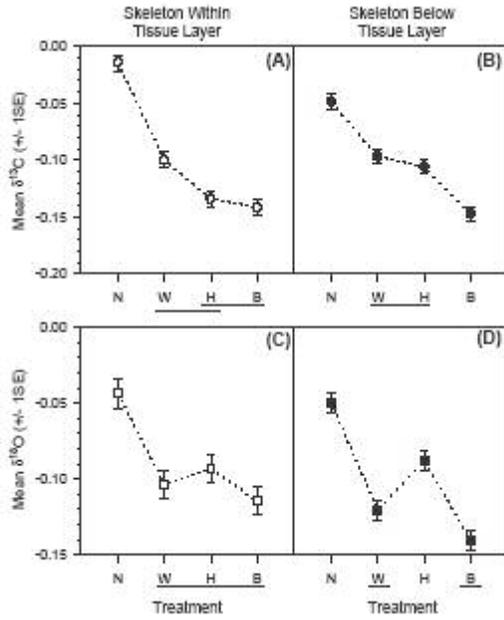
Fig. 4. Experiment #2, Effect of pre-treatments on skeletal δ^{13}C and δ^{18}O plotted on the same relative scale for: (A) and (C) coral data sets within the tissue layer [open symbols, sample size of each mean is 48 except for the bleach pre-treatment where the sample size is 47], and (B) and (D) data sets below the tissue layer [black symbols, sample size of each mean is 26]. Within each panel, treatment labels on the *x*-axis that share a line indicate that the means do not significantly differ from each other according to a posteriori Tukey tests. SE = standard error of the mean, pre-treatments as defined in Fig. 2. Statistics given in Table 5.

Table 5. Partially nested ANOVA of δ^{13}C ($n = 295$) and δ^{18}O ($n = 295$) from Experiment #2

Model	df	F	p-value	Model r^2
δ^{13}C	79	1321.17	< 0.0001	0.99
Section	1	10.25	0.002	
Treat	3	84.36	< 0.0001	
Section * treat	3	6.42	0.0004	
Geno (section)	72	1263.21	< 0.0001	
δ^{18}O	79	492.43	< 0.0001	0.99
Section	1	50.20	< 0.0001	
Treat	3	36.49	< 0.0001	
Section * treat	3	1.53	0.208	
Geno (section)	72	316.78	< 0.0001	

Fixed effects tests are over the genotype nested within sections [geno(section)] random effect. Sections are either within the tissue layer or below the tissue layer. df = degrees of freedom, p-values ≤ 0.05 are significant.

4. Discussion

4.1. Effects of pre-treatments on coral skeletal $\delta^{13}C$

The model predicts that pre-treatment of corals to remove organic "contaminants" will result in an increase in skeletal $\delta^{13}C$, but that such increases will only be analytically detectable (e.g. > 0.03‰) in samples within the tissue layer where the organic concentration is highest, and only if 100% of the organic carbon molecules fully acidify to CO_2. Except for the water pre-treated *Montipora verrucosa* from Experiment #1 and the H_2O_2 pre-treated *M. verrucosa* from Experiment #2, the average change in $\delta^{13}C$ due to all pre-treatments was always greater than the analytically detectable limit of 0.03‰ (Fig. 2 and Fig. 3). For both experiments, skeletal $\delta^{13}C$ decreased when samples were pre-treated with water, H_2O_2, or bleach. These decreases were statistically significant for H_2O_2 and bleach pre-treated corals. This is opposite of what was expected based on the model and suggests that either part of the skeleton is being oxidized during the pre-treatment stage (as suggested by Boiseau and Juillet-Leclerc, 1997 with H_2O_2), that these pre-treatments directly alter the $\delta^{13}C$ composition of the skeleton, or some combination of both. These results also indicate that organic material, which is isotopically light, is not contaminating skeletal isotopic samples with organically derived CO_2.

Decreases in skeletal $\delta^{13}C$ after pre-treatment with bleach reported here are consistent with Land et al. (1975) who found that the $\delta^{13}C$ value of two coral samples decreased after pre-treatment with bleach, and with Allison et al. (1996) who found similar results with *Porites lutea* coral samples. However, pre-treatment with H_2O_2 has been shown to result in either a decrease (Boiseau and Juillet-Leclerc, 1997) or no change (Allison et al., 1996, McConnaughey, 1989 and Watanabe et al., 2001) in skeletal $\delta^{13}C$. Differences in the effect of H_2O_2 pre-treatment on coral $\delta^{13}C$ in the literature are most likely due to species-specific differences and/or small sample sizes. Our data show that different species of corals differ in the magnitude and direction of change in $\delta^{13}C$ in response to pre-treatments and could explain the inconsistencies in H_2O_2 pre-treatment effects on coral $\delta^{13}C$ reported in the literature. This is illustrated by the significant interaction effects in both experiments. For example, in Experiment #1 *Pavona gigantea* $\delta^{13}C$ increased when pre-treated with H_2O_2 but decreased with the same pre-treatment for all other species (Fig. 2b, c). In Experiment #2, pre-treatment with H_2O_2 did not cause any change in $\delta^{13}C$ for *M verrucosa* but resulted in a large decrease in $\delta^{13}C$ for *P. clavus* (Fig. 3b, c). Overall these results indicate that within a species from a single location (within a coral data set), $\delta^{13}C$ values of skeletal samples from *different* pre-treatments are *not* comparable. Comparisons of coral $\delta^{13}C$ values from a single species and location (within a data set) with the *same* pre-treatment *are* comparable because the variation in $\delta^{13}C$ values about a single mean are small. However, comparisons of $\delta^{13}C$ values among

species and locations (among coral data sets) that have all been pre-treated the same way are not comparable because the direction and magnitude of change in $\delta^{13}C$ due to pre-treatments is highly variable among species and locations (Fig. 3c). And of course, comparisons of $\delta^{13}C$ values among species and locations (among coral data sets) with a combination of pre-treatments are not comparable because the direction and magnitude of change in the $\delta^{13}C$ values is highly variable among species, locations, and pre-treatments (Fig. 3c). In other words, $\delta^{13}C$ values from the published literature are only comparable if all coral samples in question were not pre-treated at all, or if all of the coral samples in question were from the same species and location and were pre-treated in exactly the same way.

Visual inspection of Fig. 4 reveals that pre-treatment effects on $\delta^{13}C$ values appear to be consistent for skeletal samples within and below the tissue layer. In other words, the overall shape of the curves in Fig. 4a and b appear to be similar. However, statistically their shapes are not the same as indicated by the significant interaction term (Table 5). This is most likely because the decrease in $\delta^{13}C$ in H_2O_2 pre-treated samples is greater within the tissue layer than below it by 0.02‰. Though statistically significant, the general shapes of the curves are similar and the differences between the curves are not analytically relevant. Therefore, we feel that the pre-treatment effects in skeletal samples from within and below the tissue layer are, for all practical purposes, the same. Irrespective, pre-treatment did not cause an increase in coral skeletal $\delta^{13}C$ even at the highest concentration of organic carbon within the skeleton. Therefore, the hypothesis is rejected and organic carbon within the skeleton is not a source of $\delta^{13}C$ "contamination".

4.2. Effects of pre-treatments on coral skeletal $\delta^{18}O$

Pre-treatment of coral skeletal samples with water, H_2O_2, and bleach resulted in decreases in the $\delta^{18}O$ values in both experiments. These decreases in $\delta^{18}O$ values may be due to partial dissolution of the skeleton, exposing isotopically lighter carbonate, isotopic exchange between these solutions and the skeleton, and/or replacement of $CaCO_3$ with isotopically lighter $Ca(OH)_2$. Evidence from scanning electron micrographs shows that 72 h of exposure to H_2O_2 can cause significant skeletal dissolution in the calcareous algae *Halimeda* while bleach exposure for the same time period does not (Gaffey and Bronnimann, 1993). By exposing coral samples to ^{16}O-enriched H_2O_2, Boiseau and Juillet-Leclerc (1997) determined that there is no oxygen isotopic exchange between H_2O_2 and coral aragonite. Therefore, dissolution of skeletal material is the most likely cause for the decrease in coral $\delta^{18}O$ pre-treated with H_2O_2. However, it is possible that isotope exchange between water and coral aragonite could alter the coral skeletal isotopic composition. Clearly, extensive soaking of coral samples in water can significantly alter $\delta^{18}O$ values and should be avoided. Exposure of carbonate to sodium hydroxide (a component of bleach) has been observed to cause extensive replacement of $CaCO_3$ by $Ca(OH)_2$ (Pingitore et al., 1993) and may account for the lower $\delta^{18}O$ values in the bleach pre-treated samples.

The overall decrease in skeletal $\delta^{18}O$ when pre-treated with bleach or H_2O_2 is consistent with Land et al. (1975) who reported a decrease in $\delta^{18}O$ of two coral samples that were

pre-treated with bleach, and with Boiseau and Juillet-Leclerc (1997) who reported a non-significant decrease in $\delta^{18}O$ in H_2O_2 for a single pre-treated *Porites lutea* coral head. However, others have found that bleach resulted in no change in skeletal $\delta^{18}O$ values in *Porites* (Allison et al., 1996) and no change in a single *Montastraea* coral (Watanabe et al., 2001). Pre-treatment of three coral samples with H_2O_2 resulted in an increase in $\delta^{18}O$ values (McConnaughey, 1989). Differences in the effect of bleach and H_2O_2 on coral $\delta^{18}O$ in the literature are most likely due to species-specific differences as illustrated by the significant interaction effects in Experiment #2 (Fig. 3f). In other words, the magnitude and direction of change in $\delta^{18}O$ due to pre-treatments are not the same for all species. For example, in both experiments *Montipora verrucosa* skeletal $\delta^{18}O$ values either increased or did not change when pre-treated with H_2O_2, but for all other species $\delta^{18}O$ decreased due to the same pre-treatment. Though not statistically significant, *Porites lobata* skeletal $\delta^{18}O$ values changed more due to all pre-treatments than did any of the other species in Experiment #1. Our study also shows that the magnitude and direction of change in $\delta^{18}O$ due to bleach pre-treatment are highly variable among species and coral data sets. Of particular concern is the average 0.12‰ and 0.13‰ decrease in $\delta^{18}O$ for bleach pre-treated *P. lobata* and *Pavona clavus* because it is equivalent to a > 0.5 °C difference in the interpretation of the $\delta^{18}O$ value in proxy records (Fig. 3f). Overall, the implications of these results for comparing $\delta^{18}O$ values within and among coral species, locations, and pre-treatments is the same as for $\delta^{13}C$ (see above). $\delta^{18}O$ values from the published literature are only comparable if all coral samples in question were not pre-treated at all, or if all of the coral samples in question were from the same species and location and were pre-treated in the exact same way.

Visual inspection and statistical analysis of Fig. 4 reveals that the pre-treatment effects are consistent for skeletal samples within and below the tissue layer. In other words, the pre-treatment effect for skeletal material within and below the tissue layer is the same, and organic material in the skeleton is not a source of "contamination" to the $\delta^{18}O$ value.

4.3. Genotype effect

Unlike previous studies (Cole and Fairbanks, 1990 and McConnaughey, 1989), the variations in the mean $\delta^{13}C$ and $\delta^{18}O$ of pre-treated corals in this study were no different than for non pre-treated corals. This is most likely because in our study the variability due to temporal variation in the skeletal sample was eliminated by constraining samples between known time intervals established by Alizarin Red stain lines within a data set, and only the variation due to pre-treatment was expressed. However, the direction and magnitude of change in $\delta^{13}C$ and $\delta^{18}O$ due to pre-treatments varied significantly among species and pre-treatments—an observation that was not possible in previous studies because very small sample sizes ($n = 1$–3) and examination of only one species made it difficult to tease apart pre-treatment effects from the natural variability in $\delta^{13}C$ and $\delta^{18}O$ among coral individuals and species (Allison et al., 1996, Boiseau and Juillet-Leclerc, 1997, Land et al., 1975, McConnaughey, 1989 and Watanabe et al., 2001). By comparing the standard error of the means in Experiments #1 and #2, it can be concluded that the variability among coral individuals within a data set (Experiment #2) is higher than the variability due to the pre-treatments themselves (Experiment #1).

The significant genotype effect (nested within data sets) showed that both $\delta^{13}C$ and $\delta^{18}O$ varied significantly among individual coral fragments (genotypes) within data sets. In fact, variability among genotypes in the random effect accounts for over 90% of the variation in both experiments. This means that the $\delta^{13}C$ and $\delta^{18}O$ values of a single coral colony can, by their nature, be dramatically different from the $\delta^{13}C$ and $\delta^{18}O$ of another genotypically different colony collected at the exact same location and sampled during the exact same time interval. Thus to get a reliable coral $\delta^{13}C$ or $\delta^{18}O$ value for paleoclimate or experimental purposes, the mean $\delta^{13}C$ and $\delta^{18}O$ values of multiple coral colonies needs to be calculated in order to increase the signal-to-noise ratio.

4.4. Summary

There are several main findings in this study: (1) The model predicts that skeletal $\delta^{13}C$ should *increase* after pre-treatment to remove organic "contaminants" but that such increases will only be analytically detectable (e.g. $> 0.05‰$ conservatively) in samples within the tissue layer where the organic concentration is highest. However, the experiments show that pre-treatment with water, H_2O_2, and bleach cause statistically and analytically significant *decreases* in $\delta^{13}C$ of similar magnitude in skeletal samples with both high and low organic concentrations. Thus, organic carbon in the coral skeleton, even within the tissue layer, is not a net source of contamination of the skeletal $\delta^{13}C$ value. The hypothesis is rejected and pre-treatment is not necessary to remove organic "contaminants" prior to stable isotope analysis. (2) Within a species, only $\delta^{13}C$ and $\delta^{18}O$ values from corals of similar pre-treatment are directly comparable. (3) Comparison of skeletal $\delta^{13}C$ and $\delta^{18}O$ values among species can most reliably be done when all $\delta^{13}C$ and $\delta^{18}O$ values are derived from non pre-treated skeletal samples. (4) Bleach causes the most significant and most variable change in coral $\delta^{13}C$ and $\delta^{18}O$ values and should be avoided. (5) There is no single correction factor that can be applied to H_2O_2 or bleach pre-treated coral $\delta^{13}C$ or $\delta^{18}O$ values to remove the pre-treatment effect. (6) For any coral isotopic study (experimental or proxy record), multiple coral individuals of each species must be analyzed so that the signal-to-noise ratio is reduced and true effects (e.g., experimental treatment or climate signal) are detectable above the natural variation that exists between individual corals and between species. Overall, the results indicated that for $\delta^{13}C$ or $\delta^{18}O$ of coral skeletons, pre-treatment is at best not necessary, and at worst can cause uncorrectable variability in the data and should be avoided.

Acknowledgements

References

Aharon, 1991 P. Aharon, Recorders of reef environment histories: stable isotopes in corals, giant clams, and calcareous algae, *Coral Reefs* **10** (1991), pp. 71–90.

Allison et al., 1996 N. Allison, A.W. Tudhope and A.E. Fallick, Factors influencing the stable carbon and oxygen isotopic composition of *Porites lutea* coral skeletons from Phuket, South Thailand, *Coral Reefs* **15** (1996), pp. 43–57.

Bessat et al., 1997 F. Bessat, M. Boiseau, A. Juillet Leclerc, D. Buigues and B. Salvat, Tomodensimetrie et mesures du rapport isotopique ^{18}O / ^{16}O sur *Porites lutea* a Mururoa (Polynesie francaise): une nouvelle approche pour apprehender l'influence du rayonnement sur la croissance corallienne annuelle. Comptes Rendues Academie des Sciences, *Sciences de la Vie* **320** (1997), pp. 659–665.

Boiseau and Juillet-Leclerc, 1997 M. Boiseau and A. Juillet-L eclerc, H_2O_2 treatment of recent coral aragonite: oxygen and carbon isotopic implications, *Chemical Geology* **143** (1997), pp. 171–180.

Boiseau et al., 1998 M. Boiseau, A. Juillet-Leclerc, P. Yiou, B. Salvat, P. Isdale and M. Guillaume, Atmospheric and oceanic evidences of El Niño–Southern Oscillation events in the South-Central Pacific Ocean from coral stable isotopic records over the last 137 years, *Paleoceanography* **13** (1998), pp. 671–685.

Boiseau et al., 1999 M. Boiseau, M. Ghil and A. Juillet-Leclerc, Climatic trends and interdecadal variability from South-Central Pacific coral records, *Geophysical Research Letters* **26** (1999), pp. 2881–2884.

Cardinal et al., 2001 D. Cardinal, B. Hamelin, E. Bard and J. Patzold, Sr/Ca, U/Ca and $\delta^{18}O$ records in recent massive corals from Bermuda: relationships with sea surface temperatures, *Chemical Geology* **176** (2001), pp. 213–233.

Carriquiry, 1994 J. Carriquiry, ^{18}O fractionation in the coralline aragonite of Porites lobata: implications in oceanic paleothermometry studies, *Ciencias Marinas* **20** (1994), pp. 585–606.

Carriquiry et al., 1988 J.D. Carriquiry, M.J. Risk and H.P. Schwarcz, Timing and temperature record from stable isotopes of the 1982–1983 El Niño warming event in eastern Pacific corals, *Palaios* **3** (1988), pp. 359–364.

Carriquiry et al., 1994 J.D. Carriquiry, M.J. Risk and H.P. Schwarcz, Stable isotope geochemistry of corals from Costa Rica as proxy indicator of the El Niño/Southern Oscillation (ENSO), *Geochimica et Cosmochimica Acta* **58** (1994), pp. 335–351.

Chakraborty and Ramesh, 1993 S. Chakraborty and R. Ramesh, Monsoon-induced sea surface temperature changes recorded in Indian corals, *Terra Nova* **5** (1993), pp. 545–551.

Chakraborty and Ramesh, 1997 S. Chakraborty and R. Ramesh, Environmental significance of carbon and oxygen isotope ratios of banded corals from Lakshadweep, India, *Quaternary International* **37** (1997), pp. 55–65.

Chakraborty and Ramesh, 1998 S. Chakraborty and R. Ramesh, Stable isotope variations in coral (*Favia speciosa*) from the Gulf of Kutch during 1948–1989 A.D.: environmental implications, *Proceeding of the Indian Academy of Science (Earth and Planetary Science)* **107** (1998), pp. 331–341.

Cobb et al., 2001 K.M. Cobb, C.D. Charles and D.E. Hunter, A central tropical Pacific coral demonstrates Pacific, Indian, and Atlantic decadal climate connections, *Geophysical Research Letters* **28** (2001), pp. 2209–2212.

Cobb et al., 2003a K.M. Cobb, C.D. Charles, H. Cheng and R.L. Edwards, El Niño/Southern Oscillation and tropical Pacific climate during the last millennium, *Nature* **424** (2003), pp. 271–276.

Cobb et al., 2003b K.M. Cobb, C.D. Charles, H. Cheng, M. Kastner and R.L. Edwards, U/Th-dating living and young fossil coral from the central tropical Pacific, *Earth and Planetary Science Letters* **210** (2003), pp. 91–103.

Cohen and Hart, 1997 A.L. Cohen and S.R. Hart, The effect of colony topography on climate signals in coral skeleton, *Geochimica et Cosmochimica Acta* **16** (1997), pp. 3905–3912.

Cole and Fairbanks, 1990 J.E. Cole and R.G. Fairbanks, The Southern Oscillation recorded in the $\delta^{18}O$ of corals from Tarawa Atoll, *Paleoceanography* **5** (1990), pp. 669–683.

Cole et al., 1993 J.E. Cole, R.G. Fairbanks and G.T. Shen, Recent variability in the Southern Oscillation: isotopic results from Tarawa Atoll coral, *Science* **260** (1993), pp. 1790–1793.

Cole et al., 2000 J.E. Cole, R.B. Dunbar, R. McClanahan and N.A. Muthiga, Tropical Pacific forcing of decadal SST variability in the Western Indian Ocean over the past two centuries, *Science* **287** (2000), pp. 617–619.

Druffel, 1985 E.R.M. Druffel, Detection of El Niño and decadal time scale variations of sea surface temperature from banded coral records: implications for the carbon dioxide cycle, *Geophysical Monographs* **32** (1985), pp. 111–122.

Druffel, 1997 E.R.M. Druffel, Pulses of rapid ventilation in the north Atlantic surface ocean during the past century, *Science* **275** (1997), pp. 1454–1457.

Druffel and Griffin, 1993 E.R.M. Druffel and S. Griffin, Large variations of surface ocean radiocarbon: evidence of circulation changes in the southwestern Pacific, *Journal of Geophysical Research* **98** (1993), pp. 20249–20259.

Druffel and Griffin, 1995 E.R.M. Druffel and S. Griffin, Regional variability of surface ocean radiocarbon from southern Great Barrier Reef corals. In: G.T. Cook, D.D. Harkness, B.F. Miller and E.M. Scott, Editors, *Proceedings of the 15th International ^{14}C Conference. Radiocarbon* (1995), pp. 517–524.

Druffel and Griffin, 1999 E.R.M. Druffel and S. Griffin, Variability of surface ocean radiocarbon and stable isotopes in the southwestern Pacific, *Journal of Geophysical Research* **104** (1999), pp. 23607–23613.

Druffel et al., 2001 E.R.M. Druffel, S. Griffin, T.P. Guilderson, M. Kashgarian, J. Southon and D.P. Schrag, Changes of subtropical North Pacific radiocarbon and correlation with climate variability, *Radiocarbon* **43** (2001), pp. 15–25.

Dunbar and Wellington, 1981 R.B. Dunbar and G.M. Wellington, Stable isotopes in a branching coral monitor seasonal temperature variation, *Nature* **293** (1981), pp. 453–455.

Dunbar et al., 1990 R.B. Dunbar, G.M. Wellington, M.W. Colgan and P.W. Glynn, Eastern tropical Pacific coral monitor low latitude climate of the past 400 years. In: J.L. Betancourt and V.L. Tharp, Editors, *Proceedings of the Seventh Annual Pacific Climate (CLIMAP) Workshop. Interagency Ecological Studies Program* (1990), pp. 165–178.

Dunbar et al., 1994 R.G. Dunbar, G.M. Wellington, M.W. Colgan and P.W. Glynn, Eastern Pacific sea surface temperature since 1600 A.D.: the $\delta^{18}O$ record of climate variability in Galapagos corals, *Paleoceanography* **9** (1994), pp. 291–315.

Emiliani et al., 1978 C. Emiliani, J.H. Hudson and E.A. Shinn, Oxygen and carbon isotopic growth record in a reef coral from the Florida Keys and deep-sea coral from Blake Plateau, *Science* **202** (1978), pp. 627–629.

Epstein et al., 1953 S. Epstein, R. Buchsbaum, H.A. Lowenstam and H.C. Urey, Revised carbonate-water isotopic temperature scale, *Bulletin of the Geological Society of America* **64** (1953), pp. 1315–1326.

Fairbanks and Dodge, 1979 R.G. Fairbanks and R.E. Dodge, Annual periodicity of the ^{18}O / ^{16}O and ^{13}C / ^{12}C ratios in the coral *Montastrea annualris*, *Geochimica et Cosmochimica Acta* **43** (1979), pp. 1009–1020. Abstract

Felis et al., 1998 T. Felis, J. Patzold, Y. Loya and G. Wefer, Vertical water mass mixing and plankton blooms recorded in skeletal stable carbon isotopes of a Red Sea coral, *Journal of Geophysical Research* **103** (1998), pp. 30731–30739.

Felis et al., 2000 T. Felis, J. Patzold, Y. Loya, M. Fine and A.H. Nawar, A coral oxygen isotope record from the northern Red Sea documenting NAO, ENSO, and North Pacific teleconnections on Middle East climate variability since the year 1750, *Paleoceanography* **15** (2000), pp. 679–694.

Felis et al., 2003 T. Felis, J. Patzold and Y. Loya, Mean oxygen-isotope signatures in *Porites* spp. corals: inter-colony variability and correction for extension-rate effects, *Coral Reefs* **22** (2003), pp. 328–336.

Gaffey and Bronnimann, 1993 S.J. Gaffey and C.E. Bronnimann, Effects of bleaching on organic and mineral phases in biogenic carbonates, *Journal of Sedimentary Petrology* **63** (1993), pp. 752–754.

Gaffey et al., 1991 S.J. Gaffey, J.J. Kolak and C.E. Bronnimann, Effects of drying, heating, annealing, and roasting on carbonate skeletal material, with geochemical and digenetic implications, *Geochimica et Cosmochimica Acta* **55** (1991), pp. 1627–1640.

Gagan and Chivas, 1995 M.K. Gagan and A.R. Chivas, Oxygen isotopes in western Australian coral reveal Pinatubo aerosol-induced cooling in the western Pacific warm pool, *Geophysical Research Letters* **22** (1995), pp. 1069–1072.

Gagan et al., 1994 M.K. Gagan, A.R. Chivas and P.J. Isdale, High-resolution isotopic records from corals using ocean temperature and mass-spawning chronometers, *Earth and Planetary Science Letters* **121** (1994), pp. 549–558.

Gagan et al., 1996 M.K. Gagan, A.R. Chivas and P.J. Isdale, Timing coral-based climatic histories using ^{13}C enrichments driven by synchronized spawning, *Geology* **24** (1996), pp. 1009–1012.

Goreau, 1977 T.J. Goreau, Coral skeletal chemistry: physiological and environmental regulations of stable isotopes and trace metals in *Montastrea annularis*, *Proceedings of the Royal Society of London Series B, Biological Sciences* **196** (1977), pp. 291–315.

Grottoli, 1999 A.G. Grottoli, Variability in skeletal stable isotopes and maximum linear extension in reef corals at Kaneohe Bay, Hawaii, *Marine Biology* **135** (1999), pp. 437–449.

Grottoli, 2002 A.G. Grottoli, Effect of light and brine shrimp levels on skeletal δ^{13}C values in the Hawaiian coral *Porites compressa*: a tank experiment, *Geochimica et Cosmochimica Acta* **66** (2002), pp. 1955–1967.

Grottoli and Wellington, 1999 A.G. Grottoli and G.M. Wellington, Effect of light and zooplankton on skeletal δ^{13}C values in the eastern Pacific corals *Pavona clavus* and *Pavona gigantea*, *Coral Reefs* **18** (1999), pp. 29–41.

Grottoli et al., 2004 A.G. Grottoli, L.J. Rodrigues and C. Juarez, Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event, *Marine Biology* **145** (2004), pp. 621–631.

Guilderson and Schrag, 1999 T.P. Guilderson and D.P. Schrag, Reliability of coral isotope records from the western Pacific warm pool: a comparison using age-optimized records, *Paleoceanography* **14** (1999), pp. 457–464.

Guzman and Tudhope, 1998 H.M. Guzman and A.W. Tudhope, Seasonal variation in skeletal extension rate and stable isotopic (^{13}C / ^{12}C and ^{18}O / ^{16}O) composition in response to several environmental variables in the Caribbean reef coral *Siderastrea siderea*, *Marine Ecology Progress Series* **166** (1998), pp. 109–118.

Heikoop et al., 2000 J.M. Heikoop, J.J. Dunn, M.J. Risk, H.P. Schwarcz, T.A. McConnaughey and I.M. Sandemen, Separation of kinetic and metabolic isotope effects in carbon-13 records preserved in reef coral skeletons, *Geochimica et Cosmochimica Acta* **64** (2000), pp. 975–987.

Heiss, 1996 G.A. Heiss, Annual band width variation in *Porites sp.* from Aquaba, Gulf of Aquaba, Red Sea, *Bulletin of Marine Science* **59** (1996), pp. 393–403.

Hemming et al., 1998 N.G. Hemming, T.P. Guilderson and R.G. Fairbanks, Seasonal variations in the boron isotopic composition of coral: a productivity signal?, *Global Biogeochemical Cycles* **12** (1998), pp. 581–586.

Hughen et al., 1999 K.A. Hughen, D.P. Schrag and S.B. Jacobsen, El Niño during the last interglacial period recorded by a fossil coral from Indonesia, *Geophysical Research Letters* **26** (1999), pp. 3129–3132.

Ingalls et al., 2003 A.E. Ingalls, C. Lee and E.R.M. Druffel, Preservation of organic matter in mound-forming coral skeletons, *Geochimica et Cosmochimica Acta* **67** (2003), pp. 2827–2841.

Kefu et al., 2002 Y. Kefu, L. Tungsheng, C. Tegu, Z. Juinliang and Z. Huanting, High-resolution climate recorded in the δ^{13}C of *Porites lutea* from Nansha Islands of China, *Progress in Natural Science* **12** (2002), pp. 284–288.

Klein et al., 1992 R. Klein, J. Patzold, G. Wefer and Y. Loya, Seasonal variations in the stable isotopic composition and the skeletal density pattern of the coral *Porites lobata* (Gulf of Elat, Red Sea), *Marine Biology* **112** (1992), pp. 259–263.

Klein et al., 1993 R. Klein, J. Patzold, G. Wefer and Y. Loya, Depth-related timing of density band formation in *Porites spp.* corals from the Red Sea inferred from X-ray chronology and stable isotope composition, *Marine Ecology Progress Series* **97** (1993), pp. 99–104.

Klein et al., 1997 R. Klein, A.W. Tudhope, C.P. Chilcott, J. Patzold, Z. Abdulkarim, M. Fine, A.E. Fallick and Y. Loya, Evaluating southern Red Sea corals as a proxy record for the Asian monsoon, *Earth and Planetary Science Letters* **148** (1997), pp. 381–394.

Kuhnert et al., 1999 G. Kuhnert, J. Patzold, B. Hatcher, K.-H. Wyrwoll, A. Eisenhauer, L.B. Collins, Z.R. Zhu and G. Wefer, A 200-year coral stable oxygen isotope record from a high-latitude reef off western Australia, *Coral Reefs* **18** (1999), pp. 1–12.

Kuhnert et al., 2002 H. Kuhnert, J. Patzold, B. Schnetger and G. Wefer, Sea-surface temperature variability in the 16th century at Bermuda inferred from corals records, *Palaeogeography, Palaeoclimatology, Palaeoecology* **179** (2002), pp. 159–171.

Land et al., 1975 L.S. Land, J.C. Lang and D.J. Barnes, Extension rate: a primary control on the isotopic composition of West Indian (Jamaican) scleractinian coral skeletons, *Marine Biology* **33** (1975), pp. 221–233.

Land et al., 1977 L.S. Land, J. Lang and D.J. Barnes, On the stable carbon and oxygen isotopic composition of some shallow water, ahermatypic, scleractinian coral skeletons, *Geochimica et Cosmochimica Acta* **41** (1977), pp. 169–172. Abstract

Leder et al., 1991 J.L. Leder, A.M. Szmant and P.K. Swart, The effect of prolonged "bleaching" on skeletal banding and stable isotopic composition in *Montastrea annularis*, *Coral Reefs* **10** (1991), pp. 19–27.

Leder et al., 1996 J.J. Leder, P.K. Swart, A.M. Szmant and R.E. Dodge, The origin of variations in the isotopic record of scleractinian corals: I. Oxygen, *Geochimica et Cosmochimica Acta* **60** (1996), pp. 2857–2870.

Linsley et al., 1994 B.K. Linsley, R.B. Dunbar, G.M. Wellington and D.A. Mucciarone, A coral-based reconstruction of intertropical convergence zone variability over Central America since 1707, *Journal of Geophysical Research* **99** (1994), pp. 9977–9994.

Linsley et al., 1999 B.K. Linsley, R.G. Messier and R.B. Dunbar, Assessing between-colony oxygen isotope variability in the coral *Porites lobata* at Clipperton Atoll, *Coral Reefs* **18** (1999), pp. 13–27.

Linsley et al., 2000 B.K. Linsley, L. Ren, R.B. Dunbar and S.S. Howe, El Niño Oscillation (ENSO) and decadal-scale climate variability at 10° N in the eastern Pacific from 1893–1994: a coral-based reconstruction from Clipperton Atoll, *Paleoceanography* **15** (2000), pp. 322–335.

Maier et al., 2003 C. Maier, J. Patzold and R.P.M. Bak, The skeletal isotopic composition as an indicator of ecological and physiological plasticity in the coral genus *Madracis*, *Coral Reefs* **22** (2003), pp. 370–380

McConnaughey, 1989 T. McConnaughey, ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns, *Geochimica et Cosmochimica Acta* **53** (1989), pp. 151–162.

McCulloch et al., 1994 M.T. McCulloch, M.K. Gagan, G.E. Mortimer, A.R. Chivas and P.J. Isdale, A high-resolution Sr/Ca and δ^{18}O coral record from the Great Barrier Reef, Australia, and the 1982–1983 El Niño, *Geochimica et Cosmochimica Acta* **58** (1994), pp. 2747–2754.

Morimoto et al., 2002 M. Morimoto, O. Abe, H. Kayanne, N. Kurita, E. Matsumoto and N. Yoshida, Salinity records for the 1997–98 El Niño from Western Pacific corals, *Geophysical Research Letters* **29** (2002).

Muscatine et al., 1989 L. Muscatine, J.W. Porter and I.R. Kaplan, Resource partitioning by reef corals as determined from stable isotope composition: I. δ^{13}C of zooxanthellae and animal tissue vs. depth, *Marine Biology* **100** (1989), pp. 185–193.

Nozaki et al., 1978 Y. Nozaki, D.M. Rye, K.K. Turekian and R.E. Dodge, A 200 year record of carbon-13 and carbon-14 variations in a Bermuda coral, *Geophysical Research Letters* **5** (1978), pp. 825–828.

Patzold, 1984 J. Patzold, Growth rhythms recorded in stable isotopes and density bands in the reef coral *Porites lobata* (Cebu, Philippines), *Coral Reefs* **3** (1984), pp. 87–90.

Patzold et al., 1991 J. Patzold, J.P. Heinrichs, K. Wolschendorf and G. Wefer, Correlation of stable oxygen isotope temperature record with light attenuation profiles in reef-dwelling *Tridacna* shells, *Coral Reefs* **10** (1991), pp. 65–69.

Pingitore et al., 1993 N.E. Pingitore, S.B. Fretzdorf, B.P. Seitz, L.Y. Estrada, P.M. Borrego, G.M. Crawford and K. Love, Dissolution kinetics of $CaCO_3$ in common laboratory solvents, *Journal of Sedimentary Petrology* **63** (1993), pp. 641–645.

Porter et al., 1989 J.W. Porter, W.K. Fitt, H.J. Spero, C.S. Rogers and M.W. White, Bleaching in reef corals: physiological and stable isotopic responses, *Proceedings of the National Academy of Science USA* **86** (1989), pp. 9342–9346.

Quinn et al., 1993 T.M. Quinn, F.W. Taylor and T.J. Crowley, A 173 year stable isotope record from a tropical south Pacific coral, *Quaternary Science Review* **12** (1993), pp. 407–418.

Quinn et al., 1996a T.M. Quinn, T.J. Crowley and F.W. Taylor, New stable isotope results from a 173-year coral from Espiritu Santo, Vanuatu, *Geophysical Research Letters* **23** (1996), pp. 3413–3416.

Quinn et al., 1996b T.M. Quinn, F.W. Taylor, T.J. Crowley and S.M. Link, Evaluation of sampling resolution in coral stable isotope records: a case study using records from new Caledonia and Tarawa, *Paleoceanography* **12** (1996), pp. 529–542.

Quinn et al., 1998 T.M. Quinn, T.J. Crowley, F.W. Taylor, C. Henin, P. Joannot and Y. Join, A multicentury stable isotope record from a New Caldonia coral: interannual and decadal sea surface temperature variability in the southwest Pacific since 1657 A.D., *Paleoceanography* **13** (1998), pp. 412–426.

Reynaud et al., 2002 S. Reynaud, C. Ferrier-Pages, R. Sambrotto, A. Juillet-Leclerc, J. Jaubert and J.-P. Gattuso, Effect of feeding on the carbon and oxygen isotopic composition in the tissues and skeleton of the zooxanthellate coral *Stylophora pistillata*, *Marine Ecology Progress Series* **238** (2002), pp. 81–89.

Reynaud-Vaganay et al., 1999 S. Reynaud-Vaganay, J.-P. Gattuso, J.-P. Cuif, J. Jaubert and A. Juillet-Leclerc, A novel culture technique for scleractinian corals: application to investigate changes in skeletal δ^{18}O as a function of temperature, *Marine Ecology Progress Series* **180** (1999), pp. 121–130.

Reynaud-Vaganay et al., 2001 S. Reynaud-Vaganay, A. Juillet-Leclerc, J. Jaubert and J.-P. Gattuso, Effect of light on skeletal δ^{13}C and δ^{18}O, and interaction with photosynthesis, respiration and calcification in two zooxanthellate scleractinian corals, *Palaeogeography, Palaeoclimatology, Palaeoecology* **175** (2001), pp. 393–404.

Suzuki et al., 2000 A. Suzuki, H. Kawahata, Y. Tanimoto, H. Tsukamoto, L.P. Gupta and I. Yukino, Skeletal isotopic record of a *Porites* coral during the 1998 mass bleaching event, *Geochemical Journal* **34** (2000), pp. 321–329.

Suzuki et al., 2003 A. Suzuki, M.K. Gagan, K. Fabricius, P.J. Isdale, I. Yukino and H. Kawahata, Skeletal isotope microprofiles of growth perturbations in *Porites* corals during the 1997–98 mass bleaching event, *Coral Reefs* **22** (2003), pp. 357–369.

Swart et al., 1996a P.K. Swart, R.E. Dodge and H.J. Hudson, A 240-year stable oxygen and carbon isotopic record in a coral from South Florida: implications for the prediction of precipitation in Southern Florida, *Palaios* **11** (1996), pp. 362–373.

Swart et al., 1996b P.K. Swart, G. Healy, R.E. Dodge, P. Kramer, J.H. Hudson, R.B. Halley and M.B. Robblee, The stable oxygen and carbon isotopic record from a coral growing in Florida Bay: a 160 year record of climatic and anthropogenic influence, *Palaeogeography, Palaeoclimatology, Palaeoecology* **123** (1996), pp. 219–237.

Swart et al., 1996c P.K. Swart, J.J. Leder, A.M. Szmant and R.E. Dodge, The origin of variations in the isotopic record of scleractinian coral: II. Carbon, *Geochimica et Cosmochimica Acta* **60** (1996), pp. 1885–2871.

Swart et al., 1998 P.K. Swart, K.S. White, D. Enfield, R.E. Dodge and P. Milne, Stable oxygen isotopic composition of corals from the Gulf of Guinea as indicators of periods of extreme precipitation conditions in the sub-Sahara, *Journal of Geophysical Research* **103** (1998), pp. 27885–27891.

Swart et al., 1999 P.K. Swart, G. Healy, L. Greer, M. Lutz, A. Saied, Daniel Anderegg, R.E. Dodge and D. Rudnick, The use of proxy chemical records in coral skeletons to ascertain past environmental conditions in Florida Bay, *Estuaries* **22** (1999), pp. 384–397.

Tudhope et al., 1995 A.W. Tudhope, G.B. Shimmield, C.P. Chilcott, M. Jebb, A.E. Fallick and A.N. Dalgleish, Recent changes in climate in the far western equatorial Pacific and their relationship to the Southern Oscillation; oxygen isotope records from massive corals, Papua New Guinea, *Earth and Planetary Science Letters* **136** (1995), pp. 575–590.

Tudhope et al., 1996 A.W. Tudhope, D.W. Lea, G.B. Shimmield, C.P. Chilcott and S. Head, Monsoon climate and Arabian Sea coastal upwelling recorded in massive corals from southern Oman, *Palaios* **11** (1996), pp. 347–361.

Tudhope et al., 1997 A.W. Tudhope, D.W. Lea, G.B. Shimmield, C.P. Chilcott, T.P. Scoffin, A.E. Fallick and M. Jebb, Climatic records from massive *Porites* corals in Papua New Guinea: a comparison of skeletal Ba/Ca, skeletal ^{18}O and coastal rainfall, *Proc. 8th International Coral Reef Symposium* **2** (1997), pp. 1719–1724.

Watanabe et al., 2001 T. Watanabe, M. Minagawa, T. Oba and A. Winter, Pretreatment of coral aragonite for Mg and Sr analysis: implications for coral thermometers, *Geochemical Journal* **35** (2001), pp. 265–269.

Watanabe et al., 2002 T. Watanabe, A. Winter, T. Oba, R. Anzai and H. Ishioroshi, Evaluation of the fidelity of isotope records as an environmental proxy in the coral *Montastraea*, *Coral Reefs* **21** (2002), pp. 169–178.

Watanabe et al., 2003 T. Watanabe, M.K. Gagan, T. Correge, H. Scott-Gagan, J. Cowley and W.S. Hantoro, Oxygen isotope systematics in *Diploastrea helipora*: new coral archive of tropical paleoclimate, *Geochimica et Cosmochimica Acta* **67** (2003), pp. 1349–1358.

Weber and Woodhead, 1972 J.N. Weber and P.M.J. Woodhead, Temperature dependence of oxygen-18 concentration in reef coral carbonates, *Journal of Geophysical Research* **77** (1972), pp. 463–473.

Weber et al., 1976 J.N. Weber, P. Deines, P.H. Weber and P.A. Baker, Depth related changes in the ^{13}C / ^{12}C ratio of skeletal carbonate deposited by the Caribbean reef-frame building coral *Montastrea annularis*: further implications of a model for stable isotope fractionation by scleractinian corals, *Geochimica et Cosmochimica Acta* **40** (1976), pp. 31–39.

Weil et al., 1981 S.M. Weil, R.W. Buddemeier, S.V. Smith and P.M. Kroopnick, The stable isotopic composition of coral skeletons: control by environmental variables, *Geochimica et Cosmochimica Acta* **45** (1981), pp. 1147–1153.

Wellington and Dunbar, 1995 G.M. Wellington and R.B. Dunbar, Stable isotopic signature of El Niño–Southern Oscillation events in eastern tropical Pacific reef corals, *Coral Reefs* **14** (1995), pp. 5–25.

Wellington et al., 1996 G.M. Wellington, R.B. Dunbar and G. Merlen, Calibration of stable oxygen isotope signatures in Galapagos corals, *Paleoceanography* **11** (1996), pp. 467–480.

Winters et al., 1991 A. Winters, C. Goenaga and G.A. Maul, Carbon and oxygen isotope time series from an 18-year Caribbean reef coral, *Journal of Geophysical Research* **96** (1991), pp. 16637–16678.