A novel paleo-bleaching proxy using boron isotopes and high-resolution laser ablation to reconstruct coral bleaching events

Gal Dishon[1], Jay Fisch[2], Ingo Horn[3], Karina Kaczmarek[4], Jelle Bijma[4], David F. Gruber[5], Orit Nir[1], Yotam Popovich[1] and Dan Tchernov[1]*

[1] The Leon H. Charney School of Marine Sciences, University of Haifa, Mount Carmel, Haifa 31905.

[2] Rosenstiel School for Marine and Atmospheric Sciences, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida, 33149, USA

[3] Leibniz-University of Hannover, Institute for Mineralogy, Callinstr. 3, D-30167 Hannover, Germany

[4] Alfred Wegner Institute Helmholtz center for Polar and Marine Research, Am Handelshafen 12

27570 Bremerhaven, Germany

[5] Department of Natural Sciences, City University of New York, Baruch College, Box A-0506, 17 Lexington Avenue, New York, New York 10010, USA

Correspondence to: D. Tchernov (dtchernov@univ.haifa.ac.il)

Abstract

Coral reefs occupy only ~0.1 percent of the oceans habitat, but are the most biologically diverse marine ecosystem. In recent decades, coral reefs have experienced significant global declines due to a variety of causes, one of the major being widespread coral bleaching events. During bleaching, the coral expels its symbiotic algae losing its main source of nutrition generally obtained through photosynthesis. While recent coral bleaching events have been extensively investigated, there is no scientific data on historical coral bleaching prior to 1979. In this study, we employ high-resolution femtosecond Laser Ablation Multiple Collector Inductively Coupled Plasma Mass Spectrometry (LA-MC-ICP-MS) to demonstrate a distinct biologically-induced

decline of boron (B) isotopic composition (δ^{11}B) as a result of coral bleaching. These findings and methodology offer a new use for a previously developed isotopic proxy to reconstruct paleo-coral bleaching events. Based on a literature review of published δ^{11}B data and our recorded "vital effect" of coral bleaching on the δ^{11}B signal, we also describe at least two possible coral bleaching events since the Last Glacial Maximum. The implementation of this bleaching proxy holds the potential of identifying occurrences of coral bleaching throughout the geological record. A deeper temporal view of coral bleaching will enable scientists to determine if it occurred in the past during times of environmental change and what outcome it may have had on coral population structure. Understanding the frequency of bleaching events is also critical for determining the relationship between natural and anthropogenic causes of these events.

1   Introduction

1.1   Coral bleaching

Coral bleaching occurs when environmental stress, primarily increased water temperature and high irradiance (but also decreased temperature, decreased salinity and pathogenic infections), induces breakdown of the coral-algae symbiosis and the host initiates algae expulsion (Brown, 1997). As a result, corals either temporarily lose their pigmentation or die from lack of nourishment from the algae. Bleaching is, therefore, considered an acute risk to the health of coral reefs, although some studies suggest it is an adaptive response to environmental change (Fautin and Buddemeier, 2004).

Coral bleaching was first reported in the early 20th century (Yonge et al., 1931) and heavily documented since 1979, as widespread bleaching events have been recorded with increased frequency and severity (Hoegh-Guldberg, 1999). By correlating isotopic dating of massive corals and concomitant sea surface temperatures (SSTs), it was speculated

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The authors should mention/discuss that understanding the frequency of bleaching events is critical for determining the relationship between natural and anthropogenic causes of these events

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The authors provide a pretty brief/incomplete review of the stressors that may cause bleaching events. Please expand.

that coral bleaching may have already caused mortality events by the end of the 19th century (Yu et al., 2006). While bleaching events have been proposed to occur throughout the geological record (suggested for symbiotic foraminifera during unstable SST in the upper Eocene (Wade et al., 2008)), there has been no experimentally established proxy to examine suspected paleo-coral bleaching.

1.2 Paleo bleaching proxy

Previous attempts at establishing a paleo-proxy for coral bleaching focused on carbon and oxygen isotopes (Heikoop et al., 2000; Suzuki et al., 2003) as well as skeletal ultraviolet fluorescence (Suzuki et al., 2003). However the balance between kinetic and metabolic processes, affected by factors such as coral growth rate and local light environment (i.e. shaded vs. unshaded side of a colony), complicates the use of these proxies (Suzuki et al., 2003). A correction method, suggested by Heikoop et al. (2000) to differentiate kinetic from metabolic effects, was recently refuted for the case of coral bleaching (Schoepf et al., 2014a). Boron isotopes, currently used for estimating $pH_{sw,}$ may act as an effective paleo-bleaching proxy, as kinetic effects apparently do not interfere with isotopic equilibrium during calcification (Zeebe et al., 2001).

Considerable research has been focused on paleo-techniques for estimating pH_{sw} and $pCO_{2\ atm}$ as current atmospheric CO_2 values of ~400 parts per million by volume (ppmv) are at the highest levels of the past million year timeframe (Caldeira and Wickett, 2003; Hoegh-Guldberg et al., 2007; Hönisch et al., 2012). The wide variety of proxies developed for deep time (extended to the Paleozoic era) paleo CO_2 reconstruction includes fossil plant stomata, stable carbon isotopes in paleosols and Bryophytes, and marine alkenones (reviewed for the Cretaceous time frame by Wang et al. (2014)) At time scales shorter than one million years, paleo-CO_2 and pH_{sw} analyses are derived mostly from air bubbles

in ice cores (Monnin et al., 2001; Petit et al., 1999; Vinther et al., 2009) and boron isotopic composition ($\delta^{11}B$) from carbonate skeletons of foraminifera (Foster, 2008; Hönisch and Hemming, 2005; Hönisch et al., 2009; Palmer et al., 2010; Palmer and Pearson, 2003; Sanyal et al., 1995) and corals (Douville et al., 2010; Gaillardet and Allegre, 1995; Liu et al., 2009; Pelejero et al., 2005; Shinjo et al., 2013; Wei et al., 2009). Skeletal $\delta^{11}B$ values are used for paleo pH reconstruction as they relate to ambient waters' pH. This relationship is based on the assumptions (1) that internal pH (i.e. where calcification takes place) is associated with ambient pH, and (2) that in seawater only the charged B species (borate) with lower $\delta^{11}B$ values, is incorporated into corals. The B species distribution and the $\delta^{11}B$ of the B species are pH dependent. If borate represents the only source of B in the skeletons of corals, than its $\delta^{11}B$ is equal to the $\delta^{11}B$ of the coral. Therefore, the $\delta^{11}B$ of corals is utilized as a proxy of ocean pH levels at the time of calcification.

It is important to bear in mind that the internal pH often differs from ambient pH as the combined result of geochemistry and physiology, thus changing the $\delta^{11}B_{coral}$- $\delta^{11}B_{sw}$ relationship. Environmental factors that may affect coral's internal pH include food supply, light intensity, depth or temperature elevation (e.g. Dissard et al., 2012; Hönisch et al., 2004; Reynaud et al., 2004). However, for zooxanthellate corals, the most prominent process raising the coral's internal pH, is photosynthesis carried by its' algal symbionts (Laurent et al., 2013; Venn et al., 2011; Venn et al., 2013). Thus, high photosynthesis rates will lead to elevated internal pH and therefore higher $\delta^{11}B$. This photosynthesis-pH-$\delta^{11}B$ interrelation was noted both for symbiont-bearing foraminifera (Hoenisch et al., 2003), and corals (Hemming et al., 1998).

Thermally bleached corals however show diminished symbiont photosynthesis (Glynn, 1996) causing a concomitant drop in pH measured at the corals diffusive boundary layer (DBL) (Al-Horani, 2005). While the interaction of coral biology with its internal pH (known as vital effects) has been previously quantified as ca. 0.3-0.6 pH units elevation

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11B values are largely controlled by pHsw (as the authors note) but they are also influenced by seawater temperature and salinity. This needs to be addressed in the introduction. Hence, calculations of paleo pH based on 11B records need to estimate both salinity and seawater temperature. This also needs to be carefully considered. Outside of the experiment described here, how can the authors be sure that decreases in 11B coral values are solely related to bleaching effects rather than changes in seawater temperature, pH, or salinity?
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Convince the readers by developing a mechanistic relationship between these two variables.

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in a "healthy" state (McCulloch et al., 2012; Trotter et al., 2011) , the change in physiology due to coral bleaching is hypothesized to push pH difference beyond the natural vital effect, decoupling internal and ambient pH, skewing $\delta^{11}B$ derived pH_{sw} estimations (e.g. pH_{sw} during 1998 mass bleaching from Wei et al. (2009)), and by that, enabling the use of $\delta^{11}B$ as a proxy for paleo-bleaching. This approach was recently challenged by Schoepf et al. (2014b) who reported no difference in $\delta^{11}B$ signal imprinted in the skeletons of corals that experienced short term (up to six weeks) bleaching event. We hypothesize however, that longer bleaching events, and higher resolution sampling techniques, may allow the detection of the boron-bleaching signature in coral skeletons.

In this study, we examined the signature of coral bleaching manifested in the $\delta^{11}B$ values of coral skeletons using experimentally and naturally bleached specimens. Through the use of laser ablation, high-resolution sampling of the coral skeleton deposited while in a bleached state, we quantify a boron bleaching signature. We applied this proxy to published coral $\delta^{11}B$ records to investigate the occurrence of coral bleaching since the Last Glacial Maximum.

2 Materials and methods

2.1 Experimentally induced *Porites* sp. bleaching:

2.1.1 Coral Culturing: Two adjacent *Porites* sp. colonies were collected at 10 meters depth in the Gulf of Aqaba, in the northern Red Sea (29°30'06" N, 34°55'00" E). The coral was cut into 2 X 2 cm 'nubbins' and left to recover in controlled laboratory conditions (23° C, pH of 8.17 and salinity of 40.7 psu) for three weeks prior to starting the experiment. Nubbins were separated into two groups - "control" and "heat shock" - and maintained for eight months (November (2008) to June (2009)) in open-system water tables with an input flow rate of ~2.0 L/min and constant light of an average of 150 μmol photons m^{-2} s^{-1}. For the control corals temperature varied between 22-24°C, reflecting seasonal temperature changes in the Gulf of Aqaba, while

heat shock treatment temperatures were controlled with a thermostat to
induce bleaching (30-32°C; Jan-March) and recovery (23-25°C; April-
June). Temperature fluctuations are outlined for both control and heat
shock treatments in the Supporting information (Table S1). Since the
experiment was carried in open system, (i.e. with direct connection with
the sea) pH and salinity levels remained in the natural range for the
Gulf of Aqaba, 8.20 ± 0.01 (SD) pH units and 40.60 ± 0.08 (SD) ppt
respectively throughout the experiment. Nubbins were stained at the
beginning of the experiment with Alizarin Red S to identify the start of
newly deposited skeleton during the experimental period.

Fv/Fm values were measured approximately every two days for the
first three weeks of the experiment until bleaching was seen. Twelve
dark-adapted samples were measured for each treatment using the
single turnover flash of over 10,000 µE on a fast repetition rate
fluorometer (FRRF, FIRe system, Satlantic, Halifax, Nova Scotia,
Canada).

Oxygen and pH were measured with microelectrodes in the diffusive
boundary layer (DBL) of a healthy and bleached coral during the
bleached period (Supporting information, Fig. S2). At the end of the
experiment, the tissue of all corals was removed using a high pressure
airbrush.

2.2 Natural occurring *Stylophora pistillata* bleaching:

A *Stylophora pistillata* fragment was taken from the coral documented by
Nir et al.(2014) to undergo bleaching during summer 2010. The coral
skeleton was sliced, cleaned and measured for δ ^{11}B using the
procedures described in the next section. After LA-MC-ICP-MS
measurements, coral slices were polished to a thickness of ~0.5 mm on
a microscope slide and photographed through a light microscope.
Density bands (annual growth bands) were analyzed as gray intensity of
pixels along the measurements path using ImageJ software.

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2.3 **δ¹¹B analysis**: For boron analysis, the coral skeletons were sliced and grinded to flatten the surface and then immersed in an ultra-sonicated Milli-Q water bath for 5 minutes to remove any particles. Sodium Hypochlorite (NaClO) was used to remove any organics and finally samples were washed with Milli-Q water and Acetone (full cleaning procedure is detailed in the supplementary information). In this study we used laser ablation for the determination of $\delta^{11}B$ instead of bulk analysis of a dissolved material which requires wet chemistry and rigorous cleaning steps. Laser ablation was applied on the grinded, cleaned and bleached surfaces of the sliced skeletons. From the experimentally bleached *Porites* sp. corals, a total of six coral nubbins were analyzed for $\delta^{11}B$ using UV femtosecond LA-MC-ICP-MS, two from the control treatment and four from the bleaching treatment. From the naturally bleached *S. pistillata* coral we used one branch. By using a femtosecond (fs) laser ablation system we avoided issues such as thermal fractionation and matrix effects that can occur using nanosecond lasers when using glass standards for carbonate samples (Fietzke et al., 2010; Horn and von Blanckenburg, 2007). Furthermore, the fs-laser ablation technique (ultra-short ~200 fs pulses) allows for fast and accurate determination of the isotopes in the coral skeleton with minimal melting of the material caused by heat transfer.

$\delta^{11}B$ analysis was performed using a Thermo Finnigan-Neptune MC-ICP-MS, connected to a UV fs (10^{-15} s) laser for ablation (Horn and von Blanckenburg, 2007). NIST SRM 610 was used as the reference material for bracketing. Standard error for coral fragments treatments was less than 0.30‰ (2σ).

2.4 **pH calculation based on Boron isotopic composition**

Since temperature variation in the experiment was high (23-32 °C), we had to consider the chemical effect of temperature on $\delta^{11}B$ of borate which accompanies the physiological effect of symbiont expulsion. In order to compensate for this effect, pH levels were calculated based on $\delta^{11}B$ values from the *Porites sp.* experiment using the equation:

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$$pH = pK_B - log\left(-\frac{\delta^{11}B_{sw} - \delta^{11}B_{coral}}{\delta^{11}B_{sw} - \alpha_B \times \delta^{11}B_{coral} - (\alpha_B - 1) \times 10^3}\right)$$

where the isotopic composition of sea water ($\delta^{11}B_{sw}$) is 39.5‰, α_B is the fractionation factor ($^{11-10}K_B$) between $B(OH)_4^-$ and $B(OH)_3$ of 1.0272 (Klochko et al., 2006) and the equilibrium constant pK_B was calculated for each data point with its coincident temperature and salinity following Dickson (1990) (Table S1). This pH calculation takes into account the effect of temperature on $\delta^{11}B_{Borate}$.

Seeing $\delta^{11}B$ as a pH indicator, we consider these pH values as the estimated pH at the site of calcification and present the difference of a pH value from the normal (at point 0) as ($\Delta pH_i = pH_0 - pH_i$) (Fig. 1).

2.5 Previously published $\delta^{11}B$ records examination:

Following our experimental results that showed a distinctive bleaching signature in coral's $\delta^{11}B$ values, we examined previously published coral and foraminifera's $\delta^{11}B$ records for $\delta^{11}B$ drops resembling the bleaching signature evidenced in our experiment. Our search was focused on the time frame of present day to the penultimate deglaciation (~125 kyr BP), when global temperatures were comparable to present day values (Schmidt et al., 2006). $\delta^{11}B$ and calculated pH values, as well as SST and atmospheric CO_2 data, were retrieved from relevant publications' tabulated data if available. Data of Gaillardet and Allegre (1995), which were not available, were recovered from graphics using GETDATA graph digitizer (http://getdata-graph-digitizer.com/). SST data for the open sea as close as possible to Arlington Reef (Lat. 17° S, Lon. 148° E, GBR) was taken from NOAA ERSST-3b database (http://nomads.ncdc.noaa.gov/las/getUI.do) monthly reconstruction (Smith et al., 2008). Meta data for the reviewed coral records are presented in the supporting information (table S2). Relative $\delta^{11}B$ drops ($\Delta\delta^{11}B$) in published $\delta^{11}B$ records were calculated as the difference between a specific $\delta^{11}B$ value and the average $\delta^{11}B$ of the whole data series in which it belongs. For the comparison of experimental results

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and previous (low resolution) records, the effect of sampling resolution was estimated and corrected by averaging $\delta^{11}B$ throughout the whole experimental time series (Fig. 1).

3 Results and discussion

3.1 Experimental bleaching

Oxygen and pH micro-sensor analysis in the DBL of a bleached massive *Porites* sp. revealed a decrease in pH down to 7.85 (0.32 pH units below ambient pH values) during light conditions with no increase in oxygen, as opposed to a healthy coral which exhibited an increase in oxygen production and a re-alkalization of the DBL under the same light conditions following a period of dark incubation (Supporting information, Fig S2). These measurements serve as validation of an already quantified phenomenon of reduced pH in the DBL as a result of bleaching (Al-Horani, 2005). While corals are known to elevate the pH at the site of calcification relative to ambient seawater (Venn et al., 2011) , reduced seawater pH resulting from the chemical dissolution of CO_2 has been shown to reduce the calcifying medium pH, overwhelming up-regulatory compensation (Venn et al., 2013). Theoretically, the same would hold true for biological reductions of pH in the DBL evident in micro-sensor measurements.

Corals' responses to the heat stress conditions in the experimentally induced bleaching experiment varied individually (Fig. S1), where two nubbins showed no apparent bleaching while the other two were either partially or fully bleached. Fully and partially thermally-induced bleached corals displayed a $\delta^{11}B$ drop ($\Delta\delta^{11}B$) of 2.2-3.7‰ relative to their recovered outer rim values, descending to 21.5‰, corresponds to a pH drop (ΔpH) of 0.29 units (Fig. 1). The decrease of ca. 0.3 pH units as a result of diminished photosynthesis coincides with previous measurements of internal and DBL pH in light-dark or bleaching

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manipulations (Al-Horani, 2005; Venn et al., 2011). This pH drop is also corrected for temperature effect on $\delta^{11}B_{\text{Borate}}$ (detailed in section 2.4) and therefore should be considered as the result of physiological change (i.e. coral bleaching). Due to the high resolution of sampling (~45 µm equals about two-three weeks of skeletal deposition) we were able to record low $\delta^{11}B$ values at the time of bleaching. In order to demonstrate the effect of sampling resolution and to allow comparison of our results with some lower resolution studies, we calculated the average values for the whole experiment (~7 months) consisting of unbleached acclimation, heat stress driven bleaching and recovery. In this case, partially and fully bleached corals showed $\delta^{11}B$ drop of -1.55 ‰ and -2.34 ‰ (respectively, Fig. 1). Interestingly, heat stressed corals showed a $\delta^{11}B$ drop of -0.78 ‰ even though not apparently bleached. This finding contradicts previous reports of increased $\delta^{11}B$ in elevated temperatures (Dissard et al., 2012) implying a distinctive difference between the effect of "normal" high temperatures within the natural habitat's range increasing photosynthesis and calcification rates, and the effect of heat-stress-temperature conditions depressing coral physiology.

3.2 Natural bleaching

A mesophotic (60m depth) *S. pistillata* coral documented to go through a seasonal summer bleaching (Nir et al., 2014) showed a $\delta^{11}B$ drop of $\Delta\delta^{11}B$= -5.1‰ during bleached period (Fig. 2). The "bleached" low $\delta^{11}B$ value falls on the value calculated with boron isotope equilibrium constant ($^{11-10}K_B$) of Klochko et al. (2006) (not accounted for vital effect) while the "healthy" high $\delta^{11}B$ value coincides with the normal value measured for this species at pH 8.2 (with symbionts) (Krief et al., 2010). However, the high magnitude of $\delta^{11}B$ change found for the bleached period is somewhat surprising due to the fact that baseline levels of productivity were documented to be low for this mesophotic coral (Nir et al., 2014). This result may point to other pH up-regulation processes, besides photosynthesis, that are also weakened during bleaching.

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In contrast to our findings, recent explorations of boron isotopes in bleached corals found no effect of short-term bleaching on the skeletal boron signal (Schoepf et al., 2014b). Our study may have been able to capture the depletion in $\delta^{11}B$ due to (1) the use of high resolution sampling across the coral growth axis and (2) the relatively long bleached period, allowing a coral to precipitate "new" aragonite encompassing the boron signal of bleaching. This allows for an intricate time series comparison within the same individuals enabling the detection of relative changes in the skeletal boron isotopes at multiple time points before, during and after a bleaching event sustained over approximately three months. The duration of natural coral bleaching events' varies in time scale, from a few days' to prolonged periods over several months or seasons. The boron bleaching signature would likely not be recorded in the case of short durations of bleaching, simply because not enough aragonite will have precipitated to allow analysis. However, the depletion of $\delta^{11}B$ during bleaching may be captured for longer and more pronounced bleaching events or by using fine scale measurements (e.g. laser ablation) as evidenced in our study. It is noteworthy that searching for paleo bleaching event using the boron isotope signature will only allow identification of major events (which are probably of higher environmental significance) rather than short, anecdotal events. Furthermore, the high resolution sampling implied here, which might seem irrelevant for paleo-climate studies (e.g. pH reconstruction over glacial cycles), is shown to be of crucial importance when looking for biological phenomena.

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The question is whether these timescales are environmentally relevant. These results require a discussion of what temporal scales may be resolved in the paleo record, and what events might be missing. This is key to the importance of the proxy.

3.3 Establishment of the $\delta^{11}B$ bleaching proxy

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Our experimental results showed decreased pH at the DBL accompanied by a sharp $\delta^{11}B$ drop for bleached coral held at constant ambient pH_{sw}. A field 'ground truth' for this boron bleaching signature was found in a naturally bleached mesophotic (60m depth) *S. pistillata*

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coral. Further evidence that $\delta^{11}B$ drop can serve as a bleaching proxy   338
can be gathered from a recent coral bleaching event on Australia's Great   339
Barrier Reef (GBR) (Wei et al., 2009). Using a ca. 200-year-old *Porites*,   340
Wei et al. reported low $\delta^{11}B$ values ($\delta^{11}B_{1998}$ =21.06 ‰, $\Delta\delta^{11}B_{1998}$=-2.3‰   341
and $\delta^{11}B_{2001}$ =21.93 ‰, $\Delta\delta^{11}B_{2001}$=-1.4‰, Fig. 2) during the 1998 and   342
early 2002 bleaching events. These low values, measured at times of   343
bleaching events, are similar to the low $\delta^{11}B$ values recorded in our   344
experimentally bleached coral.   345

Based on our laboratory results as well as on natural records of *Porites*   346
sp. bleaching, we define the boron bleaching proxy as $\delta^{11}B$ drop of at   347
least 1.5‰. This value was chosen by estimating the average $\Delta\delta^{11}B$ for   348
ca. one year in the experimentally bleached corals, simulating the   349
common paleo pH $\delta^{11}B$ records temporal resolution (supporting   350
information, Table S2). This value also falls outside the range of the   351
natural variability measured in *Porites lobata* coral skeleton throughout   352
two annual cycles (Hemming et al., 1998) (Fig. 3 – green boxes). In   353
applying this proxy to identify paleo-bleaching events, we also inspected   354
concomitant paleo-SST, air temperature and $pCO_{2\ atm}$ records to provide   355
support for our conclusions (Fig. 3). In cases where a drastic drop in   356
$\delta^{11}B$ is associated with high SST or severe warming rather than pCO_2 357
increase, we propose that a thermal stress may have induced bleaching   358
and resulted in the low $\delta^{11}B$ values, rather than a pH_{sw} drop.   359

It is important to note that most coral paleo-reconstruction studies focus   360
on the analysis of a single core due to the difficulty in preparation and   361
analysis of the samples. Coral bleaching however can be spatially   362
heterogeneous with neighboring individuals exhibiting variable   363
responses (Baker et al., 2008) making broad generalizations difficult.   364
Thus if a depletion in $\delta^{11}B$ values is not found where bleaching is   365
expected this does not necessarily mean that coral bleaching did not   366
occur on that reef at some point in time. For example, in a recent   367
analysis of a massive *Porites* core from Guam (Shinjo et al., 2013), no   368
bleaching signal is found in the $\delta^{11}B$ signature from the period of 1940-   369

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2000. This does not rule out bleaching in the region. In fact, slight depressions in the $\delta^{11}B$ values (-0.5 > $\Delta\delta^{11}B$ > -1 ‰) coincide with El-Nino years and may indicate physiological heat stress of the coral. Alternatively, when the boron bleaching signature is evident in fossil coral core samples, as outlined in the coming examples, based on our experimental findings it may indicate that coral bleaching has occurred.

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3.4 Coral bleaching events recorded since the Last Glacial Maximum

Over the past 800,000 years of glacial/interglacial cycles, atmospheric CO_2 oscillated between 180-280 ppmv, with an increase of less than 30 ppmv from the Holocene until the beginning of the industrial revolution (~1860). Ocean surface's pH paleo-records (based on coral's $\delta^{11}B$ measurements) exhibit higher magnitude variations in comparison to atmospheric pCO_2 (Fig. 3). These shifts have been interpreted as major oceanographic events such as alterations of ocean circulation stemming from upwelling of cold, low pH waters to the surface (Douville et al., 2010; Liu et al., 2009), decoupling the tight relation of $pCO_{2\ atm}$ and pH_{sw} on a regional scale. However, following the results presented in this paper, we suggest an alternative explanation for the $pCO_{2\ atm}$ and pH_{sw} decoupling, where a severe drop of the coral $\delta^{11}B$ values is associated with high or rapidly increasing SST rather than an increasing $pCO_{2\ atm}$, this drop may be the geochemical signature of coral bleaching and not of a pH_{sw} decline.

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Please be more precise in language - I think you mean to say "variability" and "amplitude" not "acute and radical changes".

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You state that ocean surface pH paleo records exhibit more "acute and radical changes": isn't this a result of sampling resolution or averaging? In the modern ocean these two parameters are very linked in variability, so why wouldn't they be in the paleo record?

We inspected published paleo-pH records from coral skeletons through the Eemian interglacial stage (~130 kyr BP) and identified six suspected data points (Fig. 3 - red vertical lines) that meet our boron bleaching signature definition of a depletion in $\delta^{11}B$ values greater than 1.5‰ relative to the average values measured in that series (Fig 3– dotted line). Supported with concomitant paleo climate records, we make a case for these instances that represent potential bleaching events rather than an actual ocean acidification.

3.4.1 20th century, GBR: Pelejero et al. (2005) measured a 280-year $\delta^{11}B$ record of *Porites* sp. and reported the lowest $\delta^{11}B$ value for the last three centuries at Flinders Reef, GBR, ca. 1988 (using 5-year averages) (22.99‰, 7.91 pH units) with an overall trend of low pH values associated with positive values of the Interdecadal Pacific Oscillation (IPO) index (El Niño like conditions), interpreted to result in low rates of water exchange at the Flinders Reef's lagoon. However,

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high-resolution inspection of 1987-1988 reveals that the lowest $\delta^{11}B$ value was recorded during the warmest month (February 1988, $\delta^{11}B$ = 22.26‰, stands 1.65‰ below the multi annual average) even though wind speed, normally linked to a high lagoonal water exchange, was high that month. Since this low $\delta^{11}B$ value also coincides with warm SST and the 1988 worldwide mass bleaching (Williams and Bunkley-Williams, 1990), it is likely that the reported low $\delta^{11}B$ value is due to heat stress or coral bleaching. This value was preceded by a period of lower values that commenced at the transition from cooler to warmer SST during the austral summer of 1987 and could indicate a sustained bleaching period, such as was seen in the $\delta^{11}B$ values of our experimentally bleached coral skeletons.

Additional low $\delta^{11}B$ values ($\delta^{11}B$ < 21.65‰, stands 1.67‰ below the multi annual average) were recorded for Arlington reef at two sampling points during 1937-1940(Wei et al., 2009) that coincide with a trend of rapidly warming SST(Smith et al., 2008) (Supporting information, Fig. S2), suggested to indicate pronounced bleached periods within this time frame.

3.4.2 6 Kyr BP, SCS: Liu et al. (2009) measured *Porites* sp. $\delta^{11}B$ from the South China Sea (SCS) and reported a $\delta^{11}B$ drop, at ~6 kyr BP ($\Delta\delta^{11}B$= -1.82‰) interpreted by the authors as low pH during the Holocene thermal optimum (~5-9 kyr BP). This period was suggested to be characterized by an intensified summer monsoon that resulted in strengthened upwelling and introduced low pH deep water to the surface of SCS. However, no sign of cold deep water upwelling is evidenced in the reported SST values (Yu et al., 2005). Furthermore, Mg/Ca derived SST records related to SCS upper waters (0-30m) temperature show a peak in SST around 6 kyr BP (Steinke et al., 2008) ascending to 29°C. Translating pH values to pCO_2 (using CO2sys software (Pierrot and Wallace, 2006)) shows that although Holocene pCO_2 $_{atm}$ was relatively stable (260-290 ppmv, not including the 20th century), reported pH values are equivalent to much higher pCO_2

levels, up to 571 ppmv at 6 kyr BP. This drop of $\delta^{11}B$ (at 6 kyr BP) 440
corresponds better with high SST, rather than a drastic increase in 441
pCO_2 or upwelling introducing low pH water to the surface. Therefore, 442
we propose that the low $\delta^{11}B$ values imprinted in SCS corals 6 kyr BP 443
are the result of a bleaching event. 444

3.4.3   11.5 Kyr BP, Central Pacific: Both Gaillardet and Allegre (1995) and 445
Douville et al. (2010) reported a severe drop of $\delta^{11}B$ measured in 446
central sub-equatorial pacific corals (*Acropora* sp. from Tahiti 447
(Gaillardet and Allegre, 1995) and *Porites* sp. from Marquesas Is. 448
(Douville et al., 2010)) at the end of the Younger Dryas period ~ 11.5 449
kyr BP ($\Delta\delta^{11}B$=-2.18‰). This drop deviated from the otherwise stable 450
values measured before and after this time period by more than 1.5 ‰ 451
(interpreted as pH drop ~ 0.2 (Douville et al., 2010)) (Fig. 2,). In these 452
studies, diagenesis was not examined, so the low $\delta^{11}B$ values should 453
be taken with cautious. Douville et al. (2010) attributed this dip to 454
sustained La-Nina like conditions which introduced lower pH cold 455
waters in the central sub-equatorial Pacific (Douville et al., 2010). The 456
equatorial Pacific SST gradient (ΔSST), which coincides with the 457
strength of westward low pH water advection (Koutavas et al., 2002), 458
was intensified at the end of Younger Dryas, potentially extending the 459
low pH "cold tongue" westward closer to the Tahiti Islands. 460
Nevertheless, ΔSST was only as high as modern times remaining 461
constant throughout the Holocene, while $\delta^{11}B$ returned to background 462
values (Fig. 3)(Kienast et al., 2001; Koutavas et al., 2002). The 463
dominance of La-Nina patterns at this period is further challenged by 464
alternative suggestions of El-Nino conditions prevailing during the 465
Bolling interstadial and the end of Younger Dryas (Koutavas and 466
Sachs, 2008). Furthermore, following the cold Younger Dryas period 467
was a period of rapid warming (Asami et al., 2009; Schmidt et al., 468
2006), which likely increased seasonal maximum temperatures (Fig. 3). 469
The yearly increases in temperature, the major cause of coral 470
bleaching in the modern era (Baker et al., 2008), could have resulted in 471
a wide spread coral bleaching event 11.5 kyr BP in the central sub- 472

Commented [G19]: Added following reviewer #2 advice
What about diagenesis? Could diagenesis be partly responsible for the 11B changes in the paleo-record? How can the authors rule this out?

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equatorial Pacific. This offers an alternative explanation for the low $\delta^{11}B$ values obtained for corals, rather than sustained La-Nina like conditions.

4 Conclusions

Coral bleaching exerts a considerable signature on $\delta^{11}B$ measurements taken from the scleractinian corals *S. pistilata* and massive *Porites* sp. ($\Delta \delta^{11}B = 1.5\text{-}5.1$‰). Employing high-resolution femtosecond LA-MC-ICP-MS, we provide evidence that $\delta^{11}B$ is sensitive, and highly influenced by the physiological interactions of the photosymbiont and the calcifying organism (i.e., a drop in photosynthetic activity of photosymbionts during bleaching events). Overall, our experimental results, along with previously published data, highlight the challenges of utilizing $\delta^{11}B$ as a straight forward proxy for paleo-pH$_{sw}$. To gain an accurate interpretation of $\delta^{11}B$ values, the bleaching state of the organism from which the data is derived must be considered. On the other hand, when supported with concurrent pCO$_{2\ atm}$, foraminifers' pH and SST records, this proxy opens the door to investigations that utilize $\delta^{11}B$ as a paleo-bleaching marker.

While the understanding of temporal dynamics of when/how these events are recorded in coral skeletons, still requires examination of additional modern known bleaching events, our findings provide evidence that coral bleaching may not be an exclusively modern phenomenon and we have identified at least two instances since the LGM (~20 kyr BP) prior to the industrial revolution where coral bleaching likely occurred. If short-term bleaching is indeed untraceable with $\delta^{11}B$ measurements (Schoepf et al., 2014b), then the suspected paleo-bleaching events may be a result of longer sustained bleaching events, possibly comparable with contemporary worrisome mass bleaching episodes.

Acknowledgements

Commented [G20]: This paragraph was moved here following reviewer #1 advice
2nd paragraph of the conclusions seems out of place. I think it should be modified and moved up to be the 1st paragraph of this section.

Commented [G21]: Added following reviewer #1 advice
Also, I suggest that the authors include a statement that examination of additional modern, known bleaching events will be necessary to understand the temporal dynamics of when/how these events are recorded in coral skeletons.

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Figure legends:

Fig. 1. $\delta^{11}B$ measurements of coral skeletons following different bleaching situations. (a) Dark green symbols represent control coral measurements, yellow symbols represent heat stressed corals, while bright green and white symbols represent partially and fully bleached corals respectively. Values of $\delta^{11}B = [^{11}B/^{10}B]_{sample}/[^{11}B/^{10}B]_{\text{NIST 610 standard}}$ -1 X 1000. Values are given in parts per mil (‰).standard error is within the size of symbols (≤ 0.30‰, 2σ). Dark and bright green triangles, white diamonds and yellow triangles data series were subtracted with -2‰ to compensate for a change in instrumental setup (detailed in SOM).

(b) Relative change from outer rim value for each sample ($\Delta\delta^{11}B$). All rim values (at 0 μm) were equaled to zero and the change in $\delta^{11}B$ values was plotted for each transect along the growth axis. Positive values equal an enrichment in values while negative values are depleted relative to the rim value. Dashed lines and above written values represent the average $\Delta\delta^{11}B$ for a coral bleaching state. Coral's appearance during the heated stage of the experiment is illustrated by the nubbins pictures on the right side.

(c) Relative pH change from outer rim value for each sample (ΔpH). pH values are calculated from $\delta^{11}B$ using temperature corrected pK_B in order to compensate for the temperature effect on $\delta^{11}B_{\text{Borate}}$.

> **Commented [G22]:** Panel (c) was added to figure 1

Fig. 2. (a) $\delta^{11}B$ measurements of mesophotic (60m) *Stylophora pistillata* coral documented to undergo bleaching during summer 2010(Nir et al., 2014). (b) Skeleton density, reported as the gray value of pixels along the ablation transect. Yellow shaded area represents the low density skeleton band deposited in summer 2010. Solid and dashed gray lines represent the expected $\delta^{11}B$ value for $pH_{sw}=8.2$ calculated with boron isotope equilibrium constant ($^{11-10}K_B$) of Klochko et al.(2006) (not accounted for vital effect) and an empirical coefficient specific to this species (with symbionts) by Krief et al. (2010) respectively.

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Fig. 3. 25,000 year records of paleo-ocean climate and pH (a-d) and boron isotopic compositions in coral skeletons (e-h).

Blue lines represent atmospheric pCO_2 measured in gas trapped in ice cores (Monnin et al., 2001; Monnin et al., 2004; Petit et al., 1999; Robertson et al., 2001) and direct measurements (Tans, 2012), open symbols represent SST records (triangles – *South China Sea(Steinke et al., 2008),* circles - *Western Caribbean sea* (Schmidt et al., 2006) and crosses – *modern tropics (Indian and Pacific oceans)* (Wilson et al., 2006)).

$\delta^{11}B$ derived pH from foraminifera (ocean surface in gray symbols-circles(Foster, 2008), inverted triangles (Palmer and Pearson, 2003), rectangles (Hönisch et al., 2009), diamonds (Palmer et al., 2010) and triangles (Hönisch and Hemming, 2005; Deep waters pH in blue symbols (Yu et al., 2010)) and coral (red symbols – circles (Douville et al., 2010), inverted triangles (Liu et al., 2009) and rectangles (Gaillardet and Allegre, 1995); grey (Pelejero et al., 2005) blue (Shinjo et al., 2013) and black (Wei et al., 2009) lines) is shown in (c-d). The boron isotopic compositions, on which coral pH is based, are presented in (e-f). The difference of $\delta^{11}B$ from the average value in record ($\Delta\delta^{11}B$) is shown in (g-h) together with a dotted line under which data points are suggested to represent coral bleaching. Green bars and line within represent $\delta^{11}B$ annual range and average measured at unbleached coral (Hemming et al., 1998). Red vertical lines mark the timing of suspected bleaching events. Grey and yellow shaded bars represent cold and warm periods respectively.

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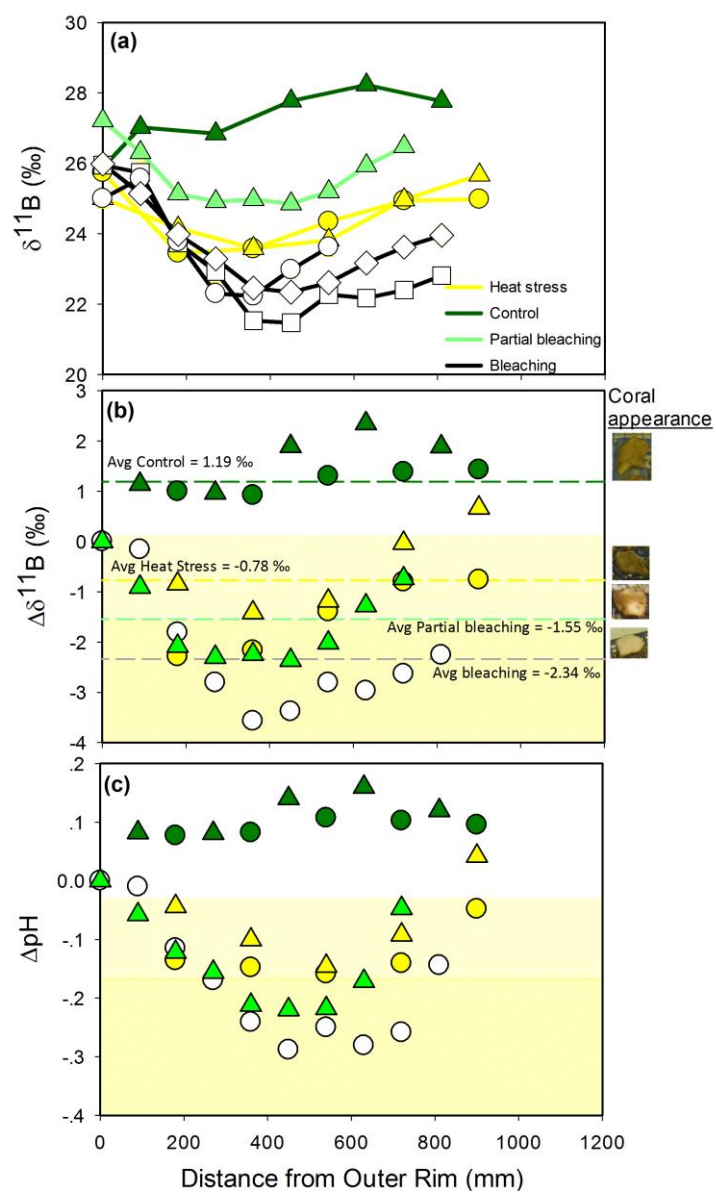773
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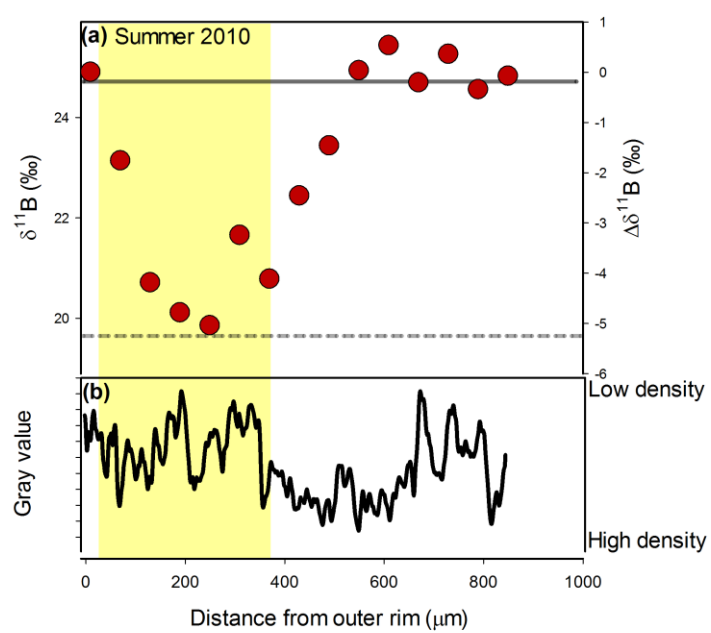780
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Figure 1

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Figure 2
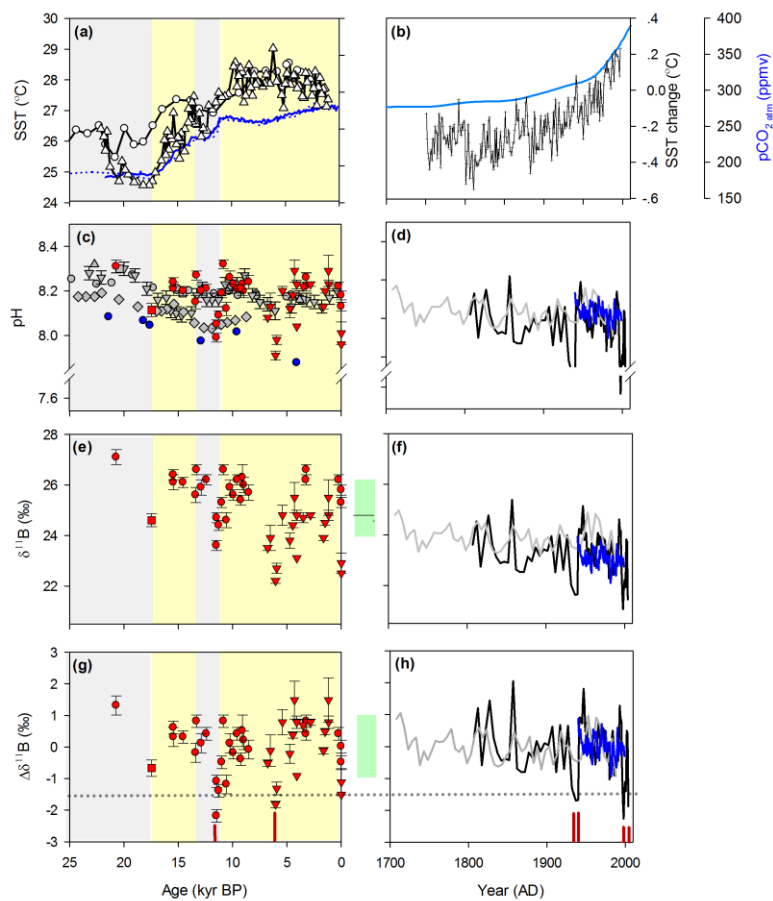
Figure 3