Biogeosciences

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Discussions

Supplement of

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

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Supplement:

Extended materials and Methods:

Sample Collection

Two colonies of *Porites* sp. were collected from the coral nursery at 10 meters depth off the shore of the Interuniversity Institute for Marine Sciences in Eilat, Israel. This area is situated in the Gulf of Aqaba, in the northern Red Sea (29°30'06" N, 34°55'00" E). The coral were brought to shore by SCUBA divers and cut into 77 pieces approximately 2 X 2 cm 'nubbins' (except for 1 of 4 X 4 cm) using a Wolf Mini Grinder (MD32/36K) with a diamond disc saw. During cutting, the coral was submerged in seawater to promote survival. All samples were 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.

Experimental Design

Coral were maintained in two 140 L open-system water tables on shore. Water was pumped to the tables directly from 30m depth at a rate of ~2.0 L/min. One table contained 25 control coral nubbins which were kept in two clear 10 L plastic boxes each containing a water inlet and an Aqua One Maxi 100 Powerhead to circulate the water at a

flow rate of 400 L/hour. Control corals stayed at the ambient water temperature throughout the experiment which fluctuated due to seasonal changes. The experimental heat stress table contained 52 coral nubbins and temperature varied throughout the experiment to induce bleaching and allow for subsequent recovery (Table S1). The water temperature was controlled using an Eliwell IC 902 electronic thermostat with single output and four Ferplast BluClima 250 W heaters (mean°C ± 0.15). This table contained two inlets and two Atman AT-201 Power Heads to circulate the water at a maximum flow rate of 800 L/hour. Coral on both tables were left to grow on crates (5 or 6 coral nubbins on each crate).

Osram Powerstar HQI-TS 150 W lights housed in Golden Light MH-150W RX7S 230V housing were suspended above each water table illuminating the tables with constant light of an average of 150 μmol photons m^{-2} s^{-1} on an 11:13 light/dark cycle. Light measurements were obtained using a Walz US-SQS spherical micro quantum sensor held at a 45° angle directly under the water surface directly above the coral nubbins. The light sensor is an integrating sphere capturing light from all angles. Corals were fed twice a week with 1-day old *Artemia nauplii* hatched on site in a separate aquarium. Both treatments were photographed once a month to record the corals' morphological status.

Coral Markings

All coral nubbins were stained at the beginning of the experiment with Alizarin Red S (Sigma A-5533), at a concentration of 20 mg/L of seawater. The coral were left in a 40 L tank for 1 full day of light, from sunrise to sunset (11 hours).   Two Atman AT-201

Powerheads were put into the tank to ensure circulation of the water. The Alizarin Red stain provided a visual marker for later identification of skeletal growth during the experiment.

Diffusive boundary layer measurements

Changes in oxygen and pH in the corals' diffusive boundary layer (DBL) were measured for a healthy coral in the control treatment as well as a bleached coral in the HS treatment to document an example of oxygen and pH changes occurring in the boundary layer (Fig. S1). Measurements were carried out simultaneously directly above the coral surface using a Presens pH-1 micro microsensor pH meter and a Presens Oxy-4 Oxygen microoptode. pH readings were taken every thirty seconds. Calibration was done using a one-point temperature compensation calculation sent by the manufacturer based on the initial calibration values of the sensor. Filtered Seawater, with a measured pH of 8.17, was used for the single point calibration. Ambient and DBL oxygen concentration was obtained every three seconds and recorded as % air saturation. A two-point calibration was performed using 0% as measured in a 100 ml solution of DDW with 1 g. of Sodium Sulfite (Sigma-Aldrich S0505) and 100% as measured from 100 ml of DDW bubbled for 20 minutes using a Schego M2K3 air bubbler constantly stirred with a M.R.C. MH-1 magnetic stirrer. The 100% calibration solution was left to stir, after bubbling, for 10 minutes to prevent super saturation of the solution.

The coral fragments were placed on several plastic blocks in a 40 L tank filled with filtered seawater. Measurements of seawater were obtained before and after the start of

the experiment to later calculate the drift of the microsensors due to photobleaching. While the oxygen optode showed little drift over the course of the experiment, the pH microsensor showed a large drift (as expected from the manufacturer manual) whose slope was added to each data point to correct for this drift in calculations.

Both microsensors were fixed above the tank using a Narishige GJ-2 micro-manipulator which allowed for a fixed position and manual movement of the sensors up or down. Using a suspended Wild Heerbrugg microscope as a visual aid, the microsensors were put into position directly above the surface of the coral. The tank was then covered with a black cloth and the coral was left in darkness for thirty minutes. After the period of dark, ~350 μmol photons m^{-2} s^{-1} of light was directed onto the coral from a Dolan-Jenner Fiber-lite light source. After another thirty minutes, the sensors were withdrawn from the coral to measure final seawater values.

Sample preparation for δ^{11}B analysis

After the experiment was completed, several coral were chosen for δ^{11}B analysis. Nine coral were chosen from the heat stress (HS) treatment; seven coral that appeared to stay bleached throughout the high temperature period of the experiment with several of them displaying recovery by the end, and two that did not appear to bleach at all over the course of the experiment. Three control coral fragments were selected.

The tissue of all coral was removed using a high pressure airbrush along with filtered seawater (0.45 μm) and collected into a 50 ml Falcon tube using a plastic bottle with two

holes cut in the sides (one for the airbrush and one for the coral) to gather the liquid. The final volume of the airbrushed sample liquid was recorded and the falcon tubes were put into -80 °C for later physiology tests.

A second round of airbrush was performed after the collection of the tissue using a higher powered airbrush to remove any remaining tissue. All skeletons were then cleaned with double deionized water (DDW) to remove salts and dried for several hours in direct sunlight.

LA-ICP-MS – Laser Ablation Inductively Coupled Plasma Mass Spectrometer

All further preparation of the samples for $\delta^{11}B$ analysis was done at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. Small fragments of each skeleton were cut by hand using a diamond saw. The surface of the samples was then grinded with a 1200 grit Silicon carbide (SiC) grinding paper mounted onto a rotating wheel consistently lubricated with Artificial Seawater. This was done to flatten the surface for easier readings of boron. The samples were then put into an ultra-sonicated Milli-Q water bath for 5 minutes to remove any particles, after which they were immersed in Sodium Hypochlorite (NaClO) for two hours to remove any organics that were on the cross-sectional surface. The samples were left in the oven at 60 °C to prevent the formation of bleach crystals on the surface of the coral. After 1 hour and 15 minutes, the oven was turned off and left to cool. The samples were then washed thoroughly over a vacuum filter. The samples were washed first with Acetone, then Milli-Q water three times, then again with Acetone, a second round of Milli-Q water

rinses and finally a wash of Acetone which would remove any water residue and then left to evaporate. This process was used to ensure that no bleach residue was left on the coral pieces which could later contaminate boron readings. The samples were put into the oven at 60 °C overnight to dry.

The following morning, the coral pieces were brought to the Leibniz-University of Hannover, Hannover, Germany for $\delta^{11}B$ analysis on a Thermo Finnigan-Neptune Multiple-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS). This machine was connected to a UV femtosecond (10^{-15} s) laser used for laser ablation of the samples. For a detailed explanation of the instrument and laser ablation system please refer to Horn and Blanckenburg *(26)*; the following is a brief description.

The MC-ICP-MS allows for simultaneous readings of low mass isotopes, such as ^{10}B and ^{11}B. It contains a nine cup collector, but only two were used during the experiment. The center cup was set at ~10.5 u with the left cup set to read ^{10}B (~10 u) and the right cup to read ^{11}B (~11 u). All cups were calibrated to capture the peak of the isotope's signal while avoiding interference from other unwanted masses. Tune-up and calibration of the machine was carried out before each day of measurements.

The samples were put into an aluminum ablation chamber mounted on a microscope stage allowing for movement of the stage and chamber. The chamber has three gas inlets and a single outlet that is connected by a tube to the MC-ICP-MS. The chamber is flushed by Helium with a flow rate of 0.6-0.7 L/min. Argon is added close to the plasma

torch at a flow rate of 0.8-0.9 L/min. An Ecoline digital camera is mounted on top of the

microscope so samples can be viewed to choose the path of ablation. The laser is

directed through a series of mirrors and an 8x objective into the chamber and ablates the

sample at varying frequencies adjusted between sample and standard in order to obtain

matching signal intensities. These frequencies were adjusted so the resulting signal of

^{11}B was close to 300 mV in each sample and standard, measured on a 10^{11}Ohm resistor.

The femtosecond (fs) laser is an in-house system built at the University of Hannover

using a Spectra Physics Hurricane I Ti-sapphire femtosecond laser producing a pulse

energy of 1 mJ at its fundamental wavelength of 785 nm. The output wavelength of 785

nm is then converted by a series of barium-beta borate crystals to produce the ultraviolet

wavelength of 196 nm in the fourth harmonic. The maximum pulse energy is 0.03 mJ at

196 nm generating an achievable energy density of 1.5 J/cm² at an estimated pulse width

of ~200 fs. This is in contrast with other laser ablation systems which utilize nanosecond

lasers causing issues of sample melting, elemental and isotopic fractionation (Horn &

von Blanckenburg, 2007). Thus, the fs-laser technique allows for fast and accurate

measurements of the isotopes in the coral skeleton with little degradation of the material

due to the ultra-short pulses used.

Samples were loaded into the chamber two at a time with the cross-sectional surface

facing upwards towards the camera and laser objective. There was at least one standard

in the chamber at all times. The standard used was a piece of National Institute of

Science and Technology Standard Reference Material 610 glass (NIST SRM 610). This

reference material has been used and measured in several studies as an appropriate

reference material for stable boron isotope analysis (Kasemann *et al.*, 2001, Le Roux *et al.*, 2004, Tiepolo *et al.*, 2006). A "standard-sample-standard" bracketing technique was used where the reference material was measured before and after the sample to correct for instrumental drift. Samples were analyzed by tracing parallel lines running along the growth axis of the coral skeleton piece. For the coral samples that bleached during the experiment less growth was observed so that the lines measured were 90 μm apart from each other. The control samples and corals that did not appear to bleach in the HS treatment of the experiment showed more skeletal growth on top of the alizarin red band and thus lines were analyzed which were 180 μm apart from each other. All transect lines started from the outer rim of the coral (end of the experiment) and moved towards the alizarin red band (beginning of the experiment). Relative values ($\Delta\delta^{11}$B) were calculated using the rim values as the reference point. This time point, post recovery, was used as the zero value as environmental conditions were known to be the same between treatments. The beginning of the experiment could not be used as the reference point because besides the alizarin red line as a coarse visual marker there was no way of definitively knowing what skeletal growth was deposited prior to the start of the experiment (prior to bleaching).

The standard was analyzed using rasters (large lined boxes) to cover larger areas of the NIST 610 glass. The NIST 610 glass was generally analyzed with a repetition rate of 78 Hz, while the sample acquisition was performed at a slightly higher repetition rate of 88-100 Hz. The mounted chamber was moved following the lines or rasters designated by a micro positioning stage of the microscope taking 50 readings of the ^{10}B and ^{11}B

intensities with an integration time of 1 second. Averages and standard errors (SE, absolute and percentage) were calculated for each analysis. Standard errors could not be used for the sample because the coral skeleton is not a homogeneous sample, so that the error estimation had to be calculated based on the standard errors of the two NIST610 standard determinations. The error propagation equation used was:

$$\text{S.E.}_{\text{sample}}= \sqrt{[(1^{\text{st}} \text{ standard } (SE_{\text{abs.}}/\text{Avg.}))^2 + (2^{\text{nd}} \text{ standard } (SE_{\text{abs.}}/\text{Avg.}))^2 + (\text{Avg}_{\text{sample}}*SE_{\text{avg. \% of two standards}}/\text{Avg}_{\text{sample}})^2]}$$

The majority of the $\delta^{11}B$ data presented in the paper was from two days of analysis on the MC-ICP-MS. X-cones were used in order to achieve the required intensities. However, X-cones degrade fast with usage and are likely to produce a significant offset in the $\delta^{11}B$ values. The x-cone was replaced with a new one on the second day. The results were significantly higher by ~ 2 ‰. To check that the readings were still accurate, we measured previously analyzed samples from the first day. The relative changes were within 1 ‰ of the previous day's readings, but the absolute values showed a consistent offset with an average enrichment of 2.31 ± 0.51 ‰. We therefore subtracted 2 ‰ from the absolute $\delta^{11}B$ values of all samples read on the second day to allow for a more reasonable comparison between the two days of data. This included the data series represented as dark and bright green triangles, white diamonds and yellow triangles in Figure 1a. It should be noted that this offset did not affect the relative changes in $\delta^{11}B$ (Figure 1b), which was the main goal of our research. Another measurement of control coral, preformed months later, revealed the same pattern of relative changes but with absolute values ca. 4‰ lower. This data series is excluded from figure 1(a) but included

in figure 1(b) demonstrating the reproducibility of $\delta^{11}B$ relative changes ($\Delta \delta^{11}B$) signal, regardless of discrepancies in absolute $\delta^{11}B$ readings caused by variability associated with day of measurement.

$\delta^{11}B$, pCO_2 and SST data compilation

Following our experimental results that showed a distinctive bleaching footprint 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 foot print" 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 (Fig. 2).

$\delta^{11}B$ 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 (Gaillardet & 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 Table S2.

Implementing the "Boron bleaching foot print"

In order to uncover the relative $\delta^{11}B$ drops (rather than absolute low $\delta^{11}B$ values) we calculated $\Delta\delta^{11}B$ 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($\Delta \boldsymbol{\delta^{11}B_i} = \overline{\boldsymbol{\delta^{11}B}} - \boldsymbol{\delta^{11}B_i}$). The relatively large

$\delta^{11}B$ drops ($\Delta \delta^{11}B < -3\permil$) measured in experimentally bleached corals were obtained

due to the high temporal resolution of measurements during the bleaching event

simulated in this study (1 data point ~ 3 weeks' growth). In order to allow the comparison

of these results with lower resolution studies (1 data point ~ 1-3 years (Table S2)) we

estimated a gross resolution sampling by averaging the whole time series including both

bleaching and recovery, relative to the outer rim value. This calculation yielded $\Delta \delta^{11}B$ of

-2.34‰ and -1.55±‰ for fully and partially bleached corals respectively (Fig. 1b). We

set the line of the "Boron bleaching footprint" as $\Delta \delta^{11}B < -1.5\permil$, outside the natural

variation recorded through two annual cycles in non-bleached coral (*Porites lobata*)

(Hemming et al., 1998) (Fig. 2 – green boxes).

By defining the "Boron bleaching foot print" as $\Delta \delta^{11}B < -1.5\permil$ we suggest that any

outlier falling more than 1.5‰ below the average $\delta^{11}B$ (assumed to be affected by pH_{sw}

and vital effects, but stable for long records) may represent a bleaching event within the

time frame of measurement.

In order to assess the probability of atmospheric CO_2 driven pH changes, we estimated

ocean surface's $p CO_2$ from pH (Total scale) values using CO2SYS software on Sea

Water scale with K1 and K2 constants from Mehrbach et al. (Mehrbach et al., 1973). This

calculation was made for the lowest pH value (pH = 7.91, 6 kyr BP (Liu et al., 2009))

with an Alkalinity, Salinity and Temperature range of 2425-2575 (μmol/kg SW), 32-40

‰ and 24-29°C respectively, and the lowest result ($pCO_2 = 586$ ppmv) is reported as a

conservative value.
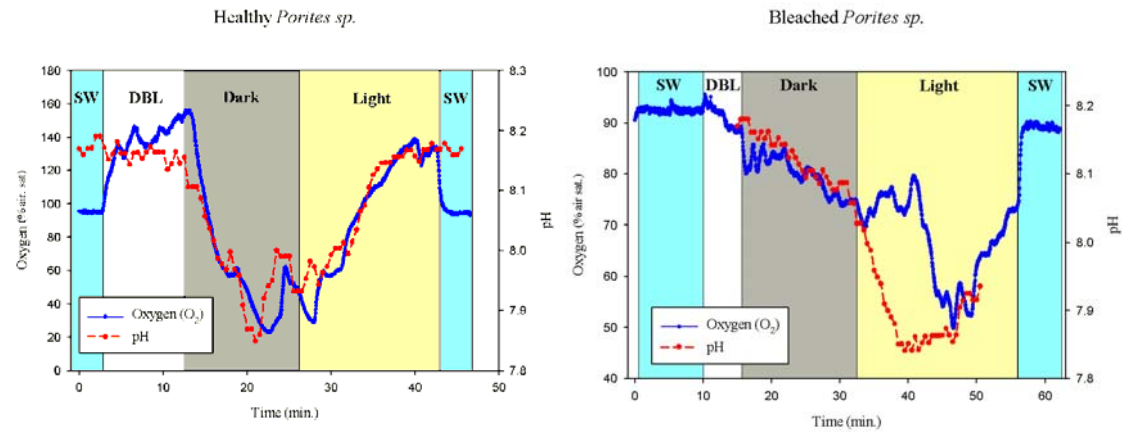
Fig. S1.

Oxygen and pH readings at the surface of a healthy (left) and bleached (right) coral.

Colored backgrounds indicate state of the experiment: Blue represents seawater readings

(SW) before and after readings at the coral surface, gray represents readings in the

diffusive boundary layer taken in the dark and yellow represents readings under light

conditions. Note: y-axes are of different scale for right and left panels.
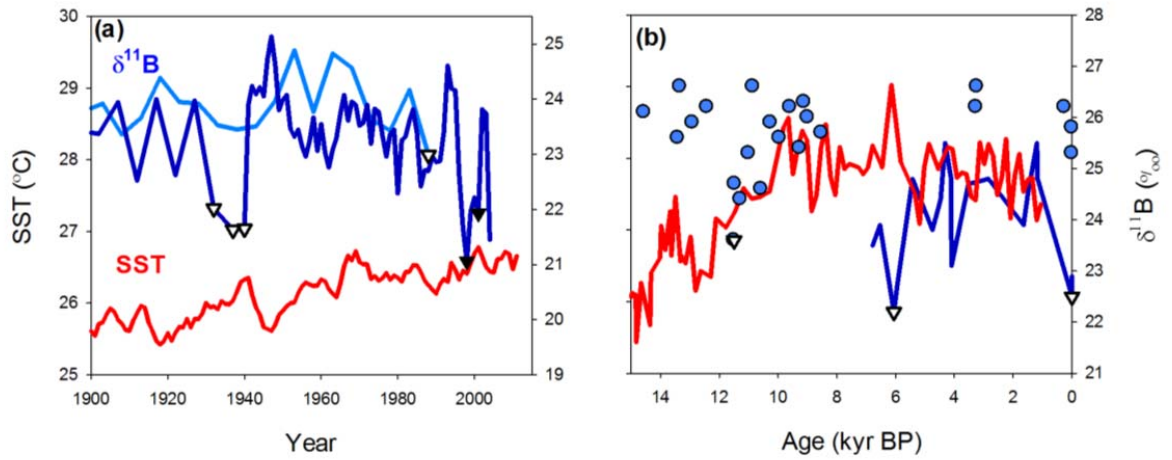
Fig. S2.

SST (red line) and δ^{11}B (blue lines and symbols) records which are suggested to contain bleaching events. (a) Dark and bright blue lines represent δ^{11}B records from Wei et al.(Wei et al., 2009) and Pelejero et al.(Pelejero et al., 2005) recorded at the GBR receptively. (b) blue line and circles lines represent δ^{11}B records from Liu et al.(Liu et al., 2009) (South China Sea) and Douville et al.(Douville et al., 2010) (Tahiti) respectively. SST was taken from Kienast et al.(Kienast et al., 2001) and NOAA ERSST monthly reconstruction averaged for 5 years (for left and right respectively). Open and black triangles represent suspected and documented bleaching events respectively.

Table S1.

Average monthly temperature is displayed for the two treatments throughout the course
of the experiment that took place from Dec 2008 to June 2009. Temperature variations in
the control treatment are reflective of seasonal temperature changes in the Gulf of Aqaba
(as measured by the Israel National Monitoring Program at the Gulf of Aqaba) while HS
treatment temperatures were controlled with a thermostat to induce bleaching (Jan-
March) and recovery (April-June).

	Nov	Dec	Jan	Feb	March	April	May	June
Control (°C)	24	23	22	22	22	22	23	24
HS treatment (°C)	24	32	32	30	30	25	23	24

Table S2.

Technical details of reviewed coral δ^{11}B records.

Author	Coral sp.	Site	Isotopic measurements technique	Time frame	Temporal resolution
Wei et al. (2009)	*Porites* sp.	Arlington Reef, GBR	Positive thermal ionization mass spectrometry (P-TIMS)	1807-1940 AD 1940-2004 AD	5 years 1 year
Pelejero et al. (2005)	*Porites* sp.	Flinders Reef, GBR	Negative Thermal Ionization Mass Spectrometer (N-TIMS)	1708-1988 AD 1987-1988 AD	5 years Ca. 1 month
Liu et al. (2009)	*Porites* sp.	South China Sea	Positive thermal ionization mass spectrometry (P-TIMS)	0-7 Kyr BP	3 years
Douville et al. (2010)	*Porites* sp.	Tahiti and Marquesas Islands	Multi collector-inductively coupled plasma mass spectrometer, (MC-ICP-MS)	0-20 Kyr BP	1-2 years
Gaillardet and Allegre (1995) ENREF_21	*Acropora* sp. and unspecified corals	French Polynesia, Sumba and Houn Peninsula	Positive thermal ionization mass spectrometry (P-TIMS)	0-117 Kyr BP	unspecified
Shinjo et al (2013)	*Porites* sp.	Guam	Multi collector-inductively coupled plasma mass spectrometer, (MC-ICP-MS)	1970-1999 AD	1 year