A novel paleo-bleaching proxy using boron isotopes	1
and high-resolution laser ablation to reconstruct coral	2
bleaching events	3
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Abstract	18 19
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	1) 20 21
experienced significant global declines due to a variety of causes, one of the major being widespread coral bleaching events. During bleaching, the coral	21 22 23
expels its symbiotic algae losing its main source of nutrition generally	24
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	25 26 27
Laser Ablation Multiple Collector Inductively Coupled Plasma Mass Spectrometry (LA-MC-ICP-MS) to demonstrate a distinct biologically-induced	28 29

decline of boron (B) isotopic composition (δ^{11} B) as a result of coral bleaching. 30 These findings and methodology offer a new use for a previously developed 31 isotopic proxy to reconstruct paleo-coral bleaching events. Based on a 32 literature review of published δ^{11} B data and our recorded "vital effect" of coral 33 bleaching on the δ^{11} B signal, we also describe at least two possible coral 34 bleaching events since the Last Glacial Maximum. The implementation of this 35 bleaching proxy holds the potential of identifying occurrences of coral 36 bleaching throughout the geological record. A deeper temporal view of coral 37 bleaching will enable scientists to determine if it occurred in the past during 38 times of environmental change and what outcome it may have had on coral 39 population structure. Understanding the frequency of bleaching events is also 40 critical for determining the relationship between natural and anthropogenic 41 causes of these events. 42

1 Introduction

1.1 Coral bleaching

Coral bleaching occurs when environmental stress, primarily increased 46 water temperature and high irradiance (but also decreased temperature, 47 decreased salinity and pathogenic infections), induces breakdown of the 48 coral-algae symbiosis and the host initiates algae expulsion (Brown, 49 1997). As a result, corals either temporarily lose their pigmentation or die 50 from lack of nourishment from the algae. Bleaching is, therefore, 51 considered an acute risk to the health of coral reefs, although some 52 studies suggest it is an adaptive response to environmental change 53 (Fautin and Buddemeier, 2004). 54

Coral bleaching was first reported in the early 20th century (Yonge et al., 55 1931) and heavily documented since 1979, as widespread bleaching 56 events have been recorded with increased frequency and severity 57 (Hoegh-Guldberg, 1999). By correlating isotopic dating of massive corals 58 and concomitant sea surface temperatures (SSTs), it was speculated 59

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that coral bleaching may have already caused mortality events by the60end of the 19th century (Yu et al., 2006). While bleaching events have61been proposed to occur throughout the geological record (suggested for62symbiotic foraminifera during unstable SST in the upper Eocene (Wade63et al., 2008)), there has been no experimentally established proxy to64examine suspected paleo-coral bleaching.65

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1.2 Paleo bleaching proxy

Previous attempts at establishing a paleo-proxy for coral bleaching 68 focused on carbon and oxygen isotopes (Heikoop et al., 2000; Suzuki et 69 al., 2003) as well as skeletal ultraviolet fluorescence (Suzuki et al., 70 2003). However the balance between kinetic and metabolic processes, 71 affected by factors such as coral growth rate and local light environment 72 (i.e. shaded vs. unshaded side of a colony), complicates the use of 73 these proxies (Suzuki et al., 2003). A correction method, suggested by 74 Heikoop et al. (2000) to differentiate kinetic from metabolic effects, was 75 recently refuted for the case of coral bleaching (Schoepf et al., 2014a). 76 Boron isotopes, currently used for estimating pH_{sw}, may act as an 77 effective paleo-bleaching proxy, as kinetic effects apparently do not 78 interfere with isotopic equilibrium during calcification (Zeebe et al., 79 2001). 80

Considerable research has been focused on paleo-techniques for 81 estimating pH_{sw} and pCO_{2 atm} as current atmospheric CO₂ values of ~400 82 parts per million by volume (ppmv) are at the highest levels of the past 83 million year timeframe (Caldeira and Wickett, 2003; Hoegh-Guldberg et 84 al., 2007; Hönisch et al., 2012). The wide variety of proxies developed 85 for deep time (extended to the Paleozoic era) paleo CO2 reconstruction 86 includes fossil plant stomata, stable carbon isotopes in paleosols and 87 Bryophytes, and marine alkenones (reviewed for the Cretaceous time 88 frame by Wang et al. (2014)) At time scales shorter than one million 89 90 vears, paleo-CO₂ 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) 91 and boron isotopic composition ($\delta^{11}B$) from carbonate skeletons of 92 foraminifera (Foster, 2008; Hönisch and Hemming, 2005; Hönisch et al., 93 2009; Palmer et al., 2010; Palmer and Pearson, 2003; Sanyal et al., 94 1995) and corals (Douville et al., 2010; Gaillardet and Allegre, 1995; Liu 95 et al., 2009; Pelejero et al., 2005; Shinjo et al., 2013; Wei et al., 2009). 96 Skeletal δ^{11} B values are used for paleo pH reconstruction as they relate 97 to ambient waters' pH. This relationship is based on the assumptions 98 (1) that internal pH (i.e. where calcification takes place) is associated 99 with ambient pH, and (2) that in seawater only the charged B species 100 (borate) with lower $\delta^{11}B$ values, is incorporated into corals. The B 101 species distribution and the δ^{11} B of the B species are pH dependent. If 102 borate represents the only source of B in the skeletons of corals, than its 103 δ^{11} B is equal to the δ^{11} B of the coral. Therefore, the δ^{11} B of corals is 104 utilized as a proxy of ocean pH levels at the time of calcification. 105

It is important to bear in mind that the internal pH often differs from 106 ambient pH as the combined result of geochemistry and physiology, thus 107 changing the $\delta^{11}B_{coral}$ - $\delta^{11}B_{sw}$ relationship. Environmental factors that 108 may affect coral's internal pH include food supply, light intensity, depth 109 or temperature elevation (e.g. Dissard et al., 2012; Hönisch et al., 2004; 110 Reynaud et al., 2004). However, for zooxanthellate corals, the most 111 prominent process raising the coral's internal pH, is photosynthesis 112 carried by its' algal symbionts (Laurent et al., 2013; Venn et al., 2011; 113 Venn et al., 2013). Thus, high photosynthesis rates will lead to elevated 114 internal pH and therefore higher $\delta^{11}B$. This photosynthesis-pH- $\delta^{11}B$ 115 interrelation was noted both for symbiont-bearing foraminifera (Hoenisch 116 et al., 2003), and corals (Hemming et al., 1998). 117

Thermally bleached corals however show diminished symbiont 118 photosynthesis (Glynn, 1996) causing a concomitant drop in pH 119 measured at the corals diffusive boundary layer (DBL) (Al-Horani, 2005). 120 While the interaction of coral biology with its internal pH (known as vital 121 effects) has been previously quantified as ca. 0.3-0.6 pH units elevation 122

in a "healthy" state (McCulloch et al., 2012; Trotter et al., 2011), the 123 change in physiology due to coral bleaching is hypothesized to push pH 124 difference beyond the natural vital effect, decoupling internal and 125 ambient pH, skewing $\delta^{11}B$ derived pH_{sw} estimations (e.g. pH_{sw} during 126 1998 mass bleaching from Wei et al. (2009)), and by that, enabling the 127 use of δ^{11} B as a proxy for paleo-bleaching. This approach was recently 128 challenged by Schoepf et al. (2014b) who reported no difference in $\delta^{11}B$ 129 signal imprinted in the skeletons of corals that experienced short term 130 (up to six weeks) bleaching event. We hypothesize however, that longer 131 bleaching events, and higher resolution sampling techniques, may allow 132 the detection of the boron-bleaching signature in coral skeletons. 133

In this study, we examined the signature of coral bleaching manifested in 134 the δ^{11} B values of coral skeletons using experimentally and naturally 135 bleached specimens. Through the use of laser ablation, high-resolution 136 sampling of the coral skeleton deposited while in a bleached state, we 137 quantify a boron bleaching signature. We applied this proxy to published 138 coral δ^{11} B records to investigate the occurrence of coral bleaching since 139 the Last Glacial Maximum. 140

2 Materials and methods

2.1 Experimentally induced *Porites* sp. bleaching: 142

2.1.1 Coral Culturing: Two adjacent Porites sp. colonies were collected at 143 10 meters depth in the Gulf of Aqaba, in the northern Red Sea 144 (29°30'06" N, 34°55'00" E). The coral was cut into 2 X 2 cm 'nubbins' 145 and left to recover in controlled laboratory conditions (23° C, pH of 8.17 146 and salinity of 40.7 psu) for three weeks prior to starting the 147 experiment. Nubbins were separated into two groups - "control" and 148 "heat shock" - and maintained for eight months (November (2008) to 149 June (2009)) in open-system water tables with an input flow rate of 150 ~2.0 L/min and constant light of an average of 150 µmol photons m⁻² s⁻ 151 ¹. For the control corals temperature varied between 22-24°C, 152 reflecting seasonal temperature changes in the Gulf of Agaba, while 153

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heat shock treatment temperatures were controlled with a thermostat to 154 induce bleaching (30-32°C; Jan-March) and recovery (23-25°C; April-155 June). Temperature fluctuations are outlined for both control and heat 156 shock treatments in the Supporting information (Table S1). Since the 157 experiment was carried in open system, (i.e. with direct connection with 158 the sea) pH and salinity levels remained in the natural range for the 159 Gulf of Aqaba, 8.20 \pm 0.01 (SD) pH units and 40.60 \pm 0.08 (SD) ppt 160 respectively throughout the experiment. Nubbins were stained at the 161 beginning of the experiment with Alizarin Red S to identify the start of 162 newly deposited skeleton during the experimental period. 163

Fv/Fm values were measured approximately every two days for the 164 first three weeks of the experiment until bleaching was seen. Twelve 165 dark-adapted samples were measured for each treatment using the 166 single turnover flash of over 10,000 μ E on a fast repetition rate 167 fluorometer (FRRF, FIRe system, Satlantic, Halifax, Nova Scotia, 168 Canada).

Oxygen and pH were measured with microelectrodes in the diffusive 170 boundary layer (DBL) of a healthy and bleached coral during the 171 bleached period (Supporting information, Fig. S2). At the end of the 172 experiment, the tissue of all corals was removed using a high pressure 173 airbrush. 174

2.2 Natural occurring Stylophora pistillata bleaching: 175

A Stylophora pistillata fragment was taken from the coral documented by 176 Nir et al.(2014) to undergo bleaching during summer 2010. The coral 177 skeleton was sliced, cleaned and measured for δ ¹¹B using the 178 procedures described in the next section. After LA-MC-ICP-MS 179 measurements, coral slices were polished to a thickness of ~0.5 mm on 180 a microscope slide and photographed through a light microscope. 181 Density bands (annual growth bands) were analyzed as gray intensity of 182 pixels along the measurements path using ImageJ software. 183

2.3 δ^{11} B analysis: For boron analysis, the coral skeletons were sliced and 184 grinded to flatten the surface and then immersed in an ultra-sonicated 185 Milli-Q water bath for 5 minutes to remove any particles. Sodium 186 Hypochlorite (NaClO) was used to remove any organics and finally 187 samples were washed with Milli-Q water and Acetone (full cleaning 188 procedure is detailed in the supplementary information). In this study we 189 used laser ablation for the determination of $\delta^{11}B$ instead of bulk analysis 190 of a dissolved material which requires wet chemistry and rigorous 191 cleaning steps. Laser ablation was applied on the grinded, cleaned and 192 bleached surfaces of the sliced skeletons. From the experimentally 193 bleached Porites sp. corals, a total of six coral nubbins were analyzed 194 for $\delta^{11}B$ using UV femtosecond LA-MC-ICP-MS, two from the control 195 treatment and four from the bleaching treatment. From the naturally 196 bleached S. pistillata coral we used one branch. By using a femtosecond 197 (fs) laser ablation system we avoided issues such as thermal 198 fractionation and matrix effects that can occur using nanosecond lasers 199 when using glass standards for carbonate samples (Fietzke et al., 2010; 200 Horn and von Blanckenburg, 2007). Furthermore, the fs-laser ablation 201 technique (ultra-short ~200 fs pulses) allows for fast and accurate 202 determination of the isotopes in the coral skeleton with minimal melting 203 204 of the material caused by heat transfer.

 δ^{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 208 was less than 0.30‰ (2 σ). 209

2.4 pH calculation based on Boron isotopic composition

Since temperature variation in the experiment was high (23-32 °C), we 211 had to consider the chemical effect of temperature on $\delta^{11}B$ of borate 212 which accompanies the physiological effect of symbiont expulsion. In 213 order to compensate for this effect, pH levels were calculated based on 214 $\delta^{11}B$ values from the *Porites sp.* experiment using the equation: 215

$$pH = pK_B - log\left(-\frac{\delta^{11}B_{sw} - \delta^{11}B_{coral}}{\delta^{11}B_{sw} - \alpha_B \times \delta^{11}B_{coral} - (\alpha_B - 1) \times 10^3}\right)$$
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where the isotopic composition of sea water ($\delta^{11}B_{sw}$) is 39.5‰, α_B is the 217 fractionation factor ($^{11-10}K_B$) between B(OH)₄⁻ and B(OH)₃ of 1.0272 218 (Klochko et al., 2006) and the equilibrium constant pK_B was calculated 219 for each data point with its coincident temperature and salinity following 220 Dickson (1990) (Table S1). This pH calculation takes into account the 221 effect of temperature on $\delta^{11}B_{Borate}$. 222

Seeing $\delta^{11}B$ as a pH indicator, we consider these pH values as the estimated 223 pH at the site of calcification and present the difference of a pH value from the 224 normal (at point 0) as ($\Delta pH_i = pH_0 - pH_i$) (Fig. 1). 225

2.5 Previously published δ^{11} B records examination:

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Following our experimental results that showed a distinctive bleaching 227 signature in coral's δ^{11} B values, we examined previously published coral 228 and foraminifera's δ^{11} B records for δ^{11} B drops resembling the bleaching 229 signature evidenced in our experiment. Our search was focused on the 230 time frame of present day to the penultimate deglaciation (~125 kyr BP), 231 when global temperatures were comparable to present day values 232 (Schmidt et al., 2006). $\delta^{11}B$ and calculated pH values, as well as SST 233 and atmospheric CO₂ data, were retrieved from relevant publications' 234 tabulated data if available. Data of Gaillardet and Allegre (1995), which 235 were not available, were recovered from graphics using GETDATA 236 graph digitizer (http://getdata-graph-digitizer.com/). SST data for the 237 open sea as close as possible to Arlington Reef (Lat. 17° S, Lon. 148° E, 238 GBR) taken from NOAA ERSST-3b 239 was database (http://nomads.ncdc.noaa.gov/las/getUI.do) monthly reconstruction 240 (Smith et al., 2008). Meta data for the reviewed coral records are 241 presented in the supporting information (table S2). Relative $\delta^{11}B$ drops 242 $(\Delta \delta^{11}B)$ in published $\delta^{11}B$ records were calculated as the difference 243 between a specific δ^{11} B value and the average δ^{11} B of the whole data 244 series in which it belongs. For the comparison of experimental results 245 and previous (low resolution) records, the effect of sampling resolution 246 was estimated and corrected by averaging δ^{11} B throughout the whole 247 experimental time series (Fig. 1). 248

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3 Results and discussion

3.1 Experimental bleaching

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Oxygen and pH micro-sensor analysis in the DBL of a bleached massive 252 Porites sp. revealed a decrease in pH down to 7.85 (0.32 pH units below 253 ambient pH values) during light conditions with no increase in oxygen, 254 as opposed to a healthy coral which exhibited an increase in oxygen 255 production and a re-alkalization of the DBL under the same light 256 conditions following a period of dark incubation (Supporting information, 257 Fig S2). These measurements serve as validation of an already 258 quantified phenomenon of reduced pH in the DBL as a result of 259 bleaching (Al-Horani, 2005). While corals are known to elevate the pH at 260 the site of calcification relative to ambient seawater (Venn et al., 2011), 261 reduced seawater pH resulting from the chemical dissolution of CO₂ has 262 been shown to reduce the calcifying medium pH, overwhelming up-263 regulatory compensation (Venn et al., 2013). Theoretically, the same 264 would hold true for biological reductions of pH in the DBL evident in 265 micro-sensor measurements. 266

Corals' responses to the heat stress conditions in the experimentally 267 induced bleaching experiment varied individually (Fig. S1), where two 268 nubbins showed no apparent bleaching while the other two were either 269 partially or fully bleached. Fully and partially thermally-induced 270 bleached corals displayed a $\delta^{11}B$ drop ($\Delta\delta^{11}B$) of 2.2-3.7% relative to 271 their recovered outer rim values, descending to 21.5‰, corresponds to a 272 pH drop (Δ pH) of 0.29 units (Fig. 1). The decrease of ca. 0.3 pH units as 273 a result of diminished photosynthesis coincides with previous 274 measurements of internal and DBL pH in light-dark or bleaching 275

manipulations (Al-Horani, 2005; Venn et al., 2011). This pH drop is also 276 corrected for temperature effect on $\delta^{11}B_{Borate}$ (detailed in section 2.4) 277 and therefore should be considered as the result of physiological change 278 (i.e. coral bleaching). Due to the high resolution of sampling (~45 µm 279 equals about two-three weeks of skeletal deposition) we were able to 280 record low δ^{11} B values at the time of bleaching. In order to demonstrate 281 the effect of sampling resolution and to allow comparison of our results 282 with some lower resolution studies, we calculated the average values for 283 the whole experiment (~7 months) consisting of unbleached acclimation, 284 heat stress driven bleaching and recovery. In this case, partially and fully 285 bleached corals showed $\delta^{11}B$ drop of -1.55 ‰ and -2.34 286 ‰ (respectively, Fig. 1). Interestingly, heat stressed corals showed a $\delta^{11}B$ 287 drop of -0.78 ‰ even though not apparently bleached. This finding 288 contradicts previous reports of increased $\delta^{11}B$ in elevated temperatures 289 (Dissard et al., 2012) implying a distinctive difference between the effect 290 of "normal" high temperatures within the natural habitat's range 291 increasing photosynthesis and calcification rates, and the effect of heat-292 stress-temperature conditions depressing coral physiology. 293

3.2 Natural bleaching

A mesophotic (60m depth) S. pistillata coral documented to go through a 295 seasonal summer bleaching (Nir et al., 2014) showed a $\delta^{11}B$ drop of 296 $\Delta \delta^{11}B$ = -5.1‰ during bleached period (Fig. 2). The "bleached" low $\delta^{11}B$ 297 value falls on the value calculated with boron isotope equilibrium 298 constant (11-10KB) of Klochko et al. (2006) (not accounted for vital effect) 299 while the "healthy" high $\delta^{11}B$ value coincides with the normal value 300 measured for this species at pH 8.2 (with symbionts) (Krief et al., 2010). 301 However, the high magnitude of $\delta^{11}B$ change found for the bleached 302 period is somewhat surprising due to the fact that baseline levels of 303 productivity were documented to be low for this mesophotic coral (Nir et 304 al., 2014). This result may point to other pH up-regulation processes, 305 besides photosynthesis, that are also weakened during bleaching. 306

In contrast to our findings, recent explorations of boron isotopes in 307 bleached corals found no effect of short-term bleaching on the skeletal 308 boron signal (Schoepf et al., 2014b). Our study may have been able to 309 capture the depletion in $\delta^{11}B$ due to (1) the use of high resolution 310 sampling across the coral growth axis and (2) the relatively long 311 bleached period, allowing a coral to precipitate "new" aragonite 312 encompassing the boron signal of bleaching. This allows for an intricate 313 time series comparison within the same individuals enabling the 314 detection of relative changes in the skeletal boron isotopes at multiple 315 time points before, during and after a bleaching event sustained over 316 approximately three months. The duration of natural coral bleaching 317 events' varies in time scale, from a few days' to prolonged periods over 318 several months or seasons. The boron bleaching signature would likely 319 not be recorded in the case of short durations of bleaching, simply 320 because not enough aragonite will have precipitated to allow analysis. 321 However, the depletion of $\delta^{11}B$ during bleaching may be captured for 322 longer and more pronounced bleaching events or by using fine scale 323 measurements (e.g. laser ablation) as evidenced in our study. It is 324 noteworthy that searching for paleo bleaching event using the boron 325 isotope signature will only allow identification of major events (which are 326 probably of higher environmental significance) rather than short, 327 anecdotal events. Furthermore, the high resolution sampling implied 328 here, which might seem irrelevant for paleo-climate studies (e.g. pH 329 reconstruction over glacial cycles), is shown to be of crucial importance 330 when looking for biological phenomena. 331

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3.3 Establishment of the δ^{11} B bleaching proxy

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Our experimental results showed decreased pH at the DBL 334 accompanied by a sharp δ^{11} B drop for bleached coral held at constant 335 ambient pH_{sw}. A field 'ground truth' for this boron bleaching signature 336 was found in a naturally bleached mesophotic (60m depth) *S. pistillata* 337

coral. Further evidence that δ^{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 δ^{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 pCO2 atm records to provide 355 support for our conclusions (Fig. 3). In cases where a drastic drop in 356 δ^{11} B is associated with high SST or severe warming rather than pCO₂ 357 increase, we propose that a thermal stress may have induced bleaching 358 and resulted in the low δ^{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 δ^{11} B signature from the period of 1940-369 2000. This does not rule out bleaching in the region. In fact, slight 370 depressions in the δ^{11} B values (-0.5 > $\Delta\delta^{11}$ B > -1 ‰) coincide with El-371 Nino years and may indicate physiological heat stress of the coral. 372 Alternatively, when the boron bleaching signature is evident in fossil 373 coral core samples, as outlined in the coming examples, based on our 374 experimental findings it may indicate that coral bleaching has occurred. 375

3.4 Coral bleaching events recorded since the Last Glacial Maximum 377

Over the past 800,000 years of glacial/interglacial cycles, atmospheric 378 CO₂ oscillated between 180-280 ppmv, with an increase of less than 30 379 ppmv from the Holocene until the beginning of the industrial revolution 380 (~1860). Ocean surface's pH paleo-records (based on coral's $\delta^{11}B$ 381 measurements) exhibit higher magnitude variations in comparison to 382 atmospheric pCO₂ (Fig. 3). These shifts have been interpreted as major 383 oceanographic events such as alterations of ocean circulation stemming 384 from upwelling of cold, low pH waters to the surface (Douville et al., 385 2010; Liu et al., 2009), decoupling the tight relation of pCO_{2 atm} and pH_{sw} 386 on a regional scale. However, following the results presented in this 387 paper, we suggest an alternative explanation for the pCO_{2 atm} and pH_{sw} 388 decoupling, where a severe drop of the coral δ^{11} B values is associated 389 with high or rapidly increasing SST rather than an increasing pCO_{2 atm}, 390 391 this drop may be the geochemical signature of coral bleaching and not of a pH_{sw} decline. 392

We inspected published paleo-pH records from coral skeletons through 393 the Eemian interglacial stage (~130 kyr BP) and identified six suspected 394 data points (Fig. 3 - red vertical lines) that meet our boron bleaching 395 signature definition of a depletion in $\delta^{11}B$ values greater than 1.5% 396 relative to the average values measured in that series (Fig 3- dotted 397 line). Supported with concomitant paleo climate records, we make a 398 case for these instances that represent potential bleaching events rather 399 400 than an actual ocean acidification.

3.4.1 20th **century, GBR:** Pelejero et al. (2005) measured a 280-year δ^{11} B 401 record of *Porites* sp. and reported the lowest δ^{11} B value for the last 402 three centuries at Flinders Reef, GBR, ca. 1988 (using 5-year 403 averages) (22.99‰, 7.91 pH units) with an overall trend of low pH 404 values associated with positive values of the Interdecadal Pacific 405 Oscillation (IPO) index (El Niño like conditions), interpreted to result in 406 low rates of water exchange at the Flinders Reef's lagoon. However, 407

high-resolution inspection of 1987-1988 reveals that the lowest $\delta^{11}B$ 408 value was recorded during the warmest month (February 1988, $\delta^{11}B =$ 409 22.26‰, stands 1.65‰ below the multi annual average) even though 410 wind speed, normally linked to a high lagoonal water exchange, was 411 high that month. Since this low $\delta^{11}B$ value also coincides with warm 412 SST and the 1988 worldwide mass bleaching (Williams and Bunkley-413 Williams, 1990), it is likely that the reported low $\delta^{11}B$ value is due to 414 heat stress or coral bleaching. This value was preceded by a period of 415 lower values that commenced at the transition from cooler to warmer 416 SST during the austral summer of 1987 and could indicate a sustained 417 bleaching period, such as was seen in the $\delta^{11}B$ values of our 418 experimentally bleached coral skeletons. 419

Additional low δ^{11} B values (δ^{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. 425

3.4.2 6 Kyr BP, SCS: Liu et al. (2009) measured *Porites* sp. δ^{11} B from the 426 South China Sea (SCS) and reported a $\delta^{11}B$ drop, at ~6 kyr BP 427 $(\Delta \delta^{11}B = -1.82\%)$ interpreted by the authors as low pH during the 428 Holocene thermal optimum (~5-9 kyr BP). This period was suggested 429 to be characterized by an intensified summer monsoon that resulted in 430 strengthened upwelling and introduced low pH deep water to the 431 surface of SCS. However, no sign of cold deep water upwelling is 432 evidenced in the reported SST values (Yu et al., 2005). Furthermore, 433 Mg/Ca derived SST records related to SCS upper waters (0-30m) 434 temperature show a peak in SST around 6 kyr BP (Steinke et al., 2008) 435 ascending to 29°C. Translating pH values to pCO₂ (using CO2sys 436 software (Pierrot and Wallace, 2006)) shows that although Holocene 437 pCO_{2 atm} was relatively stable (260-290 ppmv, not including the 20th 438 century), reported pH values are equivalent to much higher pCO₂ 439 levels, up to 571 ppmv at 6 kyr BP. This drop of δ^{11} B (at 6 kyr BP) 440 corresponds better with high SST, rather than a drastic increase in 441 pCO₂ or upwelling introducing low pH water to the surface. Therefore, 442 we propose that the low δ^{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 (Δ SST), 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, Δ SST 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 equatorial Pacific. This offers an alternative explanation for the low 473 $\delta^{11}B$ values obtained for corals, rather than sustained La-Nina like 474conditions. 475

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4 Conclusions

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Coral bleaching exerts a considerable signature on $\delta^{11}B$ measurements 478 taken from the scleractinian corals S. pistilata and massive Porites sp. (Δ 479 $\delta^{11}B = 1.5-5.1\%$). Employing high-resolution femtosecond LA-MC-ICP-480 MS, we provide evidence that $\delta^{11}B$ is sensitive, and highly influenced by 481 the physiological interactions of the photosymbiont and the calcifying 482 organism (i.e., a drop in photosynthetic activity of photosymbionts during 483 bleaching events). Overall, our experimental results, along with previously 484 published data, highlight the challenges of utilizing $\delta^{11}B$ as a straight 485 forward proxy for paleo-pH_{sw}. To gain an accurate interpretation of $\delta^{11}B$ 486 values, the bleaching state of the organism from which the data is derived 487 must be considered. On the other hand, when supported with concurrent 488 pCO_{2 atm}, foraminifers' pH and SST records, this proxy opens the door to 489 investigations that utilize δ^{11} B as a paleo-bleaching marker. 490

491 While the understanding of temporal dynamics of when/how these events are recorded in coral skeletons, still requires examination of additional 492 modern known bleaching events, our findings provide evidence that coral 493 bleaching may not be an exclusively modern phenomenon and we have 494 identified at least two instances since the LGM (~20 kyr BP) prior to the 495 industrial revolution where coral bleaching likely occurred. If short-term 496 bleaching is indeed untraceable with $\delta^{11}B$ measurements (Schoepf et al., 497 2014b), then the suspected paleo-bleaching events may be a result of 498 sustained bleaching events, possibly comparable with 499 longer contemporary worrisome mass bleaching episodes. 500

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Acknowledgements

The experimental work for this paper was conducted as part of J.F.'s M.Sc. 504 thesis while at Hebrew University in Jerusalem, Israel. The authors would like 505 to acknowledge Klaus-Uwe Richter for all of his analytical support. D.T. and 506 J.B. would like to acknowledge financial support from BMBF provided for the 507 project "Reconstruction of past bleaching events using a high resolution 508 microanalysis of δ11B" in the framework of the German Israeli collaboration 509 (03V0956). D.F.G. would like to acknowledge funding from the National 510 Science Foundation grant #0920572. G.D would like to acknowledge funding 511 from SBM and Minerva foundation. The authors would also like to 512 acknowledge the Israel National Monitoring Program for the long-term pH and 513 sea surface temperature data in the Gulf of Aqaba. We thank the two 514 anonymous referees who helped improve this manuscript. 515

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Figure legends:

Fig. 1. δ^{11} B measurements of coral skeletons following different bleaching 729 situations. (a) Dark green symbols represent control coral measurements, 730 yellow symbols represent heat stressed corals, while bright green and white 731 symbols represent partially and fully bleached corals respectively. Values of 732 $\delta^{11}B = [^{11}B/^{10}B]_{sample}/[^{11}B/^{10}B]_{NIST 610 \text{ standard}} -1 X 1000. Values are given in parts$ 733 per mil (‰).standard error is within the size of symbols (≤ 0.30 ‰, 2 σ). Dark 734 and bright green triangles, white diamonds and yellow triangles data series 735 were subtracted with -2‰ to compensate for a change in instrumental setup 736 (detailed in SOM). 737

(b) Relative change from outer rim value for each sample ($\Delta \delta^{11}$ B). All rim 738 values (at 0 μ m) were equaled to zero and the change in δ^{11} B values was 739 plotted for each transect along the growth axis. Positive values equal an 740 enrichment in values while negative values are depleted relative to the rim 741 value. Dashed lines and above written values represent the average $\Delta \delta^{11}$ B for 742 a coral bleaching state. Coral's appearance during the heated stage of the 743 experiment is illustrated by the nubbins pictures on the right side. 744

(c) Relative pH change from outer rim value for each sample (ΔpH). pH 745 values are calculated from $\delta^{11}B$ using temperature corrected pK_B in order to 746 compensate for the temperature effect on $\delta^{11}B_{\text{Borate}}$. 747

Fig. 2. (a) δ^{11} B measurements of mesophotic (60m) Stylophora pistillata coral 749 documented to undergo bleaching during summer 2010(Nir et al., 2014). (b) 750 Skeleton density, reported as the gray value of pixels along the ablation 751 transect. Yellow shaded area represents the low density skeleton band 752 deposited in summer 2010. Solid and dashed gray lines represent the 753 expected δ^{11} B value for pH_{sw}=8.2 calculated with boron isotope equilibrium 754 constant (^{11–10}K_B) of Klochko et al.(2006) (not accounted for vital effect) and 755 an empirical coefficient specific to this species (with symbionts) by Krief et al. 756 (2010) respectively. 757

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Fig. 3. 25,000 year records of paleo-ocean climate and pH (a-d) and boron 759 isotopic compositions in coral skeletons (e-h). 760 Blue lines represent atmospheric pCO₂ measured in gas trapped in ice cores 761 (Monnin et al., 2001; Monnin et al., 2004; Petit et al., 1999; Robertson et al., 762 2001) and direct measurements (Tans, 2012), open symbols represent SST 763 records (triangles - South China Sea(Steinke et al., 2008), circles - Western 764 Caribbean sea (Schmidt et al., 2006) and crosses - modern tropics (Indian 765 and Pacific oceans) (Wilson et al., 2006)). 766

 δ^{11} B derived pH from foraminifera (ocean surface in gray symbols-767 circles(Foster, 2008), inverted triangles (Palmer and Pearson, 2003), 768 rectangles (Hönisch et al., 2009), diamonds (Palmer et al., 2010) and 769 triangles (Hönisch and Hemming, 2005); Deep waters pH in blue symbols (Yu 770 et al., 2010)) and coral (red symbols - circles (Douville et al., 2010), inverted 771 triangles (Liu et al., 2009) and rectangles (Gaillardet and Allegre, 1995); grey 772 (Pelejero et al., 2005) blue (Shinjo et al., 2013) and black (Wei et al., 2009) 773 lines) is shown in (c-d). The boron isotopic compositions, on which coral pH is 774 based, are presented in (e-f). The difference of δ^{11} B from the average value in 775 record ($\Delta \delta^{11}$ B) is shown in (q-h) together with a dotted line under which data 776 points are suggested to represent coral bleaching. Green bars and line within 777 represent δ^{11} B annual range and average measured at unbleached coral 778 (Hemming et al., 1998). Red vertical lines mark the timing of suspected 779 bleaching events. Grey and yellow shaded bars represent cold and warm 780 periods respectively. 781

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Figure 2

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Figure 3