

## ***Interactive comment on “A novel paleo-bleaching proxy using boron isotopes and high-resolution laser ablation to reconstruct coral bleaching events” by G. Dishon et al.***

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### **Answer to V. Schoepf’s short comment:**

We thank V. Schoepf for these comments, especially since our results contradict Schoepf et al. (2014).

Here we address the comments:

- **Q: Do they suggest that decreases in pH in the diffuse boundary layer are reflected by d<sup>11</sup>B?**

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- **A:** Yes. Although we assume that changes in the diffuse boundary layer are likely accompanied by changes in the internal pH of the coral tissue which will, in turn, effect the pH of the calcifying fluid and thus the d<sup>11</sup>B of the skeleton during calcification.

- **Q: If so, how would that be possible given that d<sup>11</sup>B does not record seawater pH directly, but rather the increased internal pH of the calcifying fluid?**

- **A:** The empirical calibrations between seawater pH and d<sup>11</sup>B (e.g. (Honisch et al., 2004)) demonstrate the correlation between these parameters. Additionally, due to the loss of photosynthesis and the resulting unidirectional change in pH (i.e. respiration) the seawater drawn into the calicoblastic layer being used for calcification would be of a lower pH, again explaining the depleted d<sup>11</sup>B values recorded during bleaching.

- **Q: What about the hypothesis that pH-upregulation may be compromised by coral bleaching?**

- **A:** That is still a valid hypothesis. Our experiments show that d<sup>11</sup>B drops significantly upon bleaching, either caused by the loss of symbionts or by a compromised pH-upregulation. In both cases it would be indicative of coral bleaching

- **Q: it seems unfortunate that measurements of Fv/Fm were only conducted until bleaching was seen (defined as what kind of Fv/Fm values?). Since the authors also measured d<sup>11</sup>B during the recovery phase, continuous Fv/Fm measurements (or other physiological measurements such as chlorophyll a, symbiont density and calcification) would have helped to link the various observed bleaching states (i.e., the severity of bleaching) to a certain decrease in d<sup>11</sup>B.**

- **A:** We are aware of some of the shortcomings in our experiment, such as the lack of Fv-Fm and physiology measurements during the bleaching period. However,

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even without these measurements, our results can be summarized and still hold as "apparently bleached corals showed relative decrease of more than 1.5 permil in  $d^{11}\text{B}$ ", which tells the story even if we cannot quantify bleaching stages.

- Q: ". . . .bleaching in this mesophotic coral is highly unusual in that the coral paradoxically seems to perform better in a bleached state. Can the authors elaborate on a potential mechanistic link for this coral and the implications for the Porites coral?"

• A: As can be seen in (Nir et al., 2014), these mesophotic corals photosynthesized at higher rates when not bleached. However, when considering the generally low productivity rates, the strong  $d^{11}\text{B}$  drop evidenced is indeed surprising. We suggest that in this case the link may be through bleaching induced disruption of pH up-regulation mechanisms other than photosynthesis. This suggestion is now added to the revised manuscript.

- Q: One major concern is that the one control coral shown in Fig. 1a had unusually high  $d^{11}\text{B}$  values (up to 28 per mil). What about  $d^{11}\text{B}$  values of the other measured corals from the control treatment? Were they also that high? Clearly, such high values for the control corals would make it easier to define decreases in  $d^{11}\text{B}$  as a "bleaching footprint". Further, the authors state that decreases in  $d^{11}\text{B}$  of as little as 1.5 permil may be interpreted as a bleaching footprint, yet offsets between analytical sessions required a correction of 2 permil for some of their data.

• A: The definition of the boron bleaching signature in our experiment is based only on  $d^{11}\text{B}$  values relative to the "healthy" period  $d^{11}\text{B}$  of the same coral ( $d^{11}\text{B}$ ). Thus, even though we cannot fully explain the high absolute values for this control coral, this is not interfering with the  $d^{11}\text{B}$  values. The same is true for the offsets between analytical sessions.

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- Q: The authors state that in contrast to short-term coral bleaching (Schoepf et al. 2014b), longer bleaching events such as the one simulated in their experiment will be recorded by  $d^{11}\text{B}$ . Although the heat stress phase in Schoepf et al. 2014b only lasted 2.5 weeks, the corals were nevertheless physiologically bleached for much longer, yet changes in  $d^{11}\text{B}$  were not observed even 1.5 months after the heat stress phase. Can the authors elaborate on this discrepancy between the two studies, especially since two of the study species in Schoepf et al. 2014b were also Porites corals?

- A: In their study, Schoepf et al. used three species of coral that were experimentally bleached and then left to recover on the natural reef. Some of these corals remained in a bleached state for up to six weeks and did not show a significant drop in  $d^{11}\text{B}$ . Although this indeed is not a very short bleaching event, it was less prolonged than the bleaching experienced by our corals (ca. three months).

Calcification rates measured by Schoepf et al. during the experiment were quite low, at the range of  $0.5\text{-}1 \text{ mg cm}^{-2} \text{ day}^{-1}$ . We roughly estimate that these rates of calcification result in extension rates of up to  $0.5 \text{ mm year}^{-1}$ , which equals ca. 50  $\mu\text{m}$  for 6 week period. Hence, the sampling procedure in which 250-500  $\mu\text{m}$  of skeleton are "shaved" for boron isotopic analyses (as used by Schoepf et al.), will yield an average  $d^{11}\text{B}$  signal covering ca. one year (out of which only six weeks are in a bleached state).

In our paper, *we show the decrease in  $d^{11}\text{B}$  of experimentally and naturally bleached corals measured with high resolution laser ablation*. This high resolution (<45  $\mu\text{m}$ ) allows the measurement of skeleton material corresponding to several weeks of calcification, which fits the time scale of bleaching events.

We faced this sampling resolution issue when scanning published  $d^{11}\text{B}$  records for suspected bleaching events. By averaging over a year, we define a bleaching signature as one where the boron isotopic composition drops by more than 1.5 ?. When using a gross sampling resolution (250-500  $\mu\text{m}$ , which is common for paleo-pH reconstruc-

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tions), bleaching periods of ca. 6 weeks will be averaged and masked in the  $d^{11}\text{B}$  records (Schoepf et al., 2014). This composite record may still be okay when using  $d^{11}\text{B}$  as a paleo pH proxy, but is not sufficient if one is to use  $d^{11}\text{B}$  values as a paleo bleaching indicator. This highlights two important points- (1) that the  $d^{11}\text{B}$  drops identified in our paper may represent severe and possibly ecological important bleaching events, and (2) that the use of high resolution laser ablation allows to monitor even short term bleaching events.

Thus, when recommending a method for paleo-bleaching reconstruction, we suggest the implementation of high resolution laser ablation. We encourage Schoepf et al. to conduct such measurements on their bleached specimens looking for the six weeks bleaching periods experienced by their corals.

Furthermore, we propose that more pronounced events may be noticeable even without high resolution sampling (this was also noted in Schoepf et al. (2014) discussion), and can explain the  $d^{11}\text{B}$  troughs in previous publications (Douville et al., 2010; Liu et al., 2009; Pelejero et al., 2005; Wei et al., 2009). We believe that these  $d^{11}\text{B}$  drops mark severe and possibly ecological important bleaching events.

- **Q: Could the authors clarify the sample size for each treatment? The numbers differ between the Supplement and the main text and are also not consistent with the number of tracks shown in Figure 1.**
- A: Many more samples were subject to the treatments than were possible to measure for  $d^{11}\text{B}$  on the mass spectrometer. Due to the fact that only a few samples could fit into a chamber at a time and that multiple transects were run on each sample we were limited by how many samples we could run for the day. Additionally, individuals from both the bleached and control corals were run on both days of sampling to verify the relative changes in the  $d^{11}\text{B}$  signal despite apparent offsets in the absolute values that resulted from changes to the machine. While the numbers do vary for each part (number exposed, number cleaned, number

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analyzed), they reflect the actual number of coral samples that were used in each step.

- **Q: Did the authors keep track of which fragments originated from which of the two, original parent colonies?**
- A: Yes, all of the samples originated from a single parent colony, except for the two samples that experienced heat stress but did not bleach. This points to the variation that is inherent in different colonies, given that the same temperature stress resulted in a different physiological response. For the purposes of this study, we identified these as heat stressed but unbleached. Regardless of the cause (inherent genetic difference, different symbiont type etc.) the resulting  $d^{11}\text{B}$  reflects this differential response.
- **Q: Also, how robust do the authors think that their results are given that only two adjacent colonies were used for collection? Is this enough to establish a novel proxy?**
- A: Of course it would be great if others could/would repeat such a study to confirm our results and conclusions. We encourage Schoepf et al. to conduct high resolution  $d^{11}\text{B}$  measurements using LA-MC-ICP-MS on their bleached specimens. However, it is important to note that most paleo-reconstructions are done on a single or few cores and thus our results still highlight the effects of bleaching on the  $d^{11}\text{B}$  signature of a coral skeleton.
- **Q: Were pH and seawater carbonate chemistry monitored throughout the experiment?**
- A: pH and seawater carbonate chemistry were not monitored inside the growing tables. Nevertheless, the whole experiment was held in open system with direct connection to the nearby sea. pH and seawater carbonate chemistry at an

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adjacent monitoring station are monitored routinely once a month by the Israel National Monitoring Program at the Gulf of Eilat (data is publicly available online). During the experiment period, pH and alkalinity varied at the range of 8.19 to 8.21 and 2.499 to 2.516 meq/kg respectively.

- Q: Can the authors provide more information regarding the conditions under which the pH microsensor measurements were made. It is not clear whether they were made using treatment seawater in terms of temperature, nor what the flow conditions were which can significantly affect the diffuse boundary layer. How reproducible were the microprofiles? At what time point during the experiment were these measurements made? Since light levels were more than twice as high than during the experiment, wouldn't they corals have been light stressed?
  - A: Microelectrodes profiles were conducted to verify and demonstrate changes in carbonate chemistry and are reported in the supplementary information. It shows that bleaching results in decreased pH at coral's DBL. This was done only to check that we get the decrease in DBL pH for bleached or darkened corals as already documented before (e.g.(Al-Horani, 2005; Venn et al., 2011)). We added this figure.

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