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Biogeosciences Discussions

Interactive comment on "Sex-associated variations in coral skeletal oxygen and carbon isotopic composition of *Porites panamensis* in the southern Gulf of California" *by* R. A. Cabral-Tena et al.

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Response to Interactive comment on "Sex-associated variations in coral skeletal oxygen and carbon isotopic composition of Porites panamensis in the southern Gulf of California" by R. A. Cabral-Tena et al. Anonymous Referee #2

Major comments

Thank you very much for your valuable feedback. We carefully read the comments, suggestions and questions; we have taken into account all of them. First, we would

like to clarify that the aim of this paper is not to describe or solve the biochemical or physiological chemical reactions or mechanisms during coral skeletal formation that, in the end, result in the reported sex-associated variations in coral skeletal δ18O and δ13C isotopic composition. Instead, we seek to describe the sex-associated variations in coral skeletal isotopic data and to assess the implications of estimating climatic conditions if the coral sex identification is not taken into account since most of the studies in the Indo-Pacific and Caribbean had been based on unsexed massive gonochoric corals (such as Montastrea cavernosa, Porites lutea and Porites lobata). This procedure may have some serious implications in the paleoclimatic reconstructions studies made so far leading to erroneous conclusions since variability of isotopic data may have been overestimated due to the mixing of male and female data in past studies. Regarding the sex-associated variations in coral skeletal oxygen and carbon isotopic composition, we suggest two hypotheses, so they can be tested or refuted in future works, so, describing the mechanism responsible for the isotopic signal difference between sexes should be the aim of another more complex study. In other words, once the base results have been achieved (different sexes have different growth rates, calcification rates and isotopic signal) pointed in this work and in the Cabral-Tena et al. (2013) study, the next step should be to uncover the mechanisms behind it. Without the first part (since this is the first article to note or point this phenomenon), the next question cannot be answered or formulated. Also, we have no problem in show the dates of isotopic data in figure 1 as suggested by the referee in the major comments. If the Editor agree, it may be put together to other data in a table in the manuscript or as supplementary material.

Specific comments

2 Materials and methods 2.1 Collection and identification of gender Please show the map of study site. Almost readers may not be familiar with Gulfs of California and/or Mexico.

We have no problem to show the map of the study area if necessary. We also suggest

reading the Cabral-Tena et al. (2013) study since these are the same ten colonies presented in that work. The figure would be like this (see fig. 1):

2.3 Isotope analysis p 169 Micromill procedure is the bases of coral isotope study. Many of readers may wonder if powder samples are milled by machine or hand. Milling machine makes the milling to keep equal intervals in coral skeleton, but it is difficult by hand milling procedure. Please describe this process.

We rephrased as follows:

Continuous samples of aragonite powder were collected along each coral's maximum growth axis using a drill with a 0.1 mm bit. Each sample was $\sim$1 mm apart, the milling process was done by hand milling.

3.1 Skeletal growth I would like to recommend to show X-ray photographs and graph of skeletal density along growth axis. It is difficult for reader to understand the relationship between isotope compositions and skeletal growth along growth axis.

We can include new figures, X-ray photographs and graph of skeletal density along growth axis. Also we can also suggest reading the Cabral-Tena et al. (2013) study since these are the same ten colonies presented in that work. The figure would be like this (see Fig. 2).

3.2 Skeletal isotope composition and environmental data The sentences in the line between 243 and 265 should be moved into discussion section.

We agree to move these sentences to the discussion section.

line 255-257: I do not think that authors show the calculation for d18O in seawater based on the d18O of coral skeleton for current coral. Many of readers may misunderstand that this papers discussing about environmental aspects. I think that authors discuss about biological aspect for isotope variation:

This is correct, we report that the variations of Oxygen isotopic composition of sea wa-

ter vary 0.11‰ in a year, and this represents different percentages of isotopic seasonal variation in coral skeletons: 29.72% in female colonies, and 38.53% in male colonies of the average seasonal variation in δ18O. We rephrased it as follows: The expected seasonal variation of approximately 0.11‰ of δ18O in seawater (0.43 psu) represents 29.72% of δ18O seasonal variation in female colonies, and 38.53% in male colonies.

4. Discussion line 296-394: These sentences seem like review for related papers, but not essential. Please shorten.

We eliminated some information and rephrased it as follows:

Asami et al. (2004) suggest that the low correlation coefficient between δ18O and SST, and the gentle slope in the δ18O–SST calibration equations are related to small seasonal variations in SST (<3 °C), or the greater influence of δ18Osw. The seasonal variation in SST of our study area is 7.85 $\pm$ 0.77 °C, so the seasonal variation of SST is not likely to be the cause. Variations in δ18Osw represent 29.72% in female coral, and 38.53% in male coral, of the average seasonal δ18O variation. We found a significant regime shift in the δ18O data of colonies of both genders, that coincides with a regime shift in rainfall. This means that the δ18O of coral in Bahía de La Paz is influenced more by the δ18Osw than in other places in the Pacific. We found a positive relationship between skeletal δ18O and δ13C in our data. Swart et al. (1996b) suggest that this means that the maximum photoperiod in Bahía de La Paz occurs during winter (high δ18O = low SST, high δ13C = high photosynthesis). Hence, photosynthesis might be less intense until the nutrient-rich waters of winter promote the growth of zooxanthellae and restore photosynthesis intensity (Jokiel, 2004; Franklin et al., 2006).Skeletal δ13C (Fig. 2) was higher between November and January (lowest SST and PAR), and lower from June through August (highest SST and PAR), suggesting a positive relationship between δ13C and photosynthesis, and a dominant role of light-induced photosynthesis on seasonal changes of δ13C in coral. Still, the δ13C–PAR correlations were not significant, thus, photosynthesis was not stimulated or inhibited by light, and remained near its maximum efficiency during the whole year, according to Sun et al. (2008).

Other factors may be affecting photosynthesis in addition to light, such as abundance of dissolved nutrients. High concentrations of chlorophyll a occurred during periods of enrichment of 13C in the coral skeleton (November through January); however, the correlations of skeletal δ13C and chlorophyll a were not significant in any case. Trends in coral skeletal δ13C reflect seasonal variations in photosynthesis to respiration ratios in the δ13C pool of coral (McConnaughey, 1989; McConnaughey et al., 1997). Respiration normally increases with temperature and lowers 13C in coral skeletons, which is reflected in our results, high SST = low δ13C. No other environmental variables considered in this work explained this pattern in coral δ13C, driven mainly by metabolic effects as described by Sun et al. (2008) in Porites coral of the South China Sea. We found a negative correlation (r = –0.78, p = 0.001) between δ18O and the skeletal density in female colonies, this is not consistent with studies that have observed that coral skeletal high-density bands are enriched in18O (Klein et al., 1992; Al-Rousand, 2007). This may be due to a difference in timing of skeletal density bands in Porites coral species, as described by Lough and Barnes (2000). In male coral, we found a negative correlation between the δ18O and linear extension and calcification rates (r = –0.50, p = 0.045 and r = –0.44, p = 0.0008), this is consistent with the observations of other authors of Porites spp. coral (McConnaughey, 1989; Felis et al., 2003). In Porites corals, skeletal extension and calcification rates increases with SST, while skeletal density decreases (Lough and Barnes, 2000), so growth parameters of both sexes and δ18O behave as expected .No significant correlation was found between skeletal δ13C and skeletal growth parameters in either males or females, meaning that regardless of the skeletal extension rate, density or calcification rate, P. panamensis deposited a widely varying δ13C, as reported by Allison et al. (1996) in Porites coral from South Thailand, and by Swart et al. (1996b) in Montastrea annularis in Florida, USA. General consensus states that all coral skeletons contain appreciable amounts of carbon and oxygen in isotopic disequilibrium, and are depleted in 18O and 13C because of kinetic variations due to differences in coral growth. McConnaughey (1989) named this phenomenon "Vital effect". We found this to be true for all sampled coral (disequilibrium = 3.54‰

F, 3.80‰ M in δ18O; 2.81‰ F, 2.53‰ M in δ13C). McConnaughey (1989) considers kinetic depletion as a constant in coral with fast extension rates (>0.5 cm yr–1). The average yearly extension rates of all sampled coral were fast (1.05 cm yr–1 for females, and 1.27 cm yr–1 for males). Thus, we assume kinetic disequilibrium is constant in all coral. All δ18O ratios of female colonies are more enriched in 18O than the ones in male colonies, with an average difference of $\sim$0.31‰Ḟemale δ13C values were lower than the δ13C of male colonies, with an average difference of $\sim$0.28‰Ȧll coral colonies in our study grew and calcified in the same environmental conditions. Thus, differences in the isotope record between coral growing in the same environment are attributed to differences in the "Vital effect" of each colony (Linsley et al., 1999; Felis et al., 2003). Linsey et al. (1999) found differences of 0.4‰ in the δ18O records of six Porites lobata coral living in nearly identical environments, in the Clipperton atoll. Felis et al. (2003) found a 1.28‰ difference in the δ18O records of 11 coral of several Porites species, in three sites in the northern part of the Gulf of Aqaba. None of the mentioned works considered the sex of the colony as a factor explaining differences in the "Vital effect" of coral colonies. If we pool the isotopic data of both sexes together, the differences between our isotopic records are 0.38‰ in the δ18O record, and 0.29‰ in the δ13C record. If we split our data by sex, the differences in the isotopic records drop to 0.07‰ in the δ18O, and to 0.02‰ in the δ13C. In our data, the sex of the colony explains 81% (δ18O) and 93% (δ13C) of the differences in the "Vital effect" of coral colonies. Thus, the main source of differences in the isotope record is attributed to differences in the "Vital effect" associated to colony sex, for which we offer two explanations; a simple one, and a complex one: Energy expenditure during the formation of gametes causes differences in the formation of skeletal density bands, and carbon isotopic depletion in coral skeletons (Kramer et al., 1993; Gagan et al., 1994). Cabral-Tena et al. (2013), and Carricart-Ganivet et al. (2013) found sex-dependent effects on the growth parameters and timing of density band formation of coral, related to metabolic effects. We found that P. panamensis female colonies grew slower in comparison to male colonies (1.05 $\pm$ 0.04 cm yr-1 vs. 1.27 $\pm$ 0.04 cm yr-1). Faster growing coral are more depleted

in 18O and more enriched in 13C, relative to slower-growing coral (McConnaughey, 1989; Felis et al., 2003), this may be the origin of the isotope data difference between sexes (higher δ18O and lower δ13C in females), so a simplistic approach might be that since the growth rates are different between sexes, the "Vital effect" will also be different between sexes, thus explaining the differences we found in δ18O and δ13C between sexes.

In this paper, only sentences between 413 and 419 explain about the mechanism, which cause sex-associated isotope variations. I think authors should discuss this mechanism with deeper insight.

As mentioned in the major comments, the aim of this paper is not to describe or solve the biochemical or physiological chemical processes during skeletal formation that result in the reported variations in coral skeletal isotopic records associated to colony sex; we seek only to point out our findings and how this may have some serious implications in the paleoclimatic reconstructions studies made so far leading to erroneous conclusions. Also, we suggest two hypotheses, so they can be tested or refuted in future works. an

-Omata et al. (2008) attempted the isolation for both kinetic effects and metabolic effects. Please read this article.

We applied the correction factor proposed by Heikoop et al. 2000 to isolate the kinetic and metabolic effects in the δ13C of male and female colonies. We chose Heikoop et al. (2000) correction factor over Omata et al. (2008) because the temperature of skeleton precipitation was not the same during the entire study. This table summarizes our results (see Table 1). We also did the Student's T test to compare both sets of means (kinetic and metabolic) and we found significant differences between the means of male and female colonies (t498= 13.074 p< 0.000001 for Kinetic means; t498=-13.98 p< 0.000001 for Metabolic means). The overall average of δ13C in female colonies was –1.66 $\pm$ 0.38‰ and –1.38 $\pm$ 0.37‰ in male colonies (Table 1). As you can see, we

found some interesting results when applying the correction factor, both transformed δ13C and metabolic δ13C seem to be higher in males, thus supporting our hypothesis stating that an intense activity of the Ca-ATPase enzyme will result in carbon heavier skeleton. Ca-ATPase enzyme activity is related positively to energy availability in corals (Cohen and Holcomb 2009), so it would explain why both kinetic effect (skeletal growth) and metabolic effect (coral photosynthesis / respiration) are higher in male corals, since male corals grow faster than female colonies.

We can include these results in the manuscript if the Editor agree.

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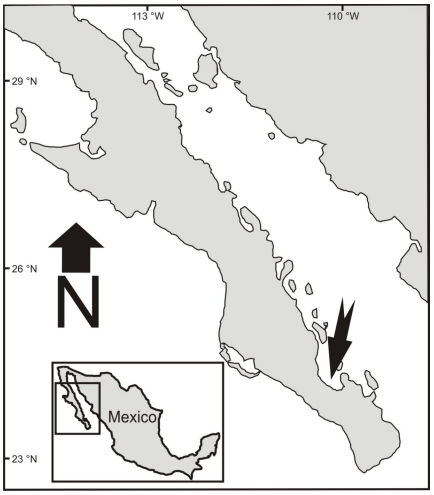

Fig. 1. Fig. 1

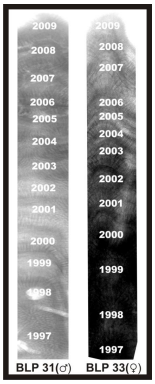

Fig. 2. Fig. 2

	Transformed $\delta^{13}C$ Females (N=200)	Transformed $\delta^{13}C$ Males (N=300)	Metabolic $\delta^{13}C$ Males (N=200)	Metabolic $\delta^{13}C$ Males (N=300)
Mean	5.082	6.30	6.23	7.43
SD	0.90	0.97	0.90	0.96

Fig. 3. Table 1