Dear Dr. S.W.A. Naqvi,

We are resubmitting to you the revised manuscript No. bg-2015-465 “Sex-associated variations in coral skeletal oxygen and carbon isotopic composition of Porites panamensis in the southern Gulf of California”. We carefully read the reviewers comments, suggestions and questions and we rewrote, delete or added paragraphs to the manuscript as needed.

In the revised manuscript the changes or additions were marked in different color according to the Anonymous Referee: gray (Anonymous Referee #1), blue (Anonymous Referee #2).

Below we address the substantive questions or suggestions of each reviewer.
Comments from Anonymous Referee #1

General Comments:

This is an interesting paper that follows from the authors’ earlier work (Cabral-Tena et al, 2013) which demonstrated that growth rates differed between male and female colonies of Porites panamensis in the southern Gulf of California. Here, they demonstrate that there are also significant differences between male and female colonies in the stable isotopic signatures of δ¹⁸O and δ¹³C and present two possible explanations for these differences in this gonochoric brooding coral species. Aside from the differences associated with sex, the study adds to our understanding of the isotopic tracers and their relationships with environmental parameters and growth characteristics. The findings also have implications for isotopic analyses and their environmental interpretation for gonochoric brooding species such as P. panamensis though the vast majority of massive Porites used in paleoclimatic reconstructions are gonochoric spawners rather than brooders (Baird et al. 2009). Although, as the authors indicate, gonochoric spawning requires less energy than brooding, it would be interesting to know whether there are also growth and isotopic differences in the commonly used massive species such as P. lobata and P. lutea. This study may be a prompt for either the authors or others to undertake such a comparison as evidence for such differences would have implications for paleoclimatic reconstructions from massive coral records. Overall, I consider this study to be sound and worthy of publication after some minor changes (mostly for clarification). The paper could also benefit from a final editing by someone with English as their first language but generally the writing is clear.

Specific comments

Page 18796, lines 2-3: delete ‘near’; add (SST) after ‘temperature’.
Page 18796, line 6: ‘lesser extent’ than what?
Page 18796, lines 7-8: make it clear that these growth differences refer to the gonochoric brooding coral P. panamensis.
Page 18796, line 9: replace ‘assess this difference’ with ‘test this’.
Page 18796, line 11: add country after ‘La Paz’.
Page 18796, line 12: photosynthetically active radiation (PAR).
Page 18796, line 18: change ‘implies’ to ‘could introduce’.
Page 18796, lines 25-26: again make it clear that these findings relate to one gonochoric brooding species though they may have implications for commonly used gonochoric spawning species such as P. lobata and P. lutea.
Page 18797: lines 2-4: Make it clear that this does not refer to all corals, only certain species; also it is not only their growth that is affected by environmental conditions but that materials (isotopic and trace elements) are incorporated into the skeleton during growth.

Page 18797, line 8: delete ‘changes’
Page 18797, line 9: change ‘events’ to ‘variability and change’.
Page 18797, line 11: change ‘from’ to ‘with’.
Page 18797, line 19: change ‘estimate’ to ‘measure’; I am not necessarily convinced that 13C has been as easy to interpret as 18O.

Page 18798, line 15: ‘upwelling events that bring nutrients to surface waters’.

Page 18798, line 21: be consistent throughout ms, here ‘vital effect’, elsewhere ‘Vital effect’; ‘constant along the growth’.

Page 18799, line 20: replace ‘recording was’ with ‘measurements were’.

Page 18800, line 4: what year were the colonies collected? Also, what was the approximate size of the colonies? Are these the same 10 colonies from La Paz presented in Cabral-Tena et al (2013)? If so, then say so.

Page 18800, line 15: replace ‘labelled’ with ‘identified as’.
Page 18800, line 17: replace ‘labelled’ with ‘identified as’.
Page 18801, line 1: replace ‘placed in’ with ‘located on’.

Page 18801, line 20: delete ‘equal’.

Page 18801, lines 21-22: What is meant by ‘different sampling resolutions’ when they were all sampled at 1 mm resolution? Is it sampling resolution in relation to different linear extension rates of the samples?

Page 18801, line 23 to Page 18802, line 2: suggest move this description of statistical analyses to separate section of Materials and Methods.

Page 18802, line 2: ‘linear’.
Page 18802, lines 4-15: provide the temporal resolution of the various data sets (e.g. daily, weekly or monthly?) and the time periods they cover.

Page 18802, line 10: indicate the time period of this comparison and temporal resolution of the data.

Page 18802, lines 15-18: delete first sentence and add the description of the Regime shift change software to the suggested new section on statistical analyses.

Page 18802, lines 21-24: Please provide details of the years covered by each of the colony growth and isotopic records. Could provide this in a Supplementary Table, possibly with all the annual growth and isotopic data?
Page 18802, Results: Please make it clear throughout the Results what the temporal resolution of the data being compared is e.g. annual, monthly, seasonal? Also whether time series or average colony values are being compared.

Page 18803, lines 6-7: Unclear what ‘strongly correlated between sexes’ means – what is being correlated here? Also, suggest using ‘significantly’ rather than ‘strongly’.

Page 18803, line 15: Refer to Fig. 1b.

Page 18804, line 2: ‘correlate with’.

Page 18804, lines 4-5: Delete first sentence and add period covered to second sentence.

Page 18804, lines 10-11: ‘small seasonal variation’ – compared to what?

Page 18805, line 4: here and elsewhere change ‘strong’ to ‘significant’.

Page 18805, line 6: delete ‘Table 4’.

Page 18805, lines 17-25: Please make it clear what the temporal resolution of these different studies is, and how they compare to this study. High correlation coefficients can always be obtained when simply correlating two annual cycles (see Lough 2004. Palaeo Palaeo Palaeo 204: 115-143).

Page 18806, line 19: ‘depleted in nutrients’.

Page 18808, line 14: ‘fast extension rates’ – fast compared to what? Compare to other reported average Porites spp. linear extension rates?

Page 18808, line 16: ‘are more enriched than in male’.

Page 18809: line 6: ‘associated with colony’.

Page 18811, line 8: delete ‘would’.

Page 18812, lines 6-9: Suggest emphasise that this study based on a gonochoric brooder and that the majority of paleoclimatic reconstructions from massive Indo-Pacific Porites spp. have been based on gonochoric spawners. Thus a fruitful area of future research would be to determine whether the sex differences the authors have identified are also characteristic of gonochoric spawners such as P. lobata and P. lutea.

Page 18820, Table 1: Indicate years covered by each series.

Page 18821, Table 2: Indicate temporal resolution of data and also time period covered by correlations.

Page 18822, Table 3: Indicate temporal resolution of data and also time period covered by correlations.

Page 18823, Table 2: Indicate temporal resolution of data and also time period covered
by correlations.

Page 18824, Figure 1: Explain the shift in the rainfall mean in the figure caption.

Page 18826, Figure 3: Is this based on all annual data for all years from each colony? If so, make this clear in figure caption.

Page 18827, Figure 4: Is this based on all annual data for all years from each colony? If so, make this clear in figure caption.

Response to Anonymous Referee #1

General comments

Thank you very much for your comments, we have taken into account all your suggestions. We carefully read the comments, suggestions and questions and we rewrote, delete or added paragraphs to the manuscript as needed. Below we address the questions or suggestions.

Specific comments

We made the necessary changes and will be included in the manuscript as follows:

Page 18796, lines 2-3: delete 'near'; add (SST) after 'temperature'.

Coral δ^{18}O variations are used as a proxy for changes in sea surface temperature (SST) and seawater isotope composition.

Page 18796, line 6: 'lesser extent' than what?

Coral growth rate is known to influence the δ^{18}O and δ^{13}C isotope record to a lesser extent than environmental variables.

Page 18796, lines 7-8: make it clear that these growth differences refer to the gonochoric brooding coral *P. panamensis*.

Recent published data show differences in growth parameters between female and male coral in the gonochoric brooding coral *Porites panamensis*.

Page 18796, line 9: replace 'assess this difference' with 'test this'.

to test this, this study describes changes in the skeletal δ^{18}O and δ^{13}C.

Page 18796, line 11: add country after 'La Paz'.

four female and six male *Porites panamensis* coral collected in Bahía de La Paz, Mexico, whose growth bands spanned 12 years.

Page 18796, line 12: photosynthetically active radiation (PAR).

The isotopic data were compared to SST, precipitation, photosynthetically active radiation (PAR).

Page 18796, line 18: change 'implies' to 'could introduce'.

A difference in the skeletal δ^{18}O could introduce an error.

Page 18796, lines 25-26: again make it clear that these findings relate to one gonochoric...
brooding species though they may have implications for commonly used gonochoric spawning species such as *P. loabta* and *P. lutea*.

Although these findings relate to one gonochoric brooding species, they may have some implications for the more commonly used gonochoric spawning species such as *Porites lutea* and *Porites lobata*.

Page 18797: lines 2-4: Make it clear that this does not refer to all corals, only certain species; also it is not only their growth that is affected by environmental conditions but that materials (isotopic and trace elements) are incorporated into the skeleton during growth.

Massive hermatypic coral are useful as recorders of oceanic conditions because their growth and skeletal materials incorporated during growth are affected by environmental variables, the calcareous material is deposited in annual density bands that allow for the determination of events over time.

Page 18797, line 8: delete 'changes'
centennial timescale of El Niño–Southern Oscillation (ENSO), the Pacific Decadal Oscillation (PDO).

Page 18797, line 9: change ‘events’ to ‘variability and change’.
pre- and post-industrial climate variability and change

Page 18797, line 11: change ‘from’ to ‘with’.
predictable way with environmental variations

Page 18797, line 19: change ‘estimate’ to ‘measure’; I am not necessarily convinced that _13C has been as easy to interpret as _18O.
skeletal δ¹³O and δ¹³C are the most common measurements because they are relatively easy to measure

Page 18798, line 15: ‘upwelling events that bring nutrients to surface waters’.
coral skeletal δ¹³C decrease during upwelling events that bring nutrients to surface waters

Page 18798, line 21: be consistent throughout ms, here ‘vital effect’, elsewhere ‘Vital effect’; ‘constant along the growth’.
We have checked all the ms and have changed all “Vital” to “vital.
This departure from equilibrium is referred to as “the vital effect” and appears to be constant along the coral growth axis

Page 18799, line 20: replace ‘recording was’ with ‘measurements were’.
Oxygen and carbon isotope measurements were used to

Page 18800, line 4: what year were the colonies collected? Also, what was the approximate size of the colonies? Are these the same 10 colonies from La Paz presented in
The specimens were collected in 2011 at depths of 3–4 m. Divers used hammer and chisel to remove the colonies from the substrate. A fragment from each colony was fixed in Davison’s solution for a histological examination and identification of sex (Howard and Smith, 1983). These are the same ten colonies presented in the Cabral-Tena et al. (2013) study.

Page 18800, line 15: replace ‘labelled’ with ‘identified as’.

The colonies were identified as female.

Page 18800, line 17: replace ‘labelled’ with ‘identified as’.

The colonies were identified as male.

Page 18801, line 1: replace ‘placed in’ with ‘located on’.

Optical density tracks were located on the maximum growth.

Page 18801, line 20: delete ‘equal’.

minimum $\delta^{18}$O value in a year to summer

Page 18801, lines 21-22: What is meant by ‘different sampling resolutions’ when they were all sampled at 1 mm resolution? Is it sampling resolution in relation to different linear extension rates of the samples?

To eliminate the effects of different sampling resolutions on the calculation of mean coral $\delta^{18}$O values due to differences in linear extension rates of each colony, the results were interpolated to create four equally spaced values per year.

Page 18801, line 23 to Page 18802, line 2: suggest move this description of statistical analyses to separate section of Materials and Methods.

A new section in Materials and Methods was written as follows:

2.5 Statistical analyses

Normality and homoscedasticity of the data were tested using Kolmogorov–Smirnov and Bartlett tests, respectively. Student’s $t$-test for independent samples with uneven variance was used to assess statistical differences in $\delta^{18}$O and $\delta^{13}$C between sexes. Pearson’s correlation test and simple linear regressions were used to estimate relationships between mean skeletal extension rate, skeletal density, and calcification rate with isotope data of both sexes. An ANCOVA test was used to assess the differences between slopes and the $y$-intercept of linear equations of $\delta^{13}$C versus $\delta^{18}$O plots of the results of male and female data.

Pearson’s correlation test and simple linear regressions were used to estimate relationships between environmental data and isotope data of both sexes. Regime shift index for environmental and isotope data were calculated with the Sequential Regime Shift Detection Software (Rodionov, 2004).
Page 18802, line 2: ‘linear’.

differences between slopes and the y-intercept of linear equations

Page 18802, lines 4-15: provide the temporal resolution of the various data sets (e.g. daily, weekly or monthly?) and the time periods they cover.

Monthly SST, PAR, and concentration of chlorophyll a data were obtained from the NOAA live access server (http://las.pfeg.noaa.gov/oceanWatch/oceanwatch.php), the environmental data spanned from 1997 to 2009

Page 18802, line 10: indicate the time period of this comparison and temporal resolution of the data.

Compared in situ and satellite data were both monthly covering from 2003 to 2007.

Page 18802, lines 15-18: delete first sentence and add the description of the Regime shift change software to the suggested new section on statistical analyses.

This was included in the new section of materials and methods.

Page 18802, lines 21-24: Please provide details of the years covered by each of the colony growth and isotopic records. Could provide this in a Supplementary Table, possibly with all the annual growth and isotopic data?

This will be included in the supplementary material.

Page 18802, Results: Please make it clear throughout the Results what the temporal resolution of the data being compared is e.g. annual, monthly, seasonal? Also whether time series or average colony values are being compared.

We detailed along all the results section that the time series is from 1997 to 2009, and the resolution of data is quarterly.

Page 18803, lines 6-7: Unclear what ‘strongly correlated between sexes’ means – what is being correlated here? Also, suggest using ‘significantly’ rather than ‘strongly’.

Page 18803, line 15: Refer to Fig. 1b.

Page 18804, line 2: ‘correlate with’.

significantly correlate with

Page 18804, lines 4-5: Delete first sentence and add period covered to second sentence.

The linear regression (Fig. 3) equations for δ¹⁸O dependence on SST (1997-2009) were:

Page 18804, lines 10-11: ‘small seasonal variation’ – compared to what?

We deleted this sentence.

Page 18805, line 4: here and elsewhere change ‘strong’ to ‘significant’.
Changes from “strong” to “significant” were made in all cases.

Page 18805, line 6: delete ‘Table 4’.

annual skeletal density was found (Table 4; $r = -0.78$, $p = 0.001$).

Page 18805, lines 17-25: Please make it clear what the temporal resolution of these different studies is, and how they compare to this study. High correlation coefficients can always be obtained when simply correlating two annual cycles (see Lough 2004. Palaeo Palaeo Palaeo 204: 115-143).

The requested information was included; the paragraph will read as follows:

Our isotope data showed a significant dependency of skeletal $\delta^{18}O$ on SST, with a low $r$ ($-0.45$ in female coral, and $-0.28$ in male coral), and a gentle slope of the $\delta^{18}O$–SST calibration equations (0.09‰ °C$^{-1}$ F; 0.11‰ °C$^{-1}$ M; Fig. 3), compared with slopes (>0.20‰ °C$^{-1}$) in Porites spp. in other areas of the Pacific: the Great Barrier Reef (Gagan et al., 1994), Costa Rica (Carriquiry, 1994), Panama (Wellington and Dunbar, 1995), and the Galapagos Archipelago (McConnaughey, 1989). These studies show high correlation coefficients (better than $-0.80$) of $\delta^{18}O$ and SST, all these studies have isotopic records varying to 5 to 40 years long, and with a high temporal resolution sampling (weekly to monthly). Our results are similar to studies reporting small correlation coefficients of $\delta^{18}O$ and SST (less than $-0.70$) and a gentle slope (<0.17‰ °C$^{-1}$) of the $\delta^{18}O$–SST calibration equations, such as at Clipperton Atoll (Linsley et al., 1999), Fiji (Le Bec et al., 2000), and Guam (Asami et al., 2004). These studies have long isotopic records (20 to 25 years) and a high temporal resolution sampling (daily to monthly) compared to our data (12 years of data with a quarterly sampling resolution).

Page 18806, line 19: ‘depleted in nutrients’.

becomes depleted in nutrients.

Page 18808, line 14: ‘fast extension rates’ – fast compared to what? Compare to other reported average Porites spp. linear extension rates?

The average yearly extension rates of all sampled coral can be considered as fast (1.05 cm yr$^{-1}$ F, and 1.27 cm yr$^{-1}$ M) in accordance with the work of McConnaughey (1989).

Page 18808, line 16: ‘are more enriched than in male’.

All $\delta^{18}O$ ratios of female colonies are more enriched in $^{18}O$ than in male colonies

Page 18809: line 6: ‘associated with colony’.

“vital effect” associated with colony sex,

Page 18811, line 8: delete ‘would’.
Page 18812, lines 6-9: Suggest emphasize that this study based on a gonochoric brooder and that the majority of paleoclimatic reconstructions from massive Indo-Pacific Porites spp. have been based on gonochoric spawners. Thus a fruitful area of future research would be to determine whether the sex differences the authors have identified are also characteristic of gonochoric spawners such as P. lobata and P. lutea.

Changes to the last paragraph of the discussion were made considering your suggestions and will read as follows:

This study provides evidence of sex-associated variations in coral skeletal δ^{18}O and δ^{13}O of *P. panamensis*. This has some implications and has to be considered when climate conditions are estimated based on comparisons of δ^{18}O and δ^{13}O values of gonochoric brooder coral genera, if sex identification is not taken into account when possible. The findings of this study are based on a gonochoric brooder species (*P. panamensis*), while the majority of paleoclimatic reconstructions in the Indo-Pacific and Caribbean have been based on massive gonochoric spawners (such as *Montastrea cavernosa*, *Porites lutea* and *Porites lobata*), so, it remains unclear if the same phenomena (sex-associated variations in coral skeletal δ^{18}O and δ^{13}O) can be observed in gonochoric spawners. This may have some serious implications in the paleoclimatic reconstructions studies made so far leading to erroneous conclusions due to errors in isotopic estimation; variability of isotopic data may have been overestimated due to the mixing of male and female isotopic data in past studies. Thus, a fruitful area of future research would be to determine whether the sex differences identified in this study are also characteristic of gonochoric spawners.

**Page 18820, Table 1: Indicate years covered by each series.**

*Table 1.* Summary of the overall average extension rate, skeletal density, calcification rate, δ^{18}O and δ^{13}C of *Porites panamensis* colonies from Bahía de La Paz, Gulf of California. Time period of data is from 1997 to 2009.

**Page 18821, Table 2: Indicate temporal resolution of data and also time period covered by correlations.**

*Table 2.* Correlation coefficients between skeletal δ^{18}O of *Porites panamensis* colonies and: Sea surface temperature, precipitation, photosynthetically active radiation and chlorophyll *a* from Bahía de La Paz. Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is quarterly. **Bold** numbers indicate significant (*p < 0.05*) correlations.

**Page 18822, Table 3: Indicate temporal resolution of data and also time period covered**
by correlations.

Table 3. Correlation coefficients between skeletal δ¹³C of *Porites panamensis* colonies and:
Sea surface temperature, precipitation, photosynthetically active radiation and chlorophyll *a*
from Bahía de La Paz. Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is quarterly. **Bold** numbers indicate significant (*p* < 0.05) correlations.

**Page 18823, Table 2: Indicate temporal resolution of data and also time period covered by correlations.**

Table 4. Correlation coefficients between skeletal extension rate, skeletal density and calcification rate, and skeletal δ¹⁸O and δ¹³C of *Porites panamensis* colonies from Bahía de La Paz. Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is yearly. **Bold** numbers indicate significant (*p* < 0.05) correlations.

**Page 18824, Figure 1: Explain the shift in the rainfall mean in the figure caption.**

**Fig. 1.** (a) Seasonal variation in δ¹⁸O composition (VPDB) from *Porites panamensis* coral colonies along the major growth axis. Blue lines represent male colonies; Red lines represent female colonies; red dotted line female colonies’ regime mean; blue dotted line, male colonies’ regime mean. (b) Satellite sea surface temperature and precipitation (1997–2009) records. Sea surface temperature (red line; °C), mean sea surface temperature (dotted red line; °C), precipitation (blue line; mm), mean precipitation (dotted blue line; mm). Note the regime shift in the precipitation mean in 2003.

**Page 18826, Figure 3: Is this based on all annual data for all years from each colony? If so, make this clear in figure caption.**

**Fig. 3.** Linear regressions between satellite derived sea surface temperature (°C) and skeletal δ¹⁸O (VPDB) of female, and male *Porites panamensis* coral from Bahía de La Paz. Time period covered by analyses is from 1997 to 2009. Temporal resolution of data is quarterly. This includes all isotopic data of all colonies. Line equations and coefficients are shown.

**Page 18827, Figure 4: Is this based on all annual data for all years from each colony? If so, make this clear in figure caption.**

**Fig. 4.** Plot of δ¹³C vs. δ¹⁸O of female (red dots), and male (blue dots) *Porites panamensis* coral from Bahía de La Paz. This includes all isotopic data of all colonies. Line equations and coefficients (red represents females; blue represents males) are shown.
Comments from Anonymous Referee #2

General Comments:

This article represents differences of oxygen and carbon stable isotope in the *Porites panamensis* for both male and female colony skeletons. I am interested in the oxygen isotope date in fig 1, which shows obvious differences in oxygen isotope for skeletons in male and female colonies.

Reading many of articles focusing on the stable isotope study in biological carbonate such as coral and foraminifera, it is important to remember the basis of stable isotope geochemistry to reconstruct the paleoclimate conditions. Why does many of biogeoscientists use the stable isotope compositions in oxygen and carbon in biological carbonates? Since Harold Urey represent the application of stable isotope in Jurassic Pee Dee Belemnite to reconstruct the paleo temperature based on the theory of isotope effect in chemical equilibrium in biological carbonate, it became possible to reconstruct paleotemperature in earth history. In each chemical reaction, stable isotope composition in both origin and product has quantitative relationship involving the parameter of reaction condition such as temperature and/or reaction rate etc. (Sharp (2006) represent these theory as text book.) Because foraminifera and shells form their skeletons in the isotope equilibrium, oxygen isotopes and temperature in seawater have quantitative relationship.

For coral skeleton, it is considered as oxygen and carbon are supplied from seawater. But their forming involves multistep chemical reactions with isotope disequilibrium in the biological body, because coral skeleton forms much faster than foraminifera and/or shells. Then isotope composition in the biological carbonate are often blinded. McConnaughey (1989a) made clear the multistep reaction in the forming of coral skeleton. He represented which chemical reactions cause the isotope disequilibrium in the forming process of coral skeleton and revealed the isotopic trends for both carbon and oxygen isotope compositions named “kinetic isotope effect”, which is called as “vital effect” before McConnaughey (1989). This paper does not seem to understand why isotope composition in biological carbonate are paleoenviromental indicator. Authors claim that isotope compositions in oxygen and carbon shows sex associated variations. However, their explanation about differences of physiology for both male and female are shown in line 411-419 only. For geochemists, this paper does not involve critical physiological chemical reaction for both male and female corals. For biologists, this paper does not involve what and
how chemical reaction makes change the isotope compositions between chemical origin and product.

I would recommend that authors add much contents of this part. Almost readers may wonder what causes the differences in physiological pathway with chemical reaction for both male and female corals. Isotope compositions in chemical product such as biological carbonate are controlled by chemical conditions in each chemical reaction, excluding isotope compositions in chemical origins. Understanding of theory in stable isotope compositions is more important than statistics analysis.

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 Gulf of California and/or Mexico.

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.

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.

3.2 Skeletal isotope composition and environmental data

The sentences in the line between 243 and 265 should be moved into 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:

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

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

Omata et al. (2008) attempted the isolation for both kinetic effects and metabolic effects.

Please read this article.

Anonymous Referee #2

General comments:
Thank you very much for your comments, we have taken into account all your suggestions. We carefully read the comments, suggestions and questions, 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 $\delta^{18}O$ and $\delta^{13}C$ isotopic composition, we seek to describe the coral skeletal isotopic data we found and assess the implications of estimating climatic conditions if the coral sex identification is not taken into account when possible since most of the studies in the Indo-Pacific and Caribbean have been based on massive gonochoric corals (such as Montastrea cavernosa, Porites lutea and Porites lobata), and how this may have some serious implications in the paleoclimatic reconstructions studies made so far leading to erroneous conclusions due to errors in isotopic estimation since variability of isotopic data may have been overestimated due to the mixing of male and female isotopic 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. Or 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.

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 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:
Micromill procedure is the base 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 ~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:

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 $d^{18}O$ in seawater based on the $d^{18}O$ 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 water vary 0.11‰ in a year, 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 $\delta^{18}O$.

We rephrased it as follows:

The expected seasonal variation of approximately 0.11‰ of $\delta^{18}O$ in seawater (0.43 psu) represents 29.72% of $\delta^{18}O$ 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 $\delta^{18}O$ and SST, and the gentle slope in the $\delta^{18}O$–SST calibration equations are related to small seasonal variations in SST (<3 °C), or the greater influence of $\delta^{18}O_{sw}$. The seasonal variation in SST of our study area is 7.85 ± 0.77 °C, so the seasonal variation of SST is not likely to be the cause. Variations in $\delta^{18}O_{sw}$ represent 29.72% in female coral, and 38.53% in male coral, of the average seasonal $\delta^{18}O$ variation. We found a significant regime shift in the $\delta^{18}O$ data of colonies of both genders, that coincides with a regime shift in rainfall. This means that the $\delta^{18}O$ of coral in Bahía de La Paz is influenced more by the $\delta^{18}O_{sw}$ than in other places in the Pacific.

We found a positive relationship between skeletal $\delta^{18}O$ and $\delta^{13}C$ in our data. Swart et al. (1996b) suggest that this means that the maximum photoperiod in Bahía de La Paz occurs during winter (high $\delta^{18}O$ = low SST, high $\delta^{13}C$ = 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 $\delta^{13}C$ (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 $\delta^{13}C$ and photosynthesis, and a dominant role of light-induced photosynthesis on seasonal changes of $\delta^{13}C$ in coral. Still, the $\delta^{13}C$–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 $^{13}$C in the coral skeleton (November through January); however, the correlations of skeletal $\delta^{13}$C and chlorophyll $a$ were not significant in any case.

Trends in coral skeletal $\delta^{13}$C reflect seasonal variations in photosynthesis to respiration ratios in the $\delta^{13}$C pool of coral (McConnaughey, 1989; McConnaughey et al., 1997). Respiration normally increases with temperature and lowers $^{13}$C in coral skeletons, which is reflected in our results, high SST = low $\delta^{13}$C. No other environmental variables considered in this work explained this pattern in coral $\delta^{13}$C, 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 $\delta^{18}$O and the skeletal density in female colonies, this is not consistent with studies that have observed that coral skeletal high-density bands are enriched in $^{18}$O (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 $\delta^{18}$O 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 $\delta^{18}$O behave as expected. No significant correlation was found between skeletal $\delta^{13}$C 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 $\delta^{13}$C, 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 $^{18}$O and $^{13}$C 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 $\delta^{18}$O; 2.81‰ F, 2.53‰ M in $\delta^{13}$C). 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 δ$^{18}$O ratios of female colonies are more enriched in $^{18}$O than the ones in male colonies, with an average difference of ~0.31‰. Female δ$^{13}$C values were lower than the δ$^{13}$C of male colonies, with an average difference of ~0.28‰. All 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 δ$^{18}$O 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 δ$^{18}$O 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 δ$^{18}$O record, and 0.29‰ in the δ$^{13}$C record. If we split our data by sex, the differences in the isotopic records drop to 0.07‰ in the δ$^{18}$O, and to 0.02‰ in the δ$^{13}$C. In our data, the sex of the colony explains 81% (δ$^{18}$O) and 93% (δ$^{13}$C) 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 $^{18}$O and more enriched in $^{13}$C, relative to slower-growing coral (McConnaughey, 1989; Felis et al., 2003), this may be the origin of the isotope data difference between sexes (higher δ$^{18}$O and lower δ$^{13}$C 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 δ$^{18}$O and δ$^{13}$C 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 to 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, and

-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 $\delta^{13}$C 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 summarizes our results:

<table>
<thead>
<tr>
<th></th>
<th>Transformed $\delta^{13}$C Females (N=200)</th>
<th>Transformed $\delta^{13}$C Males (N=300)</th>
<th>Metabolic $\delta^{13}$C Males (N=200)</th>
<th>Metabolic $\delta^{13}$C Males (N=300)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.082</td>
<td>6.30</td>
<td>6.23</td>
<td>7.43</td>
</tr>
<tr>
<td>SD</td>
<td>0.90</td>
<td>0.97</td>
<td>0.90</td>
<td>0.96</td>
</tr>
</tbody>
</table>

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 (t_{498}=13.074 p<0.000001 for Kinetic means; t_{498}=13.98 p<0.000001 Metabolic means).

The overall average of $\delta^{13}$C in female colonies was $-1.66 \pm 0.38\%o$, and $-1.38 \pm 0.37\%o$ in male colonies (Table 1).

As you can see, we found some interesting results when applying the correction factor, both transformed $\delta^{13}$C and metabolic $\delta^{13}$C 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 necessary.
Sex-associated variations in coral skeletal oxygen and carbon isotopic composition of *Porites panamensis* in the southern Gulf of California

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Con formato: Español (México)
Abstract

Coral δ¹⁸O variations are used as a proxy for changes in near sea surface temperature (SST) and seawater isotope composition. Skeletal δ¹³C of coral is frequently used as a proxy for solar radiation because most of its variability is controlled by an interrelationship between three processes: photosynthesis, respiration, and feeding. Coral growth rate is known to influence the δ¹⁸O and δ¹³C isotope record to a lesser extent than environmental variables. Recent published data show differences in growth parameters between female and male coral in the gonochoric brooding coral *Porites panamensis*, thus, skeletal δ¹⁸O and δ¹³C are hypothesized to be different in each sex. To assess this difference, this study describes changes in the skeletal δ¹⁸O and δ¹³C record of four female and six male *Porites panamensis* coral collected in Bahía de La Paz, Mexico, whose growth bands spanned 12 years. The isotopic data were compared to SST, precipitation, photosynthetically active radiation (PAR), chlorophyll *a*, and skeletal growth parameters. *Porites panamensis* is a known gonochoric brooder whose growth parameters are different in females and males. Splitting the data by sexes explained 81% and 93% of the differences of δ¹⁸O, and of δ¹³C, respectively, in the isotope record between colonies. Both isotope records were different between sexes. δ¹⁸O was higher in female colonies than in male colonies, with a 0.31‰ difference; δ¹³C was lower in female colonies, with a 0.28‰ difference. A difference in the skeletal δ¹⁸O implies an error in SST estimates of ≈ 1.0 °C to ≈ 2.6 °C. The δ¹⁸O records showed a seasonal pattern that corresponded to SST, with low correlation coefficients (−0.45, −0.32), and gentle slopes (0.09‰ °C⁻¹, 0.10‰ °C⁻¹) of the δ¹⁸O–SST relation. Seasonal variation in coral δ¹⁸O represents only 52.37% and 35.66% of the SST cycle; 29.72% and 38.53% can be attributed to δ¹⁸O variability in seawater. δ¹³C data did not correlate with any of the environmental variables; therefore, variations in skeletal δ¹³C appear to be driven mainly by metabolic effects. Our results support the hypothesis of a sex-associated difference in skeletal δ¹⁸O and δ¹³C signal, and suggest that environmental conditions and coral growth parameters affect skeletal isotopic signal differently in each sex. Although these findings relate to one gonochoric brooding species, they may have some implications for the more commonly used gonochoric spawning species such as *Porites lutea* and *Porites lobata*.
Introduction

Massive hermatypic corals are useful as recorders of oceanic conditions because their growth and skeletal materials incorporated during growth are affected by environmental variables, and the calcareous material is deposited in annual density bands that allow for the determination of events over time (Druffel, 1997; Gagan et al., 2000; Grottoli and Eakin, 2007; Lough and Barnes, 2000; Lough and Cooper, 2011). This memory of oceanographic conditions at the time of calcification, record variations at the intra-annual, inter-annual, inter-decadal, and sometimes centennial timescale of El Niño–Southern Oscillation (ENSO), the Pacific Decadal Oscillation (PDO), and pre- and post-industrial climate events variability and change (Grottoli and Eakin, 2007). Skeletal growth, isotope composition, and minor and trace element ratios in coral skeletons vary in a predictable way from environmental variations in temperature, salinity, precipitation, cloud cover, fresh water discharge, upwelling, and pH (Dunbar and Wellington, 1981; Bernal and Carriquiry, 2001; Hönisch et al., 2004; Grottoli and Eakin, 2007). Among the proxies used in coral skeletons (trace element ratios, $\delta^{18}O$, $\delta^{13}C$, $\delta^{11}B$, $\delta^{15}N$), skeletal $\delta^{18}O$ and $\delta^{13}C$ are the most common measurements because they are relatively easy to measure and interpret (Dunbar et al., 1994; Linsley et al., 1994; Swart et al., 1996a; Tudhope et al., 1996; Charles et al., 1997; Schrag, 1999).

Most of the variability in skeletal $\delta^{18}O$ in calcifying organisms, including coral, results from a combination of temperature-induced isotopic fractionation of local seawater $\delta^{18}O$ ($\delta^{18}O_{sw}$) that depends on changes in precipitation and oceanic evaporation, which affect salinity (Epstein et al., 1953). Depletion in carbonate $\delta^{18}O$ occurs as temperature increases in inorganic and biogenic carbonates (Allison et al., 1996). In tropical and subtropical oceans, variations in salinity caused by evaporation, rainfall, or river run-off affect skeletal $\delta^{18}O$ and need to be considered when establishing a skeletal $\delta^{18}O$-SST relationship (Cole and Fairbanks, 1990; Carriquiry et al., 1994; Al Rousan et al., 2007; Sazzad et al., 2010).

Variations of skeletal $\delta^{13}C$ are controlled mainly by an interrelationship between photosynthesis, respiration, and feeding. During high photosynthesis, zooxanthellae fixation of $^{12}CO_2$ increases, which leads to an increase in $^{13}CO_2$ in the coral carbon pool. Hence, coral skeletons formed during periods of high photosynthesis contain greater amounts of $^{13}C$ (Swart, 1983; McConnaughey, 1989; McConnaughey et al., 1997). During seasons with lower photosynthetic activity or when the photosynthesis to respiration ratio falls, coral skeletons...
would have lesser amounts of $^{13}$C. Changes in the photosynthesis–respiration ratio are
influenced by photoperiods, photo-intensity, and temperature; where longer photoperiods and
higher temperatures promote higher photosynthesis–respiration ratios (higher $^{13}$C). If
maximum solar radiation occurs during summer, skeletal $\delta^{13}$C will be inversely related to
$\delta^{18}$O; if the maximum photoperiod occurs during colder seasons, $\delta^{13}$C and $\delta^{18}$O will be
positively related (Swart et al., 1996b). Since zooplankton have generally low isotope levels,
compared to coral skeletons and zooxanthelae, an increase in the heterotrophic activity of
coral should reduce the $\delta^{13}$C of coral skeletons (Grottoli and Wellington, 1999). Felis et al.
(1998), and Bernal and Carriquiry (2001) demonstrated that levels of coral skeletal $\delta^{13}$C
decrease during upwelling events that bring nutrients to surface waters, with high
concentrations of zooplankton related to decreasing zooxanthellae photosynthetic activity, and
an increase in coral heterotrophic feeding (Cole et al., 1993; Quinn et al., 1993).

The $\delta^{18}$O and $\delta^{13}$C in coral skeletons are depleted in $^{18}$O and $^{13}$C, in comparison to
inorganic aragonite precipitated under isotope equilibrium (Weber and Woodhead, 1972;
McConnaughey, 1989). This departure from equilibrium is referred to as “the vital effect” and
appears to be constant along the coral growth axis (Land et al., 1975; McConnaughey,
1989; Barnes and Lough, 1992; Barnes et al., 1995; Wellington et al., 1996). Isotope
disequilibrium of coral skeletons results from coral precipitating their skeletons too quickly to
attain isotope equilibrium (McConnaughey, 1989). Hence, all coral skeletons contain
appreciable amounts of carbon and oxygen, which have not been allowed to equilibrate with
the ambient conditions and are isotopically depleted.

Variations in coral skeletal growth parameters (skeletal density, extension, and
calcification rate) are possible sources of deviation from oxygen and carbon isotope
fractionation, which affect the external controls of the isotopes (Allison et al., 1996; Lough et
al., 1996; Barnes et al., 1995; Cohen and Hart, 1997). Skeletal growth parameters in coral
have sex-based differences in some gonochoric species (Cabral-Tena et al., 2013; Carricart-
Ganivet et al., 2013), so it is possible for the sex of a coral colony to be another cause of
deviation in oxygen and carbon isotope fractionation. The influence of metabolic effects, such
as reproduction, is another factor affecting the $\delta^{18}$O and $\delta^{13}$C signal in skeletons (Kramer et
al., 1993; Gagan et al., 1994; Barnes et al., 1995; Taylor et al., 1995; Allison et al., 1996;
Cohen and Hart, 1997; Lough et al., 1996; Swart et al., 1996b).

The stony coral *Porites panamensis* has a wide distribution along the eastern tropical
Pacific, from Mexico to Ecuador, and tolerates a wide range of environmental conditions,
including low temperature and high-turbidity that are often stressful to other coral species (Halfar et al., 2005; Reyes-Bonilla et al., 2007). This coral has extension rates ranging from 0.4 to 1.2 cm yr\(^{-1}\), along the coast of Mexico and Costa Rica (Guzmán and Cortés, 1989; Halfar et al., 2005; Cabral-Tena et al., 2013), where extension and calcification rates are different in males and females (Cabral-Tena et al., 2013). \textit{P. panamensis} is a gonochoric brooder with reproductive activity throughout the year (Glynn et al., 1994; Carpizo-Ituarte et al., 2011; Rodríguez-Troncoso et al., 2011).

This study describes changes in the skeletal isotopic oxygen and carbon record of six male and four female \textit{P. panamensis} coral, collected in Bahía de La Paz, with growth density banding covering 12 years. Oxygen and carbon isotope recording was used to assess a possible sex-associated variation in the coral skeletal \(\delta^{18}O\) and \(\delta^{13}C\) signal related to differences in the "vital effect" of colonies between sexes. The isotopic record was compared to surface seawater temperature (SST), rainfall, photosynthetically active radiation (PAR), concentration of chlorophyll \(a\), and skeletal growth data.

2 Materials and methods

2.1 Collection and identification of gender

Ten colonies of \textit{Porites panamensis} were collected in Bahía de La Paz (Fig. 1; 24°N, 110°W) during the main reproductive period (March) of this genus (Glynn et al., 1994; Carpizo-Ituarte et al., 2011; Rodriguez-Troncoso et al., 2011). The specimens were collected in 2011 at depths of 3–4 m. Divers used hammer and chisel to remove the colonies from the substrate. A fragment from each colony was fixed in Davison’s solution for a histological examination and identification of sex (Howard and Smith, 1983). These are the same ten colonies presented in the Cabral-Tena et al. (2013) study.

Coral fragments were first decalcified for 24 h in a solution containing 10% HCl, 0.7 g EDTA, 0.008 g sodium potassium tartrate, and 0.14 g sodium tartrate in 1 liter of distilled water (Glynn et al., 1994). The tissue was then rinsed under running water until free of acid, and placed in 70% ethanol until processed by conventional histological techniques (Humason, 1979). Transverse 8 µm sections were prepared with a rotator manual microtome, and stained with hematoxylin and eosin. After staining, the samples were studied under a compound microscope. The colonies were labeled as female if any planulae or oocytes
were observed, regardless of their stage of development; the colonies were labeled as male if any spermatocytes were observed in the slide section.

2.2 Growth parameters

From each colony, three slices (7–8 mm thick) were cut along the major growth axis. Slices were air-dried and X-rayed with a digital mammograph machine (Senographe 600T, GE Healthcare, Little Chafont, UK). Images were made at 36 kVp for 980 mAs and 30 cm source-to-subject distance. X-ray films were digitized with a Kodak DirectView Classic CR System, at 75 dpi resolution. An aragonite step-wedge was included on each X-radiograph as a reference for calculating skeletal density. The step-wedge was built from eight blocks cut from a shell of *Tridacna maxima*; each block had an area of 2.5 cm² and varied in thickness from 0.09 to 1.18 cm. Optical density tracks were placed located on the maximum growth axis in the digital X-radiography of each slice; density was measured using the ImageJ 1.44 image processing program (http://imagej.nih.gov/ij). A data series of absolute density versus distance was generated and dated backwards for each slice, using photodensitometry (Carricart-Ganivet and Barnes, 2007). The coral year starts in the summer, with the highest SST at the sampling site (Hudson et al., 1976). The maximum and minimum density for each year (1993 through 2009) were identified in each density series.

2.3 Isotope analysis

After the skeletal growth analysis, one slice covering the most extensive chronological extension of each of the ten colonies was selected for isotope analysis. 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 ~1 mm apart. The milling process was done by hand milling.

Aragonite powder was analyzed using an isotope ratio mass spectrometer (Delta V Plus, Thermo Scientific, Waltham, MA) with an automated system for carbon analysis in an acid bath (Finnigan Gas Bench II, Thermo Electron, Madison, WI). Each isotope sample had <0.05‰ error. Reference NBS-19 (International Atomic Energy Agency, Vienna, Austria)
was used as the isotope standard. The seasonal pattern of δ¹⁸O was used to establish chronology. This is supported by the consistent pattern of annual density-band pairs described for *Porites* by Lough and Barnes (2000). Chronologies were established by designating the minimum δ¹⁸O value in a year equal to summer (consistent with maximum SST). To eliminate the effects of different sampling resolutions on the calculation of mean coral δ¹⁸O values due to differences in linear extension rates of each colony, the results were interpolated to create four equally spaced values per year. Normality and homoscedasticity of the data were tested using Kolmogorov–Smirnov and Bartlett tests, respectively. Student’s *t* test for independent samples with uneven variance was used to assess statistical differences in δ¹⁸O and δ¹³C between sexes. Pearson’s correlation test and simple linear regressions were used to estimate relationships between mean skeletal extension rate, skeletal density, and calcification rate with isotope data of both sexes. An ANCOVA test was used to assess the differences between slopes and the y-intercept of linear equations of δ¹³C versus δ¹⁸O plots of the results of male and female data.

Heikoop et al. (2000) correction factor was applied 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.

2.4 Environmental data

Monthly SST, PAR, and concentration of chlorophyll *a* data were obtained from the NOAA live access server (http://las.pfeg.noaa.gov/oceanWatch/oceanwatch.php), the environmental data spanned from 1997 to 2009, and *in situ* thermograph temperature data (2003–2007) from the Marine Observatory for the Mexican Pacific region (Sicard-González et al., 2012). This information was used to compare satellite and *in situ* temperature data. Compared *in situ* and satellite data were both monthly covering from 2003 to 2007. Both temperature records (satellite and *in situ* measurements) from Bahía de La Paz showed the same seasonal signal and a close fit (*r* = 0.90, *p* < 0.05). This result supports the use of satellite SST data for coral skeletal δ¹⁸O calibration. Monthly rainfall data (1997–2009) were obtained from the Servicio Meteorológico Nacional (http://smn.cna.gob.mx/). Some sea surface salinity data was obtained from previous published data in the study area (Obeso-Niebla, 2007). δ¹⁸O<sub>sw</sub> was
calculated from the δ\textsuperscript{18}O relationship with the salinity equation for the Eastern Pacific (Fairbanks et al., 1997).

### 2.5 Statistical analyses

Normality and homoscedasticity of the data were tested using Kolmogorov–Smirnov and Bartlett tests, respectively. Student’s t-test for independent samples with uneven variance was used to assess statistical differences in δ\textsuperscript{18}O and δ\textsuperscript{13}C between sexes and to compare both sets of means obtained using the Heikoop et al. (2000) correction factor (kinetic and metabolic δ\textsuperscript{13}C). Pearson’s correlation test and simple linear regressions were used to estimate relationships between mean skeletal extension rate, skeletal density, and calcification rate with isotope data of both sexes. An ANCOVA test was used to assess the differences between slopes and the y-intercept of linear equations of δ\textsuperscript{13}C versus δ\textsuperscript{18}O plots of the results of male and female data.

Pearson’s correlation test and simple linear regressions were used to estimate relationships between environmental data and isotope data of both sexes. Regime shift index for environmental and isotope data were calculated with the Sequential Regime Shift Detection Software (Rodionov, 2004).

### 3 Results

#### 3.1 Skeletal growth

All specimens were collected in March, a period of low SST in Bahía de La Paz. All X-radiographs had a low-density annual growth band in the apex of the slice. This means that \textit{P. panamensis} form a low-density band in winter. Annual growth bands in each colony were dated and the sampling resolution for isotope analysis was determined.

The average yearly extension rate was 1.05 ± 0.04 cm yr\textsuperscript{-1} for female colonies, and 1.27 ± 0.04 cm yr\textsuperscript{-1} for male colonies. The average skeletal density was 0.94 ± 0.01 g cm\textsuperscript{-3} for females, and 0.95 ± 0.01 g cm\textsuperscript{-3} for males. The average calcification rate was 0.97 ± 0.04 g cm\textsuperscript{-2} yr\textsuperscript{-1} for females, and 1.24 ± 0.03 g cm\textsuperscript{-2} yr\textsuperscript{-1} for males. Figure 2 shows X-ray photographs and of skeletal density along the growth axis.
3.2 Skeletal isotope composition and environmental data

The δ¹⁸O quarterly records of female and male coral colonies show a seasonal pattern (Fig. 43) that was strongly significantly correlated between sexes (r = 0.45, p > 0.000001), thus both sexes showed the same seasonal pattern. δ¹⁸O in female colonies, was higher than in male colonies (Fig. 34). The overall average δ¹⁸O in female colonies was –2.89 ± 0.33‰, and –3.20 ± 0.37‰ in male colonies (Table 1). Overall, the δ¹⁸O average of females is significantly higher than that of males (t₀₉₈ = 9.34, p > 0.000001). Quarterly δ¹⁸O time series data of all colonies showed a “regime shift” of the mean in 2004, from –2.75 to –3.14‰, with a regime shift index (RSI) of –0.69 (p = 0.008) in female colonies, and from –3.08 to –2.42‰ with a RSI of –0.65 (p = 0.003) in male colonies. This coincides with a regime shift in the rainfall mean of 2003, changing from 15.76 to 30.25 mm, with a RSI of 0.30 (p = 0.01), as seen in Figure 3b.

The quarterly δ¹³C time series showed a cyclic pattern in female and male colonies (Fig. 42), that was correlated between both genders sexes (r = 0.19, p = 0.005), thus both sexes showed the same seasonal pattern. The skeletal δ¹³C of female colonies was lower than the skeletal δ¹³C of male colonies (Fig. 42). The overall average of δ¹³C in female colonies was –1.66 ± 0.38‰, and –1.38 ± 0.37‰ in male colonies (Table 1). The overall average of δ¹³C in females is significantly lower than in males (t₀₉₈ = –8.01, p > 0.000001). No regime shift was found in the δ¹³C data of either sex.

The δ¹⁸O skeletal data series corresponds to the SST (Fig. 34). Table 2 shows correlation coefficients between the δ¹⁸O isotope data of coral colonies and environmental variables. The correlation coefficient between the isotope average time series data and SST was –0.45 (p = 0.000003) for female colonies, and –0.32 (p = 0.0005) for male colonies; the r-to-Z transformation showed that both correlation coefficients are equally strong significant (Z = –1469; p = 0.07). No significant correlation was found between the δ¹⁸O skeletal data sets and the rainfall data. The δ¹³C skeletal data series did not significantly correlate with any of the environmental data variables in any of the colonies (Table 3). The temporal resolution of compared data (isotopes vs. environmental data) is quarterly in all cases.

The relationship between δ¹⁸O and satellite-derived SST for 13 years (1997-2009) was calibrated. The linear regression (Fig. 3) equations for δ¹⁸O dependence on temperature were:

\[
\text{SST} = 7.0889 - 5.7193(\delta^{18}O), \quad (s^2 = 0.23, \quad p = 0.000001) \text{ for female coral, and }
\]

\[
\text{SST} = 7.1008 - 5.7195(\delta^{18}O), \quad (s^2 = 0.23, \quad p = 0.000001) \text{ for male coral.}
\]
SST = 14.739 – 2.9246 (δ^{18}O) + 0.10; p = 0.00007) for male coral.

The annual range of δ^{18}O was the difference between the highest δ^{18}O measurement in January–March, and the lowest in July–September (1997–2008). The colonies had a small seasonal variation. The average amplitude was 0.37 ± 0.15‰ in female colonies, and 0.28 ± 0.72‰ in male colonies. Satellite data of SSTs had an average amplitude cycle of 7.35 ± 0.72°C, and rainfall had an average annual amplitude of 1.11 °C in female colonies, and 2.80 °C in male colonies. This is 52.37% in female colonies, and 35.66% in male colonies of the seasonal range of the SST. The expected variation of approximately 0.11‰ of δ^{18}O in seawater (0.43 psu) is 29.72% in female colonies, and 38.53% in male colonies of the average seasonal variation in δ^{18}O.

Heikoop et al. (2000) correction factor results are shown in Table 4. The overall average of δ^{13}C in female colonies was –1.66 ± 0.38‰, and –1.38 ± 0.37‰ in male colonies. Student’s T test showed that both sets of means (kinetic and metabolic) are significantly different between male and female colonies (t_{498} = 13.074 p< 0.000001 for Kinetic means; t_{498} = –13.98 p< 0.000001 Metabolic means).

3.3 Skeletal isotopic composition and skeletal growth

The analysis showed that high density bands are depleted in δ^{18}O and δ^{13}C, which are deposited during summer; low density bands are enriched in δ^{18}O and δ^{13}C, which are deposited during winter. In female colonies, a strong significant negative correlation between the mean annual
coral $\delta^{18}$O and annual skeletal density was found (Table 54; $r = -0.78$, $p = 0.001$) (Table 4). This suggests that denser skeletons are more depleted in $\delta^{18}$O, compared to less dense skeletons, and no significant correlation was found between $\delta^{18}$O and other skeletal growth parameters in female colonies; no significant correlations between mean annual coral $\delta^{13}$C and any growth parameters were found. In male colonies, there was a strong significant negative correlation between mean annual coral $\delta^{18}$O and the annual linear extension and calcification rates (Table 54; $r = -0.50$ and $-0.44$, $p = 0.045$ and 0.0008). This suggests that faster growing and calcifying colonies are more depleted in $\delta^{18}$O. No significant correlation was found between $\delta^{18}$O and skeletal density in male colonies; no significant correlation between any coral growth parameter and mean annual coral $\delta^{13}$C was found.

4 Discussion

Our isotope data showed a significant dependency of skeletal $\delta^{18}$O on SST, with a low $r$ (–0.45 in female coral, and –0.28 in male coral), and a gentle slope of the $\delta^{18}$O–SST calibration equations (0.09‰ °C$^{-1}$ F; 0.11‰ °C$^{-1}$ M; Fig. 25), compared with slopes (>0.20‰ °C$^{-1}$) in *Porites* spp. in other areas of the Pacific: the Great Barrier Reef (Gagan et al., 1994), Costa Rica (Carriquiry, 1994), Panama (Wellington and Dunbar, 1995), and the Galapagos Archipelago (McConnaughey, 1989). These studies show high correlation coefficients (better than –0.80) of $\delta^{18}$O and SST, all these studies have isotopic records varying two to 40 years long, and with a high temporal resolution sampling (weekly to monthly). Our results are similar to studies reporting small correlation coefficients of $\delta^{18}$O and SST (less than –0.70) and a gentle slope (<0.17‰ °C$^{-1}$) of the $\delta^{18}$O–SST calibration equations, such as at Clipperton Atoll (Linsley et al., 1999), Fiji (Le Bec et al., 2000), and Guam (Asami et al., 2004). These studies have long isotopic records (20 to 25 years) and a high temporal resolution sampling (daily to monthly) compared to our data (12 years of data with a quarterly sampling resolution).

Asami et al. (2004) suggest that the low correlation coefficient between $\delta^{18}$O and SST, and the gentle slope in the $\delta^{18}$O–SST calibration equations are related to small seasonal variations in SST (<3 °C), or the greater influence of $\delta^{18}$O$. \}$ The seasonal variation in SST of our study area is 7.85 ± 0.77 °C, and the variation in $\delta^{18}$O accounts for only 52.37% in female coral, and 35.66% in male coral, of the seasonal range, so the seasonal variation of SST is not likely to be the cause. Variations in $\delta^{18}$O$_sw$ represent 29.72% in female coral, and 38.53% in
male coral, of the average seasonal $\delta^{18}O$ variation. We found a significant regime shift ($p < 0.01$) in the $\delta^{18}O$ data of colonies of both genders, that coincides with a regime shift ($p = 0.01$) in rainfall (which changes the $\delta^{18}O_{sw}$). We think that a greater influence of $\delta^{18}O_{sw}$ is the most likely source of our findings. This means that the $\delta^{18}O$ of coral in Bahía de La Paz is influenced more by the $\delta^{18}O_{sw}$ than in other places in the Pacific.

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The linear regression (Fig. 5) equations for $\delta^{18}O$ dependence on SST (1997-2009) were:

- For female coral: $\text{SST} = 7.0889 - 5.7193 (\delta^{18}O)$, ($r^2 = 0.23$, $p = 0.00003$)
- For male coral: $\text{SST} = 14.739 - 2.9246 (\delta^{18}O)$ ($r^2 = 0.10$, $p = 0.00007$)

The annual range of $\delta^{18}O$ was the difference between the highest $\delta^{18}O$ measurement in January–March, and the lowest in July–September (1997–2008). The average amplitude was 0.37 ± 0.15‰ in female colonies, and 0.28 ± 0.72‰ in male colonies. Satellite data of SSTs had an average amplitude cycle of 7.85 ± 0.77 °C, and rainfall had an average annual amplitude of 3.55 ± 16.07 mm. Using the calculated gradients of 0.09‰ °C$^{-1}$ for female colonies, and 0.10‰ °C$^{-1}$ for male colonies, the average seasonal variation of $\delta^{18}O$ would reflect a temperature change of 4.11 °C in female colonies, and 2.80 °C in male colonies. This is 52.37% in female colonies, and 35.66% in male colonies of the seasonal range of the SST.

The expected seasonal variation of approximately 0.11‰ of $\delta^{18}O$ in seawater (0.43 psu) represents 29.72% of $\delta^{18}O$ seasonal variation in female colonies, and 38.53% in male colonies.

The departure from isotope equilibrium of our samples was estimated with the equations by Grossman and Ku (1986), for $\delta^{18}O$, and Romanek et al. (1992) for $\delta^{13}C$. We found that the theoretical $\delta^{18}O$ value of coral aragonite that precipitates at equilibrium with
seawater is –0.65‰, which means that our samples of coral have an average departure from isotope equilibrium of ~3.54‰ in females, and ~3.80‰ in males. For δ¹³C, we found a theoretical value of –1.15‰ for coral aragonite that precipitates at equilibrium with seawater. This means that average departure from isotope equilibrium is ~2.81‰ in females, and ~2.53‰ in males.

We found a positive relationship between skeletal δ¹⁸O and δ¹³C in our data, where r = 0.42 in females, and r = 0.58 in males. 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). When the SST peaks in the summer and surface seawater generally becomes depleted in nutrients, zooxanthellae disperse (Hoegh-Guldberg, 1999; Barton and Casey, 2005). 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 in both genders 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 regressions and correlations were not significant, meaning that photosynthesis was not stimulated or inhibited by light, and remained near its maximum efficiency during the whole year, according to Sun et al. (2008), in Porites in southern China. They suggest that 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 relative enrichment of ¹⁴C in the coral skeleton (November through January), when fixation by algae of the
isotopically lighter carbon enriches δ^{13}C in coral skeletons (Allison et al., 1996); however, the correlations of skeletal δ^{13}C and chlorophyll a were not significant in any case.

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Trends in coral skeletal δ^{13}C reflect seasonal variations in metabolic effects, that is, modifications of photosynthesis to respiration ratios in the δ^{13}C pool of coral. Higher coral respiration reduces coral δ^{13}C (McConnaughey, 1989; McConnaughey et al., 1997).

Respiration normally increases with temperature and lowers δ^{13}C in coral skeletons, which is reflected in our results, high SST = low δ^{13}C. No other environmental variables considered in this work explained this pattern in coral δ^{13}C, 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 δ^{18}O and the skeletal density in female colonies, i.e. More dense skeletons are depleted in δ^{18}O. This is not consistent with studies that have observed that coral skeletal high-density bands are enriched in δ^{18}O (Klein et al., 1992; Al-Rousand, 2007). This may be due to a difference in timing of skeletal density bands in different Porites coral species, as described by Lough and Barnes.
In male coral, we found a negative correlation between the $\delta^{18}O$ and linear extension and calcification rates ($r = -0.50$, $p = 0.045$, and $r = -0.44$, $p = 0.0008$), meaning that the faster a colony grows and calcifies, the more it is depleted in $\delta^{18}O$. This is consistent with the observations of other authors of *Porites* spp. coral (McConnaughey, 1989; Felis et al., 2003). In *Porites* corals, SST is a dominating control of variations in growth parameters and of $\delta^{18}O$; the skeletal extension and calcification rate increases with SST, while skeletal density decreases (Lough and Barnes, 2000), so the growth parameters of both sexes and $\delta^{18}O$ behave as expected; that is, an increase in SST = a decrease in density = $\delta^{18}O$ enrichment in females, and an increase in SST = an increase in extension and calcification rate = $\delta^{18}O$ enrichment in males. No significant correlation was found between skeletal $\delta^{13}C$ 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 $\delta^{13}C$, 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.

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General consensus states that all coral skeletons contain appreciable amounts of carbon and oxygen in isotopic disequilibrium, and are depleted in $\delta^{18}O$ and $\delta^{13}C$ because of
kinetic variations due to differences in coral growth. Larger isotopic disequilibrium occurs when coral grows faster (Land et al., 1975; McConnaughey, 1989; Aharon, 1991). McConnaughey (1989) named this phenomenon “Vital effect”. We found this to be true for all-sampled coral (disequilibrium = 2.54‰ 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. 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 = 2.54‰ 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 can be considered as fast (1.05 cm yr⁻¹ F, and 1.27 cm yr⁻¹ M) in accordance with the work of McConnaughey (1989). Thus, we assume kinetic disequilibrium is constant in all coral.

All δ¹⁸O ratios of female colonies are more enriched in ¹⁸O than in male colonies, with an average difference of ~0.31‰ (female average minus male average). Female δ¹³C values were lower than the δ¹³C of male colonies, with an average difference of ~0.28‰. All coral colonies in our study grew and calcified in the same environmental conditions (SST, δ¹⁸O, PAR, Chlorophyll a, etc.). 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 δ\(^{18}\)O records of six *Porites* lobata coral living in nearly identical environments (2 km of each other), in the Clipperton atoll. Felis et al. (2003) found a 1.28‰ difference in the δ\(^{18}\)O records of 11 coral of several *Porites* species (not detailed by the authors), 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 δ\(^{18}\)O record, and 0.29‰ in the δ\(^{13}\)C record (similar to the observations of Felis et al., 2003). If we split our data by sex, the differences in the isotopic records drop to 0.07‰ in the δ\(^{18}\)O, and to 0.02‰ in the δ\(^{13}\)C. In our data, the sex of the colony explains 81% (δ\(^{18}\)O) and 93% (δ\(^{13}\)C) 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 with 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 ± 0.04 cm yr\(^{-1}\) vs. 1.27 ± 0.04 cm yr\(^{-1}\)). Faster growing coral are more depleted in \(^{18}\)O and more enriched in \(^{13}\)C, relative to slower growing.
coral (McConnaughey, 1989; Felis et al., 2003), this may be the origin of the isotope data difference between sexes (higher δ\(^{18}\)O and lower δ\(^{13}\)C 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 δ\(^{18}\)O and δ\(^{13}\)C between sexes.

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A more complex explanation for this sex-associated difference in coral isotopic data could result from the role Ca-ATPase (enzyme strongly associated with coral calcification) activity has in the mechanism of the "vital effect". Adkins et al. (2003), and Rollion-Bard et al. (2003) found that the Ca-ATPase activity in deep sea and symbiotic coral establishes a pH gradient between the coral cell wall and the extracellular calcifying fluid (ECF). The pH gradient (more basic in the ECF) promotes a passive CO\(_2\) flux into the ECF and controls the mixing of carbon with isotopically heavier signature from the seawater-dissolved inorganic carbon, thus, the intense activity of Ca-ATPase will result in a carbon heavier skeleton. Oxygen isotopes also respond to the pH of the ECF, proportions of the dissolved carbonate species are pH dependent. At low pH the dominant species is H\(_2\)CO\(_3\), at intermediate pH it is HCO\(_3^\) and at high pH, CO\(_3^{2-}\) is the dominant species. McCrea (1950) demonstrated that the δ\(^{18}\)O of carbonates is related to the proportion of HCO\(_3^\) and CO\(_3^{2-}\) in the solution (CO\(_3^{2-}\) is isotopically lighter). Thus, pH controls the relative fractions of dissolved HCO\(_3^\) and CO\(_3^{2-}\) in the ECF and the kinetics of their isotopic equilibration with water, before carbonate precipitation. An intense activity of Ca-ATPase will result in oxygen lighter skeletons. According to this theory, a higher activity of the Ca-ATPase enzyme will result in carbon
heavier skeletons and oxygen lighter skeletons. Cohen and Holcomb (2009) mention that the activity of ATPase depends on the amount of energy available for the calcification for coral. Cabral-Tena et al. (2013) suggest it is possible that male *P. panamensis* have more available energy for calcification, which would mean males have a higher activity of the Ca-ATPase, which results in enriched C$^{13}$ and depleted O$^{18}$ skeletons, in comparison to female skeletons, as seen in our data ($-1.66\%$ F vs. $-1.38\%$ M $\delta^{13}C$, $-2.89\%$ F vs. $-3.20\%$ M $\delta^{18}O$). This complex mechanism of the origin of the “Vital effect” might explain why we found a sex-associated variation in coral skeletal oxygen and carbon isotopic composition of *Porites panamensis*.

Kramer et al. (1993), and Gagan et al. (1994) suggested that energy expenditure during the formation of gametes may cause differences in the isotopic depletion in coral skeletons; Kramer et al. (1993) observed depletions in isotope data during reproductive seasons, regardless of the sex of the coral, and found minimum $\delta^{13}C$ values in skeletons of *Oribicella faveolata* during spawning seasons (summer), although this phenomenon was also observed in other coral species which produce gametes the whole year (*O. faveolata* has only one reproductive event per year). The results obtained by Kramer et al. (1993) were inconclusive, but suggested a lag effect of isotope signal, associated with the initiation and duration of the reproductive cycle. It is possible that the sex-associated variation we found in isotope data is due to the reproductive strategy of *P. panamensis*. *P. panamensis* is a gonochoric brooding species with reproductive and larval release events through the whole year in the Pacific coast of Mexico (Carpizo-Ituarte et al., 2011; Rodriguez-Troncoso et al., 2011). Energy costs of reproduction in gonochoric spawners are lower than in gonochoric brooding species where energy is required not only for egg production, but also for larval development (Szmant, 1986). This implies that there should be sex-associated variations in the coral skeletal isotope data of other gonochoric brooding coral, as some massive *Porites* (which can be spawners or brooders; Glynn et al., 1994; Baird et al., 2009).

We found some interesting results when applying the Heikoop et al. (2000) correction factor to isolate the kinetic and metabolic effects in the $\delta^{13}C$ of male and female colonies, both transformed $\delta^{13}C$ and metabolic $\delta^{13}C$ seem to be higher in males, thus supporting the 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.

Considering δ¹⁸O of coral skeletons is used to estimate SST in different sites and conditions, the next part of the discussion seeks to exemplify what would a difference in δ¹⁸O between sexes would represent in terms of errors in SST estimation. Using the widely accepted paleotemperature equations for calcite (Epstein et al., 1953) and aragonite (Grossman and Ku, 1986), a ~0.31‰ difference between sexes would represent an error in SST estimates of ~1.47 °C and ~1.33 °C. Using accepted SST–coral δ¹⁸O relationships from different regions of the Pacific, derived from Porites spp., the δ¹⁸O difference between sexes would represent an error of ~1.75 °C (Red Sea; Al-Rousand et al., 2003), ~1.71 °C (Great Barrier Reef; Gagan et al., 1994), ~1.31 °C (Costa Rica; Carriquiry, 1994), ~1.39 °C (Central and Eastern Tropical Pacific; Druffel, 1985), ~1.47 °C (The Galapagos; McConnaughey, 1989), and ~1.47 °C in SST estimates, for the commonly admitted paleotemperature calibration in coral (0.21‰ per °C).

δ¹³C of coral skeletons has been used as a proxy for the photosynthetic activity of zooxanthellae (mainly driven by light). Until now, no general rule applies to how much δ¹³C means how much radiance (like the dependence of δ¹⁸O to SST resulting in paleotemperature equations), but a difference of ~0.28‰ in coral δ¹³C between sexes should be taken into account for this kind of applications, since it may influence the descriptions of the variability in δ¹³C of coral skeletons. δ¹³C of coral skeletons is also used to correct the δ¹⁸O data when estimating the SST at which coral grew, by using the regression line equations obtained from the δ¹³C vs. δ¹⁸O plots (Smith et al., 2000). When we compared the regression line equations obtained from the δ¹³C vs. δ¹⁸O plots of both sexes, the ANCOVA showed that both the slope (F₄₉₈ = 9.619, p=0.002) and the y-intercept (F₄₉₈ = 222.5, p<0.00001) are different between equations (fig 64). Also, Fisher’s r-to-z transformation (z=–2.34, p=0.01) showed that the δ¹³C vs. δ¹⁸O correlation coefficients are significantly different between sexes, i.e. the relationship in δ¹³C vs. δ¹⁸O is different in both sexes; this has important implications because it could add a variability source to the use of the δ¹³C vs. δ¹⁸O regression line as corrector for δ¹⁸O data, if the sex of the colony is not taken into account in the analysis.

This study provides evidence of sex-associated variations in coral skeletal δ¹⁸O and δ¹³C of P. panamensis. This has some implications and has to be considered when climate
conditions are estimated based on comparisons of δ¹⁸O and δ¹³C values of gonochoric coral genera, if sex identification is not taken into account when possible. This study provides evidence of sex-associated variations in coral skeletal δ¹⁸O and δ¹³C of *P. panamensis*. This has some implications and has to be considered when climate conditions are estimated based on comparisons of δ¹⁸O and δ¹³C values of gonochoric brooder coral genera, if sex identification is not taken into account when possible. The findings of this study are based on a gonochoric brooder species (*P. panamensis*), while the majority of paleoclimatic reconstructions in the Indo-Pacific and Caribbean have been based on massive gonochoric spawners (such as *Montastrea cavernosa*, *Porites lutea* and *Porites lobata*), so, it remains unclear if the same phenomena (sex-associated variations in coral skeletal δ¹⁸O and δ¹³C) can be observed in gonochoric spawners. This may have some serious implications in the paleoclimatic reconstructions studies made so far leading to erroneous conclusions due to errors in isotopic estimation; variability of isotopic data may have been overestimated due to the mixing of male and female isotopic data in past studies. Thus, a fruitful area of future research would be to determine whether the sex differences identified in this study are also characteristic of gonochoric spawners.

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**Author contributions**

RACT and EFB conceived and designed the study; RACT, AHRD and AS processed isotopically the material. RACT, AS, HRB and EFB analyzed the data. All authors discussed the results and wrote the manuscript.
<table>
<thead>
<tr>
<th>Colony</th>
<th>Sex</th>
<th>Avg Ext (cm yr⁻¹)</th>
<th>Avg Den (g cm⁻³)</th>
<th>Avg Cal (g cm⁻² yr⁻¹)</th>
<th>Avg δ¹⁸O (‰)</th>
<th>Avg δ¹³C (‰)</th>
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<tr>
<td>BLP32</td>
<td>F</td>
<td>1.06 ± 0.32</td>
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<td>-2.94 ± 0.35</td>
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<td>-1.67 ± 0.38</td>
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<td>F</td>
<td>1.10 ± 0.19</td>
<td>0.94 ± 0.02</td>
<td>1.03 ± 0.17</td>
<td>-2.87 ± 0.31</td>
<td>-1.66 ± 0.39</td>
</tr>
<tr>
<td>BLP31</td>
<td>M</td>
<td>1.21 ± 0.61</td>
<td>0.90 ± 0.08</td>
<td>1.21 ± 0.44</td>
<td>-3.19 ± 0.38</td>
<td>-1.39 ± 0.37</td>
</tr>
<tr>
<td>BLP34</td>
<td>M</td>
<td>1.35 ± 0.30</td>
<td>0.98 ± 0.04</td>
<td>1.33 ± 0.29</td>
<td>-3.25 ± 0.38</td>
<td>-1.37 ± 0.37</td>
</tr>
<tr>
<td>BLP35</td>
<td>M</td>
<td>1.59 ± 0.31</td>
<td>0.95 ± 0.01</td>
<td>1.61 ± 0.28</td>
<td>-3.19 ± 0.37</td>
<td>-1.39 ± 0.37</td>
</tr>
<tr>
<td>BLP37</td>
<td>M</td>
<td>1.28 ± 0.34</td>
<td>0.96 ± 0.03</td>
<td>1.23 ± 0.34</td>
<td>-3.21 ± 0.39</td>
<td>-1.39 ± 0.38</td>
</tr>
<tr>
<td>BLP38</td>
<td>M</td>
<td>0.83 ± 0.36</td>
<td>0.88 ± 0.02</td>
<td>0.75 ± 0.33</td>
<td>-3.19 ± 0.37</td>
<td>-1.39 ± 0.38</td>
</tr>
<tr>
<td>BLP39</td>
<td>M</td>
<td>1.39 ± 0.40</td>
<td>1.00 ± 0.02</td>
<td>1.40 ± 0.40</td>
<td>-3.18 ± 0.37</td>
<td>-1.38 ± 0.37</td>
</tr>
<tr>
<td>Avg F</td>
<td>F</td>
<td>1.05 ± 0.04</td>
<td>0.94 ± 0.01</td>
<td>0.97 ± 0.04</td>
<td>-2.89 ± 0.33</td>
<td>-1.66 ± 0.38</td>
</tr>
<tr>
<td>Avg M</td>
<td>M</td>
<td>1.27 ± 0.04</td>
<td>0.95 ± 0.01</td>
<td>1.24 ± 0.03</td>
<td>-3.20 ± 0.37</td>
<td>-1.38 ± 0.37</td>
</tr>
</tbody>
</table>

Table 1. Summary of the overall average extension rate, skeletal density, calcification rate, δ¹⁸O and δ¹³C of *Porites panamensis* colonies from Bahía de La Paz, Gulf of California. The period of data is from 1997 to 2009.
Table 2. Correlation coefficients between skeletal $\delta^{18}$O of *Porites panamensis* colonies and:

Sea surface temperature, precipitation, photosynthetically active radiation and chlorophyll $a$ from Bahía de La Paz. **Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is quarterly.** **Bold** numbers indicate significant ($p < 0.05$) correlations.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Sex</th>
<th>SST r</th>
<th>SST p</th>
<th>Precipitation r</th>
<th>Precipitation p</th>
<th>PAR r</th>
<th>PAR p</th>
<th>Chlorophyll $a$ r</th>
<th>Chlorophyll $a$ p</th>
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</thead>
<tbody>
<tr>
<td>BLP32</td>
<td>F</td>
<td>-0.36</td>
<td>0.007</td>
<td>0.10</td>
<td>0.44</td>
<td>-0.41</td>
<td>0.02</td>
<td>-0.08</td>
<td>0.55</td>
</tr>
<tr>
<td>BLP33</td>
<td>F</td>
<td>-0.35</td>
<td>0.01</td>
<td>0.07</td>
<td>0.58</td>
<td>-0.40</td>
<td>0.03</td>
<td>-0.11</td>
<td>0.44</td>
</tr>
<tr>
<td>BLP36</td>
<td>F</td>
<td>-0.37</td>
<td>0.006</td>
<td>0.08</td>
<td>0.55</td>
<td>-0.42</td>
<td>0.02</td>
<td>-0.11</td>
<td>0.42</td>
</tr>
<tr>
<td>BLP40</td>
<td>F</td>
<td>-0.38</td>
<td>0.006</td>
<td>0.08</td>
<td>0.54</td>
<td>-0.41</td>
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<tr>
<td>BLP31</td>
<td>M</td>
<td>-0.28</td>
<td>0.04</td>
<td>0.05</td>
<td>0.68</td>
<td>-0.36</td>
<td>0.05</td>
<td>-0.06</td>
<td>0.64</td>
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<tr>
<td>BLP34</td>
<td>M</td>
<td>-0.26</td>
<td>0.06</td>
<td>0.06</td>
<td>0.65</td>
<td>-0.31</td>
<td>0.09</td>
<td>-0.08</td>
<td>0.53</td>
</tr>
<tr>
<td>BLP35</td>
<td>M</td>
<td>-0.29</td>
<td>0.03</td>
<td>0.06</td>
<td>0.67</td>
<td>-0.36</td>
<td>0.05</td>
<td>-0.06</td>
<td>0.65</td>
</tr>
<tr>
<td>BLP37</td>
<td>M</td>
<td>-0.28</td>
<td>0.04</td>
<td>0.06</td>
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<td>-0.34</td>
<td>0.06</td>
<td>-0.07</td>
<td>0.60</td>
</tr>
<tr>
<td>BLP38</td>
<td>M</td>
<td>-0.29</td>
<td>0.03</td>
<td>0.06</td>
<td>0.67</td>
<td>-0.36</td>
<td>0.04</td>
<td>-0.05</td>
<td>0.68</td>
</tr>
<tr>
<td>BLP39</td>
<td>M</td>
<td>-0.28</td>
<td>0.04</td>
<td>0.05</td>
<td>0.69</td>
<td>-0.36</td>
<td>0.05</td>
<td>-0.06</td>
<td>0.64</td>
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</table>
Table 3. Correlation coefficients between skeletal δ¹³C of *Porites panamensis* colonies and:

Sea surface temperature, precipitation, photosynthetically active radiation and chlorophyll *a* from Bahía de La Paz. **Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is quarterly.** **Bold** numbers indicate significant (*p* < 0.05) correlations.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Sex</th>
<th>SST</th>
<th>Precipitation</th>
<th>PAR</th>
<th>Chlorophyll <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>BLP32</td>
<td>F</td>
<td>0.19</td>
<td>-0.07</td>
<td>0.62</td>
<td>0.10</td>
</tr>
<tr>
<td>BLP33</td>
<td>F</td>
<td>0.17</td>
<td>-0.04</td>
<td>0.73</td>
<td>0.11</td>
</tr>
<tr>
<td>BLP36</td>
<td>F</td>
<td>0.17</td>
<td>-0.06</td>
<td>0.63</td>
<td>0.09</td>
</tr>
<tr>
<td>BLP40</td>
<td>F</td>
<td>0.15</td>
<td>-0.07</td>
<td>0.62</td>
<td>0.08</td>
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<tr>
<td>BLP31</td>
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<td>-0.01</td>
<td>0.89</td>
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<td>BLP35</td>
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<td>-0.02</td>
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<tr>
<td>BLP37</td>
<td>M</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.93</td>
<td>0.08</td>
</tr>
<tr>
<td>BLP38</td>
<td>M</td>
<td>0.003</td>
<td>-0.01</td>
<td>0.93</td>
<td>0.09</td>
</tr>
<tr>
<td>BLP39</td>
<td>M</td>
<td>0.02</td>
<td>-0.02</td>
<td>0.88</td>
<td>0.24</td>
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</table>
Table 4. Heikoop et al. (2000) correction factor results comparing transformed and metabolic skeletal $\delta^{13}C$ of *Porites panamensis* colonies from Bahía de La Paz.

<table>
<thead>
<tr>
<th></th>
<th>Transformed $\delta^{13}C$ Females ($N=200$)</th>
<th>Transformed $\delta^{13}C$ Males ($N=300$)</th>
<th>Metabolic $\delta^{13}C$ Females ($N=200$)</th>
<th>Metabolic $\delta^{13}C$ Males ($N=300$)</th>
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<tbody>
<tr>
<td>Mean</td>
<td>5.082</td>
<td>6.30</td>
<td>6.23</td>
<td>7.43</td>
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<tr>
<td>SD</td>
<td>0.90</td>
<td>0.97</td>
<td>0.90</td>
<td>0.96</td>
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Table 5. Correlation coefficients between skeletal extension rate, skeletal density and calcification rate, and skeletal $\delta^{18}O$ and $\delta^{13}C$ of *Porites panamensis* colonies from Bahía de La Paz. Time period covered by correlations is from 1997 to 2009. Temporal resolution of data is annual. Bold numbers indicate significant ($p < 0.05$) correlations.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Sex</th>
<th>Ext vs $\delta^{18}O$</th>
<th>Den vs $\delta^{18}O$</th>
<th>Cal vs $\delta^{18}O$</th>
<th>Ext vs $\delta^{13}C$</th>
<th>Den vs $\delta^{13}C$</th>
<th>Cal vs $\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>BLP32</td>
<td>F</td>
<td>0.34</td>
<td>0.24</td>
<td>-0.81</td>
<td><strong>0.001</strong></td>
<td>0.31</td>
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<tr>
<td>BLP33</td>
<td>F</td>
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<td>0.22</td>
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<td>0.21</td>
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<td><strong>0.003</strong></td>
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<tr>
<td>BLP40</td>
<td>F</td>
<td>0.40</td>
<td>0.18</td>
<td>-0.73</td>
<td><strong>0.008</strong></td>
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<td>0.18</td>
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<tr>
<td>BLP31</td>
<td>M</td>
<td><strong>0.61</strong></td>
<td>0.018</td>
<td>-0.13</td>
<td>0.69</td>
<td><strong>-0.51</strong></td>
<td>0.008</td>
</tr>
<tr>
<td>BLP34</td>
<td>M</td>
<td><strong>0.62</strong></td>
<td>0.018</td>
<td>-0.19</td>
<td>0.53</td>
<td><strong>-0.54</strong></td>
<td>0.005</td>
</tr>
<tr>
<td>BLP35</td>
<td>M</td>
<td><strong>0.67</strong></td>
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<td><strong>-0.49</strong></td>
<td>0.011</td>
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<tr>
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<td>0.021</td>
<td>-0.20</td>
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<td><strong>-0.48</strong></td>
<td>0.019</td>
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<tr>
<td>BLP38</td>
<td>M</td>
<td><strong>0.60</strong></td>
<td>0.023</td>
<td>-0.15</td>
<td>0.58</td>
<td><strong>-0.47</strong></td>
<td>0.001</td>
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<tr>
<td>BLP39</td>
<td>M</td>
<td><strong>0.63</strong></td>
<td>0.011</td>
<td>-0.24</td>
<td>0.39</td>
<td><strong>-0.51</strong></td>
<td>0.008</td>
</tr>
</tbody>
</table>
**Figure Captions**

**Fig. 1.** Map showing Location of coral sampling site in Bahía de La Paz, México.

**Fig. 2.** Negative X-Radiographs showing skeletal growth density band pairs of two *Porites panamensis* corals (one male and one female) of Bahía de La Paz. The numbers on the image mark the years of the corresponding high density bands.

**Fig. 3.** (a) Seasonal variation in δ\(^{18}\)O composition (VPDB) from *Porites panamensis* coral colonies along the major growth axis. Blue lines represent male colonies; Red lines represent female colonies; red dotted line female colonies’ regime mean; blue dotted line, male colonies’ regime mean. (b) Satellite sea surface temperature and precipitation (1997–2009) records. Sea surface temperature (red line; °C), mean sea surface temperature (dotted red line; °C), precipitation (blue line; mm), mean precipitation (dotted blue line; mm). Note the regime shift in the precipitation mean in 2003.

**Fig. 4.** (a) Seasonal variation in δ\(^{13}\)C composition (VPDB) from *Porites panamensis* coral colonies along the major growth axis. Blue lines represent male colonies; Red lines represent female colonies; red dotted line female colonies’ regime mean; blue dotted line, male colonies’ regime mean. (b) Satellite chlorophyll \(a\) and PAR (1997–2009) records. Clorophyll \(a\) (red line; mg l\(^{-1}\)), mean chlorophyll \(a\) (dotted red line; mg l\(^{-1}\)), photosynthetically active radiation (blue line; E m\(^{-2}\) Day\(^{-1}\)), photosynthetically active radiation (dotted blue line; E m\(^{-2}\) Day\(^{-1}\)).

**Fig. 5.** Linear regressions between satellite derived sea surface temperature (°C) and skeletal δ\(^{18}\)O (VPDB) of female, and male *Porites panamensis* coral from Bahía de La Paz. Time period covered by analyses is from 1997 to 2009. Temporal resolution of data is quarterly. This includes all isotopic data of all colonies. Line equations and coefficients are shown.

**Fig. 6.** Plot of δ\(^{13}\)C vs. δ\(^{18}\)O of female (red dots), and male (blue dots) *Porites panamensis* coral from Bahía de La Paz. This includes all isotopic data of all colonies. Line equations and coefficients (red represents females; blue represents males) are shown.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6. Scatter plot showing the relationship between Coral $\delta^{18}O$ and Coral $\delta^{13}C$.

Red line:
- Equation: $y = 0.3629x - 2.2949$
- $r^2 = 0.1793$

Blue line:
- Equation: $y = 0.5891x - 2.387$
- $r^2 = 0.3417$