Sex-associated variations in coral skeletal oxygen and carbon isotopic composition of *Porites panamensis* in the southern Gulf of California

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**Abstract.** Coral $\delta^{18}O$ variations are used as a proxy for changes in sea surface temperature (SST) and seawater isotope composition. Skeletal $\delta^{13}C$ 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 $\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$ and $\delta^{13}C$ are hypothesized to be different in each sex. To test this, this study describes changes in the skeletal $\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$, and of $\delta^{13}C$, respectively, in the isotope record between colonies. Both isotope records were different between sexes. $\delta^{18}O$ was higher in female colonies than in male colonies, with a 0.31 ‰ difference; $\delta^{13}C$ was lower in female colonies, with a 0.28 ‰ difference. A difference in the skeletal $\delta^{18}O$ could introduce an error in SST estimates of $\approx 1.0$ to $\approx 2.6 \degree C$. The $\delta^{18}O$ records showed a seasonal pattern that corresponded to SST, with low correlation coefficients ($-0.45$, $-0.32$), and gentle slopes ($0.09$, $0.10 \% \degree C^{-1}$) of the $\delta^{18}O$–SST relation. Seasonal variation in coral $\delta^{18}O$ represents only 52.37 and 35.66 % of the SST cycle; 29.72 and 38.53 % can be attributed to $\delta^{18}O$ variability in seawater. $\delta^{13}C$ data did not correlate with any of the environmental variables; therefore, variations in skeletal $\delta^{13}C$ appear to be driven mainly by metabolic effects. Our results support the hypothesis of a sex-associated difference in skeletal $\delta^{18}O$ and $\delta^{13}C$ signal, and suggest that environmental conditions and coral growth parameters affect skeletal isotopic signals 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*.

1 Introduction

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 (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 calcifica-
tion records 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 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 with 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, δ18O, δ13C, δ14B, δ15N), skeletal δ18O and δ13C are the most common measurements because they are relatively easy to measure (Dunbar et al., 1994; Linsley et al., 1994; Swart et al., 1996a; Tudhope et al., 1996a; Charles et al., 1997; Schrag, 1999).

Most of the variability in skeletal δ18O in calcifying organisms, including coral, results from a combination of temperature-induced isotopic fractionation of local seawater δ18O (δ18Osw) that depends on changes in precipitation and oceanic evaporation, which affect salinity (Epstein et al., 1953). Depletion in carbonate δ18O 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 δ18O and need to be considered when establishing a skeletal δ18O-SST relationship (Cole and Fairbanks, 1990; Carriquiry et al., 1994; Al-Rousan et al., 2007; Sazzad et al., 2010).

Variations of skeletal δ13C are controlled mainly by an interrelationship between photosynthesis, respiration, and feeding. During high photosynthesis, zooxanthellae fixation of 12CO₂ increases, which leads to an increase in 13CO₂ in the coral carbon pool. Hence, coral skeletons formed during periods of high photosynthesis contain greater amounts of 13C (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 13C. Changes in the photosynthesis–respiration ratio are influenced by photoperiods, phot-intensity, and temperature; where longer photoperiods and higher temperatures promote higher photosynthesis–respiration ratios (higher 13C). If maximum solar radiation occurs during summer, skeletal δ13C will be inversely related to δ18O; if the maximum photoperiod occurs during colder seasons, δ13C and δ18O will be positively related (Swart et al., 1996b). Since zooplankton have generally low isotope levels, compared to coral skeletons and zooxanthellae, an increase in the heterotrophic activity of coral should reduce the δ13C of coral skeletons (Grottoli and Wellington, 1999). Felis et al. (1998) and Bernal and Carriquiry (2001) demonstrated that levels of coral skeletal δ13C 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 δ18O and δ13C in coral skeletons are depleted in 18O and 13C, 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 gonochorics (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 δ18O and δ13C 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). *P. panamensis* is a gonochoric brooder with reproductive activity throughout the year (Glynn et al., 1994; Carpiço-Iturbe 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 *P. panamensis* coral, collected in Bahía de La Paz, with growth density banding covering 12 years. Oxygen and carbon isotope measurements were used to assess a possible sex-associated variation in the coral skeletal δ18O and δ13C signal related to differences in the “vital effect” of colonies between sexes. The isotopic record was compared to surface seawater temperatures (SST), rainfall, photosynthetically active radiation (PAR), concentration of chlorophyll *a*, and skeletal growth data.
Materials and methods

2.1 Collection and identification of sex

Ten colonies of *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 a 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 10 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 (Hummason, 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 identified as female if any planulae or oocytes were observed, regardless of their stage of development; the colonies were identified 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 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 10 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 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.

The 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; Simons, 2015). The environmental data spanned from 1997–2009, 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 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 $\delta^{18}O$ calibration. Monthly rainfall data (1997–2009) were obtained from the Servicio Meteorológico Nacional (http://smn.cna.gob.mx/CLICOM, 2015). Some sea surface salinity data were obtained from previous published data in the study area (Obeso-Niebla, 2007). $\delta^{18}O_{sw}$ was calculated from the $\delta^{18}O$ 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 $\delta^{18}O$ and $\delta^{13}C$ between sexes and to compare both sets of means obtained using the Heikoop et al. (2000) correction factor (kinetic and metabolic $\delta^{13}C$). 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. 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. A Regime shift index for environmental and isotope data was 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 seasonally 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 $P. \text{panamensis}$ 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 $1.05\pm0.04$ for female colonies, and $1.27\pm0.04$ for male colonies. The average skeletal density was $0.94\pm0.01$ for females, and $0.95\pm0.01$ g cm$^{-3}$ for males. The average calcification rate was $0.97\pm0.04$ for females, and $1.24\pm0.03$ g cm$^{-2}$ yr$^{-1}$ for males. Figure 2 shows X-ray positive prints for two of the samples.

3.2 Skeletal isotope composition and environmental data

The $\delta^{18}O$ quarterly records of female and male coral colonies show a seasonal pattern (Fig. 3) that was significantly correlated between sexes ($r = 0.45$, $p>0.000001$), thus both sexes showed the same seasonal pattern. $\delta^{18}O$ in female colonies, was higher than in male colonies (Fig. 3). The overall average $\delta^{18}O$ in female colonies was $-2.89\pm0.33$ and $-3.20\pm0.37\%e$ in male colonies (Table 1). Overall, the $\delta^{18}O$ average of females is significantly higher than that of males ($t_{408} = 9.34$, $p<0.00001$). Quarterly $\delta^{18}O$ time series of all colonies showed a “regime shift” of the mean in 2004, from $-2.75$ to $-3.14\%e$, with a regime shift index (RSI) of $-0.69$ ($p = 0.008$) in female colonies, and from $-3.08$ to $-2.42\%e$ with an 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 an RSI of 0.30 (p = 0.01), as seen in Fig. 3b.

The quarterly δ¹³C time series showed a cyclic pattern in female and male colonies (Fig. 4), that was correlated between both 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. 4). 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 > 0.00001). No regime shift was found in the δ¹³C data of either sex.

The δ¹⁸O skeletal data series corresponds to the SST (Fig. 3). 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 = 0.00003) for female colonies, and −0.32 (p = 0.0005) for male colonies; the r-to-z transformation showed that both correlation coefficients are equally 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 variables in any of the colonies (Table 3). The temporal resolution of compared data (isotopes vs. environmental data) is quarterly in all cases.

Heikoop et al. (2000) correction factor results are shown in Table 4. The overall average of δ¹³C 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 (t₀⁹₈ = 13.074, p < 0.000001 for Kinetic means; t₀⁹₈ = −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 ¹⁸O and ¹³C, which are deposited during summer; low density bands are enriched in ¹⁸O and ¹³C, which are deposited during winter. In female colonies, a significant negative correlation between the mean annual coral δ¹⁸O and annual skeletal density was found (Table 5; r = −0.78, p =
Our isotope data showed a significant dependency of skeletal \( \delta^{18}O \) on SST, with a low \( r (\approx -0.45 \) in female coral, and \( -0.28 \) in male coral), and a gentle slope of the \( \delta^{18}O \)-SST calibration equations (0.09 \% \text{C}^{-1} F; 0.11 \% \text{C}^{-1} M; \text{Fig. 5}), compared with slopes \( >0.20 \% \text{C}^{-1} \) in \textit{Porites} spp. in other areas of the Pacific: the Great Barrier Reef (Gagan et al., 1994), Costa Rica (Carriquiry, 1994), Panama (Welling-ton 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 between 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 (\( <0.17 \% \text{C}^{-1} \)) 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 δ18O and SST, and the gentle slope in the δ18O–SST calibration equations are related to small seasonal variations in SST (<3 °C), or the greater influence of δ18Osw. The seasonal variation in SST of our study area is 7.85 ± 0.77 °C, so the seasonal variation of SST is not likely to be the cause. Variations in δ18Osw represent 29.72 % in female coral, and 38.53 % in male coral, of the average seasonal δ18O variation. We found a significant regime shift in the δ18O data of colonies of both genders, that coincides with a regime shift in rainfall. This means that the δ18O of coral in Bahía de La Paz is influenced more by the δ18Osw than in other places in the Pacific.

The linear regression (Fig. 5) equations for δ18O dependence on SST (1997–2009) were SST = 7.0889 – 5.7193 (δ18O), (r² = 0.23, p = 0.00003) for female coral, and SST = 14.739 – 2.9246 (δ18O) (r² = 0.10, p = 0.00007) for male coral.

The annual range of δ18O was the difference between the highest δ18O 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 SST data 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 δ18O 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.

Table 4. Heikoop et al. (2000) correction factor results comparing transformed and metabolic skeletal δ13C of Porites panamensis colonies from Bahía de La Paz.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Sex</th>
<th>SST</th>
<th>Precipitation</th>
<th>PAR</th>
<th>Chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLP32</td>
<td>F</td>
<td>0.19</td>
<td>-0.07</td>
<td>0.62</td>
<td>-0.11</td>
</tr>
<tr>
<td>BLP33</td>
<td>F</td>
<td>0.17</td>
<td>-0.04</td>
<td>0.73</td>
<td>-0.12</td>
</tr>
<tr>
<td>BLP36</td>
<td>F</td>
<td>0.17</td>
<td>-0.06</td>
<td>0.63</td>
<td>-0.16</td>
</tr>
<tr>
<td>BLP40</td>
<td>F</td>
<td>0.15</td>
<td>-0.07</td>
<td>0.62</td>
<td>-0.11</td>
</tr>
<tr>
<td>BLP31</td>
<td>M</td>
<td>0.005</td>
<td>0.97</td>
<td>-0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>BLP34</td>
<td>M</td>
<td>0.03</td>
<td>0.79</td>
<td>-0.02</td>
<td>0.86</td>
</tr>
<tr>
<td>BLP35</td>
<td>M</td>
<td>0.01</td>
<td>0.93</td>
<td>-0.02</td>
<td>0.84</td>
</tr>
<tr>
<td>BLP37</td>
<td>M</td>
<td>0.01</td>
<td>0.92</td>
<td>-0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>BLP38</td>
<td>M</td>
<td>0.003</td>
<td>0.98</td>
<td>-0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>BLP39</td>
<td>M</td>
<td>0.02</td>
<td>0.88</td>
<td>-0.02</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Figure 5. Linear regressions between satellite-derived sea surface temperature (°C) and skeletal δ13O (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.

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of the SST. The expected seasonal variation of approximately 0.11‰ of δ¹⁸O in seawater (0.43 psu) represents 29.72% of δ¹⁸O seasonal variation in female colonies, and 38.53% in male colonies.

The departure from isotope equilibrium of our samples was estimated with the equations by Grossman and Ku (1986), for δ¹⁸O, and Romanek et al. (1992) for δ¹³C. We found that the theoretical δ¹⁸O 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. 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. 4) was higher in both sexes between November and January (lowest SST and PAR), and lower from June through August (highest SST and PAR), suggesting a positive relationship between δ¹³C and photosynthesis, and a dominant role of light-induced photosynthesis on seasonal changes of δ¹³C in coral. Still, the δ¹³C–PAR correlations were not significant, thus, photosynthesis was not stimulated or inhibited by light, and remained near its maximum efficiency during the whole year, according to Sun et al. (2008). Other factors may be affecting photosynthesis in addition to light, such as abundance of dissolved nutrients. High concentrations of chlorophyll a occurred during periods of enrichment of ¹³C in the coral skeleton (November through January); however, the correlations of skeletal δ¹³C and chlorophyll a were not significant in any case.

Trends in coral skeletal δ¹³C reflect seasonal variations in photosynthesis to respiration ratios in the δ¹³C pool of coral (McConnaughey, 1989; McConnaughey et al., 1997). Respiration normally increases with temperature and lowers ¹³C in coral skeletons, which is reflected in our results, high SST = low δ¹³C. No other environmental variables considered in this work explained this pattern in coral δ¹³C, driven mainly by metabolic effects as described by Sun et al. (2008) in Porites coral of the South China Sea.

We found a negative correlation (r = −0.78, p = 0.001) between δ¹⁸O and the skeletal density in female colonies, this is not consistent with studies that have observed that coral skeletal high-density bands are enriched in ¹⁸O (Klein et al., 1992; Al-Rousand, 2007). This may be due to a difference in timing of skeletal density bands in Porites coral species, as described by Lough and Barnes (2000). In male coral, we found a negative correlation between the δ¹⁸O and extension and calcification rates (r = −0.50, p = 0.045 and r = −0.44, p = 0.0008), this is consistent with the observations of other authors of Porites spp. coral (McConnaughey, 1989; Felis et al., 2003). In Porites corals, skeletal extension and calcification rates increases with SST, while skeletal density decreases (Lough and Barnes, 2000), so growth parameters of both sexes and δ¹⁸O behave as expected. No significant correlation was found between skeletal δ¹³C and skeletal growth parameters in either males or females, meaning that regardless of the skeletal extension rate, density or calcification rate, P. panamensis deposited a widely varying δ¹³C, as reported by Allison et al. (1996) in Porites coral from South Thailand, and by Swart et al. (1996b) in Montastrea annularis in Florida, USA.
General consensus states that all coral skeletons contain appreciable amounts of carbon and oxygen in isotopic disequilibrium, and are depleted in $^{18}\text{O}$ and $^{13}\text{C}$ 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 $^{18}\text{O}$; 2.81 % F, 2.53 % M in $^{13}\text{C}$). McConnaughey (1989) considers kinetic depletion as a constant in coral with fast extension rates ($>0.5 \text{ cm yr}^{-1}$).

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 $^{18}\text{O}$ ratios of female colonies are more enriched in $^{18}\text{O}$ than in male colonies, with an average difference of $\sim0.31 \%e$. Female $^{13}\text{C}$ values were lower than the $^{13}\text{C}$ of male colonies, with an average difference of $\sim0.29 \%e$. 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).

Linsley et al. (1999) found differences of 0.4 %e in the $^{18}\text{O}$ records of six Porites lobata coral living in nearly identical environments at Clipperton atoll. Felis et al. (2003) found a 1.28 %e difference in the $^{18}\text{O}$ 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 %e in the $^{18}\text{O}$ record, and 0.29 %e in the $^{13}\text{C}$ record. If we split our data by sex, the differences in the isotopic records drop to 0.07 %e in the $^{18}\text{O}$, and to 0.02 %e in the $^{13}\text{C}$. In our data, the sex of the colony explains 81 % ($^{18}\text{O}$) and 93 % ($^{13}\text{C}$) 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 Carricarte-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 $^{18}\text{O}$ and more enriched in $^{13}\text{C}$, relative to slower-growing coral (McConnaughey, 1989; Felis et al., 2003), this may be the origin of the isotope data difference between sexes (higher $^{18}\text{O}$ and lower $^{13}\text{C}$ 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 $^{18}\text{O}$ and $^{13}\text{C}$ between sexes.

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 as 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 $^{18}\text{O}$ 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 ($-1.66 \%e$ F vs. $-1.38 \%e$ M $\Delta^{13}\text{C}$; $-2.89 \%e$ F vs. $-3.20 \%e$ M $\Delta^{18}\text{O}$). 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 $^{13}\text{C}$ 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 reproduc-
tive strategy of *P. panamensis*. *P. panamensis* is a gonochorich 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 gonochorich spawners are lower than in gonochorich 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 gonochorich 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 δ¹³C of male and female colonies. Both transformed δ¹³C and metabolic δ¹³C seem to be higher in males, thus supporting the hypothesis stating that an intense activity of the Ca-ATPase enzyme will result in a 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 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 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 (Red Sea; Al-Rousand et al., 2003), ~1.71 (Great Barrier Reef; Gagan et al., 1994), ~1.31 (Costa Rica; Carrquiry, 1994), ~1.39 (Central and Eastern Tropical Pacific; Druffel, 1985), ~1.47 (The Galapagos; McConnaughey, 1989), and ~1.47°C in SST estimates, for the commonly used paleotemperature calibration in coral (0.21‰°C⁻¹).

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 application, 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. 6). 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 a 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 gonochorich brooder coral genera, if sex identification is not taken into account when possible. The findings of this study are based on a gonochorich brooder species (*P. panamensis*), the majority of paleoclimatic reconstructions in the Indo-Pacific and Caribbean have been based on massive gonochorich 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 gonochorich 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 gonochorich spawners.
Data availability

Monthly SST, PAR, and concentration of chlorophyll a records are respectively available at http://coastwatch.pfeg.noaa.gov/erddap/griddap/erdBStsstd1day.html, http://coastwatch.pfeg.noaa.gov/erddap/griddap/erDMH1part0mday.html and http://coastwatch.pfeg.noaa.gov/erddap/griddap/erDMBchlamladay.html. Monthly rainfall data are accessible from the Servicio Meteorológico Nacional at http://clicom-mex.cicese.mx/mapa.html, data from the 3074-LA PAZ (DGE), BCS climatological station were used.

Author contributions. Rafael A. Cabral-Tena and Eduardo F. Balart conceived and designed the study; Rafael A. Cabral-Tena, Angel H. Ruvalcaba-Díaz and AS processed isotopically the material. Rafael A. Cabral-Tena, AS, Héctor Reyes-Bonilla and Eduardo F. Balart analyzed the data. All authors discussed the results and wrote the manuscript.

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References


Schrag, D. P.: Rapid analysis of high-precision Sr/Ca ratios in coral and other marine carbonates, Paleoenvironment, 14, 97–102, 1999.