

# Fluctuation in total phenols and photosynthetic pigments content in *Pocillopora capitata* (Cnidaria, Scleractinia) under different environmental conditions

Fluctuación en el contenido total de fenoles y pigmentos fotosintéticos en *Pocillopora capitata* (Cnidaria, Scleractinia) bajo diferentes condiciones ambientales

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**Abstract.** Coral reefs are ecosystems with high biological diversity that, under anthropogenic and climatic stress, produce chemical defense compounds. Different indicators have been used to measure the health of coral systems. The concentration of total phenols could be used for this purpose due to their antioxidant response during harsh environmental conditions, although this stress indicator has received little attention. The total content of phenols, chlorophyll- $\alpha$ , chlorophyll  $c_1 + c_2$  and  $\beta$ -carotenes was evaluated in *Pocillopora capitata* corals collected at three sites of the Central Mexican Pacific under multiple stress conditions in two different seasons of the year. Total phenol content, chlorophyll- $\alpha$ , chlorophyll  $c_1 + c_2$ , and  $\beta$ -carotene concentrations ranged from 1.49 to 4.43  $\mu\text{g GAE mL}^{-1}$ , 1.28 to 2.08  $\mu\text{g cm}^{-2}$ , 0.15 to 0.43  $\mu\text{g cm}^{-2}$ , and 0.20 to 0.77  $\mu\text{g cm}^{-2}$ , respectively. The content of total phenols and  $\beta$ -carotenes between seasons had statistically significant differences, where the total content of phenols and pigments tended to be higher in the rainy season (October) than in the dry season (February), as these compounds are sensitive to environmental fluctuations and may therefore serve as indicators of such changes.

**Key words:** Hermatypic corals, oxidative stress, chlorophyll, phenols

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## INTRODUCTION

Healthy coral reefs constitute important ecosystems as a habitat for marine fauna and as a source of food, coastal protection by acting as marine barriers and income from tourism and fishing for communities, recreation, in addition to other cultural goods and services (Hoegh-Guldberg *et al.* 2017). However, these valuable systems are under severe pressure worldwide due to anthropogenic activities, exposure to natural disturbances, and global climate change. The predicted collapse of the world's coral reefs is expected to have disastrous social, cultural, economic, and ecological consequences (Spalding & Brown 2015).

Reef-building corals (Cnidaria, Scleractinia) house dinoflagellate endosymbionts of the genus *Symbiodinium* (Gert Hansen & Daugbjerg 2009). One key function of reef-building corals is the formation of calcareous structures that provide habitat for high marine biodiversity (EPA 2024)<sup>1</sup>. The relationship between coral and *Symbiodinium*

<sup>1</sup>Basic Information about Coral Reefs. EPA, United States Environmental Protection Agency, Washington DC.  
<<https://www.epa.gov/coral-reefs/basic-information-about-coral-reefs>>



can break down and even zooxanthellae detach from corals due to high temperatures above 29 °C and UV radiation which could cause coral bleaching and death (Ding *et al.* 2022). To prevent cellular damage, coral-symbiont associations produce low-molecular-weight antioxidants including pigments (such as  $\beta$ -carotene); vitamins as ascorbic acid (vitamin C) and  $\alpha$ -tocopherol (vitamin E); phenolic compounds (flavonoids, tocopherols, and others); antioxidants enzymes like catalase and superoxide dismutase (SOD) (Safafar *et al.* 2015). These chemicals provide ecological and physiological information that allows quantification of stress levels or the degree of ecological response (Vermeij & Bak 2002). Polyphenols are organic molecules with one or more hydroxyl group attached to a phenyl ring. Nearly 8,000 compounds of this family have been reported, subdivided into 3 main classes, flavonoids, stilbenoids, and phenolic acids radical (Papuc *et al.* 2017). The effectiveness of phenols in inhibiting the oxidative processes is related to their free radicals scavenging capacity due principally to the transfer of H-atom from their active OH group(s) to the free radical ( $\text{Ar-OH} + \text{R} \rightarrow \text{ArO} + \text{RH}$ ) or by electrochemical oxidation ( $\text{Ar-OH} \rightarrow \text{ArO}^\cdot + \text{e}^- + \text{H}^+$ ) (Papuc *et al.* 2017). Polyphenols preserve corals' health by protecting them from oxidative stress, UV radiation, pathogens, and harsh climatic conditions (Pandey & Rizvi 2009). Antioxidants have been proposed as biomarkers of several marine organisms subjected to oxidative stress (Gopeechund *et al.* 2020). Photosynthetic pigments such as chlorophyll-*a*, chlorophyll  $c_1 + c_2$ , and  $\beta$ -carotene, are compounds that absorb visible solar energy or photosynthetic active radiation ( $\lambda$ , 400-700 nm; PAR) (Safafar *et al.* 2015). Visible light is necessary for the photosynthetic activity and carbon fixation by the zooxanthellae population in the coral ecosystem (Falkowski & Raven 2007). This symbiont photoacclimates its photosynthetic performance in responses to changes in light availability. Corals exposed to lower irradiation (deeper waters) contain more chlorophyll within larger chloroplasts, resulting in greater light-capture efficiency, whereas corals exposed to higher irradiation (shallow waters) contain less chlorophyll distributed among a greater number of smaller chloroplasts. The genus *Pocillopora*, which includes *Pocillopora capitata* (Verrill, 1864), is among the dominant scleractinian corals inhabiting rocky substrates and shallow reefs at depths of 1-5 m in the tropical eastern Pacific Ocean. (Medellín-Maldonado *et al.* 2016). Since the phenols and pigment content of corals is sensitive to even small natural variations in environmental conditions, it is hypothesized that colonies of *P. capitata* would respond to stressful environmental conditions by changing their polyphenols and pigments content. This study places

particular emphasis on total phenol content, as these metabolites have been seldom studied in corals.

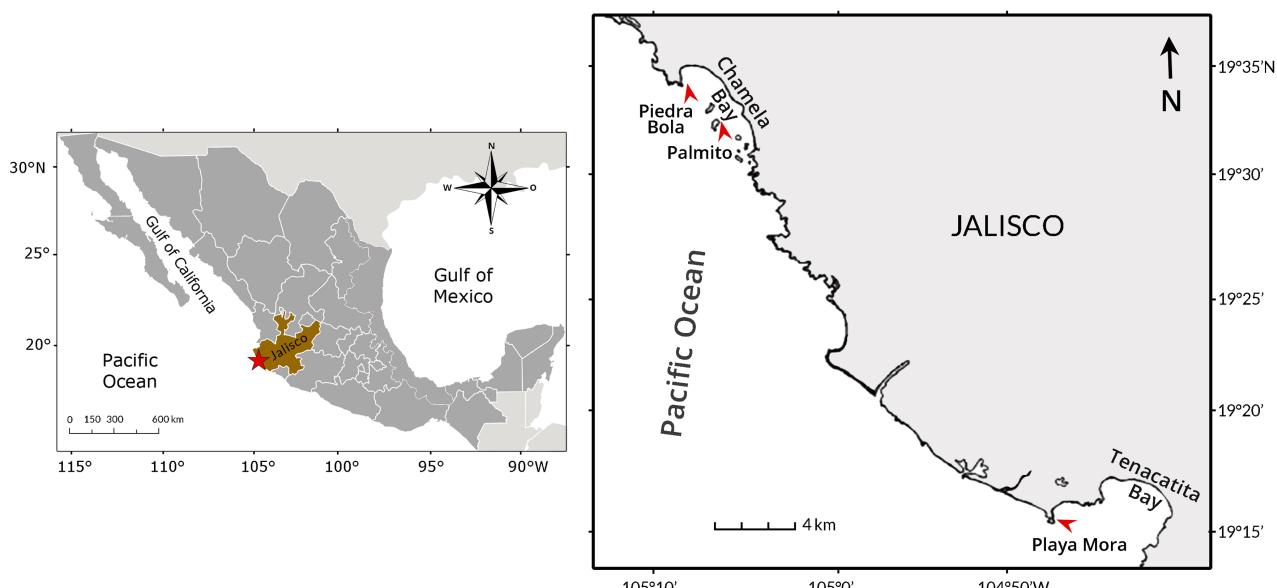
The aim of this work was to determine possible variations in the biochemical indicators of photosynthetic pigments (chlorophyll and carotenoids) and total phenols of *P. capitata* from three coral communities of the Mexican Central Pacific under different environmental conditions during the dry (February) and rainy (October).

## MATERIALS AND METHODS

The branches of the coral *Pocillopora capitata* were sampled during February (winter) and October (autumn) 2020 in the coral communities of Piedra Bola ( $19^{\circ}34'38''\text{N}$ - $105^{\circ}08'0.1''\text{W}$ ); and Palmito ( $19^{\circ}33'25''\text{N}$ - $105^{\circ}06'18''\text{W}$ ) in Chamele Bay and Playa Mora ( $19^{\circ}16'50.2''\text{N}$ - $104^{\circ}52'17.7''\text{W}$ ) in Tenacatita Bay, Jalisco, Mexico (Fig. 1). The study area has a sub-humid to semi-arid climate, with rainfall during July to October. Tide level and water surface temperatures are highest in summer-autumn and lowest in late winter (March) and early spring (April). Due to their proximity to the coast and shallow depth, sublittoral coral communities are particularly susceptible to human activities as well as to natural disturbances such as tropical storms and El Niño-Southern Oscillation (ENSO) events, including the 1997-1998 episode and subsequent occurrences (Galván-Villa *et al.* 2011). In each coral community SCUBA diving was performed at a depth between 1.8 and 3.0 m to randomly select eight branches approximately 10 cm long from base to distal end using chisel, hammer, and gloves. The total number of branches was kept at a temperature of 4 °C during transfer to the laboratory to be refrigerated at -20 °C for further processing at the Centro Universitario de Ciencias Biológicas y Agropecuarias of Universidad de Guadalajara. Upon arrival, the samples were frozen and stored at -20 °C until analysis. At each sampling, temperature, salinity, and pH were recorded using a YSI 55 multiparameter probe. In addition, three water samples were collected at the depth of each coral community using a 1 L Niskin bottle to determine nitrate, nitrite, and ammonium concentrations according to Greenberg *et al.* (1992), McAlpine & Soule (1933), and Mackereth *et al.* (1978), respectively. Also, tidal levels and radiation were collected from the records of the Marine Secretary (SEMAR 2017)<sup>2</sup>; rainfall and environmental temperature were collected from the records of INIFAP station (INIFAP 2017)<sup>3</sup>, both located at the port of Manzanillo  $19^{\circ}03.45'\text{N}$ - $104^{\circ}18.08'\text{W}$ , near the sampling sites (86 km from Tenacatita, and 115 km from Chamele Bay).

<sup>2</sup>SEMAR. 2017. Estación mareográfica de Manzanillo, Secretaría de Marina, Colima. [http://oceanografia.semar.gob.mx/Templates/grafnum\\_manzanillo.html](http://oceanografia.semar.gob.mx/Templates/grafnum_manzanillo.html)

<sup>3</sup>INIFAP. 2017. Red de estaciones. Estación V. Carranza. Manzanillo, Colima. México. <https://clima.inifap.gob.mx/lnmysr/Estaciones/Mapa>



**Figure 1. Sampling sites in Chamela and Tenacatita Bay, Jalisco, Mexico** / Sitios de muestreo en la bahía de Chamela y en la bahía de Tenacatita, Jalisco, México

Total phenolic content was determined using the spectrophotometric method described by Singleton *et al.* (1999). Decalcified tissues (150 mg) were extracted twice (sequentially) at 25 °C for 2 h with 2 mL of methanol-water (50% v/v) and gentle agitation (200 rpm). Slurries were then centrifuged (4,000 rpm for 15 min) and the liquid phase was filtered. Then, 20 µL of the extract were added to 1.58 mL of HPLC-grade water, mixed with 100 µL of 1N Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate), and 300 µL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). After vortexing and incubating for 90 min in the dark, the absorbance at 765 nm was measured in a Metash V-5100 spectrophotometer. A blank was prepared in the same way as the samples but replacing the extract with methanol-water. A calibration curve ranging from 0 to 500 µg mL<sup>-1</sup>, with 100 µg mL<sup>-1</sup> intervals, was constructed using gallic acid as the standard to calculate phenolic content, taking into account extract volume and specimen weight. The phenolic content in the samples is expressed as gallic acid equivalent (GAE) per gram of dry sample. This test was done in triplicate. For the analysis of photosynthetic pigments (Chl-*a*, Chl *c<sub>1</sub>* + *c<sub>2</sub>*, and carotenoids) coral fragments were suspended in 10 mL of acetone-distilled water (9/1, v/v) at 2 °C for 24 h and subsequently centrifuged at 6,000 rpm for 15 min. The supernatant absorbance at 664, 647, and 630 nm was then measured in the spectrophotometer and the chlorophyll concentrations were calculated using Jeffrey & Humphrey (1975) equations. Carotenoids were determined

under dark conditions. Tissues were dissolved in 10 mL of chloroform (100%) for 48 h at 2 °C, centrifuged at 6,000 ppm for 10 min, and the absorbance at  $\lambda = 430$  nm measured. The carotenoid concentrations were calculated using the peridinin molar extinction coefficient reported by Borneman (2001). The data were standardized considering the extract volume and fragment surface area according to the following formula:

$$\text{Conc} \left( \frac{\mu\text{g}}{\text{cm}^2 \text{coral}} \right) = \frac{C_{\text{extr}} V_{\text{extr}} DF}{A}$$

where A=Area, C<sub>extr</sub>= extract concentration, DF=dilution factor, V<sub>extr</sub>= extract volume

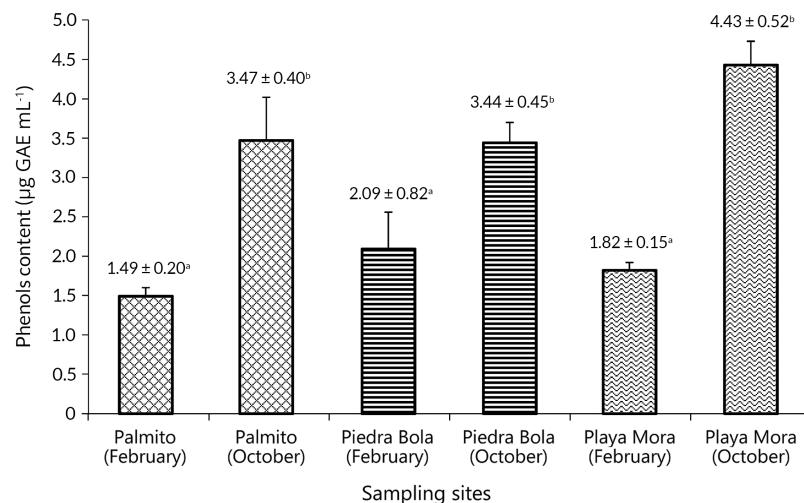
The experimental design included two categorical factors: sampling site with three levels (Playa Mora, Piedra Bola, and Palmito) and season with two levels (February and October), with three replicates per factor combination. Response variables included the concentrations of chlorophyll-*a*, chlorophyll *c<sub>1</sub>* + *c<sub>2</sub>*, carotenoids and polyphenols. Normality of the data was assessed using the Anderson-Darling test. For all response variables (Chl-*a*, Chl *c<sub>1</sub>* + *c<sub>2</sub>*, and carotenoids), *P*-values exceeded the significance level ( $\alpha = 0.01$ ), indicating that the null hypothesis of normality was not rejected. The data were then analyzed using a multifactorial analysis of variance (ANOVA) to evaluate the effects of the factors and differences among means, followed by Tukey's HSD *post-hoc* test.

## RESULTS AND DISCUSSION

This study analyzed the total phenols and photosynthetic pigments concentration in endosymbiotic algae of *P. capitata* under different natural conditions and seasons. Seawater temperature, salinity, and pH were higher in October than in February but were similar among sites. The temperature ranged from 25.7 °C (February) to 29.4 °C (October), while the salinity and pH varied from 34 to 36.1, and 7.09 to 8.07, respectively. Nutrients in the water column associated with nitrogen varied with the season and sampling site, without any tendency. The average ammonium, nitrite and nitrate content varied from 0.1 to 0.5 mg L<sup>-1</sup>, 0.56 to 0.85 mg L<sup>-1</sup>, and 0.05 to 0.66 mg L<sup>-1</sup>, respectively. Regarding oceanographic variables, differences were observed between sampling periods: tidal level was 66 cm higher in October, ambient temperature increased by 2.72 °C in October, whereas solar radiation was 56.2 W m<sup>-2</sup> higher in February, according to official records (SEMAR 2017<sup>2</sup>, INIFAP 2017<sup>3</sup>).

Both sampling site and season had a significant effect on coral's total phenolic content ( $P= 0.00001$ , 95% confidence intervals) (Fig. 2). Regardless of the season, the polyphenol concentration in *P. capitata* colonies was about twice as much in October than in February. No effect of the sampling site ( $P = 0.1040$ ) or the site/season interaction ( $P = 0.1144$ ) was observed. Nevertheless, samples from Playa Mora had the highest phenolic content ( $4.43 \pm 0.52 \mu\text{g GAE mL}^{-1}$ ) in October while Palmito samples had the lowest concentration ( $1.49 \pm 0.20 \mu\text{g GAE mL}^{-1}$ ) during February. Most available studies on phenolic compounds focus on marine algae as primary sources of phenols, flavonoids and other antioxidant metabolites. For example, the macroalgae *Ulva fasciata* (green) contains  $10.5 \pm 0.46 \text{ mg GE g}^{-1} \text{ dw}$  and *Corallina officinalis* (red) contains  $6.0 \pm 0.02 \text{ mg GE g}^{-1} \text{ dw}$  (Abou-

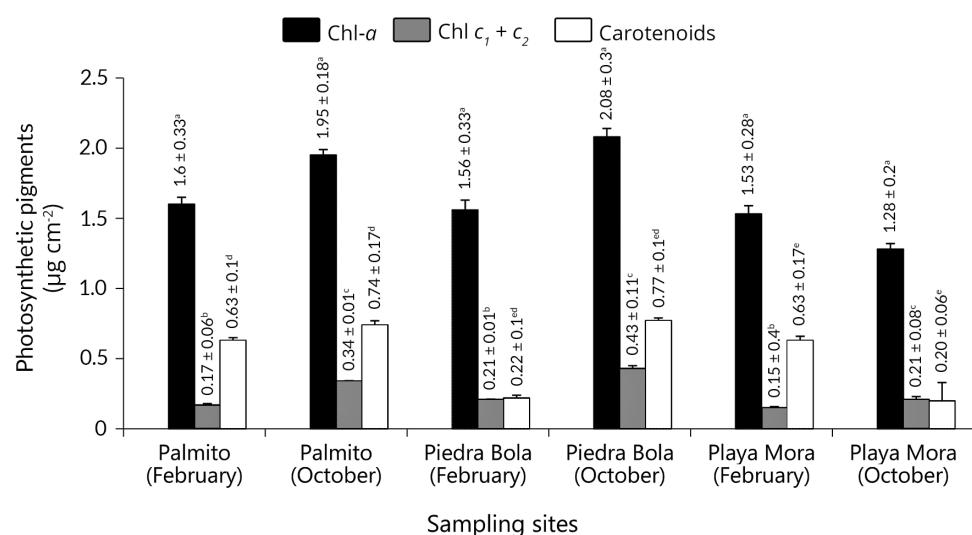
Gabal *et al.* 2021). Likewise, Schneider *et al.* (2022) report an increase in the concentration of phenolic compounds in the red macroalga *G. cornea* exposed to UV-vis radiation emitted by a sodium lamp from around  $11 \text{ mg g}^{-1} \text{ dw}$  to  $17 \text{ mg g}^{-1}$  because of the stress caused by radiation and the photoprotective role of these compounds. However, there are few reports on phenolic content as a bioindicator of the health status of coral systems because information on this subject in corals is scarce. Louis *et al.* (2016) use these antioxidant compounds to evaluate the variation of light between sites and times of the year in the endosymbiont alga *Symbiodinium* of *Acropora muricata*, with results like those of the present study, where the concentration of total phenols is higher in summer than in winter, but not between experimental sites. They suggest that high light intensity and water temperature during summer may increase the biosynthesis of phenolic compounds and antioxidant activity, possibly as a defense mechanism against increased ROS production. This pattern may represent a physiological stress response in otherwise unbleached corals. In brown algae (class Phaeophyceae), phlorotanins, fucolls, phloretols, fucophloretols, fuhalols and sulphited phlorotannins have been identified within phenolic compounds content (Soo-Jin *et al.* 2005). Phlorotannins have been shown to counteract oxidative stress caused by UV-B light in shallow water macroalgae (Soo-Jin & You-Jin 2009). Phenols in plants, corals, or algae, prevent the chemical chain reaction, using hydrogen donation towards free radicals or by electrochemical oxidation, stabilizing them. The resultant phenolic radicals are less reactive due to their resonant aromatic core; they distribute and dislocate the unpaired electron terminating the chain reaction (Sivakumar *et al.* 2011). Analogously, *Pocillopora capitata* may exhibit responses similar to those observed in brown algae, providing protection against solar radiation as well as against predators such as fish, mollusks, and crustaceans.



**Figure 2. Total phenolic concentration ( $\mu\text{g GAE mL}^{-1}$ ) in corals collected at Palmito, Piedra Bola and Playa Mora in February (winter) and October (autumn) 2020. Bars represent the mean + standard deviation of three replicates. Bars with different letters indicate significant differences ( $P \leq 0.05$ ) / Concentración de fenoles totales ( $\mu\text{g EAGmL}^{-1}$ ) en corales colectados en Palmito, Piedra Bola y Playa Mora en febrero (invierno) y octubre (otoño). Las barras representan el promedio + desviación estándar de tres réplicas. Las barras con letras diferentes indican diferencias significativas ( $P \leq 0,05$ )**

In relation to photosynthetic pigments, Chl-*a* concentrations were statistically similar among sites and seasons ( $P > 0.05$ ), which suggests that the levels of this pigment are typical of corals, at least under these conditions. Similarly, chlorophyll *c*<sub>1</sub> + *c*<sub>2</sub> concentrations showed a pattern comparable to that polyphenols, with significant differences between sampling season ( $P = 0.0188$ ) but not differences among sites ( $P = 0.1600$ ) and no significant site  $\times$  season interaction ( $P = 0.5015$ ). Carotenoid concentrations did not vary significantly between seasons ( $P = 0.3836$ ) but showed significant differences among sampling sites ( $P = 0.0202$ ), as well as a significant site  $\times$  season interaction ( $P = 0.0003$ ). Overall, per collection site the increasing order of pigment concentration was Piedra Bola > Palmito > Playa Mora. The highest concentration was found in October. The highest values were recorded in samples from Piedra Bola in October, with  $2.08 \pm 0.30 \mu\text{g Chl-}a \text{ cm}^{-2}$ ,  $0.43 \pm 0.11 \mu\text{g Chl }c_1 + c_2 \text{ cm}^{-2}$ , and  $0.77 \pm 0.12 \mu\text{g carotenoids cm}^{-2}$ . The lowest values were observed in Playa Mora, with  $1.28 \pm 0.20 \mu\text{g Chl-}a \text{ cm}^{-2}$  in October,  $0.15 \pm 0.04 \mu\text{g Chl }c_1 + c_2 \text{ cm}^{-2}$  in February, and  $0.20 \pm 0.06 \mu\text{g carotenoids cm}^{-2}$  in October (Fig. 3). Regardless of site or season, this study found little variation in the levels of photosynthetic pigments quantified. In comparison with the present results, a study conducted on reef banks at different zones and depths in Tenacatita Bay (Liñán-Cabello *et al.* 2006)

reported similar chlorophyll-*a* values ( $1.7 \mu\text{g cm}^{-2}$ ) and higher  $\beta$ -carotenes concentrations ( $1.4 \mu\text{g cm}^{-2}$ ) in normal corals (without signs of discoloration), whereas lower values were recorded in bleached corals ( $0.3 \mu\text{g cm}^{-2}$  for chlorophyll-*a* and  $0.2 \mu\text{g cm}^{-2}$  for carotenoids). These values are also lower than those reported for *Pocillopora capitata* in Manzanillo Bay, where chlorophyll-*a* concentrations of  $3\text{--}10 \mu\text{g cm}^{-2}$  and  $\beta$ -carotenes concentrations of  $10\text{--}60 \mu\text{g cm}^{-2}$  have been recorded under normal conditions (Flores-Ramírez & Liñán-Cabello 2007). Conversely, in this study *P. capitata* contained higher pigment levels than *Acropora japonica* Veron, 2000, *Acropora secale* (Studer, 1878), and *Acropora hyacinthus* (Dana, 1846) corals (Maoka *et al.* 2011). The carotenoids xanthophylls, diatoxanthin (yellow or brown), peridinin, pyrrhoxanthin are associated with chlorophyll and they are produced by the dinoflagellate symbiont zooxanthellae. Corals absorb carotenoids without metabolic alteration, peridinin and pyrrhoxanthin mainly, and it is considered that they play a significant role in coral reproduction (Maoka *et al.* 2011). Under high solar radiation stress, reactive oxygen species (ROS), mainly singlet oxygen, are produced in chlorophyll and interact with antioxidant compounds such as carotenoids. When this stress persists, zooxanthellae are expelled from host coral cells, leading to discoloration due to a reduction in their concentration (Smith *et al.* 2005).



**Figure 3. Concentrations of photosynthetic pigments by sampling site and sampling period. Bars with different letters indicate significant differences ( $P \leq 0.05$ ; Tukey's HSD post-hoc test) / Concentración de los pigmentos fotosintéticos por sitio y período de muestreo. Las barras con letras diferentes indican diferencias significativas ( $P \leq 0,05$ ; prueba post-hoc de Tukey)**

Corals of genus *Pocillopora* have the capacity to adapt to environmental fluctuations (currents, low tides, turbulent water, incident light) (Borneman 2001). Carotenoids and phenols are antioxidants of endogenous origin with biological function in a diverse number of organisms (Liñán-Cabello *et al.* 2010). It is recognized that carotenoids can remove reactive oxygen species (ROS) in aquatic organisms (Liñán-Cabello *et al.* 2010). Overall, phenol and carotenoid levels were higher in October, possibly as a result of seasonal fluctuations, particularly increases in temperature (25.7 °C in February *vs.* 29.4 °C in October), salinity (34-36.1), and pH (7.09-8.07). Environmental factors influence key functional processes in corals, such as photosynthesis, photoacclimatization, and the metabolic contribution of zooxanthellae (Lesser 2000). Phenolic compounds may be sufficiently sensitive to respond to environmental fluctuations and, under extreme conditions, exert an important antioxidant function (Lesser 2000). These compounds help improve tolerance to oxidative stress by protecting tissues from the damaging effects of free radicals so they can be considered a useful biochemical indicator to evaluate coral oxidative stress.

## STATEMENTS

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### AUTHOR CONTRIBUTIONS

J. Jesús Vargas Radillo and Lucía Barrientos-Ramírez contributed to manuscript writing and data management and served as corresponding authors. Mario A. Ruiz-López contributed to manuscript writing and data management. Mariel Torres-Ortiz conducted the experimental work. Ernesto López-Uriarte and Ramón Reynoso-Orozco contributed to manuscript review.

### DATA AVAILABILITY

Data are available upon request from the corresponding authors (JVR, LBR).

### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

### USE OF AI

No artificial intelligence tools were used in this work.

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