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Two biomarkers of gene expression plasticity in *Pocillopora* corals from the Carrizales reef, Mexican Tropical Pacific

Dos biomarcadores de la plasticidad de la expresión génica en corales *Pocillopora* del arrecife Carrizales, Pacífico Tropical Mexicano

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ABSTRACT

Background. Gene expression (GE) plasticity is an acclimation response that allows organisms to adjust rapidly to environmental changes, providing an adaptive advantage. GE biomarkers are emerging as a valuable tool for linking the organism's physiological plasticity with the synergetic effects of large-scale climatic conditions and local impacts such as temperature and nutrients. **Objectives.** In this study, we investigate the GE plasticity of the 70-kDa heat shock protein (*hsp70*) and the carbonic anhydrase enzyme (CA) to confirm the ability of those two genes as biomarkers of the Cellular Stress Response and Cellular Homeostasis Response, respectively. **Methods.** Using qPCR, we evaluate the GE plasticity of coral colonies from *Pocillopora capitata*, *Pocillopora damicornis*, and *Pocillopora verrucosa* at the Carrizales reef (Colima coast of Mexico) naturally exposed to environmental changes in the Sea Surface Temperature (SST), productivity and nutrients using the cellular density of Symbiodiniaceae and chlorophyll content as health indices. **Results.** Our results clearly show GE plasticity in the *hsp70* for *Pocillopora verrucosa* and *Pocillopora damicornis* related to a daily environmental change in temperature and nutrients. On the other hand, the CA gene expression shows no change in response to daily variations. However, there was a significantly high expression of CA and a lower expression of *hsp70* in *Pocillopora capitata*. Furthermore, we found no significant differences in the health indices, suggesting some degree of physiological plasticity in *Pocillopora* corals like its extensive morphological plasticity that could reflect different adaptation capacities to low temperatures and high nutrients during the spring season in the central Mexican Pacific. **Conclusions.** Evaluating the phenotypic plasticity (morphology and molecular physiology) could help identify coral colonies with a more significant potential to survive environmental stressors. The latter is an essential consideration for managing, conserving, and restoring coral reefs in the Mexican Pacific.

Keywords: coral acclimatization, molecular physiology, phenotypic plasticity

RESUMEN

Antecedentes. La plasticidad de la expresión génica (GE) es una respuesta inmediata de aclimatación al cambio ambiental que puede proporcionar una ventaja adaptativa. Los biomarcadores de GE están emergiendo como una herramienta valiosa para vincular la plasticidad fisiológica del organismo con los efectos sinérgicos del cambio climático y el impacto local como la temperatura y nutrientes. **Objetivos.** Investigamos la plasticidad de la expresión de genes que codifican para la proteína de choque térmico de 70-kDa (*hsp70*) y la enzima anhidrasa carbónica (CA) para confirmar su utilidad como biomarcadores de la respuesta de estrés y de homeostasis celular, respectivamente. **Métodos.** Evaluamos la GE mediante qPCR en colonias de corales *Pocillopora capitata*, *Pocillopora damicornis* y *Pocillopora verrucosa* del arrecife Carrizales (Colima, México) expuestas a un cambio natural en la temperatura de la superficie del mar (SST), productividad primaria y nutrientes utilizando la densidad de Symbiodiniaceae y el contenido de clorofila como indicadores

de salud. **Resultados.** La plasticidad de la GE de *hsp70* en *Pocillopora damicornis* y *Pocillopora verrucosa* se asocia con la variación diaria de temperatura y nutrientes, mientras que el gen de la CA no muestra cambios de expresión relacionada con esta variabilidad. Sin embargo, en *Pocillopora capitata* se encontró una expresión significativamente mayor de CA y una menor expresión de *hsp70*. Estos resultados reflejan un grado de plasticidad fisiológica en corales *Pocillopora* similar a la extensa plasticidad morfológica dentro de este género, lo que podría sugerir diferentes capacidades de adaptación a la temporada primaveral de bajas temperaturas y alto contenido de nutrientes en la región. **Conclusiones.** Evaluar la plasticidad fenotípica (morfología y fisiología molecular) podría ser útil para identificar colonias de corales con un mayor potencial de sobrevivencia al estrés ambiental. Lo anterior resulta relevante para la conservación, manejo y restauración de los arrecifes de coral del Pacífico mexicano.

Palabras clave: aclimatación coralina, fisiología molecular, plasticidad fenotípica

INTRODUCTION

Coral reefs currently face the challenges of increased sea surface temperatures and severe changes in ocean chemistry due to global warming and ocean acidification (Hughes *et al*., 2017) under an unprecedented climate change crisis (Barnes *et al*., 2022). In addition, accelerated industrialization, urbanization, and agriculture have played a significant role in coral reefs' degradation through eutrophication, sedimentation, and turbidity (Suggett & Smith, 2020; Donovan *et al*., 2021). Those environmental challenges, if extreme and prolonged, cause signs of severe stress effects on coral calcification, and massive bleaching and mortality due to the loss of the endosymbiotic relationship between corals and Symbiodiniaceae (Eakin *et al*., 2019).

Recent studies, however, have shown that differential changes in gene expression, via physiological plasticity, between and within coral species (Rivera *et al*., 2021; Strader & Quigley, 2022) could contribute to emergent stress responses such as thermal tolerance (van Oppen & Oakeshott, 2020; Avila-Magaña *et al*., 2021) and resistance to ocean acidification (Yuan *et al*., 2019; Scucchia *et al*., 2021); with particular links between specific environmental stressors (nutrient/thermal) that could benefit or synergistically affect heat-stressed corals at the cellular level (Rodríguez-Casariego *et al*., 2020; Montalbetti *et al*., 2021; Thummasan *et al*., 2021) resulting in tolerant and susceptible populations that show distinct transcriptional resilience and acclimation potential (Savary *et al*., 2021; Drury *et al*., 2022). The latter highlights the ability of qPCR-based gene expression biomarkers to elucidate gene expression plasticity (Poli *et al*., 2017) and their potential as molecular tools to assess and predict coral reef health and function under climate change scenarios (Hook *et al*., 2014; Palumbi *et al*., 2014; Zoccola *et al*., 2016), with further applications on coral reef restoration and conservation as an aid in determining what readily quantifiable phenotypes are most indicative of resilience (Parkinson *et al*., 2020; Kenkel & Wright, 2022).

Molecular markers (biomarkers) are defined as early detectable changes in the expression of one or several genes that indicate physiological effects or alterations (Smith *et al*., 2009), and those gene expression biomarkers have been widely used to measure environmental and anthropogenic impacts on marine species (Hook *et al*., 2014; Tarrant *et al*., 2019). As the earliest steps of an organism's response to environmental stress occur at the molecular level comprising the cellular stress response (CSR), gene expression analyses of the CSR provide a valuable tool to link an organism's physiology with large-scale climatic conditions (Kenkel *et al*., 2014). The CSR is activated only by severe stress, which causes a proportional increase in macromolecular damage and exceeds the elastic limit of cellular homeostasis (Kültz, 2020); once cell damage control has taken place and cells have been repaired, a complementary response called the cellular homeostasis response (CHR) is triggered to reestablish homeostasis under the new environmental conditions (Kültz, 2005). Therefore, gene expression analyses of the CSR and CHR genes have been proposed as a standard metric for quantifying stress and evaluating an organism's condition *in situ* (Evans & Hofmann, 2012).

The CSR genes include those coding for molecular chaperones of the Hsp family (*hsp110, hsp90, hsp70, hsp40, hsp60,* and small Hsps), antioxidants, redox enzymes, and enzymes that mitigate stress-induced damage to cellular components such as membranes, proteins, and DNA (Kültz, 2005; Shitaoka *et al*., 2021); while the CHR genes include those coding for Ca^{2+} homeostasis enzymes, ribosomal proteins, cytoskeleton and, in the case of calcifying/photosynthetic organisms (such as corals), extracellular matrix proteins and the carbonic anhydrase enzyme family (Kenkel *et al*., 2013, 2018). The immediate up-regulation of the Hsps family is a ubiquitous, vital, and dynamic response associated with thermal stress tolerance and bleaching resistance (Barshis *et al*., 2013; Palumbi *et al*., 2014; Poli *et al*., 2017; Zhang *et al*., 2018), in which the coral holobiont expresses the genes at high levels at all times in stressful conditions/locations (Mayfield *et al*., 2013, 2019). Furthermore, the carbonic anhydrase (CA) isoforms are a potential biomarker family for global and local impacts in calcifying organisms (Zebral *et al.,* 2019), such as ocean acidification and contamination effects in corals (Kenkel *et al*., 2014, 2018; Zoccola *et al.,* 2016).

Before the expression of any gene can be applied as a universal biomarker, however, there must be an understanding of its promises and limitations concerning natural environmental sensitivity, species-specificity response, environmental history, and current anthropogenic impacts and how expression patterns relate to the physiological and ecological consequences of stress tolerance and resilience due to those varying factors (Kenkel *et al*., 2014; Rivera *et al.,* 2021; Drury *et al*. 2022). For instance, the *hsp70* gene expression can change drastically with a variety of environmental stress such as extreme temperatures (high or low), high light intensity and salinity changes, nutrient enrichments, and cellular stress caused by coral bleaching and pathogen invasion in corals (Seveso *et al.,* 2016; Zhang *et al.,* 2018; Dellisanti *et al*., 2022). Furthermore, when nutrient enrichment is coupled with thermal stress, severe damage occurs to the coral holobiont of *Pocillopora damicornis* (Linnaeus, 1758) (compared with the individual effects) due to the Hsps upregulation-induced apoptosis and bleaching mechanism amplification by high nitric oxide (NO) production (Thummasan *et al*., 2021).

This study aims to confirm and validate the ability of the *hsp70* and CA genes as biomarkers of differential stress responses and acclimation potential in corals under natural high nutrient concentrations and low thermal variations. For this, we performed a field study of the *hsp70* and CA (qPCR-based) gene expression on *Pocillopora capitata* Verrill,

1864, *P. damicornis*, and *Pocillopora verrucosa* (Ellis & Solander, 1786) (*Pocillopora* mtORF type 1; *sensu* Pinzón & LaJeunesse, 2011) from the Mexican Pacific. We mainly chose those biomarkers because they have proven effective in identifying gene expression plasticity in *Pocillopora* (Delgadillo-Nuño *et al.*, 2020). Therefore, we hypothesize that equally healthy corals assessed through Symbiodiniaceae density and chlorophyll content will have some level of gene expression plasticity as part of a differential acclimation and adaptation potential, having the highest plasticity in the most abundant corals at the Carrizales reef, which could explain its differences in coverage and frequency (Reyes-Bonilla *et al.*, 2013).

Such new information will highlight the importance of including the evaluation of phenotypic plasticity (morphology/molecular physiology) and temporal variability (daily/seasonal) to accurately predict and anticipate *Pocillopora* coral's response to future conditions. Moreover, knowing which readily quantifiable *Pocillopora* phenotypes could have lower or higher resilience under global change scenarios should be an essential consideration for the experimental designs, management plans, and conservation and restoration efforts of coral reefs in the Mexican Pacific.

MATERIALS AND METHODS

Study area. The Carrizales coral reef is located on the central Mexican Pacific at the Colima coast (19°05´42" N, 104°26'21" W) (Fig. 1), which is on the list of Priority Marine Regions (RMP) as part of the Punta Graham-El Carrizal PMR#27 (Arriaga-Cabrera *et al.*, 1998); such regions are a framework to propose new natural protected areas in Mexico due to their high biodiversity potential and value (Arriaga-Cabrera *et al.*, 2009). Accordingly, the Carrizales reef is a relatively well-developed, undisturbed, and ecologically rich coral community (Liñán-Cabello & Michel-Morfin, 2018) dominated by branching corals (*Pocillopora* spp.) at shallow and mid-shallow depths (1 to 8 m), with a change in dominance by encrusting and submassive corals (*Porites* spp. and *Pavona* spp.) in deeper waters (8 to 10 m), with fewer records of *Psammocora* spp. (Reyes-Bonilla *et al.*, 2013), similar to other coral reefs in the central Mexican Pacific (Hernández-Zulueta *et al.*, 2017). Coral species occupy many microenvironments within the reef (Reyes-Bonilla *et al.*, 2013), with highly variable temporal conditions (Liñán-Cabello *et al.*, 2016; Delgadillo-Nuño *et al.,* 2020). We focused on low temperatures and high nutrients during the spring-dry season (Muñiz-Anguiano *et al.*, 2017) to reduce the specific conditions of poor light quality and high turbidity and sedimentation during the rainy season.

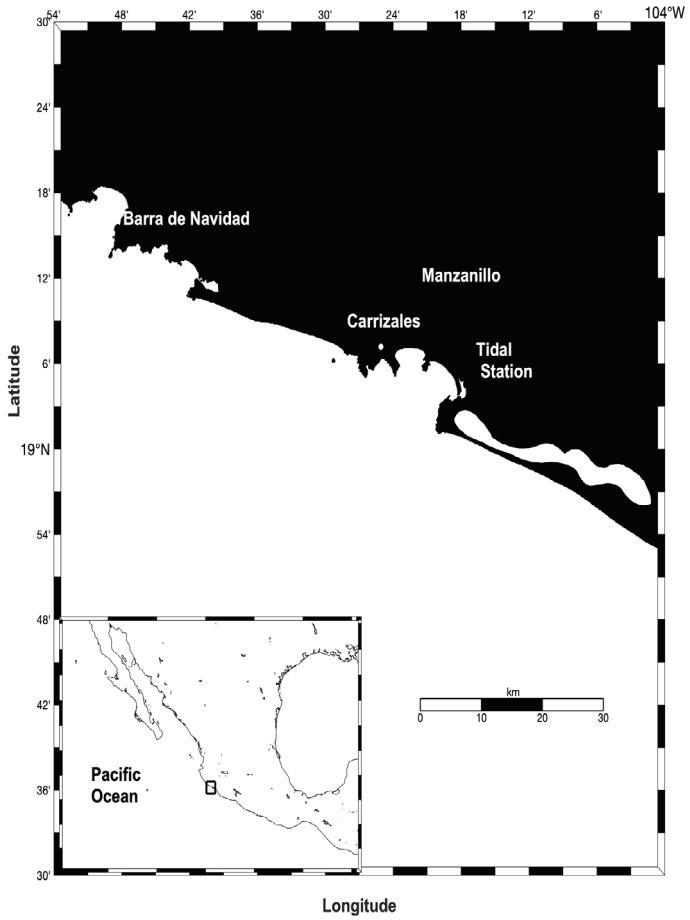

Figure 1. Location of the Carrizales reef on the coast of Colima, central Mexican Pacific.

Morphospecies identification. During the last week of April 2016, we used SCUBA diving in the Carrizales reef to visually identify the three most abundant *Pocillopora* morphospecies based on the macro-morphology of the colonies (after Schmidt-Roach *et al*., 2014), namely *P. capitata*, *P. damicornis*, and *P. verrucosa* nominal species (after Veron *et al*., 2016). However, considering the discrepancies between morphological and molecular identification (Gélin *et al*., 2017; Johnston *et al*., 2017), those are regarded here, according to Pinzón & LaJeunesse (2011), as *Pocillopora* morphospecies of a single genetic lineage (mtORF type 1). No other phenotypic characteristics besides macro skeletal morphology were considered in our study (i.e., color phenotypes or rare morphologies). Nevertheless, all colonies were recorded *in situ* (photographically) for further revision.

Sampling design. After visual identification, we carried out two samplings separately on April 22 and 24, 2016. To reduce the effects of light intensity on the *hsp70* and CA gene expression and Symbiodiniaceae indexes (Delgadillo-Nuño *et al.,* 2020) and ensure as much as possible that environmental variation corresponded to temperature and nutrient conditions, all samplings were conducted at midday, from the top of coral colonies at 3 m depth within the main body of the reef avoiding any microenvironments such as large rocks, caves, sandy patches, and isolated or separated colonies (Reyes-Bonilla *et al.,* 2013). We collected 72 coral fragments (~1 cm^2) from 36 different colonies of *Pocillopora* morphospecies that occurred in sympatric groups (i.e., one coral colony of *P. capitata*, *P. damicornis*, and *P. verrucosa* were next to the other); each group of sympatric morphospecies was established as an independent experimental sampling unit (n = 6) and those were separated at least 5 to 10 meters to reduce the potential of having collected clones.

Hydrographic analysis. We collected three samples of seawater (200 mL) on each of the sampling days (April 22 and 24, 2016) at 2 m depth on the Carrizales reef and 1 m close to the coral colonies for the determination of dissolved inorganic nutrients (aliquots of 50 mL) according to the methods described by Strickland & Parsons (1972) and Grasshoff *et al*. (2009) using a segmented flow autoanalyzer (Skalar SanPlus II). These parameters were used as a proxy of the environmental variation at the local scale independent of the microenvironment. Given the lack of temperature measurements in the sampling site, we used sea surface temperature records in the study area made by the Red Mareográfica at Manzanillo station of the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) (http://redmar.cicese.mx/emmc/DATA/MNZN). Additionally, for the mesoscale, we used temperature and chlorophyll data from satellite images (8 days compound at processing level 3) from the MODIS Aqua sensor (http://oceancolor.gsfc.nasa.gov).

Total RNA isolation. The excess of RNALater from coral fragments stored at -20 °C was removed, and coral fragments were crushed with a sterile porcelain mortar and pestle. Immediately, ~100 mg of the slurry was placed in 1.5 mL microcentrifuge tubes with 1 mL of TRIzol Reagent (Life Technologies©) and homogenized using a mechanical disruptor (FastPrep®24, MP Biomedicals, Santa Ana, California, USA). We isolated total RNA from each sample following the manufacturer's specifications and, according to Anderson *et al.* (2016), up to 2 μL of 6 M HCl was added to avoid the neutralization reaction that occurs between the calcium carbonate of the skeleton and the acidity of TRIzol. Total RNA was eluted in 50 μL of RNase-free water. The quantity and quality of the nucleic acid were analyzed using a Nanodrop® spectrophotometer and through visual inspection in 1.5 % agarose gel. DNA contamination was removed from the RNA samples by treatment with the enzyme DNase I (RNase-Free), following the manufacturer's specifications (Invitrogen, Thermo Fisher Scientific Inc). RNA samples were stored at -80 °C until further processing to avoid degradation.

Gene expression. For the analysis of qPCR-based gene expression biomarkers (*hsp70* and CA), we used sequence-specific primers for *Pocillopora* corals obtained from the literature (Mayfield *et al*., 2013) and the 18S ribosomal RNA gene (18S rRNA) as an internal control designed from *Pocillopora* sequences on the GenBank (accession No. HM013849.1). The set of primers was as follows: 1) *hsp70* (Forward: 5'-CCGCCGGTGGGGTAATGA-3', Reverse: 5'-CTTGTCGCGTTTCTTCT-CG-3'), 2) CA (Forward: 5'-AGGATGATGAGGAGGATGAGG-3', Reverse: 5'-ATAGCAGGGAGGGGTGGTAA-3'), and 3) 18S rRNA (Forward: 5'-GGTG-GTTGAGATGGATGG-3', Reverse: 5'-ACGTAGGCAGGCACC-3'). Before the gene expression analysis of the biomarkers, we performed a reverse transcription of RNA (200 ng) into complementary DNA (cDNA), using the High-Capacity cDNA Reverse Transcription Kit and following the manufacturer's specifications (Applied Biosystems, Life Technologies, CA). Aliquots of 20 ng μL^{-1} of cDNA were then used for the qPCR amplifications, carried out in 20 μL of the total volume containing 6.3 μL of 2X SYBR Green Master Mix (Applied Biosystems), 0.6 μL of one set of primers (10 mmol L^{-1}), 5.0 μL of the cDNA sample (20 ng μL^{-1}), and 6.9 μL of RNase/DNase-free water in a StepOnePlus™ thermocycler (Applied Biosystems), with the following thermocycling conditions: 1) "hot start" at 95 °C for 10 min, 2) amplification of 40 cycles at 95 °C for 15 s followed by 60 s at 60 °C, and 3) fusion at 95 °C for 15 s followed by 60 s at 60 °C and 15 s at 95 °C. Results expressed the relative mRNA expression of target genes and internal control using the $2^{-\Delta\Delta Ct}$ quantification method (Schmittgen & Livak, 2008).

Health indices. For Symbiodiniaceae cells extraction, coral fragments previously preserved in 10 mL of 10 % formaldehyde were thoroughly rinsed with distilled water and incubated in 10 mL of 4M NaOH at 37.5 °C until the tissue was removed from the skeleton (modified from Zamoum & Furla, 2012). The cell density of Symbiodiniaceae was quantified from an aliquot of 10 μL using a Neubauer hemocytometer (n = 8 replicates). Results express Symbiodiniaceae cells per unit of surface area occupied by the living tissue in the coral skeleton (cells cm^{-2}), obtained by measuring each fragment with millimeter precision calipers. For Chlorophyll (Chl *a*) extractions, we used ~ 100 mg of frozen fragments crushed in a mortar and placed in microcentrifuge tubes with 1.5 mL of 100 % methanol stored in the dark for 24 h (4 °C). The resulting slurry was sonicated for 15 s and then centrifuged at 1 500 g for 5 min (4 °C), and the supernatant was used immediately for quantification. Pigment measurements were performed in duplicates with a Spectronic® Genesys™ 5 spectrophotometer (Thermo Fisher Scientific), using a 96 wells microplate. Chl *a* concentration was calculated at 664 nm (Jeffrey & Humphrey, 1975), using the extinction coefficient 90 L gm^{-1} cm^{-1} (Vernon, 1960), with the recommended turbidity correction. Results expressed pigment concentration per unit of surface area (Chl *a* μg cm^{-2}).

Statistics. We used R software (R Core Team, 2013) to perform all statistical analyses; we proved normality with the Shapiro-Wilk test and homoscedasticity with Bartlett's test for all data (95 % confidence intervals). As normality and homoscedasticity assumptions were true for nutrients and gene expression data, a two-sample unpaired t-test was used to analyze significant differences in nutrients between days

($P > 0.05$) and a two-way analysis of variance (ANOVA) followed by multiple comparisons (Tukey's test) to evaluate significant differences of gene expression between days and morphospecies ($P > 0.05$). In the cases when normality or homoscedasticity assumptions were false (Symbiodiniaceae cell density and Chl *a* concentration), a non-parametric two-way Friedman test and pairwise sign test were used to evaluate significant differences between days and morphospecies ($P > 0.05$). Additionally, we performed a principal component analysis (PCA) using the built-in R functions prcomp to identify differences between morphospecies in Symbiodiniaceae cell density, Chl *a* concentration, and changes in the differential expression of *hsp70*, and CA genes. A logarithmic transformation was applied to eliminate the data variation due to the unit difference.

RESULTS

Remote sensing showed low SST (22 °C) and high productivity (80 mg m^{-3}) along the central Mexican Pacific (including the Manzanillo coast) during the first week of April 2016 (March 29-April 05). Then, the SST increased to 26 °C, while productivity decreased (10 mg m^{-3}) during the second week (April 06-April 13). The SST continued to change between 22 and 26 °C during the third (April 14-April 21, 2016) and fourth week (April 22-April 29), but productivity remained low until the end of April 2016 (Fig. 2). In the same way, the temperature records close to the Carrizales reef showed a thermal change from 22 to 24 °C in nine days (April 01 to 09), followed by an increase from 24 to 26 °C in another nine days (April 10 to 18). Later, the temperature dropped from 26 to 24 °C in only three days (April 19 to 21) and suddenly decreased from 24 °C on the first day of sampling (April 22) to reach almost 20 °C on the second day of sampling (April 24), to increase again in the next day at 24 °C and remain until the end of April 2016 (Fig. 3). The results of the t-test showed significant variations in dissolved inorganic nutrients ($P < 0.05$), with higher nitrogen concentrations on April 22, while phosphorus and silicates were higher on April 24, 2016 (Table 1).

For the qPCR-based biomarkers, the relative *hsp70* mRNA expression of *P. damicornis* and *P. verrucosa* morphospecies showed significant differences between the two days of sampling ($P < 0.05$). Furthermore, both morphospecies showed significantly higher values of relative mRNA expression ($P < 0.05$) compared to those of *P. capitata* morphospecies (Fig. 4A). On the other hand, the relative mRNA expression of CA did not show significant differences between the sample days for none of the three *Pocillopora* morphospecies. However, the expression of CA in *P. capitata* morphospecies showed significantly ($P < 0.05$) higher values of relative mRNA expression compared to those of *P. damicornis* morphospecies and *P. verrucosa* morphospecies (Fig. 4B). Regarding the health indices, there were no significant differences in Symbiodiniaceae density or chlorophyll between sampling days and *Pocillopora* morphospecies (Fig. 5A and B). Finally, the principal component analysis showed a clear separation between *P. damicornis* and *P. verrucosa* morphospecies from *P. capitata* morphospecies. The first two principal components (PCs) explained 63.5 % of the variation (Fig. 6).

DISCUSSION

Interpreting how or whether a coral holobiont phenotype (i.e., a morphotype, morphospecies, or ecomorph) can employ gene expression plasticity to ensure short and long-term survival (Rivera *et al*., 2021) will be critical for understanding global and local impacts of climate change and coastal anthropogenic activities (Hughes *et al*., 2017; Donovan *et al*., 2021) across diverse taxa (Suggett & Smith, 2020; Barnes *et al*., 2022) and in determining which phenotypes are most indicative of resilience to those impacts (Parkinson *et al.,* 2020; Rivera *et al*., 2021). In this sense, our study proves some degree of physiological plasticity through diversity in the cellular response between the three *Pocillopora* morphospecies and provides determining information on their distinctive acclimation responses which could suggest different capacities for genetic or biochemical adaptation (Bernhardt & Leslie, 2013; Stillman & Paganini, 2015) in *Pocillopora* morphospecies of the Carrizales reef.

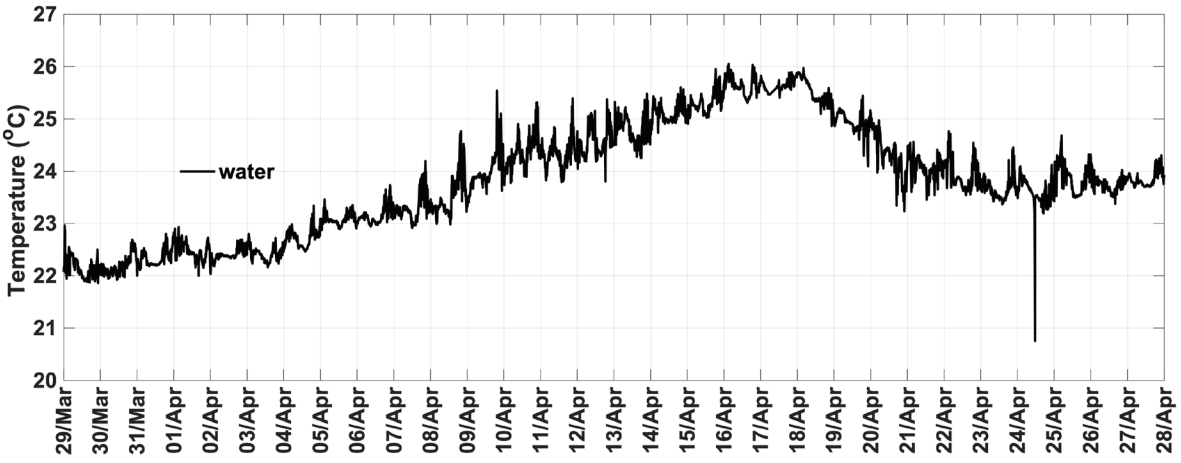

Figure 2. Seawater temperature measurements at the Tidal Station on Manzanillo Bay, Colima, Mexico. Recorded each minute on April 2016 from the Redmar, Coastal and Meteorological Station of CICESE.

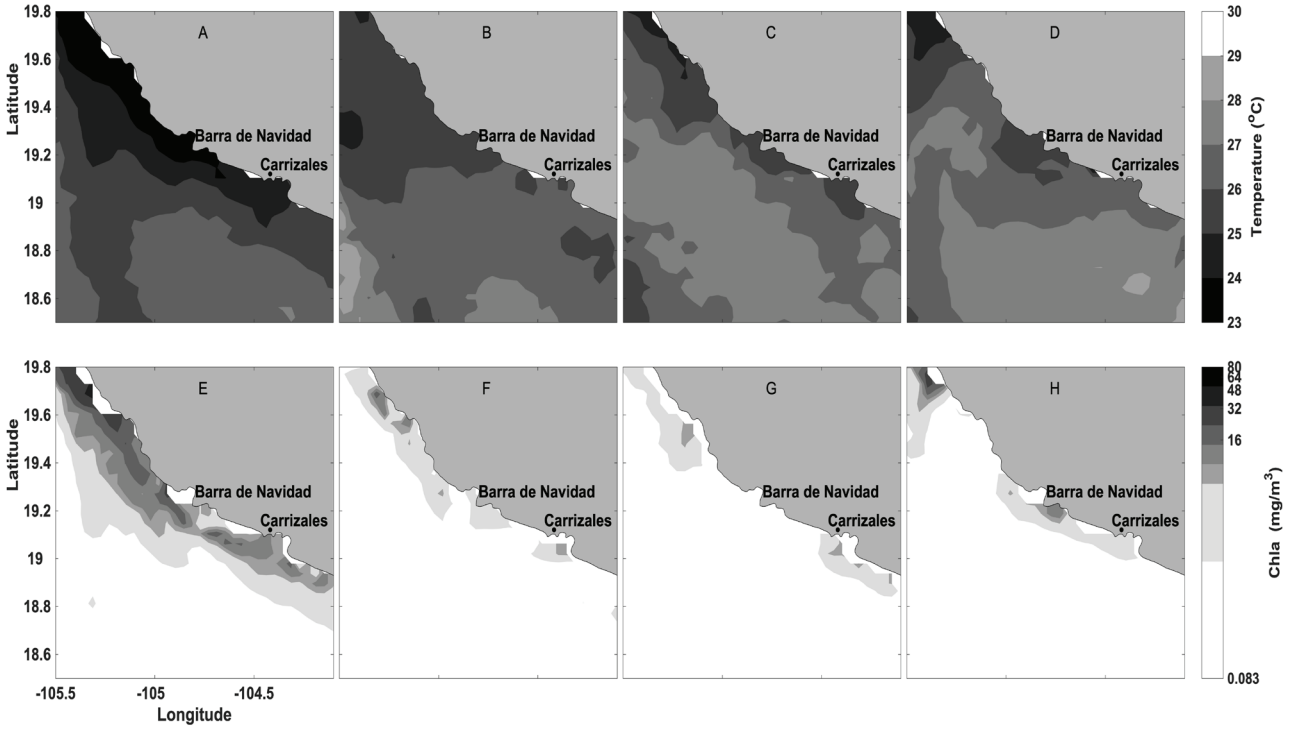

Figure 3. Satellite images of SST (A, B, C and D) and chlorophyll (E, F, G and H) from the MODIS Aqua sensor. Each column shows a data compound of 8 days (processing level 3) for the central Mexican Pacific in April 2016. The dot marks the location of the Carrizales reef.

First, we observed a significant increase in the *hsp70* gene expression in *Pocillopora* morphospecies (Fig. 4A), clearly associated with the short-term temperature change within the sampling days (±2 °C in 3 days, followed by ±4°C in 24 h) at Manzanillo coast (Fig. 2) coupled with high nutrients concentration at Carrizales reef, particularly in Nitrogen (7-9 µMoles) (Table 1); without significant changes in the symbiotic condition whatsoever (Fig. 5A and B). Both the changes in temperature (up or down) and the high nutrient concentrations can cause positive expression of Hsp proteins (Seveso *et al*., 2016; Thummasan *et al*., 2021), and these conditions occur naturally during the spring season at the Carrizales reef (Muñiz-Anguiano *et al.,* 2017). Therefore, as expected, the prominent molecular response in *Pocillopora* morphospecies was an induction of the *hsp70* gene related to local environmental changes in the short term. However, the magnitude and direction of this plasticity were different between *P. damicornis/P. verrucosa* and *P. capitata* (Fig. 6), suggesting two different levels of *hsp70* gene expression plasticity in *Pocillopora* morphospecies: higher plasticity (*P. damicornis/P. verrucosa*) and lower or reduced plasticity (*P. capitata*).

Table 1. Comparison of nutrient concentrations (Average ± SD) in Carrizales reef between April 22 and April 24, 2016 (n = 3 samples).

Nutrient	April 22	April 24	*t*	*p*-value
$NO_3 + NO_2$ (µMoles)	9.20 + 0.14	7.62 + 0.28	8.7860	0.0005
NH_4 (µMoles)	0.28 + 0.03	0.22 + 0.02	3.3793	0.0139
PO_4 (µMoles)	1.16 + 0.23	2.41 + 0.12	-8.4408	0.0005
DIN:PO_4 ratio	8.43 + 1.45	3.26 + 0.20	4.9741	0.0038
SiO_2 (µMoles)	12.25 + 0.56	14.58 + 0.51	-5.3431	0.0030

As we hypothesized, *Pocillopora* morphospecies would have some degree of physiological plasticity at the molecular level when exposed to environmental stress (thermal/nutrients). In terms of gene-by-gene expression analysis, plasticity occurs after the environmental change (stress signal) when the expression of a gene significantly increases to reach a peak of expression under the new stress condition (Hédouin & Berteaux-Lecellier, 2014; Rivera *et al*., 2021), in this case, thermal and nutrients stresses. Higher *hsp70* levels in *Pocillopora* corals are generally related to a protective response toward environmental and cellular stressors (Poli *et al*., 2017; Zhang *et al.,* 2018; Dellisanti *et al.,* 2022), with the tolerant phenotypes exhibiting higher expression levels than the susceptible ones.

This is true when stress-tolerant populations show higher baseline expressions before the stress condition and less (induction) positive change in gene expression after (Poli *et al*., 2017), which confers protection from frequent stresses through a pre-emptive response (front-loading) and by maintaining cellular integrity under constant pressure (Brener-Raffalli *et al.,* 2022). While low baseline expression and a high induction are observed in sensitive populations (Rivera *et al*., 2021), this has been observed in high thermal regimens mainly (Kenkel *et al*., 2013; Palumbi *et al.,* 2014; Poli *et a*l., 2017). However, this is not the case in our results, in which a lower baseline expression of the *hsp70*

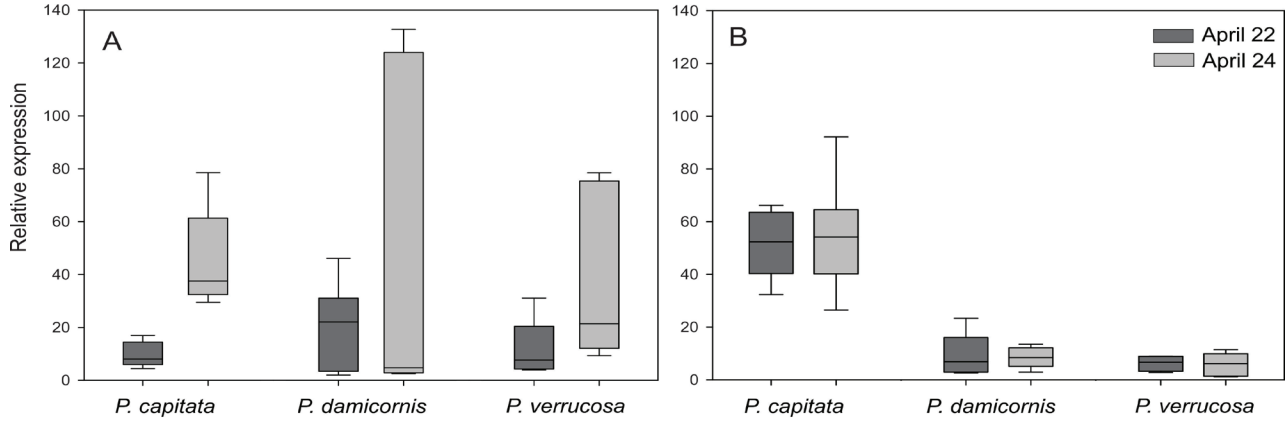

Figure 4. mRNA relative expression in *Pocillopora* morphospecies from the Carrizales reef on April 22 and 24, 2016; A) heat shock protein (*hsp70*) and B) carbonic anhydrase (CA).

gene was shown before the condition without stress for all the morphospecies studied, with the sole difference in the magnitude of the hsp70 gene expression between *P. damicornis/P. verrucosa* and *P. capitata* after the environmental change.

In our study, the lower plasticity of the *hsp70* gene expression could suggest some tolerance to the combination of low temperature and high nutrient stress in *P. capitata* morphospecies by a lowered induction of the *hsp70* gene expression. This is a more general pattern also associated with genes involved with thermal stress (Bay & Palumbi, 2017) as part of a cellular response that appears muted (dampening) under the new stress condition, which allows for reducing the energy requirement of the stress response and utilizes it in cellular maintenance and homeostasis (Rivera *et al.*, 2021). The latter is supported by a higher constitutive expression of the CA gene through the sampling days in *P. capitata* morphospecies, which suggests that CA gene expression could have been sustained for more extended periods to maintain cellular homeostasis during the highly variable conditions, of low temperature and high nutrients, on the Carrizales reef during the spring season.

Broadly, the positive regulation of the CA (as an adaptive mechanism) sustains all the vital metabolic processes in the coral holobiont, such as photosynthesis, calcification, cellular homeostasis, and growth simultaneously (Bertucci *et al.*, 2013). This process of adaptive metabolism by increasing CA gene expression in *P. capitata* morphospecies could help to cope with the energy requirements of an environmental stress regime (Kültz, 2003), reflecting a higher resilience potential by long-term physiological acclimation (Kenkel *et al.*, 2014, 2020), since higher induction of *hsp70* gene comes at a substantial energy cost (Kenkel *et al.*, 2013; Poli *et al.*, 2017). Therefore, the differences in basal expression of the CA gene and the levels of *hsp70* gene induction reveal some physiological plasticity between *P. capitata* and *P. damicornis/P. verrucosa*, probably through diversity in the cellular response of the morphospecies, which could confer slight differences in stress tolerance and resilience.

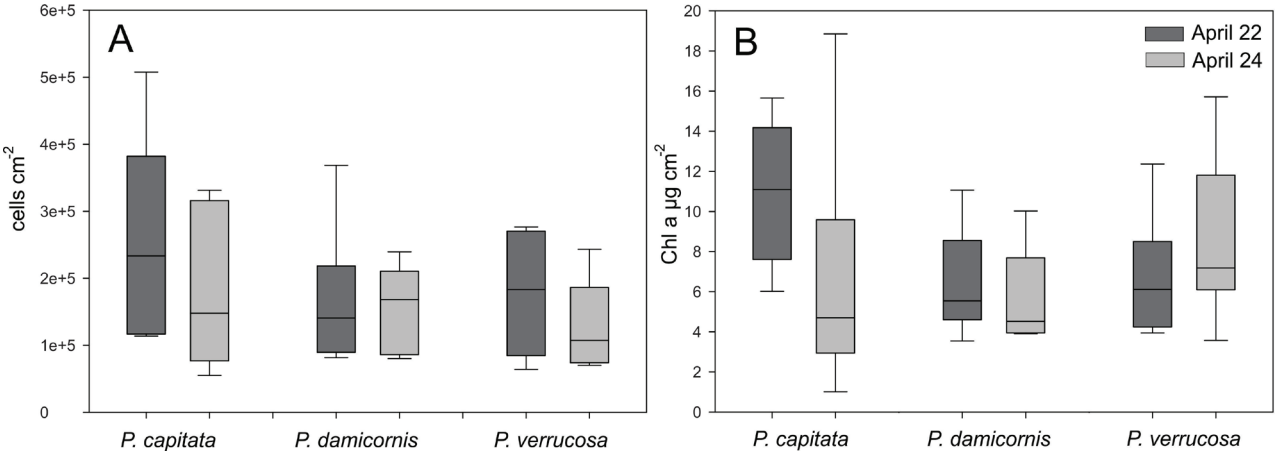

Figure 5. Health indexes in *Pocillopora* morphospecies from the Carrizales reef on April 22 and 24, 2016; A) Symbiodiniaceae density and B) Chlorophyll (*a* + *c2*).

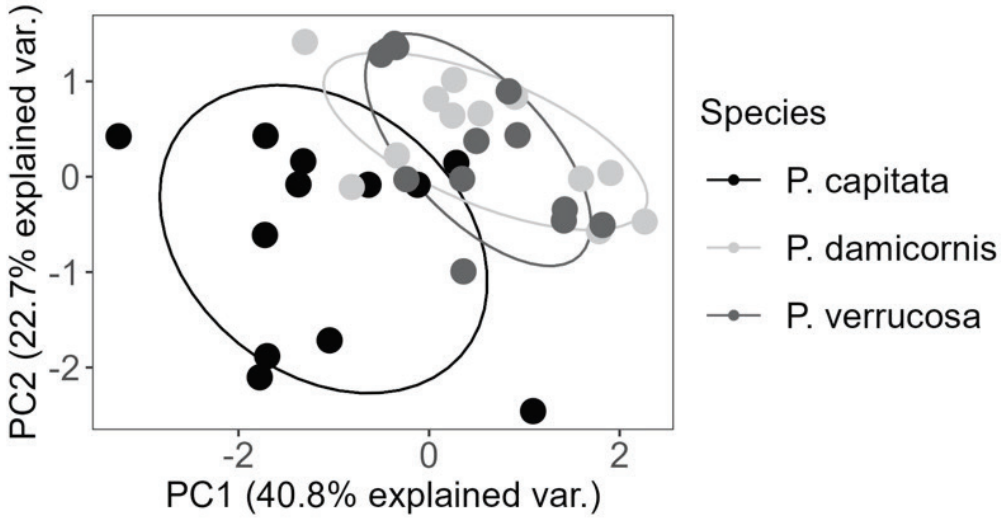

Figure 6. Principal component analysis (PCA) of the *hsp70* and CA mRNA relative expression, Symbiodiniaceae density and Chlorophyll content in *Pocillopora* morphospecies on April 22 and 24, 2016 from the Carrizales reef.

Finally, the results suggest that there was no substantial effect on the ability of Symbiodiniaceae to capture light (Brown, 1997; Douglas, 2003), translocate nutrients to coral host cells (Saxby *et al*., 2003; Hoegh-Guldberg *et al*., 2005; Smith *et al*., 2005), and maintain metabolic homeostasis of the holobiont (Hinrichs *et al*., 2013), and this is another important consideration in our study since the coral bleaching machinery-induced apoptosis seems absent. That is, no evidence of Symbiodiniaceae and Chl *a* loss, coral de-pigmentation, or tissue detachment due to the oxidative stress-induced mechanism, which increases ROS levels, damages the photosynthetic machinery, alters the ionic balance in the symbiont thylakoid membranes and disrupts the symbiosome microenvironment (Rodríguez-Casariego *et al.,* 2020; Thummasan *et al*., 2021). Therefore, no differences in energy supply are inferred, and the *hsp70* and CA gene expression levels could be considered within the acclimation or adaptation potential with the sole difference in the response thresholds of *P. capitata* and *P. damicornis/P. verrucosa*.

In conclusion, the most prominent result of this study could be interpreted as a diversity in the CSR and CHR of *Pocillopora* morphospecies that affected the patterns of magnitude and direction of physiological plasticity (high or reduced), as shown by the constitutive expression and induction of the CA and *hsp70* genes, respectively. We demonstrated that *P. capitata* and *P. damicornis/verrucosa* could have specific tolerance to cope with the natural stressful conditions in the Carrizales reef. However, this only partially explains the differences in coverage and frequency between the three morphospecies in the Carrizales reef (Reyes-Bonilla *et al*., 2013; Hernández-Zulueta *et al*., 2017) and might seem contradictory for the spatial differences of the three morphospecies through the Mexican Central Pacific (see supplementary material on Hernández-Zulueta *et al*., 2017) so, further study of complete transcriptional response (i.e., RNAseq) is needed for a better understanding of how *Pocillopora* morphospecies are (more or less) resilient to environmental changes and anthropogenic impacts. Considering that the *Pocillopora* morphospecies studied here are potential early colonizers and ecological activators of impacted reefs (Buitrago-López *et al*., 2020) and some of the most critical and principal reef-builders of the Tropical Eastern Pacific (Cabral-Tena *et al*., 2020); our results could be used for linking phenotypic plasticity (morphological and physiological) with some degree of diversity in the cellular response of *Pocillopora* corals, and this could have important implications for future experimental designs and the ecological success of management plans, and conservation and restoration efforts of coral reefs in the Mexican Pacific.

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