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ARTICLE

Response to pH stress in the reef-building coral *Pocillopora capitata* (Anthozoa: Scleractinia)

Respuestas al estrés de pH en el coral petreo *Pocillopora capitata* (Anthozoa: Scleractinia)

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Resumen.- Para evaluar la respuesta metabólica del coral simbiótico *Pocillopora capitata* a la reducción del pH del agua de mar en un sistema *in vitro*, 112 ramas de *P. capitata* se obtuvieron de la comunidad de corales La Boquita (LB) sin exhibir algún daño aparente de blanqueamiento. Se evaluaron 2 tratamientos de pH: a) 7,85/7,95 pH (Tratamiento C_1), b) 7,60/7,70 (Tratamiento C_2) y 8,00/8,40 pH tratamiento de control (CC). Las ramas de coral fueron asignadas aleatoriamente a unidades experimentales (n= 38 por tratamiento). Las muestras fueron tomadas en 2 tiempos para el análisis bioquímico y para evaluar cualitativamente los aspectos microestructurales por microscopía electrónica de barrido: tiempo inicial, muestra tomada en 12 h (T_1); tiempo final, muestras del último día de la exposición (T_f, día 7 del experimento). A diferencia del tejido simbionte, el análisis bioquímico del tejido del coral reveló que *P. capitata* muestra una respuesta inmediata reflejada en los coeficientes de ARN/ADN y proteína/ADN, así como en las concentraciones de ARN y proteína, particularmente en el tratamiento de C_2 en las horas iniciales del experimento. El análisis microestructural cualitativo identificó principalmente efectos en el tratamiento C_2, que fue influenciado por la presencia de disgregaciones micro-superficiales en las regiones terminales de las fibras esqueléticas.

Palabras clave: *Pocillopora capitata,* coral, ARN/ADN, Proteina/ADN, fibras esqueléticas, estrés

Abstract.- To evaluate the metabolic response of the symbiotic coral *Pocillopora capitata* to reduced seawater pH in an *in vitro* system, 112 branches of *P. capitata* with no apparent damage from bleaching were collected from the La Boquita (LB) coral community. Two pH treatments were evaluated: a) pH 7.85/7.95 (Treatment C_1), b) 7.60/7.70 (Treatment C_2), and 8.00/8.40 pH control treatment (CC). The coral branches were randomly assigned to experimental units (n= 38 per treatment). Samples were taken at 2 separate times for biochemical analysis and qualitative assessment of microstructural aspects with scanning electron microscopy: initial time, sample taken at 12 h (T_1); end time, samples from the final day of exposure (T_f, day 7 of the experiment). Unlike the symbiont tissue, the biochemical analysis of the host tissue revealed that *P. capitata* displayed an immediate response as reflected in the RNA/DNA and protein/DNA ratios as well as in the concentrations of RNA and protein, particularly in the initial hours of the experiment in treatment C_2. Qualitative microstructural analysis primarily identified effects in treatment C_2 which was influenced by the presence of micro surface detachments in the terminal regions of the skeletal fibers.

Key words: *Pocillopora capitata,* coral, RNA/DNA, Protein/DNA, skeletal fibers, stress

INTRODUCTION

Coral reefs currently face the challenge of adaptation/ acclimatization due to increased ocean temperatures and changes in the physical chemistry of the oceans, including an increase in the dissolution of CO_2 (Hoegh-Guldberg *et al.* 2007, Baker *et al.* 2008, Veron *et al.* 2009). Increases in atmospheric CO_2 concentrations that are predicted to occur over the next 100 years will be a major challenge to the growth of coral reefs (Hoegh-Guldberg *et al.* 2007). Of the CO_2 emitted into the atmosphere by human activities, 30% is absorbed by the ocean (Kleypas & Langdon 2006), causing a decrease in surface water pH, which is a major threat to reefs. The increasing ocean surface temperature associated with increasing CO_2 concentrations will lead to an increase in the frequency and severity of coral bleaching with negative consequences for their survival, growth, and reproduction (Hoegh-Guldberg 1999). Similarly, the acidification of the oceans could cause coral bleaching, a result that would be fatal to coral ecosystems.

In corals, transport of dissolved inorganic carbon from the marine environment to the dinoflagellate involves a large number of metabolic pathways that allow the compound to be used by the anthozoa and dinoflagellate symbionts (Yellowlees *et al.* 2008). Moreover, variations in the dissolved carbon concentration can affect coral physiology (Harrould-Kolieb & Savitz 2009). DNA and RNA content and the RNA/DNA ratio have been used for nearly 40 years to determine cellular activities such as increased cell size, increased cell number, and RNA and protein synthesis activity (Sulkin *et al.* 1975). Specifically, the RNA/DNA ratio evaluates the rate of RNA synthesis, which is related to the ability of an organism to produce proteins; however, in some cases, the increase in protein synthesis may reflect stress responses (Buckley & Szmant 2004). In this instance, the RNA/DNA ratio can also be a biomarker of physiological stress.

According to Buckley & Szmant (2004), the ability of corals to survive global environmental change depends on the physiological mechanisms of acclimatization and adaptation. Gates & Edmunds (1999) used the RNA/DNA in coral in the coral *Madracis mirabilis* were able to observe that this ratio varies by a factor of 5 to 10 between clonal genotypes, suggesting that there are large intrinsic metabolic differences across individuals even of the same species; this is most likely due to the dynamic capacity for the exchange of different endosymbiotic dinoflagellate clades of the coral

In the massive corals *Porites lobata* and *P. lutea*, the RNA/DNA ratios may be higher under conditions of high turbidity and sedimentation; however, the ratio decreases with depth in the reef. These behaviors may indicate genetic variations (in metabolic function) to permit adaptation to the environment or may reflect marginal acclimation of metabolism in response to the environmental situation (Bak & Meesters 2000, Meesters *et al.* 2002). It is clear that responses to environmental factors are highly similar between different species and genera of hermatypic corals. By contrast, in 2 species of coral affected by bleaching, *Porites lutea* and *Acropora formosa*, the physiological response to stress resulted in changes in the pigmentation composition, zooxanthellae density, mitotic index (MI) of zooxanthellae, RNA/DNA ratios, and protein profiles in healthy corals. In semi-milled and bleached corals, the MI-zooxanthellae, the RNA/DNA ratio and protein profile in both species increased as bleaching increased; high concentrations of nucleic acids and proteins were attributed to the increased expression of new stress responses proteins (Harithsa *et al.* 2005). Another study acknowledged that the influence of macroalgal cover on *Acropora millepora*, was influenced by decreases in pH, changes in dissolved oxygen saturation, and increased concentrations of dissolved inorganic and organic carbon, exhibited a steady increase in the RNA/DNA ratio during the experiment, which, according to Hauri *et al.* (2010), may represent the synthesis of proteins in response to stress.

Based on the above-mentioned studies, the RNA/DNA ratio is considered a suitable marker for evaluating the metabolism and physiology of a wide variety of coral species and may represent a valuable tool for the diagnosis of the physiological effects and as an indicator of the response time to several effectors of stress (including those associated with climate change) to study how the decline in ocean pH could affect coral populations.

The objective of this investigation was to study the effect of the decrease in pH as an indicator of increased CO_2 concentration; in symbiont and *P. capitata*, we determined the RNA/DNA and protein/DNA ratios, RNA content and total protein content. The physiological response capacity may differ between host and symbiont, and this could lead to bleaching as well as short-term effects in calcification patterns.

MATERIALS AND METHODS

STUDY SITE AND SAMPLE COLLECTION

Samples of the coral *Pocillopora capitata* (Verrill, 1864), were collected from La Boquita reef (LB) in the Bay of Santiago in Manzanillo, Colima, México.

A jetty was constructed in 2003, resulting in the exchange of water masses between the sea and the lagoon adjacent to the northwest margin of the reef (19º06'13.85"-19º06'12.08"N, and 104º23'49.180"-104º23'45.810"W). As a result, the reef is subjected to intense and constant stressors including input of sediment and warm water with high salinity.

In July 2010, 112 individual branches obtained from a different coral colonies of *P. capitata* that showed no apparent bleaching damage were collected from LB at 2.2 m depth within 100 m of the jetty. The branches (~ 8 cm) were collected and transported to the Facultad de Ciencias Marinas (FACIMAR, Universidad de Colima, México) laboratory in 10 L buckets containing ambient seawater, immediately transferred to the aquarium and kept at 25ºC

and 35 of salinity for 3 days under an intensive system of sea water exchange in order to minimize the influence of collection stress prior to experimentation.

Experimental design

The experimental system consisted of 3 experimental units (50 x 35 x 10 cm). Each container had a volume of 17.5 L of seawater, and each unit was installed in a system to control the pH via Venturi reactor-diffuser powered by pressurized CO_2. Each unit was installed with a water filtration device (RESUN®, HF-2001 340 L h^{-1}, mechanical filtration, chemical and biological) and was maintained with artificial light (210 μmol m^{-2} s^{1}) at an average temperature of 29°C. The pH levels were monitored 5 times a day using a senION Hatch® potentiometer model 378. Two criteria that correspond to the acidification scenarios predicted for 50 and 100 years by the Intergovernmental Panel on Climate Change (Anthony *et al.* 2008) were considered: a) pH 7.85-7.95 (Treatment C_1); b) pH of 7.60-7.70 (Treatment C_2); and c) pH of 8.00-8.40 (control Treatment CC). According to the IPCC (2007), these values represent the current levels of CO_2 in atmosphere of 380 ppm of CO_2 and projected intermediate (high category IV, 520-700 ppm), and high-end (above category VI, 1000-1300 ppm) values.

The coral branches were randomly assigned to experimental units (n= 38 per treatment). For biochemical and scanning electron microscopy analysis, samples were taken in triplicate for each treatment at 2 separate time points: initial time, sample taken at 12 h (T_1); and end time, samples from the final day of exposure (T_f, day 7 experiment).

Purification of nucleic acids and total protein

Nucleic acids were purified and quantified using a matrix of nucleic acid binding conditions (Karp 2010). In particular, a commercial kit, AllPrep™ DNA/RNA/Protein Kit (QIAGEN Inc., Valencia, CA, USA), that permits selective binding of genomic DNA was used, likewise separation of total RNA under the RNeasy® principle and the chemical precipitation of total protein was done of same tissue.

The coral tissue from each sample was removed from the skeleton with compressed air and weighted (wet tissue) in analytical balance. According to the modification proposed by Buckley & Szmant (2004), the removed wet tissues was homogenized at 4 to 8°C with 1 ml of filtered seawater in a sterile 0.45 μm matrix and the homogenate was centrifuged at 1,537 g for 5 min at 4°C to precipitate the *Symbiodinium* cells. The aqueous phase (host) was transferred to a fresh tube and centrifuged at 8,854 g for 15 min at 4°C to precipitate the host cells. Both precipitates were stabilized in AllProtect® DNA/RNA/Protein solution (QIAGEN Inc., Valencia, CA, USA). The concentration of RNA, DNA and protein was determined for each precipitate according to the AllPrep™ DNA/RNA/Protein protocol (QIAGEN Inc., Valencia, CA, USA). Absorbance was measured at 260-nm, and RNA and DNA dilution buffers were used in the measurement. To quantify total protein in corals according to Gates & Edmunds (1999), we used the method of Bradford (1976) and the Coomassie® Protein Assay Kit Reagent (Pierce, Thermo Fisher Scientific Inc., Rockford, IL, USA). The absorbance was measured at 595-nm, and concentrations were determined from a standard calibration curve. The concentration of total RNA/DNA is expressed in μg mg^{-1} tissue and mg mg^{-1} of wet tissue in the case of protein.

Skeletal micromorphology

The microstructure of the freshly deposited skeleton collected at T_1 and T_f from different treatments was examined by scanning electron microscopy (SEM) using a low vacuum (JEOL JSM-6390LV). The sample preparation and analysis were performed according to Marubini *et al.* (2003). Briefly, after washing the bare skeletons several times with distilled water, the skeletons were dried at room temperature. The microstructure of the coral skeleton was observed by SEM scans at low vacuum (30 Pa). Three nubbins were randomly selected from each treatment for 3D-observations with SEM (more than 50 per nubbin) focused on the distal tips of crystal fibers across septa. Only the study of microstructures considered additional control treatment (CL) in which the specimens of *P. capitata* were from a region located about 7.5 km; this region is not subjected to intense and constant stressors.

Statistical analysis

To test for normality, independence, and homogeneity of the data, Bartlett and Kolmogorov–Smirnov tests were applied (Zar 1999). A one-way ANOVA was used to assess the significant differences. Treatment and initial-final time of the experiment were fixed factors and with interaction (treatment x time effects) followed by Tukey tests. A t-test was used to assess the significant differences between treatment and time. Significance was determined at $P = 0.05$. All the tests were performed using R Software V 3.0.1 (The R Foundation for Statistical Computing 2013).

Results

Anthozoa

RNA and total protein concentration were used as indicators of physiological response in the anthozoan species *Pocillopora capitata* to exposure to pH < 8. The RNA/DNA ratio was also used as an indicator of metabolism, and the protein/DNA ratio was used as an indicator of protein metabolism.

The concentrations of molecular markers and their ratios at T_1 and T_f are present in Table 1. Significant decreases in RNA ($P < 0.05$) and highly significant decreases the RNA/DNA and Protein/DNA ratios ($P < 0.01$) occurred only in the C_2 treatment, while the C_1 and CC treatments did not yield this behavior. However, when we performed the comparative analysis with each molecular and biochemical indicator and only considered the concentrations of T_1 in respect to T_f (Figs. 1 a-d), this data showed a trend to be higher the RNA/DNA and protein/DNA ratios, RNA and proteins concentrations, in T_1 of C_2; however in this treatment (C_2), the RNA/DNA, protein/DNA ratios, and RNA concentration at the T_f was significantly lower in respect to T_1.

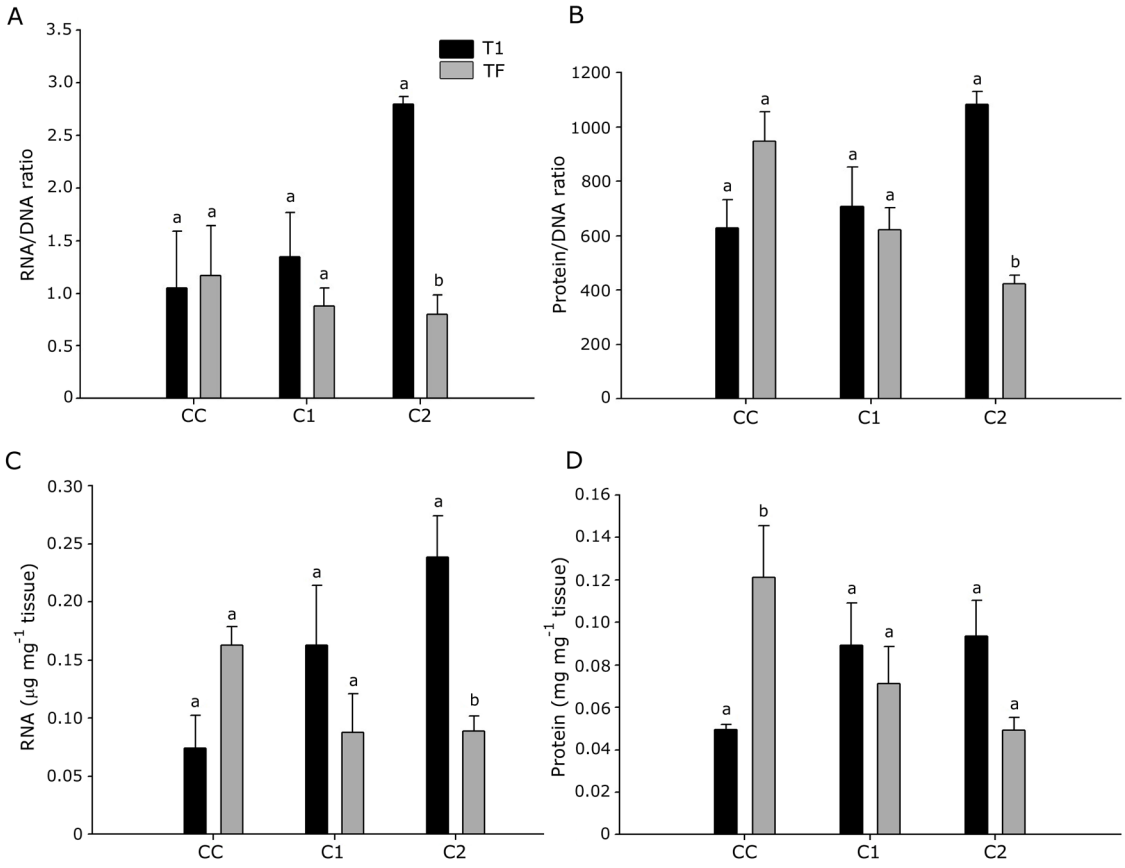

Figure 1. Mean values of biochemical markers and their ratios in the coral tissue at initial time (black) and final time (grey) a) RNA/DNA ratio, b) protein/DNA ratios, c) RNA content and d) protein content. Data are shown as the mean ± SE (n= 3). Different symbols denote significant differences between treatments ($P < 0.05$). C_1: treatment 7.85/7.95 pH; treatment C_2: 7.60/7.70 pH; CC: control treatment 8.00/8.40 pH / Valores promedio de marcadores bioquímicos y de sus relaciones en el tejido del coral en el momento inicial (negro) y el tiempo final (gris) a) la relación ARN/ADN, b) relaciones proteína/ADN, c) el contenido de ARN y d) contenido de proteína. Los datos se muestran como media ± EE (n= 3). Diferentes símbolos denotan diferencias significativas entre los tratamientos ($P < 0,05$). C_1: tratamiento 7,85/7,95 pH; tratamiento C_2: 7,60/7,70 pH; CC: tratamiento de control 8,00/8,40 pH

Table 1. Average values of RNA/DNA and protein/DNA ratios, total amounts of RNA and protein, in *Pocillopora capitata* (Cnidaria) and *Symbiodinium* sp. (Zooxanthellae), during pH-low levels experiment at initial time (T_1) and final time (T_f). C_1: treatment 7.85/7.95 pH; treatment C_2: 7.60/7.70 pH; CC: control treatment 8.00/8.40 pH. Means with different superscript are significantly different ($P < 0.05$) / Valores promedio de las relaciones ARN/ADN y proteína/ADN, concentración total de ARN y proteínas, en *Pocillopora capitata* (Cnidaria) and *Symbiodinium* sp. (Zooxanthellae), durante niveles experimentales bajos de pH a tiempo inicial (T_1) y tiempo final (T_f). C_1: tratamiento 7,85/7,95 pH; tratamiento C_2: 7,60/7,70 pH; CC: tratamiento de control 8,00/8,40 pH. Valores medios con diferente literal son significativamente diferentes ($P < 0,05$)

Parameter (Units)	T1			TF		
	CC	C1	C2	CC	C1	C2
Cnidarian						
RNA/DNA ratio	1.05±0.94[a]	1.35±0.74[a]	2.79±0.12[a]	1.17±0.83[a]	0.88±0.30[a]	0.80±0.32[b]
Protein/DNA ratio	627.97±182.16[a]	706.98±249.95[ab]	1081.97±82.65[a]	946.36±188.30[a]	622.10±142.09[ab]	422.59±57.10[b]
RNA (µg mg-1 tissue)	0.07±0.05[a]	0.16±0.09[a]	0.24±0.06[a]	0.16±0.03[a]	0.09±0.06[a]	0.09±0.03[b]
Protein (mg mg-1 tissue)	0.05±0.00[a]	0.09±0.03[a]	0.09±0.03[a]	0.12±0.04[a]	0.07±0.03[a]	0.05±0.01[a]
	CC	C1	C2	CC	C1	C2
Zooxanthellae						
RNA/DNA ratio	1.02±0.54[a]	0.87±0.42[a]	0.42±0.05[a]	1.93±0.12[a]	0.59±0.17[b]	0.56±0.04[b]
Protein/DNA ratio	818.60±606.78[a]	558.14±176.87[a]	364.02±43.12[a]	840.89±220.45[a]	289.58±66.10[b]	383.74±158.48[b]
RNA (µg mg-1 tissue)	0.07±0.03[a]	0.05±0.03[a]	0.06±0.02[a]	0.15±0.06[a]	0.11±0.03[a]	0.06±0.05[a]
Protein (mg mg-1 tissue)	0.06±0.03[a]	0.03±0.01[a]	0.05±0.02[a]	0.06±0.03[a]	0.05±0.02[a]	0.04±0.02[a]

Dinoflagellate endosymbionts

Regarding the behavior of the symbiont, Table 1 shows that significant differences between the initial and final time were apparent only in the C_2 and C_1 treatments in the RNA/DNA ratio. A decreasing trend in the protein/DNA ratio was observed in treatment C_2, but this trend was not significant.

Comparison of each relationship and molecular indicator of T_1 in respect to T_f of symbiont in each treatment are presented in Figures 2 a-d. Generally, there is a tendency to decrease the values in the RNA/DNA, protein/DNA ratios and the concentration of RNA and protein in C_I and C_2 treatments compared to the control treatment without being significantly different; however, the RNA/DNA ratio in T_f-CC was the only that showed a significantly higher ratios compared to other treatments at different times ($P < 0.05$). This differs from previously described responses for the host in the initial phase of the experiment, in which higher concentrations were observed in the treatment C_2 time T_1.

Skeletal micromorphology

Skeletal micromorphology was observed by SEM as a qualitative marker of the physiological effects of decreased pH on *P. capitata* (Figs. 3 a-d). The effects of C_2 and C_1 on the surface relief pattern could be observed (Figs. 3 a-b). At T_1, effects of C_2 could only be detected at isolated sites, characterized mainly by micro surface detachments in the superficial regions of the skeletal, at the end of the experiment we observe more detachments in treatment C_2 in contrast with areas without detachments and increased rugosity of coral control treatment collected from distant location. The Figure 3 c shows the observation of a greater abundance of ridges distributed in the spines and in the basis of those same.

Discussion

Increased CO_2 and the consequent decrease in pH may affect the physiology and health of various species of coral, rapidly leading to alterations in metabolic processes, productivity, the balance of dinoflagellate endosymbionts, and calcification, which could result in bleaching and, finally, cessation of growth (Anthony *et al.* 2008).

When *Pocillopora capitata* was exposed to the lowest pH level (C_2), the RNA/DNA ratio increased significantly, reflecting mainly an increase in the concentration of RNA with respect to the variability of DNA that is considered less variable (Fig. 1c). Bak & Meesters (2000) characterized the behavior of the RNA/DNA ratio in *Porites* spp. and recognized that this increase in the metabolic ratio represents an intensification that was influenced by an

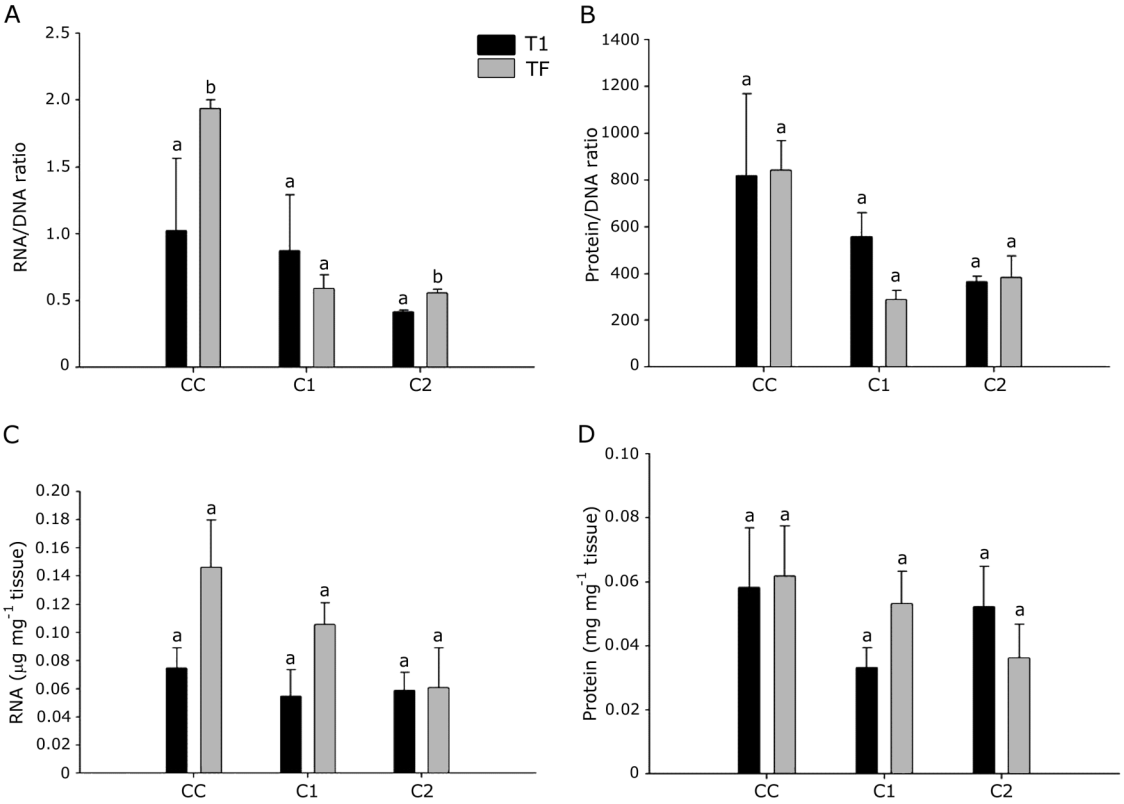

Figure 2. Mean values of biochemical markers and their ratios in *Symbiodinium* sp. at initial time (black) and final time (grey): a) RNA/DNA ratio, b) protein/DNA ratios, c) RNA content and d) protein content. Data are shown as the mean ± SE (n= 3). Different symbols denote significant differences between treatments ($P < 0.05$). C_1: treatment 7.85/7.95 pH; treatment C_2: 7.60/7.70 pH; CC: control treatment 8.00/8.40 pH / Valores promedio de marcadores bioquímicos y sus proporciones en *Symbiodinium* sp. en el momento inicial (negro) y el tiempo final (gris): a) proporción de ARN/ADN, b) coeficientes de proteína/ADN, c) el contenido de ARN y d) contenido de proteína. Los datos se muestran como media ± EE (n= 3) Diferentes símbolos denotan diferencias significativas entre los tratamientos ($P < 0,05$). C_1: tratamiento 7,85/7,95 pH; C_2: tratamiento: 7,60/7,70 pH; CC: tratamiento de control 8,00/8,40 pH

increase in the reef's turbidity. According to Messters *et al.* (2002), there is an inverse relationship between the RNA/DNA ratio and the penetration of light radiation; the metabolic adjustment to sedimentation and turbidity corresponds to genetic adaptation or physiological acclimatization in *Porites* spp. Under physiological conditions that are favorable to coral, such as clear water and high light penetration, high RNA/DNA ratios would be expected; however, the effect of increased turbidity on metabolism suggests that the RNA/DNA ratio may be sensitive to both short-term and long-term changes in water quality (Cooper *et al.* 2009).

According to Buckley & Szmant (2004), high RNA/DNA ratios may represent a greater investment in protein synthesis that reflects a response aimed at trying to repair and compensate for the effects of the prevailing stress. Our results also supported an increase in host metabolism, at least at the beginning of the experiment, because the C_2 treatment exhibited a greater protein concentration which is also directly reflected in the protein/DNA ratio. However, after 7 days of the experiment, the intensity of the metabolic relationships reflected in the RNA/DNA and protein/DNA ratios decreased as the RNA and protein concentrations decreased (Figs. 1 a-d). In this regard, there is some variability in the observations related to stress effectors. Mayfield *et al.* (2011) reported that thermal stress increased *Seriatopora hystrix* protein metabolism between 12 and 24 h, followed by a significant decrease

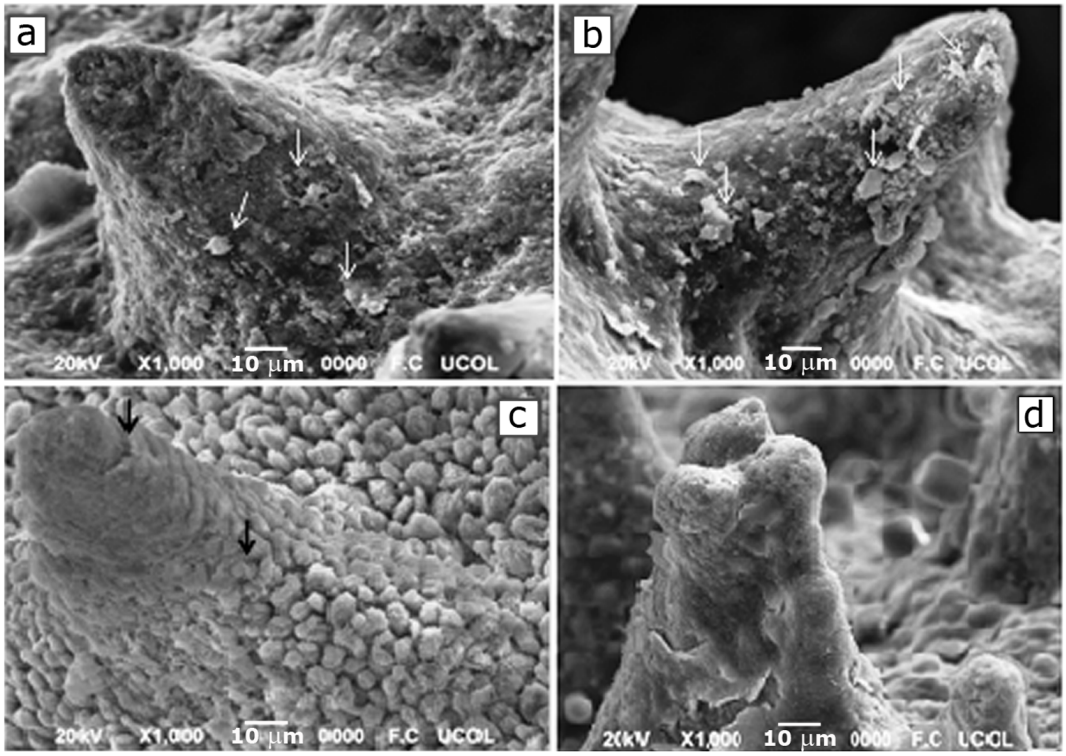

Figure 3. Scanning electron micrographs of micro surface detachments in the terminal regions of the skeletal fibers of *P. capitata* exposed at T_1 a) C_2 treatment-T_1 b) C_2 treatment-T_f, c) *P. capitata* from CL and d) CC treatment-T_f. White arrows show micro surface detachments and black arrows show normal roughness patterns. C_1: treatment 7.85/7.95 pH; treatment C_2: 7.60/7.70 pH; CC: control treatment 8.00/8.40 pH; CL: station sampling control / Micrografías de barrido electrónico de desprendimientos micro-superficiales en las regiones terminales de las fibras esqueléticas de *P. capitata* expuestos en la T_1 a) tratamiento C_2-T_1 b) tratamiento C_2-T_f , c) *P. capitata* de CL y d) tratamiento CC-T_f. Las flechas blancas muestran desprendimientos micro-superficiales y flechas negras muestran patrones de rugosidad normal. C_1: tratamiento 7,85/7,95 pH; C_2: tratamiento 7,60/7,70 pH; CC: tratamiento de control 8,00/8,40 pH; CL: estación control de muestreo

at 48 h. By contrast, in the coral *Acropora millepora*, Hauri *et al.* (2010) reported the persistence of extensive metabolism for up to 240 h after physicochemical stress. In other studies of reefs with marginal conditions, species of *Acropora* and *Porites* corals maintained high levels of RNA/DNA for a period of one to 7 days (Messters *et al.* 2002, Harithsa *et al.* 2005). With respect to the specific case of exposure to increased CO_2 and decreased pH, in a review of marine invertebrates, Pörtner (2008) recognized an important effect of metabolic depression as a result of various causes, including the reduction in the rate of ion exchange (Pörtner *et al.* 2000), the maintenance of ammonia excretion rates (Pörtner *et al.* 1998), as well as the metabolism of amino acids and/or the rates of protein synthesis, which are mediated by acid-based intracellular variables (Langenbuch *et al.* 2006).

Likewise, our results from the end of the experiment demonstrated that the increased coral metabolism recorded in the C_2 treatment decreased highly significantly ($P < 0.001$), as reflected by the RNA/DNA and protein/DNA ratios, which were associated with an significant decrease in the concentrations of RNA ($P < 0.017$), which allows us to infer that the coral metabolic response is possibly a response designed to repair and/or stabilize physiological processes, offset energy costs, and allow the body to acclimate to the changing conditions (Hawkins 1991, Gates & Edmunds 1999). In this respect, it is known that corals can synthesize a series of proteins in response to stress; therefore, the increase in molecular markers such as the RNA/DNA ratio and protein content in coral may be related to an increase in synthesis of new proteins to counteract stress such as thermal stress inducing heat

shock proteins or HSPs (Harithsa *et al.* 2005, Hauri *et al.* 2010). In addition to their role in overall cellular protection, HSPs have been reported to enhance 'thermotolerance', or the ability to recover from stress and the ability to cope with subsequent stress processes in various organisms (Tomanek 2010, Sokolova *et al.* 2011), including the octocoral *Dendronephthya klunzingeri* (Wiens *et al.* (2000). These stress factors have occurred in the coral community LB, as documented by Liñan-Cabello *et al* (2008, 2010b) and will be subsequently discussed in this study.

Moreover, the initial dinoflagellate stress response was different from that observed in host; instead of an increase in symbiont metabolism at T_1, a significant decrease in the RNA/DNA and protein/DNA ratios were observed in treatment C_2 compared to treatments C_1 and CC ($P < 0.05$, Figs. 2 a-b) without significant changes in RNA and protein. This apparent lack of metabolic response is in agreement with other studies that have suggested that symbiont photosynthetic metabolism is not favored by the enrichment of dissolved inorganic carbon, and the photosynthetic capacity is unaffected by changes in CO_2 and pH in the short and medium term (Langdon *et al.* 2003, Reynaud *et al.* 2003). In this regard, Muehllehner & Edmunds (2008) note that, while the decrease in pH can negatively affect the calcification of the coral *Pocillopora meandrina*, no deficiency in endosymbiotic photosynthetic capacity occurs upon exposure for 14 days to pH 7.8 at low temperatures of 29°C and lower. In this regard, Marubini *et al.* (2008) recognized that the lack of a response in *Symbiodinium* spp. may be associated with low permeability to CO_2 in the membrane of *Symbiodinium* and/or the peri-symbiotic membrane, coupled with considerable control of the supply of carbon by the host. This confirms the possible deficiencies previously established in relation to the metabolic response of *Symbiodinium* to increasing dissolved CO_2 related to a short-term decrease in pH and CO_3^{2-} (Marubini *et al.* 2003, Langdon *et al.* 2003, Reynaud *et al.* 2003). Despite the identification of the metabolic response signs of *Symbiodinium* referred by Kaniewska *et al.* (2012) to ocean acidification, the present study confirms deficiencies of a specific short and medium-term metabolic response of *Symbiodinium* sp. to increased CO_2. These differences in the responsiveness between host and symbiont were recently confirmed by Soriano-Santiago *et al.* (2013) in an *in vitro* experiment using low pH, they observed a change in oxidative stress markers within the first few hours, but this response did not prevent cellular damage.

At the T_f time point, the metabolism of *Symbiodinium* was characterized by significantly lower RNA/DNA and protein/DNA ratios in C_1 and C_2 treatments (Fig. 2 a-b). For its part, the slight increase in the RNA/DNA ratio in the C_2 treatment at the end of the experiment compared to the initial time, similar to that observed in the control treatment, could be due to the different response capabilities of the endosymbiont community. Different clades of *Symbiodinium* sp. could be present in different host species and in a single host that inhabits various environments, leading to differences in symbiont photosynthetic characteristics (Leggat *et al.* 1999). In this respect, recently Cunning *et al.* (2013) recognized that *Pocillopora* are the corals typically associated with *Symbiodinium* in clade C and/or D, with clade D associations having greater thermal tolerance and resistance to bleaching. Thus, the acclimatization response of the specimens from LB may be influenced by the activity of a specific symbiont clade that has allowed it to respond to the stress environment prevailing in the locality. In turn, this could permit specimens to demonstrate a greater responsiveness to pH treatments, giving rise to the highest survival in days for specimens from LB (10) compared with the survival of 7 days recently reported by Delgadillo-Nuño (2012) for specimens of *P. capitata* from another locality of Colima coast, in an experimental system *in vitro*, similar to conditions of acidification used in this experiment. In the presence of medium pH conditions (7.85-7.95), host tissue exhibited a significant increase in the RNA and protein content similar to that presented in the C_2 treatment of LB of our study. Similarly, the symbiont did not exhibit any significant metabolic changes upon treatment. Thus, there were clearly differences in the metabolic responses of host and symbionts in specimens from LB and corals from other areas with less history of environmental and/or anthropogenic stress. Notwithstanding the different responses to low pH treatments, the specimens exposed to low pH treatment showed signs of bleaching and died at 10 days. Anthony *et al.* (2008) reported that low pH could disrupt the major route of CO_2 accumulation or electron transport, destabilizing the intracellular proton gradient and directly affecting the ability of symbiont to fix carbon, in addition to disrupting photosynthetic processes.

It has previously been reported that specimens of *P. capitata* from LB, in addition to exhibiting short-term adjustments such as the amount of photosynthetic pigment and/or sensitivity to the photosynthetic

response reported in other coral species, exhibit a series of short-term enzymatic responses aimed at countering the effects of ROS, thereby promoting adaptation to shallow coastal environments which typically exhibit high levels of UVR and thermal variability (Flores-Ramirez & Liñán-Cabello 2007, Liñán-Cabello *et al.* 2010a). According to Freire *et al.* (2011), changes in HSP (and other proteins) expression can be detected within a few hours (1-6 h), corresponding to the duration of most physiological events that occur in tidal cycles. Recently, different signs of stress in *Pocillopora* spp. corals have been reported as a result of the inadequate location of the artificial structure intercommunication of Juluapan Lagoon in the Bay of Santiago where the LB coral community is located; this exposes the coral community to tidal effects, rapidly influencing turbidity, sediment, nutrients, temperature and osmolarity (Liñán-Cabello *et al.* 2008, 2010b). The environmental history of the LB coral community may have generated a physiological alertness that promotes a rapid metabolic response, but, at the prevailing low pH conditions, may have interfered with carbon concentration mechanisms in the photorespiration machinery and/or the direct impact of metabolic acidosis as a consequence of the decrease of seawater pH (Leggat *et al.* 1999, Gattuso *et al.* 1999, Kim *et al.* 2004), which led to the bleaching and deaths of our specimens.

According to our microscopic observations, the abundance of thorns bordering the LB control treatment (Fig. 3 d) contrasts sharply with the submitted bounds of control *P. capitata* obtained from the locality CL (Fig. 3 c). In this regard, Brown et al. (1997) study the effect of the dynamic action on the layout and arrangement of the surface ultrastructure. According to these authors, specimens of *P. damicornis* that were located on a larger dynamic envelope wave-influenced side and shape of the skeletal spines. Thereby, spines are more present in corallites on branch tips exposed to wave action. In this sense, we can considerer the lack of LB's roughness could be a sign of microstructural deterioration associated with continuous exposure to environmental variability, as this population is also in a shallow location (0.5-2.5 m). The area is highly exposed to ocean dynamics; therefore, it should exhibit a greater roughness pattern.

Differences in the patterns of abundance of ridges distributed in the spines and in the basis of those observed under high magnification were a consequence of the micro surface detachments in the terminal regions of C_2 with respect to C_1 and CC (Figs. 3 a, b and d). This coincides with reports by Marubini *et al.* (2003), who used SEM to study the suppression of the growth of scleractinian coral environments induced by low carbonate ion concentrations and recognized differences in the size of available microcrystalline units; the magnitude of the effects varied depending on the species: the fibers were most reactive in *Acropora verweyi*, less in *Turbinaria reniformis*, and intermediate in *Galaxea fascicularis* and *Pavona cactus*. In corals, aragonite saturation state control via ion supply for calcification and skeletal growth involves a large number of metabolic pathways; however, temperature and pCO_2 affect growth physiology and calcification (Tambutté *et al.* 2011). Therefore, the present results of skeletal surface and metabolic responses suggest this effect in *P. capitata*, but further experiments are needed to better estimate the effects and adaptation capacity of *Pocillopora* spp. from LB.

The RNA/DNA ratio was a useful tool to observe rapid changes in active host metabolism. Of the two treatments used, the treatment at the lower pH resulted in increased metabolic activity, primarily the host, which was directly related to the protein/DNA ratio and RNA and protein concentrations. This response was identified in the early hours of the experiment but its incidence subsequently decreased. In the case of *Symbiodinium*, a metabolic response similar to the host response was not apparent. Qualitative microstructural analysis primarily revealed effects from the C_2 treatment in the first hours of testing with an influence on the micro surface detachments in terminal regions of the skeletal fibers.

Observed pH changes may directly affect coral calcification or indirectly affect calcification through coral metabolism, they may affect symbionts by inducing a decrease in zooxanthellae photorespiration and productivity or bleaching, as both of these processes can affect coral calcification.

Further studies using other quantitative indicators are needed to confirm that decreases in pH may be associated with skeletogenesis in scleractinian corals.

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