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Molecular evidence of thermal stress in two massive coral species in a remote reef system in the southwestern Gulf of Mexico during an extreme marine heatwave

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Abstract

Coral reefs are facing escalating impacts from intensifying marine heatwaves, causing unprecedented thermal stress, widespread mass bleaching, and mortality events. While many reef systems are declining, some coral communities, particularly on remote reefs, maintain a high conservation status, suggesting the presence of acclimation mechanisms that may be essential for coral survival under climate change. In this study, we assessed the spatial and temporal expression of heat shock protein 70 (HSP70) in colonies of two massive reef-building corals, Orbicella faveolata and Montastraea cavernosa, during the global thermal stress episode (2023–2024) and the consecutive year (2025) in Cayo Arenas, a remote reef system in the southwestern Gulf of Mexico. During the study period, the corals were exposed to severe thermal stress. In situ estimated Degree Heating Weeks (DHW) reached a maximum in 2023 (19.3) and decreased to 17.6 and 15.5 °C-weeks in 2024 and 2025, respectively. Both species showed progressively elevated HSP70 expression, suggesting sustained activation of cellular stress responses. The observed increase in HSP70 levels reflects differential physiological strategies, including a constitutive elevation of baseline HSP70 expression in M. cavernosa and stronger signatures of cumulative stress under repeated heat exposure in O. faveolata. Given the reduced recovery intervals and anticipated recurrence of extreme thermal conditions, even thermotolerant corals seem to be driven towards their functional limits. Understanding the transcriptional plasticity responses that contribute to physiological resilience is fundamental to understanding the impact of climate change on coral reef biodiversity and function.

Introduction

Coral reefs are among the ecosystems most vulnerable to the climate crisis (Hughes et al. 2017; van Woesik et al. 2022). Over the past four decades, rising sea surface temperatures and intensifying marine heatwaves (Sully et al. 2019; Lachs et al. 2023), in synergy with anthropogenic pressures such as nutrient enrichment in the water column, marine chemical pollution, and the proliferation of emerging diseases, have driven their worldwide structural and functional degradation (Edmunds et al. 2014; Hughes et al. 2017).

In 2023, coral reefs experienced an unprecedented global thermal stress event, characterized by the most intense marine heatwave ever recorded, resulting from the amplification of pre-existing thermal anomalies under the influence of the El Niño Southern Oscillation (ENSO) (Raghuraman et al. 2024; Gunnarson et al. 2025). In reef ecosystems, this event was reflected in record-breaking values of degree heating weeks (DHW) (Reimer et al. 2024; Goreau et al. 2024), a metric which integrates the intensity and duration of thermal anomalies above the local bleaching threshold over a 12-week period (Liu et al. 2013).

During 2023 and 2024, DHW values far exceeded the critical thresholds associated with the breakdown of coral-dinoflagellate symbiosis (DHW > 8) and reached unprecedented levels. Consequently, widespread bleaching and mass mortality events have been documented in the Pacific and Caribbean

Sea (e.g. Doherty et al. 2025; Alvarado et al. 2025; Bon et al. 2025; López et al. 2024; Birkart & Álvarez-Filip 2025).

At the cellular level, thermal stress compromises coral stability by disrupting proteostasis, increasing Reactive Oxigen Species (ROS) production, and impairing immunity and metabolic homeostasis. Under severe stress conditions, coral–Symbiodineaceae symbiosis breaks down, generating an energetic deficit that may ultimately result in host mortality (Han et al. 2024; Suggett et al. 2017; Helgoe et al. 2024).

In response, corals modulate their gene expression as a central mechanism of physiological adjustment. This response involves the differential regulation of hundreds of transcripts, particularly those associated with oxidative stress, transcriptional control, heat shock response, apoptosis, and extracellular matrix organisation. (Kenkel et al. 2013; Louis et al. 2017; Bellantuono et al. 2012). Transcriptional plasticity is a key component of coral thermal tolerance, enabling rapid molecular adjustments that support acclimation and resilience (Brener-Raffalli et al. 2022; Palumbi et al. 2014; Rivera et al. 2021).

The growing understanding of these transcriptomic responses has driven increasing interest in the use of gene expression biomarkers for the early detection of physiological stress in reef-building corals (Kenkel et al. 2011; Palumbi et al. 2014; Louis et al. 2017). These molecular tools can support the identification of thermotolerant individuals, populations, and species, thereby informing targeted interventions and improving the effectiveness of restoration and conservation efforts (van Oppen et al. 2015; Louis et al. 2017).

Among the most widely recognized molecular biomarkers of environmental stress in reef-building corals are genes that encode heat shock proteins (HSPs), particularly HSP70 and HSP90 (Kenkel et al. 2011; Leggat et al. 2011; Rosic et al. 2011). The expression levels of these genes are strongly modulated in response to a wide variety of environmental stressors, including thermal and light stress (Leggat et al. 2011; Kenkel et al. 2011), exposure to pollutants (Nishioka et al. 2024; Ven et al. 2009), and disease (Rosenzweig et al. 2019; Seveso et al. 2020). Despite increasing evidence on coral stress biomarkers, field-based assessments of HSP70 expression during extreme marine heatwaves remain limited, particularly in remote reefs with low local anthropogenic influence. This limits our ability to disentangle the thermal effects from chronic local stressors.

Under heat stress, HSPs prevent protein aggregation, facilitate the refolding of misfolded proteins, and promote the degradation of damaged polypeptides (Sørensen et al. 2003; Louis et al. 2017). Their role in maintaining proteome integrity makes them central regulators of the chain of events that lead to bleaching (Thummasan et al. 2021). Consistent experimental (Leggat et al. 2011; Desalvo et al. 2008; Kenkel et al. 2011; Han et al. 2024; Skutnik et al. 2020) and observational (Louis et al. 2020; Oliver & Palumbi 2011; Poli et al. 2017) studies suggest that elevated or rapid HSP70 expression is associated with enhanced thermal tolerance in several coral species. Therefore, high basal expression achieved

through preconditioning represents an important acclimatory mechanism to recurrent thermal stress (Barshis et al. 2013; Rivera et al. 2021; Dilworth et al. 2024).

However, given the central role of HSPs in cellular protection, their use in observational studies is highly sensitive to both natural environmental variability and local anthropogenic pressures, which may interact to modulate their expression levels (Kenkel et al. 2013; Drury et al. 2022). Reef systems with limited human access and disturbance offer ideal environmental conditions where the reduction of local chronic stressors minimizes confounding effects and enables more robust attribution of molecular responses to thermal stress. These systems, often located in remote regions, have been proposed as potential climate refuges. Reduced anthropogenic disturbance aids the establishment of acclimation mechanisms that are essential for coral persistence in the face of climate change (Roberts et al. 2002; Hoegh-Guldberg et al. 2018).

In this study, we assessed the spatial and temporal HSP70 expression during the unprecedented global thermal stress episode (2023–2024) and the subsequent year in colonies of two massive reef-building corals, *Orbicella faveolata* and *Montastraea cavernosa*, in Cayo Arenas, southwestern Gulf of Mexico. This remote reef system, located approximately 200 km from the coast, is characterized by a high prevalence of massive corals, limited anthropogenic pressure, and no documented disease outbreaks to date (Frías-Vega et al. 2025; Álvarez-Filip et al. 2026).

Materials and Methods

Study area and sampling design

Cayo Arenas is a platform reef system belonging to a series of emergent reefs and shallow coral banks distributed along the Campeche Bank in the southwestern Gulf of Mexico (Tunnell et al. 2010) (Fig.1). The remoteness of Cayo Arenas (~200 km offshore) and its limited accessibility have historically reduced direct anthropogenic pressure. This is consistent with the well-preserved condition of the reef and the dominance of reef-building scleractinian corals, including large colonies of species such as *Orbicella* spp., *Colpophyllia natans*, *Diploria labyrinthiformis*, and *Siderastrea* spp. (Pérez-Cervantes et al. 2017; Álvarez-Filip et al, 2026; Rioja-Nieto et al. under review).

Cayo Arenas exhibits a pronounced seasonal Sea Surface Temperature (SST) variability, from around 29˚C during June to September to colder waters (around 23.5˚C) from January to April. This thermal regime is influenced by regional oceanographic dynamics, particularly the Yucatan Current (Loop Current), which flows from east to west across the Gulf of Mexico and generates circulation patterns in the area, contributing to the observed intra-annual variability in SST (Bessonov et al. 1968; Sánchez-Pelcastre et al. 2023).

During August 2023, 2024, and 2025, encompassing the marine heatwave of 2023-2024 that caused the Fourth Global Bleaching Event (Reimer et al. 2024), six sites at Cayo Arenas (Fig. 1) were sampled. The sites were distributed across three depth categories: 7–9 m (A15 and A1), 11–12 m (A16 and BT), and

17–19 m (A4 and A14) to evaluate the role of depth in modulating heat stress responses. Except for the northernmost site (A15), all sites were protected by emerged land formations (e.g. an island and keys) and located within the “reef lagoon”.

At each sampling site, 10 coral tissue nubbins (~1 cm²) were randomly collected from colonies of *O. faveolata* and *M. cavernosa* within ~480 m² permanent plots(n = 60 nubbins per species per year). These species were selected based on their ecological relevance as reef-building corals and their dominance in the study area (Pérez-Cervantes et al., 2017). Different collection methods were employed based on skeletal morphology, using side cutters for *O. faveolata* and a chisel and hammer for *M. cavernosa*, ensuring clean excisions while minimizing damage and disturbance to the colonies. Colonies did not show any visible signs of bleaching or disease at the time of sampling. Despite random annual sampling, the inspection of orthomosaics obtained from the parcels using Underwater Digital Photogrammetry (Rioja-Nieto et al. under review), indicated resampling of some colonies. Six *M. cavernosa* (A16=1, A15=4, BT=1) and nine *O. faveolata* (A16=5, A15=4) in 2024 and two *M. cavernosa* (A16=1, A15=1) and *O. faveolata* (A16=2) in 2025.

Samples were immediately preserved in pre-labelled Eppendorf tubes containing n RNA stabilization solution (Colín-García et al. 2019) and preserved at −80 °C, until they were processed in the Cellular and Molecular Biology Laboratory at the ENES Unidad Mérida, Universidad Nacional Autónoma de México (UNAM).

Thermal regime and heat stress characterization

To contextualize the thermal conditions of 2023–2025, SST data from 1986 to 2026 were obtained from the Coral Reef Watch (CRW NOAA 2026). These data were used to characterize the long-term thermal regime of the Cayo Arenas reef system. Historical heat stress was characterized by calculating DHW (°C-week), which integrates both the intensity and duration of anomalously warm conditions (Liu et al. 2013), providing a continuous assessment of thermal stress from 1986 to 2026.

In addition, during the study period, HOBO data loggers were used to record in situ water column temperatures at four reef sites A14 (19m), A16, BT (11-12m), and A1 (8m) (Fig. 1), allowing the characterization of fine-scale thermal conditions. Data loggers, which were replaced during each sampling period, were programmed to record temperature at 30-minute intervals. The datasets comprised from July 2023 to September 2025.

Paired t-tests and Pearson’s correlation analyses were used to compare in situ temperatures between sites and against satellite-derived SST (NOAA Coral Reef Watch, 2026). Additionally, DHW were calculated for 2023–2025 using mean in situ temperatures, enabling comparison between remotely sensed and site-specific thermal stress conditions.

Molecular Analyses

RNA was extracted following the protocol described by Colín-García et al. (2024). Approximately 10 mg of coral tissue was homogenized in TRIzol reagent for cell lysis. Phase separation was achieved by adding chloroform and centrifuging. Total RNA was then precipitated using isopropanol, washed with ethanol to remove impurities, and resuspended in RNase-free water. The quantity of RNA was assessed using a NanoDrop spectrophotometre (Thermo Fisher Scientific) and by visual inspection on a 1% agarose gel. Residual genomic DNA was removed by treating the samples with RNase-free DNase I (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. Purified RNA samples were stored at −80 °C.

cDNA synthesis used 4 µL of total RNA at 100mM/µL, using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) following the manufacturer's protocol and stored at −20 °C until further use. Gene expression of HSP70 and elongation factor 1 alpha (EF1α) was quantified using real-time polymerase chain reaction (qPCR), with EF1α serving as the housekeeping gene for normalisation. EF1α was selected as the reference gene based on previous studies demonstrating its stability in *Acropora* spp. under thermal stress (Kenkel et al., 2011). Reactions were prepared using 1 µL/10mM of gene-specific primer mix per target. Primer sequences were as follows (Rosic et al. 2011): HSP70, 5'-CCC GCT TGT TGG AGG AAA TA-3' (forward) and 5'-GAT ACC CAT TTG GGA GGT GAA G-3' (reverse); EF1α, 5'-GGGTGGCGTAGTTTCTTTGTT-3' (forward) and 5'-CCGAAACTCTCTGCTTTGCTC-3' (reverse). Primer efficiency was validated for both species and targets using a 5-point dilution series (10^{-1} to 10^{-5}). HSP70 efficiencies were 98% in both species.

qPCR assays were performed using the Maxima SYBR Green/ROX qPCR Master Mix Kit (Thermo Fisher Scientific). All samples were analysed in three technical replicates. The cycling conditions comprised an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 s. Melt curve analysis was performed to verify the specificity of the amplification. The results were expressed as the relative mRNA expression of the target genes and the housekeeping gene using the 2^(−ΔCt) quantification method (Schmittgen & Livak 2008).

Statistical analysis

Given the non-normal distribution of gene expression data (Shapiro Wilk test, $p<0.05$), non-parametric analyses were applied. The normalised relative expression of HSP70 was compared between years using the Kruskal–Wallis test for each species (n=60 colonies per species). In addition, we evaluated the interannual differences for each sampling site within each species (n = 10 colonies per site per species) using the same approach. When significant differences were detected, Dunn's post hoc pairwise comparisons were performed, with p-values adjusted using the Benjamini–Hochberg correction. All statistical analyses and graphical representations were conducted in R (v. 4.4.1) using the ggplot2 package for data visualization.

Results

Thermal regime and heat stress characterization

Cayo Arenas exhibited pronounced seasonal variability in SST based on the 1986–2026 values recorded. The lowest monthly mean temperatures occurred in February (23.88 ± 0.70 °C), whereas the highest values were observed during August–September (29.51 ± 0.61 °C).

The highest annual maximum SST within the 1986–2026 time series, was recorded during August–September of the last three years, reaching 30.61 °C (2023), 30.67 °C (2024), and 30.58 °C (2025). These values exceeded the historical maximum monthly mean (28.97 °C) by +1.64, +1.70, and +1.61 °C, respectively.

Thermal stress analyses based on DHW derived from SST (NOAA CRW) indicate that these years represent unprecedented conditions within the historical thermal regime of the Cayo Arenas reef system (Fig.2a). The peak DHW reached 17.72 in 2023, 15.49 in 2024, and 15.10 °C weeks in 2025, surpassing all previous records since 1986. Notably, the previous maximum DHW value recorded for the Cayo Arenas reef system was 8 °C-weeks in 2004, which has doubled during recent events

In situ temperature records showed limited spatial variability across the reef system (Fig. 2b). Although paired comparisons between the sensors were statistically significant ($p < 0.05$), the magnitude of these differences was, in most cases, in the order of hundredths of a degree, with a maximum difference of 0.11 °C and was not related to depth (Supplementary Table 1). These results suggest a well-mixed and thermally homogeneous water column within the upper 20 m.

Comparisons of satellite-derived SST and daily mean in situ temperatures showed a strong agreement in temporal variability (Pearson's correlation coefficient $r > 0.99$). However, the in-situ values were higher ($p < 0.05$, difference= ≤ 0.22°C) (Supplementary Table 1). Consequently, the in-situ data indicated higher thermal stress accumulation. A maximum DHW of 19.3 was observed in November 2023, which decreased to 17.6 and 15.5 °C-weeks for the same month in 2024, and 2025, respectively. Data collection was performed each year prior to the onset of thermal stress (Fig. 2b).

Variation in the relative expression of HSP70

The species showed different HSP70 expression patterns (Fig. 3). In *M. cavernosa*, the Kruskal–Wallis test revealed significant differences in HSP70 expression over time ($x^2=83.40$, d.f.=2, $p<0.05$), where Dunn's post hoc pairwise comparisons indicated significant differences between all years ($p < 0.05$). For this species, expression steadily increased ~5-fold relative to the reference gene in 2025. In *O. faveolata*, HSP70 relative expression also varied between years ($x^2=46.20$, d.f.=2, $p<0.05$). However, no differences were observed between 2023 and 2024 ($p>0.05$). The highest HSP70 relative expression levels were observed in 2025 with an increase ~4-fold relative to the reference gene.

At the site level, *M. cavernosa* showed a marked temporal upregulation of HSP70 (all expression data are Log_2-transformed fold-changes relative to the reference gene; Fig. 4). In 2023, HSP70 was downregulated at A15, A1, BT, and A14. From 2023 to 2024, expressions at A15 and A14 increased (5.0 ± 0.3-fold; $p < 0.05$) and remained at those elevated levels in 2025. In contrast, A1 and BT expressions

peaked in 2025. BT was the only site where every pairwise year comparison was different ($p < 0.05$). Sites A16 and A4 already exhibited HSP70 upregulation in 2023 ($Log_2 > 0$). In A4 no differences were observed between 2023 and 2024, but an increase occurred in 2025 (~5-fold; $p<0.05$). In A16, no differences ($p>0.05$) in HSP70 expression levels were observed over time (Fig.4).

O. faveolata also exhibited an overall increase in HSP70 expression over time (Fig 4), although inter-colony variability and downregulation in 2023, particularly in A15, A1, and A4, was observed, where median values remained close to the baseline levels. At these sites, as well as at A14, no differences were detected between 2023 and 2024, whereas the expression in 2025 was significantly higher ($p<0.05$). At A16, HSP70 expression was downregulated in 2023 and 2025, with a significant increase ($p<0.05$) observed in 2024. In contrast, BT was the only site showing upregulation relative to the reference gene in 2023 (~3-fold), followed by a significant increase ($p<0.05$) in 2025. In both species, 2025 yielded the highest and least variable HSP70 expression levels, particularly at BT and A4, indicating a more uniform transcriptional response after repeated thermal stress.

Discussion

We evaluated the modulation of HSP70 expression under natural conditions during consecutive heat-stress episodes. Our results showed increased expression levels in both species following the 2023 and 2024 thermal anomalies, with higher values observed in 2025. The observed responses occurred under the most intense marine heatwave on record (Reimer et al. 2024; Goreau et al. 2024). Thermal stress was the highest in 2023 and decreased towards 2025, always remaining at high risk of mortality for multiple species DHW > 12 °C-weeks (NOAA Coral Reef Watch 2026).

Given that HSP70 plays a central role as a molecular chaperone responsible for maintaining proteome integrity, elevated HSP70 expression in the host has been associated with increased stress tolerance (Rivera et al. 2021; Dilworth et al. 2024; Seveso et al. 2018; Brener-Raffalli et al. 2022; Poli et al. 2020). Our observations align with the thermal preconditioning hypothesis: repeated exposure to sublethal heat stress induces molecular and physiological responses that enhance tolerance to subsequent thermal stress events (van Oppen et al. 2015; Brown & Barott 2022; Hughes et al. 2019). This environmental memory is particularly relevant for sessile organisms, such as corals, as it enables them to respond more efficiently to recurrent disturbances (Hackerott et al. 2021; Barshis et al. 2013; Brener-Raffalli et al. 2022; Kenkel & Matz 2013; Palumbi et al. 2014; Stick et al. 2026).

Environmental memory has been widely documented in the context of marine heatwaves. Several authors have reported that surviving corals can develop increased tolerance to thermal stress within as little as one year after exposure (e.g. Brown & Barott 2022; Li et al. 2024). This is often reflected in reduced bleaching severity during subsequent marine heatwaves compared to the initial event, despite experiencing similar or even higher thermal stress (Brown & Barott, 2022; Hughes et al. 2019; DeCarlo et al. 2019; Hackerott et al. 2021).

Although the underlying mechanisms driving memory responses in corals remain poorly understood, the regulation of gene expression has been suggested to play a key role in enhancing coral resistance to bleaching (Hackerott et al. 2021; Drury et al. 2022; Brown & Barott 2022). In this context, HSPs are particularly relevant as they contribute to maintaining protein structure and mitigating the harmful effects of excess ROS produced during episodes of heat stress (Louis et al. 2025; Louis et al. 2017).

A limitation of this study is the lack of sampling during or immediately after peak thermal stress, precluding direct assessment of acute bleaching responses. However, coral coverage assessments between 2023-2024 provide indirect evidence of coral persistence, *M. cavernosa* coverage remained stable, whereas *O. faveolata* showed a small decrease despite record thermal stress (Rioja-Nieto et al. under review).

Massive coral species, including *O. faveolata* and *M. cavernosa*, are characterized by slow growth rates, large polyps and substantial energy reserves, traits that may provide a high acclimatory capacity under stress (Brown et al. 2002; Glass et al. 2026). Doherty et al (2025) reported widespread bleaching but low mortality in massive species during the 2023 heatwave in Little Cayman.

Montastraea cavernosa exhibited a progressive upregulation of HSP70 across the three year study period. Expression increased from downregulation in 2023 to ~3-fold in 2024, reaching ~5-fold in 2025. This cumulative response pattern is consistent with increasing thermal memory. Repeated exposure to heat stress appears to progressively enhance the molecular defense mechanisms of corals, resulting in amplified HSP70 responses. Notably, *M. cavernosa* has been characterized as highly thermotolerant, capable of sustaining calcification and metabolic function via heterotrophic feeding under stress conditions (Lesser et al. 2020; Manzello et al. 2015), which may contribute to its upregulation of HSP70.

While HSP70 upregulation was progressive at most sites, only A16 and A4 maintained constitutively elevated expression across all three years (Fig. 4). This pattern is consistent with observations that colonies with high baseline HSP70 expression often show attenuated inducibility under thermal stress, a constitutive defense strategy that may trade rapid stress responses for cellular protection (Barshis et al. 2013; Bellantuono et al. 2012; Rivera et al. 2021; Poli et al. 2017). Colin-García et al (2024) reported that colonies of *M. cavernosa* with initially high expression levels tend to exhibit only modest increases under thermal stress, whereas colonies with lower baseline expression display more pronounced changes in HSP70 expression.

In contrast *O. faveolata* showed pronounced spatial and temporal heterogeneity in HSP70 expression. Between 2023 and 2024, responses varied markedly. Site A16 showed upregulation, whereas other sites remained stable or declined with no significant differences. Notably, high inter-colony variability in expression levels was observed in 2023. The sustained or elevated HSP70 in 2025 may reflect either ongoing acclimation or, alternatively, physiological dysregulation when stress exceeds critical thresholds. Chronic upregulation of HSP70 incurs substantial metabolic costs (Sørensen et al. 2003; Brown & Barott 2022), creating a trade-off between cellular protection and energy allocation. Prolonged elevation of these protective mechanisms may therefore constrain long-term acclimation capacity and

fitness. Grottoli et al (2014) described a complex acclimation response in *O. faveolata*, involving declines in Symbiodiniaceae density, energy reserves, and calcification following repeated thermal stress, yet with full recovery over time. Importantly, inter colony variability in HSP70 expression converged in 2025 in both species, suggesting increased synchrony in stress perception and response. This convergence could indicate either: heightened protective demands under chronic thermal stress, or saturation of acclimation capacity as colonies approach physiological limits, as documented in other organisms under persistent thermal stress (Tomanek & Sanford 2003; Barua et al. 2008; Cho et al. 2021).

Contrary to studies reporting depth dependent variation in HSP70 expression (Poli et al., 2017; Louis et al., 2020), no clear patterns were detected here. This likely reflects the relatively homogeneous thermal structure of Cayo Arenas, where minimal vertical temperature gradients limit the range of local thermal microhabitats. However, Site A16 showed distinct temporal expression patterns in both species, with *M. cavernosa* maintaining stable upregulation, while O. faveolata peaked in 2024 before declining in 2025. This site is in the westernmost portion of Cayo Arenas and is likely one of the most sheltered areas of the reef. It is also characterized by a predominance of calcareous substrate with low structural complexity, where coral colonies are spatially dispersed. Microhabitat conditions can strongly influence coral thermal tolerance at fine spatial scales (Voolstra et al. 2020; Brown et al. 2024). Reduced structural complexity may increase light exposure by limiting shading between colonies, potentially exacerbating thermal stress and accelerating the expulsion of Symbiodiniaceae (Ellis et al. 2024; Krämer et al. 2022). Holobionts can also exhibit photoacclimatory responses that may enhance thermal tolerance through transcriptional regulation and adjustments to the photosynthetic apparatus and microbiome, including shifts in symbiont clade composition within host tissues (Travesso et al. 2023; Hackerott et al. 2021; Gomez-Campo et al. 2024). Emerging evidence suggests that specific coral-associated bacterial taxa contribute to stress tolerance (Glass et al. 2026; Marangon et al. 2025), though the functional mechanisms and taxonomic identity of these protective taxa remain poorly understood for *M. cavernosa* and *O. faveolata*.

DHW values recorded during 2023–2025 confirm that the Cayo Arenas reef system experienced unprecedented thermal stress, far exceeding historical conditions. Despite occurring during the Fourth Global Bleaching Event (Reimer et al. 2024), no drastic decline in coral cover has been documented at this site (Álvarez-Filip et al. 2026). This contrasts with other reef systems in the Gulf of Mexico and Caribbean closer to the coast, where thermal stress has caused widespread bleaching and mortality (Birkart & Álvarez-Filip 2025; Álvarez-Filip et al. 2026), suggesting that low local anthropogenic pressure may contribute to maintaining coral resistance to thermal stress (Donovan et al. 2020; Ouédraogo et al. 2021; Zaneveld et al. 2026). The synergistic interaction between global and local stressors can exceed the physiological adjustment capacity of corals, leading to critical depletion of the energy reserves required for recovery and stress response (Downie et al. 2024; Brown and Barott 2022).

In coastal reefs, local anthropogenic disturbances, such as nutrient enrichment, pollution, and overfishing, can disrupt competitive dynamics, reduce recruitment, and impair physiological performance, thereby increasing coral susceptibility to marine heatwaves (Zaneveld et al. 2016; Walker &

van Woesik 2026). These local stressors also alter microbial communities, facilitating the proliferation of opportunistic and pathogenic bacteria, increasing coral disease susceptibility and mortality especially during the warmer periods (Zaneveld et al. 2016; Miller et al. 2009). In this context, the remoteness of reefs such as Cayo Arenas may provide a form of passive conservation by limiting the intensity of these local pressures, potentially buffering corals against the synergistic effects of thermal stress (Baumann et al. 2022; Limmon et al. 2023).

Seasonal temperature variability in Cayo Arenas may also contribute to coral performance. SST differences between cooler and warmer months reach ~ 5 °C, with summer conditions approaching the local MMM (28.9 °C), and thermal stress onset typically occurring towards the end of summer, when seawater temperatures reach their seasonal maximum following prolonged periods of elevated atmospheric temperatures (Skutnik et al. 2020). Variable thermal regimes have been shown to enhance coral thermal tolerance by promoting acclimation and preconditioning prior to marine heatwaves (Ainsworth et al. 2016; Sully et al. 2019; Brown et al. 2024; Ferrara et al. 2024; Drury et al. 2022). Additionally, cooler months (December–May) may provide critical recovery windows for these colonies, facilitating the restoration of their energy reserves and overall physiological function. In contrast, sustained warm conditions can elevate metabolic costs even outside peak summer periods, progressively depleting energy reserves before the onset of maximum thermal stress (Anthony & Hoegh-Guldberg 2003; Cornet et al. 2026). Remote sites, with variable thermal regimes and low anthropogenic disturbance could become climate refuges for corals under expected environmental change.

This study documents progressive HSP70 upregulation in two dominant massive coral species during the three consecutive summers of recent marine heatwaves. These in situ observations provide rare insights into thermal acclimation dynamics in natural reef populations, revealing both species specific and site-specific variation in stress response capacity. However, the intensification of marine heatwaves, exemplified by the extreme thermal anomaly of 2023 and the projected recurrence of similarly intense conditions during the 2026 ENSO event (NOAA CPC 2026), reduces recovery windows and could drive even thermotolerant species closer to their physiological limits, constraining their energy replenishment and acclimation capacity (Brown & Barott 2022; Louis et al. 2025). Chronic exposure to thermal stress may therefore lead to progressive sensitization, reducing coral fitness over time and compromising the long-term stability of these populations (Bellantuono et al. 2012; Hughes et al. 2018; Brown & Barott 2022).

Remote reefs offer an important opportunity to determine that reducing localized human impacts can promote resilience to climate change (Baumann et al. 2022), emphasizing that effective conservation strategies require both global reductions in greenhouse gas emissions and mitigation of local stressors to maintain the acclimation capacity of reef-building coral communities (Walker & van Woesik 2026; Álvarez-Filip et al. 2026; Zaneveld et al. 2016; Downie et al. 2024). Cayo Arenas was recently included into a newly established Marine Protected Area (MPA). This is an important step towards regional coral conservation, as long as the MPA establishment is accompanied with sufficient resources for effective management.

Declarations

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Ethics

This work did not require ethical approval from an animal welfare committee. Authorizations PPF/DGOPA-039/24 from Secretaría de Agricultural Ganadería, Desarrollo Rural Pesca y Alimentación (SAGARPA), SPARN/DGVS/1053/23, and SBRA/DGVS/06650/25 from Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), and F00.9.DPNAGMS/041/2025 from Comisión Nacional de Áreas Naturales Protegidas (CONANP) allowed the collection of samples for analyzes.

Statements and Declarations

The authors declare have no competing interests to declare that are relevant to the content of this article.

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Figures

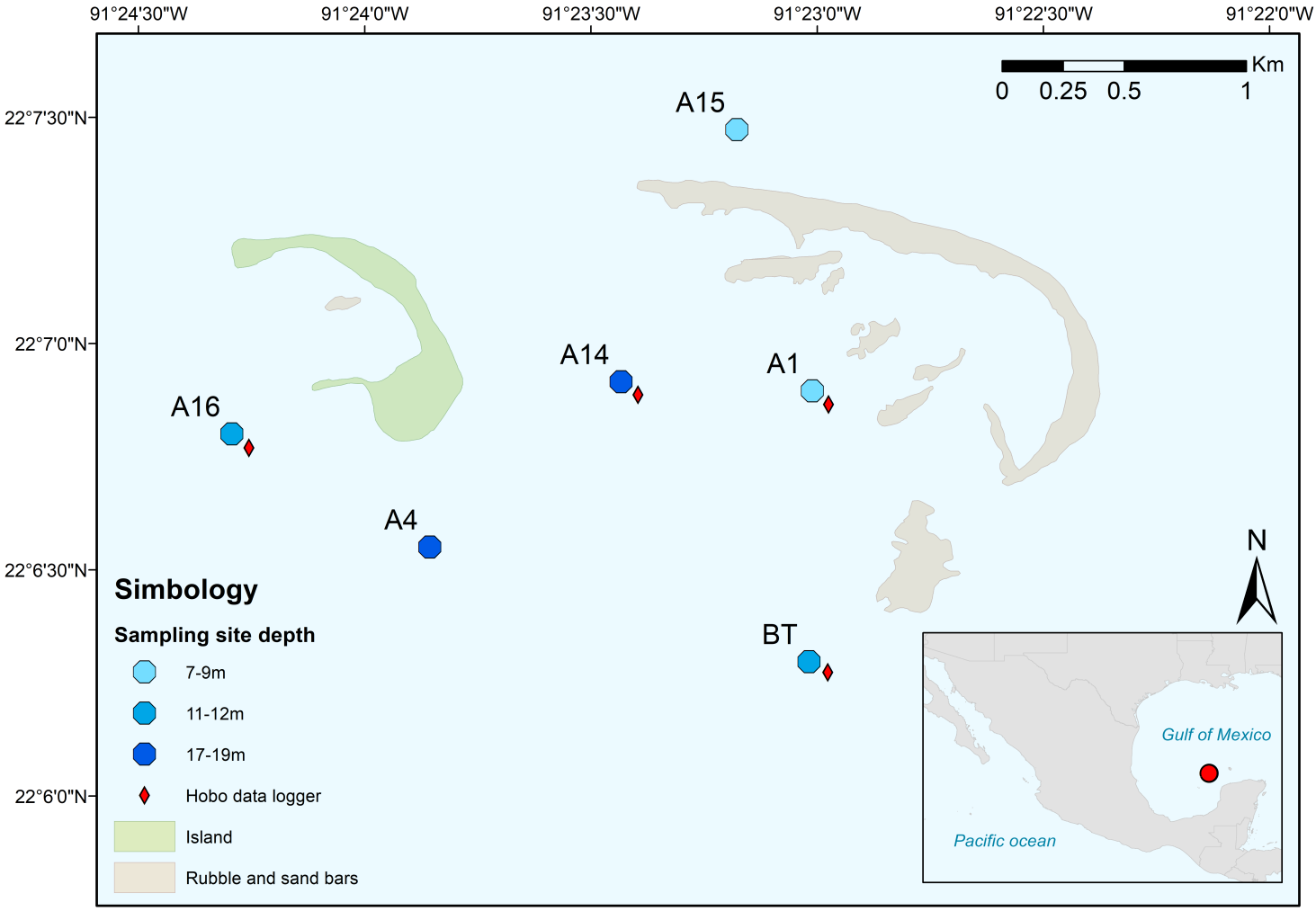


Figure 1

Location of sampling sites and in situ temperature loggers (HOBO) at Cayo Arenas reef in the Gulf of Mexico), distributed across depth categories.

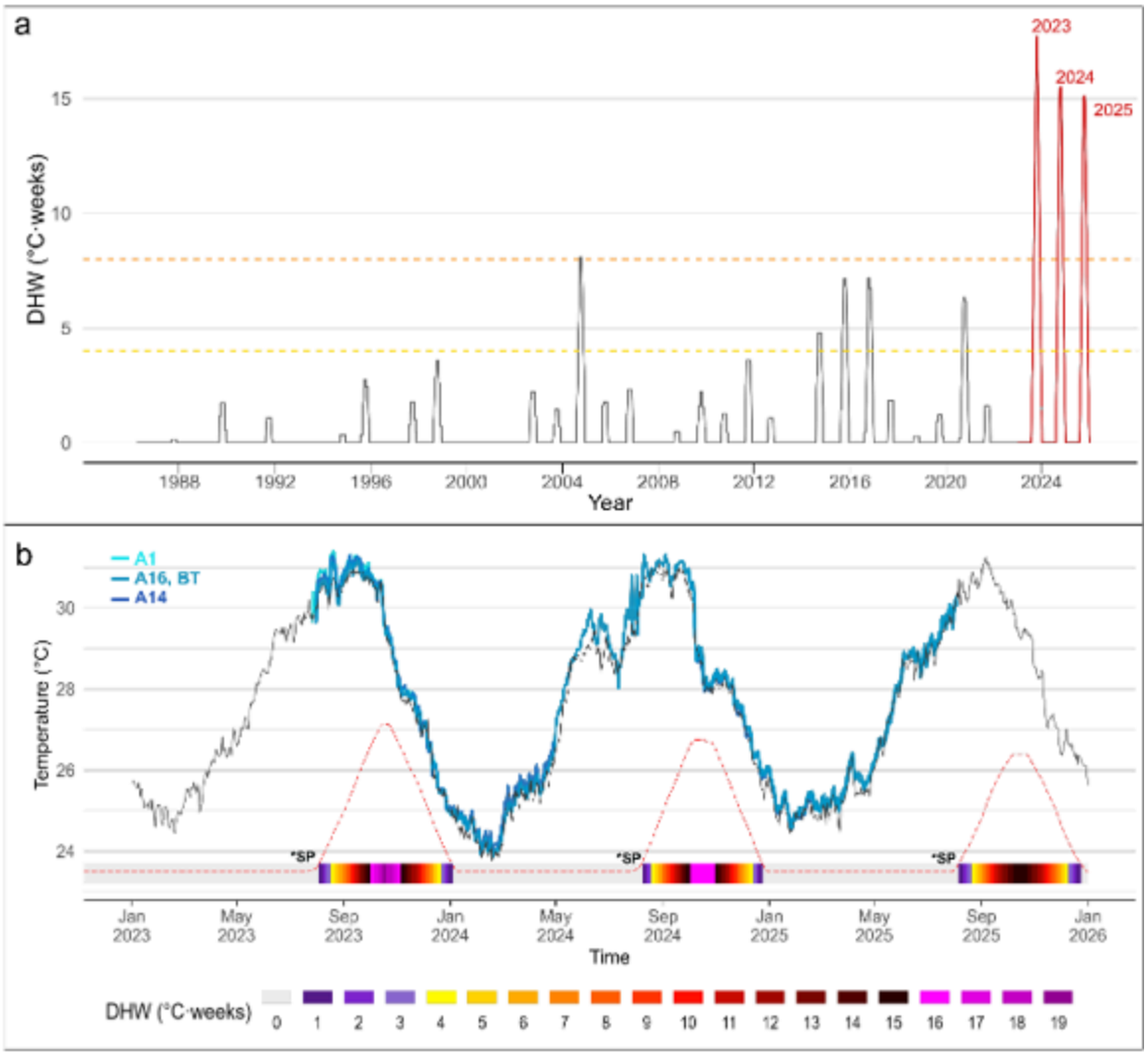


Figure 2

a) Long-term variability in accumulated thermal stress based on historical daily DHW, derived from satellite-based SST (NOAA Coral Reef Watch, 2026) from 1986 to 2026 at Cayo Arenas reef in the Gulf of Mexico. b) Temperature time series from January 2023 to January 2026, including in situ records (blue lines), satellite-derived SST (black line), and in situ–derived DHW (red dashed line and bars). (*SP) indicates the time when data was collected.

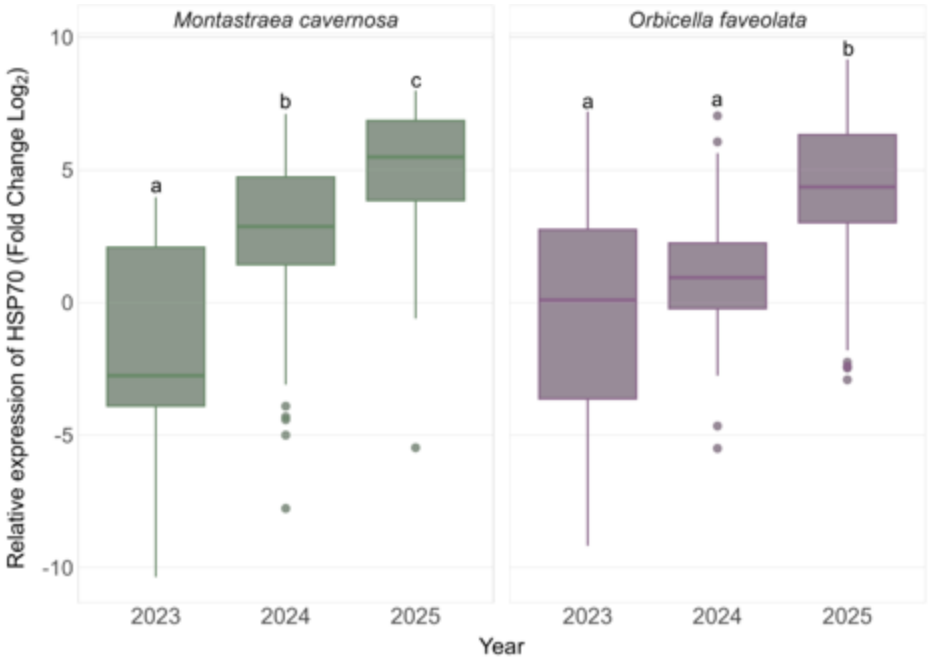


Figure 3

Interannual variation in HSP70 relative expression in *M. cavernosa* and *O. faveolata* from Cayo Arenas (2023–2025). Different letters indicate significant differences between years for each species (Dunn's test, $p<0.05$).

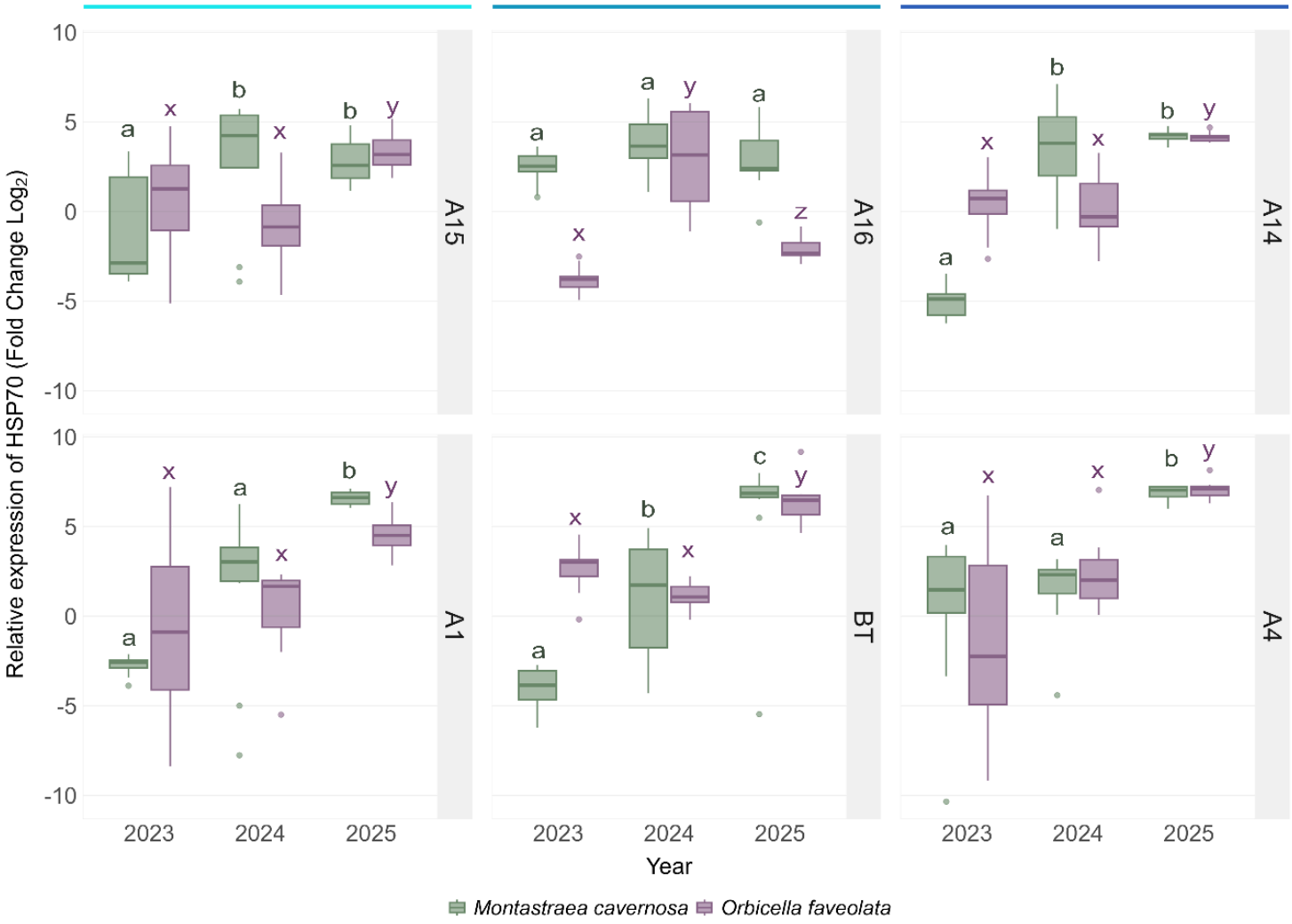


Figure 4

HSP70 relative expression across six sampling sites in Cayo Arenas (2023–2025). Different letters indicate significant differences between years within each species at each site (Dunn's test, $p < 0.05$). Letters (a-c) correspond to *M. cavernosa*, while letters (x-z) correspond to *O. faveolata*. Blue bars denote depth categories: light blue, 7–9 m (A15 and A1); medium blue, 11–12 m (A16 and BT); and dark blue, 17–19 m (A14 and A4).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterialRojasCanoD2026CA.docx