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RESEARCH

Marine Heatwave Caused Differentiated Dysbiosis in Photosymbiont Assemblages of Corals and Hydrocorals During El Niño 2015/2016

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Abstract
Reef corals have been threatened by climate change, with more frequent and intense bleaching events leading to extensive coral mortality and loss of coral cover worldwide. In the face of this, the corals' photosymbiont assemblages have received special attention as a key to better understand the bleaching process and its recovery. To assess the effects of thermal anomalies, the coral *Mussismilia harttii* and the hydrocoral *Millepora alcicornis* were monitored through the El Niño 2015/2016 at a Southwestern Atlantic (SWA) coral reef. A severe bleaching event (57% of colonies bleached) was documented, triggered by a < 3 °C-week heatwave, but no mortality was detected. The hydrocoral was more susceptible than the scleractinian, displaying bleaching symptoms earlier and experiencing a longer and more intense bleaching event. The composition of photosymbionts in the *M. alcicornis* population was affected only at the rare biosphere level (< 5% relative abundance), with the emergence of new symbionts after bleaching. Conversely, a temporary dysbiosis was observed in the *M. harttii* population, with one of the dominant symbiodiniaceans decreasing in relative abundance at the peak of the bleaching, which negatively affected the total β-diversity. After colonies' complete recovery, symbiodiniaceans' dominances returned to normal levels in both hosts. These results highlight critical differences in how the two coral species cope with bleaching and contribute to the understanding of the role of photosymbionts throughout the bleaching-recovery process.

Keywords Zooxanthellae · Symbiodiniaceae · Coral bleaching · South Atlantic reefs · Thermal anomalies · Global climate change

Introduction

In the past decades, reef corals have been constantly threatened by climate change with more frequent and intense thermal anomalies [1]. Thermal stresses of just 1 °C above a region's maximum monthly average temperature can lead to coral bleaching [2], a phenomenon in which the symbiosis between reef corals and dinoflagellates from the family Symbiodiniaceae is broken [3]. Corals can deal with short and isolated bleaching

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occurrences, but long and intense events have led to extensive coral mortality and loss of coral cover worldwide [1, 2, 4].

Global climate change impacts (e.g., increasing sea surface temperature) have been intensified by pulse heat stress events, such as El Niño, the warm phase of the El-Niño Southern Oscillation (ENSO) [1]. In particular, the ENSO 2015/2016 summed with local thermal anomalies triggered the third global-scale coral bleaching event between 2015 and 2017 [1, 5]. In the face of this alarming scenario, the diversity and dynamics of corals' photosymbiotic assemblages have received special attention as a key to better understand the process of coral bleaching and its recovery [6, 7]. Consequently, knowledge of symbiodiniaceans' diversity has increased drastically over the past few years, with new Symbiodiniaceae genera now being recognized [8], with each of them encompassing a high diversity of physiologically distinguished lineages or species [7, 9]. Since some corals can harbor multiple genera of Symbiodiniaceae, with distinct levels of thermotolerance [10], and this assemblage could be highly dynamic over time and space [11, 12], these photosymbionts can display a relevant role in the holobiont thermal adaptation [7].

According to the Adaptive Bleaching Hypothesis [13, 14], symbiosis breakdown could allow the coral's tissue to be colonized by a different set of symbiodiniaceans [15]. During bleaching recovery, symbiont assemblage could re-establish itself in a different composition better adapted to the new thermal condition [15]. The reorganization of the photosymbiont assemblage altering dominant lineages, could be a mechanism by which corals might adapt to climate change [9].

Brazilian coral reefs are the only reefs in the South Atlantic Ocean whose generally turbid waters harbor a coral fauna with a high degree of endemism [16], including the four massive reef-building scleractinian species of the genus *Mussismilia* Ortmann, 1890, as well as amphi-Atlantic species, such as the branched hydrocoral *Millepora alcicornis* Linnaeus, 1758. Those species are of special interest due to their ecological relevance as reef-builders and three-dimensional complexity promoters in Southwestern Atlantic (SWA) reefs and their conservation status. This is because the scleractinian coral *Mussismilia harttii* (Verrill, 1868) is an endangered species facing losses of coral cover and population decline in some coastal reefs [17], while the common *M. alcicornis* is the only branching species in the region and the main generator of structural complexity, but has been very susceptible to coral bleaching [18].

Bleaching has been recorded for SWA reefs since the 1990s, and such events are becoming more frequent and widespread, being strongly correlated with El Niño years [17–20]. Nevertheless, SWA reefs have been highlighted as less susceptible to mortality from bleaching than the Caribbean and Indo-Pacific reefs due to characteristics, such as water turbidity, the depth range of coral species, the dominance of more resilient massive corals, and the generalism of symbiosis patterns, including the dominance of generalist host species and symbiont lineages [21]. In addition, many studies have revealed a high diversity of symbiodiniaceans associated with SWA corals, following Caribbean patterns [22–25]. However, the dynamics of these diverse photosymbiont assemblages during bleaching events are still poorly understood.

To assess the effect of thermal anomalies predicted by the NOAA Coral Reef Watch Program (CRW), during the ENSO 2015/2016, over the composition of symbiodiniacean assemblages, we monitored for 6 months the bleaching status and the profile of Symbiodiniaceae ITS2 rDNA phylotypes, assessed through next-generation sequencing, in the populations of the hydrocoral *Millepora alcicornis* and the scleractinian coral *Mussismilia harttii* at three reef sites in a model coral reef at the SWA.

Methods

Study Area

The study was carried out at Recife de Fora Marine Protected Area (no-take zone), Porto Seguro, Brazil (Fig. 1, 16 23.931′S, 038 59.193′W), a reef of 17.5 km^2. North of the Abrolhos Reef Complex, the South Atlantic center for coral diversity, Recife de Fora is a biogenic reef that harbors almost all the coral species occurring in this province [16] and is a shallow (< 15 m depth; [26]) and easily accessible reef, 3.5 km offshore the Porto Seguro Bay. Because of these characteristics, Recife de Fora has been used as a model coral reef for ecological studies in Brazilian reef systems [26–30].

Monitoring Bleaching and Symbiotic Assemblages

Three sites, Mourão (16 25′02.6″ S, 038 58′55.8″ W), Funil (16 24′39.0″ S, 038 59′06.1″ W), and Taquaruçu (16 24′25.3″ S, 038 58′41.0″ W) were monitored over 6 months from December 15th, 2015 to June 15th, 2016 by biweekly expeditions (totaling 12 sampling days). Mourão is a region located west of the reef plateau, characterized by an extensive coral cover found at depths ranging from 2 to 6 m, where corals of the genera *Mussismilia* and *Millepora* are common. The Funil region, situated south of the reef plateau, consists of multiple reef patches with depths and fauna similar to Mourão's. Taquaruçu, on the other hand, is a tidal pool located on the eastern side of the reef. It features a sandy bottom and is characterized by the shallowest depths, ranging from 2 to 4 m. In this area, coral species such as *Mussismilia harttii* and *Millepora alcicornis* can be easily observed along the pool's walls. The sites were monitored for bleaching through 20 × 1 m transects placed randomly at

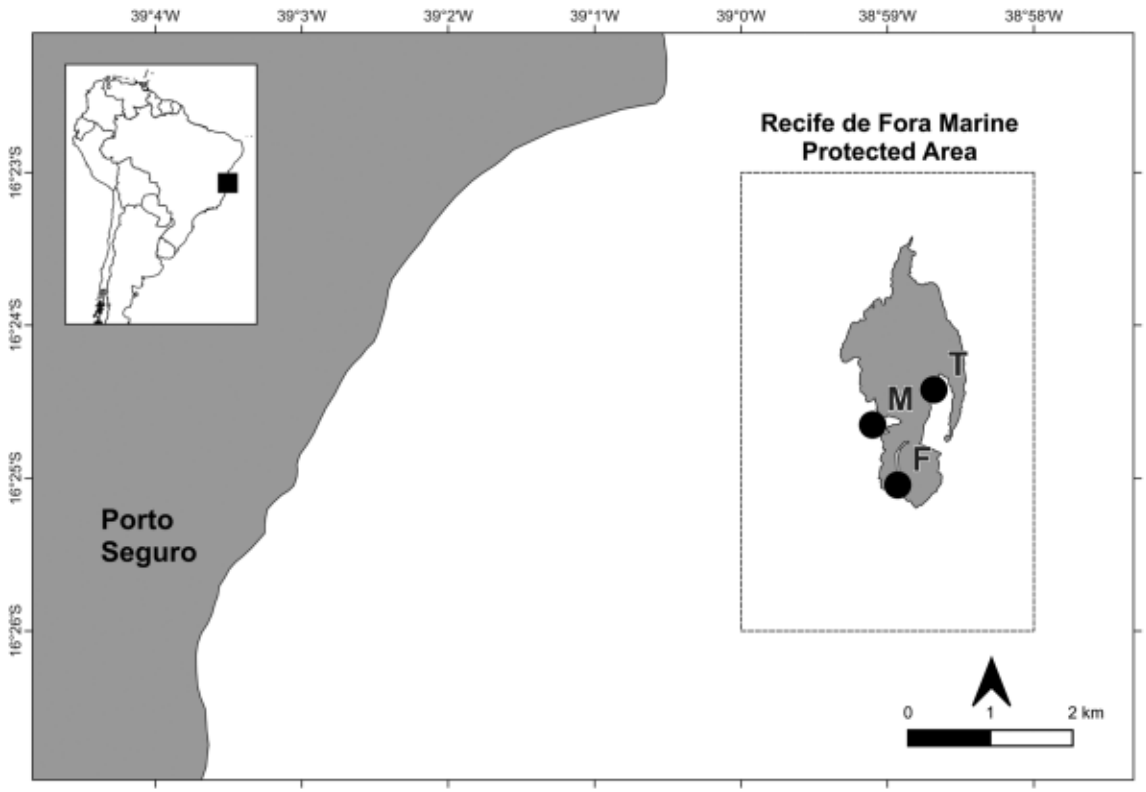

Fig. 1 The three monitored sites at Recife de Fora, Porto Seguro, Bahia, Brazil: Taquaruçu (T), Mourão (M), and Funil (F). The dashed square indicates Recife de Fora Marine Protected Area

approximately 2 m depth during the low tide at each site. All *M. harttii* and *M. alcicornis* coral colonies inside the transects were recorded and classified according to their color following the Coral Health Chart color scale (https://coralwatch.org/; [31]). A fragment of ~ 0.5 cm^2 was sampled from four randomly selected individuals of each species per site with a hammer and chisel. Tissue samples were stored in liquid nitrogen and afterward transferred to CHAOS solution (4 M guanidine thiocyanate, 0.1% N-lauroylsarcosine sodium, 10 mM Tris pH 8.0, 0.1 M 2-mercaptoethanol) and maintained at room temperature until analyzed.

Thermal Stress Assessment

Water temperatures at each monitored site were measured by in situ temperature data loggers (HOBO Pendant model UA-002-64, onset®), continuously recording every 15 min from December 20th, 2015, to June 20th, 2016. The long-term maximum of the monthly mean sea surface temperature (MMM) from NOAA's 5 km virtual station, Recife de Fora, Brazil, was assessed and combined with in situ temperature data to calculate the cumulative heat stress as the sum of the positive anomalies ≥ 1 °C of the daily mean temperature exceeding the MMM. Degree heating days (DHD) were calculated according to the NOAA Coral Reef Watch Program (CRW; https://coralreefwatch.noaa.gov/product/5km/methodology.php#dhw) as the accumulation of temperature anomalies exceeding the MMM over the last 84 days (12 weeks) and converted to degree heating weeks (DHW) by dividing it by 7 days [32, 33]. We, therefore, used the long-term regional MMM of 26.87 °C for our analyses.

Sample DNA Extraction, Processing, and Sequencing

Total DNA was extracted by phenol:chloroform:isoamyl alcohol protocol [34]. Extracted samples were purified with ProNex Size-Selective Purification System (Promega®) for DNA fragments of 600 bp, according to the manufacturer's instructions. PCR amplified the region of the internal transcribed spacer 2 (ITS2) rDNA with the specific primers SYM_VAR_5.8S2 (5′ GAATTGCAGAACTCCGTGAACC 3′) and SYM_VAR_REV (5′ CGGGTTCWCTTGTYTGAC TTCATGC 3′; [35]) linked to Illumina adapters (forward: 5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG 3′; reverse: 5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G 3′; following the 16S Metagenomic Sequencing Library Preparation Illumina protocol). The target amplicon was approximately 400 bp. The PCR reaction contained 1 µl of DNA (~ 5 ng/µl), 0.2 µM of each primer, 0.025 U/µl of *Pfu* DNA polymerase (Promega®),

1X *Pfu* reaction buffer with $MgSO_4$, 200 µM dNTP mix (Promega®) and molecular grade water up to a total reaction volume of 30 µl. PCR cycles were as follows: 98 °C for 2 min, 35 cycles of 98 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min.

Triplicate PCRs for each sample were pooled, and PCR clean-up was performed with the ProNex System to select 350 bp amplicons. Illumina indexing primers (Nextera® XT Index Kit—96 indexes, 384 samples) were added to 5 µl of mixed purified PCR product, and a new PCR of 50 µl was run to incorporate unique barcodes for each sample. This PCR reaction contained 5µl of cleaned PCR product, 5 µl of Illumina Indexed Primer 1 (i5), 5 µl of Illumina Indexed Primer 2 (i7), 1 µl of HotStar*Taq* (Qiagen®), 10 µl of 1X HotStar*Taq* Buffer with dNTP, and 24 µl PCR grade water for a total reaction volume of 50 µl. PCR cycles were as follows: 95 °C for 3 min, 8 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s, and lastly, 72 °C for 5 min. PCR clean-up was completed one last time with the ProNex System, and the resulting products were quantified by fluorometry with Qubit dsDNA HS Assay Kit by Qubit Fluorometric Quantification (ThermoFisher®). Indexed and purified samples were pooled in equal molar amounts (4 nM). The two obtained amplicon libraries, with 96 samples each, were sequenced on the Illumina MiSeq platform using a MiSeq Reagent Kit v2 (500-cycles) paired-end run with 25% PhiX at the Integrated Functional Genomics Unit at Instituto de Biodiversidade e Sustentabilidade - NUPEM/UFRJ, Brazil.

Bioinformatic Data Processing

Illumina's real-time analysis was run during sequencing using the default settings to remove clusters with the least reliable data. Demultiplexed *fastq* files were generated with Illumina's BaseSpaceFS (version 1.5.964), and reads were processed in FastQC online platform. Samples with less than 200 reads ($N = 4$) were removed from the dataset ($N = 182$ samples remained, including at least three random colonies of each species per location and collection time; Table S1). All remaining sample reads were processed with the Geneious software, where data were cleaned of primers, adapters, and indexes with the BBDuk plugin and were filtered by quality (> 30) and length (> 200 bp). Chimeric reads were removed using UCHIME v4.2.40, and the remaining reads were clustered into contigs with 98% similarity using de novo assembler for each sample separately. Contigs encompassing more than 10 reads per sample were classified into operational taxonomic units (OTUs) according to a Symbiodiniaceae ITS2 rDNA reference database containing sequences downloaded from the GenBank (NCBI) and SymPortal (symportal.org). To remove non-symbiodiniaceans' sequences, a minimum sequence cover of 75% was applied to classify the contigs, and classifications with > 98% of similarity with the reference sequence were accepted to avoid incorrect identifications. When a contig was classified in more than one OTU with the same percentage of similarity, all corresponding OTUs were attributed to the contig (e.g., C1/C3), and when similarity was lower than 98%, a prefix TL- (from "too-low similarity") was added to the OTU (e.g., TL-B23/B42). When similarity was lower than 95% with any reference sequence, the contig was named "unclassified." Differences in library sizes across samples were adjusted by rarefaction using the smallest sample (1024 reads) as the threshold. The rarefied samples were used to compare Symbiodiniaceae assemblage composition between monitored sites and host species during the collection period.

Statistical Analyses

All analyses were performed in the R statistical environment (version 1.4.1717; [36]), and the significance level adopted was 95% ($\alpha = 0.05$). Permutational multivariate analysis of variance (PERMANOVA) with the function *adonis* from "vegan" compared the Bray-Curtis Index dissimilarity of relative abundance of Symbiodiniaceae assemblage composition of each host species population for the effect of sites and sampling period. For significant results, pairwise PERMANOVAs were performed with the function *pairwise adonis* from "vegan," both with 999 permutations. Indicator species analysis (ISA) was performed with the function *multipatt* from the package "indicspecies" to identify symbiodiniacean lineages found more often in one group than another, considering the groups indicated by PERMANOVA. For β-diversity metrics, differences in symbiodiniaceans assemblages from each host species were compared through the relative abundance of Symbiodiniaceae ITS2 phylotypes and visualized in a non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis Index dissimilarity with the function *metaMDS* from "vegan."

Results

All three reef sites at Recife de Fora were hit by the marine heatwave and suffered bleaching during the summer of 2015/2016 (as seen by [30]; Fig. 2; Fig. S1). Although bleaching was severe, no mortality was recorded for either *Mussismilia harttii* or *Millepora alcicornis*. The sea surface temperature (SST) at two of the monitored sites exceeded the long-term maximum of the monthly mean SST (MMM) in late December and early February and stayed above the MMM for 30 and 33 consecutive days at Funil and Mourão, respectively, between March and April (Fig. 2a). Although SST at Taquaruçu never rose over the regional MMM, this site recorded the most intense bleaching status for both monitored species (100% of *M. alcicornis*, $N = 4$–9 between

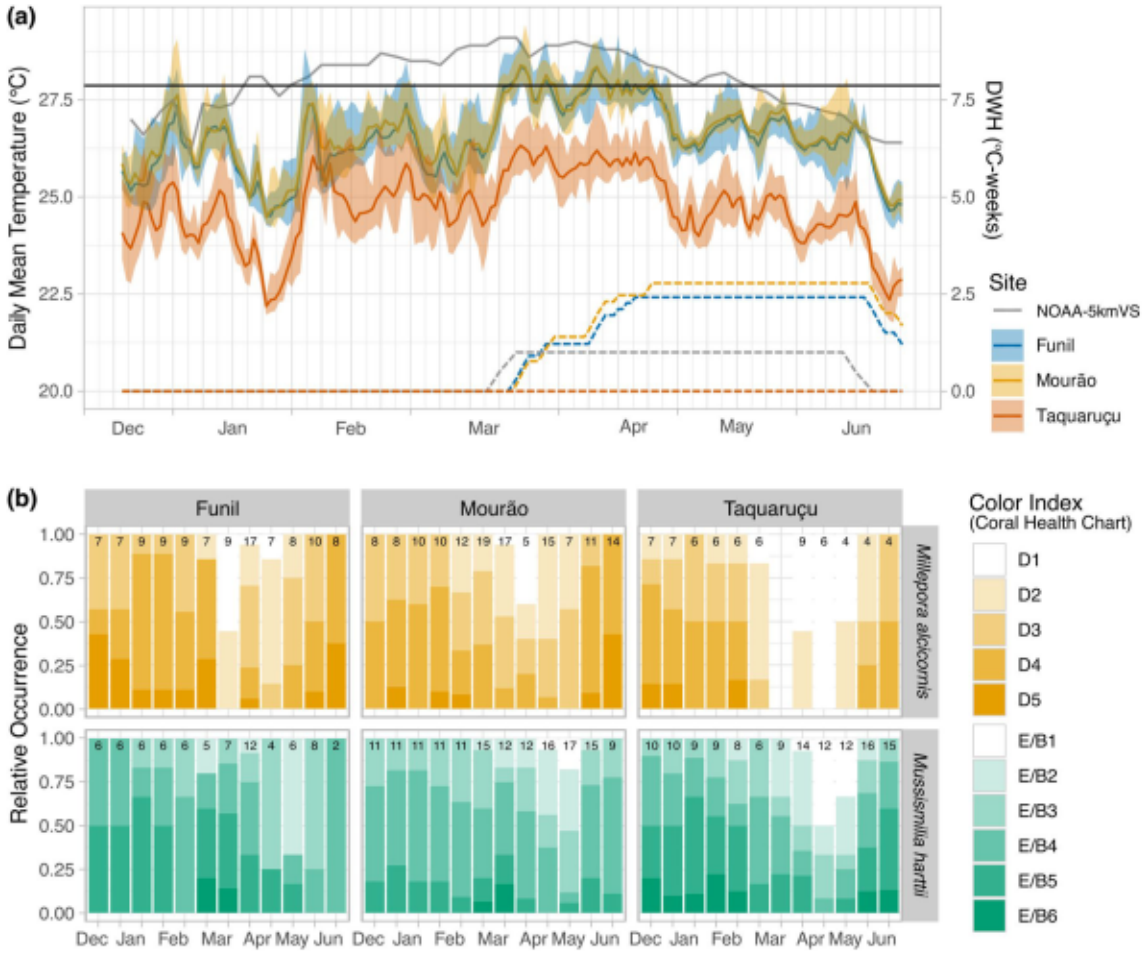

Fig. 2 **a** Daily mean water temperature (°C; solid lines) and accumulated heat stress (degree heating weeks, DHW; dashed lines) during the 6-month monitoring period (December 2015 to June 2016) measured in three sites at Recife de Fora Marine Protected Area (Porto Seguro, Bahia, Brazil) and from NOAA's 5 km virtual station, Recife de Fora. Shadows indicate the daily maximum and minimum temperature. The dark continuous line is the bleaching threshold (long-term maximum of the monthly mean sea surface temperature (MMM) + 1 °C). **b** Relative occurrence of *Millepora alcicornis* and *Mussismilia harttii* colonies in three sites according to color index based on Coral Health Chart along the monitoring period. Sample size indicated for each bar

March and May, and 66.67% of *M. harttii* colonies, $N = 12$ in final-April; Fig. 2b).

Although the degree heating weeks (DHW) did not reach the bleaching threshold (4 °C-weeks DHW, according to Coral Reef Watch Program - CRW), bleaching occurrences were recorded between March and May at all monitored sites, with a peak in final April to May, with 57–55% of the colonies bleached ($N = 63$ and 54, for final April and May, respectively). *Millepora alcicornis* was more susceptible to bleaching, displaying bleaching symptoms about 1 month before *M. harttii*. Additionally, *M. alcicornis* experienced a longer and more intense event, for 3 months (March–May), with 75% of colonies bleached at the peak of the event ($N = 28$ colonies in final-April), whereas *M. harttii* colonies experienced bleaching for 2 months (April–May) with 47% of them bleached ($N = 32$ colonies in final-April). In June, recovery was observed with a drop to 0.08% of bleached colonies in early June ($N = 64$) and to zero in final June ($N = 52$; Fig. 2b). We have considered "bleached" all colonies with a color index of 1 or 2 (e.g., Fig. S1), since it was a two-point reduction on the most common color index of the coral population (4 for both species; [31]).

After filters and data cleaning, 709,341 sequences were obtained from the two sequencings, library size varied between 1394 and 13,939 sequences per sample (Table S1). Symbiodiniaceae ITS2 rDNA phylotype profiles were distinct between host species (Fig. 3) and responded differently to environmental changes. *Millepora alcicornis* was dominated by *Breviolum* phylotype B23, and no change in its dominance was observed throughout the bleaching event (Fig. 3). Conversely, *M. harttii* was dominated by *Cladocopium* phylotype C3 at Funil and Mourão, while at Taquaruçu colonies were co-dominated by the same C3 and *Symbiodinium linucheae* (ITS2 phylotype A4) (Trench & Thinh) LaJeunesse 2018 (Fig. 3). However, in

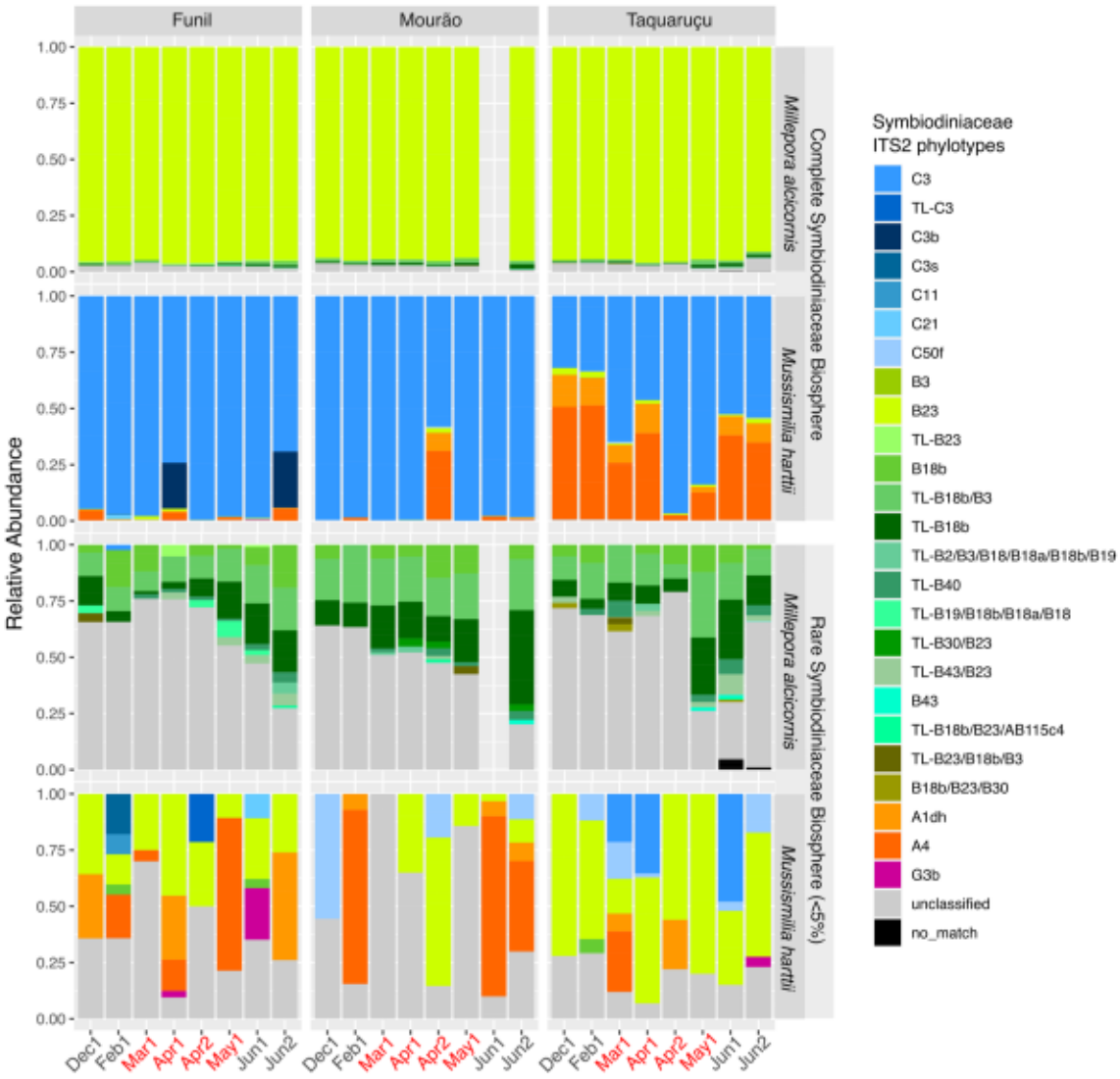

Fig. 3 Relative abundance of Symbiodiniaceae ITS2 rDNA phylotypes representatives of complete and rare biospheres (relative abundance < 5%) associated with the hydrocoral *Millepora alcicornis* and the coral *Mussismilia harttii* at the three sampled sites along the 6-month monitoring period (December 2015 to June 2016) at Recife de Fora Marine Protected Area (Porto Seguro, Bahia, Brazil). Sampling periods with bleaching incidence are in red

M. harttii there was a change in dominance during the bleaching period, where C3 was replaced by A4 at Mourão and A4 by C3 at Taquaruçu. After recovery, the symbiont assemblage resumed its original composition (Fig. 3).

In general, β-diversity analysis (non-metric multidimensional scaling, nMDS, and Table S2) showed that photosymbiont assemblages of *M. alcicornis* were distinct between pre- and post-bleaching, with post-bleaching assemblages forming a more heterogeneous group (Fig. 4). Conversely, *M. harttii* colonies collected before bleaching were more divergent from one another than colonies collected during the bleaching event (Fig. 4). After recovery, colonies returned to their original assemblages, and β-diversity was recovered, but there was no significant difference in symbiodiniacean phylotypes' relative abundance between collection periods for this host (Table 1).

The rare biospheres of photosymbionts (< 5% relative abundance) did not suffer marked changes, except for the presence of B43 and the increase of B18b-related phylotypes in *M. alcicornis* during and after bleaching (Fig. 3), but the relative abundance of *M. alcicornis*' photosymbionts changed significantly throughout the monitored period (PERMANOVA: $F_7 = 2.4861$, $p < 0.01$; Table 1). Indicator species analysis showed that three OTUs were significantly associated with both bleaching and recovery periods in *M. alcicornis*: TL-B43/B23 (stat = 0.452, $p = 0.0026$), TL-B40 (stat = 0.451, $p = 0.0022$) and TL-B18b (stat = 0.559, $p < 0.0001$), being present only from March onwards.

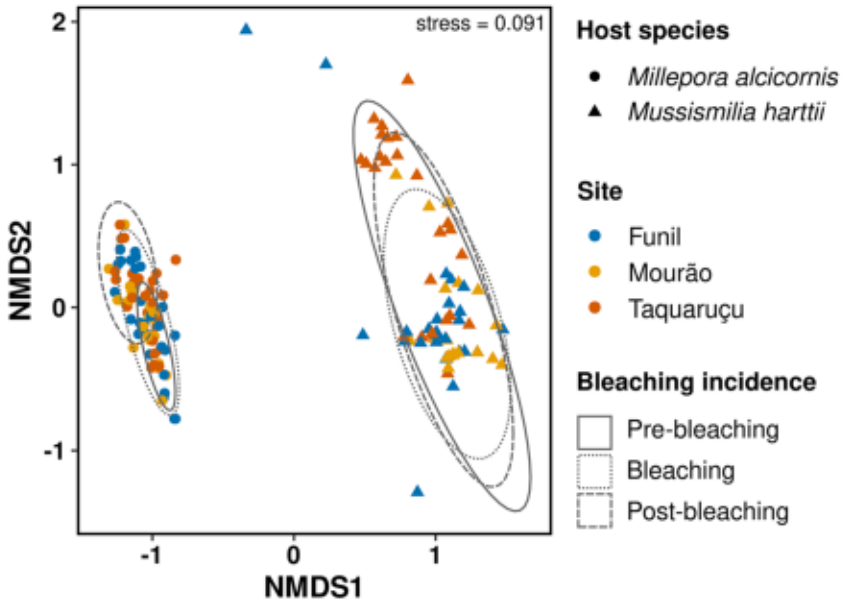

Fig. 4 Non-metric multidimensional scaling (nMDS) biplot based on Bray-Curtis index dissimilarity showing the configuration of the relative abundances of Symbiodiniaceae ITS2 rDNA phylotypes within different coral host species along the sampling months grouped by bleaching incidence (ellipses)

Table 1 PERMANOVA based on Bray-Curtis index dissimilarity of the Symbiodiniaceae composition present in *Millepora alcicornis* and *Mussismilia harttii* from three sites at Recife de Fora, Brazil, over 6-month monitoring (8 sampling periods). Pairwise PERMANOVA only for the "collection site" factor

Factor	Df	Sum Sq	F	R^2	*p*-value
Millepora alcicornis					
Sampling period	7	0.0047	2.4861	0.16353	**0.001**
Collection site	2	0.0028	5.1621	0.09701	**0.001**
Period:Site	13	0.0034	0.9763	0.11926	0.516
Funil *vs* Mourão	1	0.0018	8.2160	0.1279	**0.003**
Funil *vs* Taquaruçu	1	0.0018	5.5412	0.0845	**0.003**
Mourão *vs* Taquaruçu	1	0.0004	1.1018	0.0193	0.969
Mussismilia harttii					
Sampling period	7	0.4795	0.7978	0.0433	0.617
Collection site	2	2.9135	16.9661	0.2633	**0.001**
Period:Site	14	1.8336	1.5253	0.1657	0.089
Funil *vs* Mourão	1	0.0551	1.1939	0.0192	0.999
Funil *vs* Taquaruçu	1	2.3137	18.8833	0.2456	**0.003**
Mourão *vs* Taquaruçu	1	2.2420	21.5365	0.2674	**0.003**

p-values in bold are significative (< 0.05)

The effect of the collection site was significant for symbiodiniaceans' relative abundance from both host species (PERMANOVA: *M. alcicornis* - $F_7 = 5.1621$, $p < 0.01$; *M. harttii* - $F_7 = 16.966$, $p < 0.01$; Table 1). Pairwise PERMANOVA showed that only Mourão was not different from Taquaruçu for *M. alcicornis* symbionts, and only Funil was not different from Mourão for *M. harttii* (Table 1). Indicator species analysis showed that 7 OTUs associated with *M. alcicornis* were significantly associated with reef sites: *Breviolum* TL-B23 and TL-B19/B18b/B18a/B18 at Funil (stat = 0.524/0.335, $p = 0.0001/0.0096$, respectively), *Breviolum* TL-B30/B23 at Mourão (stat = 0.269, $p = 0.0242$), *Breviolum* TL-B40 at Taquaruçu (stat = 0.286, $p = 0.0167$), *Breviolum* TL-B43/B23 grouped Funil+Taquaruçu (stat = 0.265, $p = 0.0283$), and *Breviolum* TL-B18b/B23 and TL-B18b grouped Mourão+Taquaruçu (stat = 0.376/0.281, $p = 0.0009/0.0233$, respectively). Regarding the photosymbionts of *M. harttii*, four were significantly associated with reef sites: *Symbiodinium* A4, *Symbiodinium* A1dh, and *Breviolum* B23 at Taquaruçu (stat = 0.578/0.574/0.422, respectively, $p < 0.0001$), and *Cladocopium* C3 grouping Funil+Mourão (stat = 0.507, $p < 0.0001$).

Discussion

The coral bleaching event during the summer of 2015/2016 was severe at Recife de Fora (> 50% of the colonies bleached; [37]), as other reefs of south Bahia [18, 38] even with relatively low thermal stress estimations (< 4 °C-weeks degree heating weeks; DHW), but no coral mortality was recorded. Bleaching occurrences without DHW values ≤ 4 °C-weeks have already been recorded, being attributed to the accumulation of thermal anomalies lower than 1 °C persisting over months [33, 39], or to the synergic effect of thermal and luminous stress in shallow reefs [40, 41], or even that NOAA's long-term MMM is not adequate, as a threshold, to estimate thermal stress on corals at a local scale. However, this is an indication that CRW general bleaching threshold may not be suitable as a bleaching predictor in Southwestern Atlantic (SWA) reefs, as reported for other regions [39], and consistent long-term bleaching and temperature monitoring coupled with analyses of historical coral bleaching are necessary to improve bleaching threshold prediction for SWA corals.

Although no thermal anomalies were recorded at Taquaruçu, the higher bleaching occurrence at this site could have resulted from the high levels of irradiance experienced by corals at this shallow tide pool [40]. Unlike the other two studied sites that receive warm water washed over the reef during tidal rises, this one faces the open sea and receives cooler water during high tides, decreasing the daily mean SST locally. So, even though the stress faced by the corals was higher at this site, the DHW calculation based on NOAA 5 km virtual station long-term MMM was not representative of this stress in this locality.

As expected, *Mussismilia* spp. were less susceptible to bleaching due to morphological characteristics and high heterotrophy capability [20, 21]. On the contrary, milleporids are historically classified as "losers" been highly susceptible to bleaching and mortality [18, 42, 43], although its heterotrophic feeding can also supply energetic demands during bleaching [44, 45], and no mortality was observed here. According to Marangoni et al. [30], who assessed the oxidative stress of the samples analyzed herein, *M. alcicornis* had shown an oxidative stress condition before and during bleaching, when total antioxidant capacity (TAC) and lipid damage increased. On the other hand, *M. harttii* naturally operated with maximal capacity against reactive oxygen species formation, with no distinction between healthy and bleached colonies, but TAC decreased in March, before the peak of bleaching, which might indicate an imbalance in the oxidative status before the occurrence of bleaching. Also, oxidative stress biomarkers indicated a recovery, resuming healthy levels by June 2016 [30]. In addition, *M. harttii* was able to increase heterotrophic activity 15 to 30 days before bleaching symptoms appeared, while a lower heterotrophic activity was observed for *M. alcicornis* [29], which may have also influenced the difference in bleaching intensity between these two species.

The dominant symbiodiniaceans found in *M. harttii* at Recife de Fora (*Cladocopium* phylotype C3 and *Symbiodinium linucheae,* ITS2 phylotype A4) have already been described in association with other *Mussismilia* spp. [22, 23] and recently with *M. harttii* (Maia et al. *in prep*). Interestingly, *Symbiodinium* spp. has already been identified in *Mussismilia* spp. from well-lit environments [22, 23], such as Taquaruçu shallow tide pool. In contrast, *Cladocopium* spp. were frequently found in more turbid habitats [23], such as Funil and Mourão, the washed patch reefs on the inner and southern edges of Recife de Fora. Nevertheless, it is difficult to attribute putative function or assume physiological characteristics to OTUs identified as "too-low similarity" (TL-), particularly for the genus *Breviolum*, which presents a high diversity of physiologies [10] and for which several new lineages have been discovered in the Southwestern Atlantic (SWA; [25]). However, the temporal changes in the relative abundance of *Symbiodinium* A4 in *M. harttii* colonies during the bleaching period have never been observed. They may have helped the holobiont to deal with the thermal stressful period.

Changes in photosymbionts dominance due to bleaching events could represent a way for coral adaptation to climate change [7], and those changes have already been observed [9, 46]. However, they must be long-lasting and reach most of the coral population. However, without permanent changes in dominance, we found no evidence of adaptation after bleaching for *M. harttii*, at least regarding this dominant portion of the photosymbiotic assemblage. In this case, the changes in photosymbiotic assemblage observed in *M. harttii* during bleaching seem more related to a dysbiosis process. The incidence of coral bleaching by itself has been defined as a dysbiosis simply due to the breakdown of the relationship of the coral with the most common photosymbionts [47, 48]. Moreover, depending on the path taken by the holobiont, distinct dysbiosis profiles could be determinants for coral death or survival after bleaching. In this sense, the temporary dominance changes in symbiont relative abundances in the population of *M. harttii* during the bleaching peak may be a result of the metabolic imbalance inherent to bleaching [47], or a strategy to maintain essential functions of the holobiont during stress [48].

The presence of *Breviolum* phylotype B23 in *M. alcicornis* was recently described as dominant for this reef region [25], but the rare biosphere of photosymbionts of this hydrocoral had never been assessed until now. Contrasting with *M. harttii*, post-bleaching samples of *M. alcicornis* were more heterogeneous and different from pre-bleaching samples. As we did not observe changes in dominance in the composition of assemblages before, during or after bleaching, this result indicates that changes in the rare biosphere was mainly responsible for these observed differences over time. The emergence of new symbionts through switching or shuffling after bleaching has already been reported for branched corals [49] and has been attributed to coral acclimation/adaptation to the new conditions of the environment. In this case, the dysbiosis process may confer certain advantages to the holobiont (adaptive dysbiosis; [48]). Background photosymbiotic lineages can play a relevant role in host fitness and resilience [50] since coral tissue repopulation can start by multiplying remaining symbionts [51]. However, its influence on holobiont physiology and its contribution to host recovery is poorly understood [50].

The disruption of the homeostasis between coral and symbionts by thermal stress can also lead to an outbreak of opportunistic symbiont lineages. Symbiodiniaceans from the genus *Durusdinium* have frequently been defined as opportunistic or thermotolerant [10] due to their wide distribution, physiologic characteristics, and documented replacement of healthy coral's symbionts after induced thermal stress [9, 46] and in Caribbean coral populations after bleaching [52]. The dominance effects of opportunistic symbiodiniaceans in

coral fitness is still not well known, but a few studies have shown that they have reduced coral growth rates [53]. To date, *Durusdinium* spp. has never been found in the SWA. Some *Symbiodinium* spp. have also been considered opportunistic for some Caribbean corals [54]. However, since *S. linucheae* (phylotype A4) is commonly found in healthy corals in the SWA (this study and [22–25]), we believe that its increased dominance in *M. harttii* tissue at Taquaruçu may be due to better fitness of this phylotype during the bleaching period when compared to the co-dominant *Cladocopium* C3.

The present study documented the distinct responses of a coral and a hydrocoral facing a severe bleaching event in a model reef in the Southwestern Atlantic during the ENSO 2015/2016, with no mortality records. The factors contributing to holobionts' survival are still poorly understood. However, they could be attributed to the host mechanisms of oxidative stress mitigation [29, 30] and feed mode balancing [20], but also to adjustments in photosymbiont assemblages composition during and after bleaching or all this together. Even subtle changes in photosymbiont composition, such as the documented dysbiosis, may contribute to the acclimatization of the holobiont [50]. Nevertheless, assessing symbionts' taxonomic and physiologic diversity is essential to better comprehend the role of symbiodiniaceans in host resilience in the face of climate change. These findings follow the observed resilience of Brazilian coral reefs to bleaching [21], since both species were able to deal with heat stress without mortality. However, the recently documented vulnerability of those species, mainly *M. alcicornis*, to more severe heat anomalies [18] reinforces the urgent need for monitoring programs and research focused on SWA corals to determine priorities for coral reef conservation and management.

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Author Contribution All authors contributed to the study's conception and design. Amana G. Garrido, Laís F. Machado, Cristiano M. Pereira, and Douglas P. Abrantes performed material preparation, data collection, and analysis. Carla Zilberberg and Emiliano N. Calderon led the funding acquisition. Amana G Garrido wrote the manuscript's first draft, and all authors commented on previous versions. All authors read and approved the final manuscript.

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Data Availability The molecular data generated and analyzed during the current study are available in the NCBI Sequence Read Archive database under the BioProject ID PRJNA943220.

Declarations

Ethics Approval This study was performed under the sampling permission (# 47714-1) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) / Chico Mendes Institute for Biodiversity Conservation (ICMBio), under the Instruction Normative n° 03/2014 of System Authorization and Information on Biodiversity (SISBIO).

Competing Interests The authors declare no competing interests.

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