



# Experimental evidence of temperature-induced bleaching in two fluorescence morphs of a Red Sea mesophotic coral

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**Abstract** Coral bleaching, as one of the major threats to the well-being of coral reefs worldwide, has been extensively studied. However, corals from mesophotic coral ecosystems (MCEs), found at 30 to 150 m depth and considered as a potential refuge, have not yet been well studied experimentally under thermal stress. As mesophotic corals are also highly fluorescent, and fluorescence under heat stress is known to undergo change, we examined the involvement of fluorescence during heat-induced bleaching, by incorporating both high- and low-fluorescence morphs in our experiments. We collected colonies of the mesophotic coral *Alveopora ocellata* and subjected them to elevated temperatures in both winter and summer in controlled laboratory experiments. We followed their physiological response and their bleaching at a high sampling resolution (every 48 h following the temperature

ramping period). We found that *A. ocellata* bleached after a short-term (14 days) thermal stress of +4 °C above ambient sea temperature during the summer, but did not bleach during the winter despite the elevated temperature (+5 °C; max temperature of 32 °C and 28 °C, respectively). After experiencing temperatures higher than 29.5 °C, the peak summer temperature, the corals gradually lost their algal symbionts during the summer experiment, while exhibiting an increase in symbiont density during the winter experiment. A similar response was also observed in chlorophyll a concentration, host fluorescence intensity, and maximal quantum yield of PSII ( $F_v/F_m$ ). Throughout the experiments (in both seasons and treatments), the high-fluorescence corals presented lower zooxanthellae densities, higher cellular chlorophyll a concentration, and up to sixfold higher fluorescence. The differences found between the two morphs suggest that fluorescence may be favorable under thermal stress, strengthening the possibility of using coral fluorescence as a noninvasive monitoring tool for early detection of bleaching. This demonstration of a bleaching process in a mesophotic coral indicates the vulnerability of MCEs to the increase, in recent decades, in the frequency and intensity of temperature anomalies.

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## Introduction

Mass coral bleaching has constituted one of the major threats to coral reefs in the recent decades. Events of extensive breakdown of the symbiotic relationship between corals and their photosynthetic endosymbionts (i.e.

“zooxanthellae”, family Symbiodiniaceae) have become increasingly frequent and severe in the last three decades (Hughes et al. 2018). As the deterioration of shallow reefs continues, and our ability to explore deeper habitats increases, the interest in mesophotic coral ecosystems (MCEs) is rising. MCEs are light-dependent communities usually found between 30–40 m and 150 m depth (Hinderstein et al. 2010). These reefs can be considered, in part, as an extension of their shallow counterparts, based on their species diversity and community structure overlapping with those of adjacent shallow reefs, and the existence of species that are considered “depth generalists” (Semmler et al. 2017; Kramer et al. 2019; Tamir et al. 2019). Moreover, genetic examination has revealed a possible connectivity between shallow and mesophotic coral communities in some species and locations (Van Oppen et al. 2011; Bongaerts et al. 2017; Studivan and Voss 2018). However, in view of our knowledge today that the extent of coral overlapping may be biased due to the use of traditional taxonomy which is based mainly on shallow species diversity (Bongaerts and Smith 2019), and that in corals and other taxa, shallow and mesophotic communities may differ significantly (Kahng et al. 2010; Slattery et al. 2011; Rocha et al. 2018; Dumalagan et al. 2019), MCEs are also being considered as unique habitats that should be studied and protected. Regardless of the magnitude of connectivity between shallow and mesophotic depths, deeper reefs are assumed to be less affected by temperature anomalies (Glynn 1996), storms (Highsmith et al. 1980; Woodley et al. 1981), and other anthropogenic stressors (i.e., pollution, diving pressure, and urban development; reviewed by Bongaerts et al. 2010) as they are mostly located farther away from shore and from human impacts and experience more stable abiotic conditions. The potential connectivity and reduced stress pressure in MCEs formed the basis for development of the “deep reef refugia hypothesis” (DRRH), which suggests that the mesophotic community could serve as a refuge for shallower corals or as a source of replenishment for the degraded shallow reefs (Bongaerts et al. 2010).

MCEs are considered more pristine and stable than shallow waters; therefore, the risk of mass bleaching in the former is supposedly reduced (Glynn 1996; Hughes and Tanner 2000). However, in recent years, both small-scale and mass bleaching events in MCEs have been reported in various studies, albeit many of them are only qualitative: Honduras (Laverick and Rogers 2018), US Virgin Islands (Smith et al. 2016), Papua New Guinea (reviewed by Longenecker et al. 2019), the Caribbean (Lang et al. 1988; Bunkley-Williams et al. 1991), Philippines (Cabaitan et al. 2019), Australia (Bridge et al. 2019), the Chagos Archipelago (Sheppard et al. 2017), and the Red Sea (Eyal et al. 2019).

The Gulf of Eilat/Aqaba (GoE/A) is one of the northernmost reefs in the world and was suggested to serve as a potential thermal refuge for corals (Fine et al. 2013). Studies have demonstrated a high tolerance of corals under thermal stress (Bellworthy and Fine 2017; Grottoli et al. 2017; Krueger et al. 2017), and mass bleaching has not been documented in shallow reefs, whereas many other reefs have been affected worldwide. To date, all experiments on the thermal tolerance of corals in the GoE/A were performed on shallow-water corals, mostly the comprehensively studied and abundant species *Stylophora pistillata* (Fine et al. 2013; Bellworthy and Fine 2017; Krueger et al. 2017; Bellworthy et al. 2019); consequently, we sought here to examine the resilience to thermal stress of a mesophotic coral from the GoE/A.

The conversion of light (mostly from short to longer wavelength) by fluorescent proteins (FPs) is a common phenomenon in scleractinian corals, and the role of FPs has been investigated under thermal stress. Previous studies have examined the regulation of green fluorescent proteins (GFPs) during thermal stress (DeSalvo et al. 2008; Smith-Keune and Dove 2008; Hume et al. 2013; Roth and Deheyn 2013; Bollati et al. 2020). These reported responses indicate a specific regulation of FPs during thermal stress, suggesting that fluorescence could serve as an early detection tool for coral bleaching. Fluorescence polymorphism among corals was also recorded and was suggested to be involved in the fine-tuning of the internal light environment within coral tissue, providing a mechanism for photoacclimation or photoprotection along a depth gradient (Gittins et al. 2015; Quick et al. 2018). Since many mesophotic corals present fluorescence polymorphism (Eyal et al. 2015; Roth et al. 2015; Ben-Zvi et al. 2019), we also incorporated in the current study high-fluorescence and low-fluorescence morphs of a mesophotic coral species. *Alveopora ocellata* is highly abundant and exclusively found at the well-characterized (i.e., environmental conditions and community composition), mesophotic depths in the GoE/A (Eyal-Shaham et al. 2016; Tamir et al. 2019). *A. ocellata* presents a green fluorescence emission and colonies may express high or low levels of fluorescence, making it a model species for further examination of the potential of fluorescence to serve as a bleaching indicator, as well as for exploring the physiological responses of mesophotic corals to high temperature.

## Materials and methods

### Coral collection and field survey

Eight colonies of the coral *A. ocellata* were collected during an open-circuit technical dive at 45 m on the

mesophotic reef in front of the Interuniversity Institute for Marine Sciences in Eilat (IUI), Israel. These reefs are well monitored and well characterized (i.e., coral community structure, temperature, sedimentation rates, and light quality and quantity; Eyal et al. 2019; Tamir et al. 2019). While temperature ranges do not differ significantly between shallow and mesophotic reefs in Eilat (20.7–30.3 °C and 20.9–27.8 °C, respectively), light is dramatically reduced in the mesophotic depths (200–2000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and 1–200  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , for shallow and mesophotic depths, respectively; Eyal et al. 2019). Upon collection, coral fluorescence was measured using a JAZ spectrophotometer system (Ocean Optics, USA) and a 450 nm torch for excitation (Night Sea, USA). Out of the four colonies, two were highly fluorescent (HF) and therefore appearing as green, while two were barely fluorescent (LF), displaying a more reddish color. Corals were fragmented into 1 cm<sup>2</sup> pieces ( $n = 8$ –16 for each colony) and glued to round cement plugs. Fragments were kept prior and during the experiments in open-water aquaria system under a lighting filter (“Lagoon blue,” Lee Filters), which creates a light environment similar of that found at 45 m in the Gulf of Eilat/Aqaba (EMS 1). The fragments were given a month to recover prior to each experiment. Ten-meter-long line transects ( $n = 8$ ) were performed at the site of collection. Colonies of *A. ocellata* were classified as high or low fluorescing by their ambient light appearance (green vs. red, respectively). Each morph’s abundance was calculated from the total number of *A. ocellata* colonies on the transect.

## Experimental design

Following one month of recovery, corals were assigned to one of two treatments (EMS 2): high temperature (HT) or ambient sea temperature (AT). This experiment was repeated twice: once during summer (August–September), when average ( $\pm$  SD) ambient sea temperature was  $27.9 \pm 0.69$  °C (ranging from 26.7 °C to 29.5 °C); and again during winter (January), when average ( $\pm$  SD) ambient sea temperature was  $22.99 \pm 0.16$  °C (ranging from 22.6 °C to 23.5 °C; The Israel National Monitoring Program at the Gulf of Eilat). These seasons and temperatures were chosen in order to cover the entire temperature range corals experience in the GoE/A, thus testing whether the ramping of the temperature or the peak temperature is the cause for bleaching of the mesophotic corals. Each treatment contained three 2-L glass containers, each of them continuously supplied with fresh seawater at a rate of 1.5 L/min, using a pump. Temperature in the HT containers was gradually ramped by 1 °C per day to reach +

4 °C or + 5 °C above ambient sea temperature (i.e., 32 °C and 28 °C for summer and winter, respectively), using submersible water heaters (250 W, Aqua One). Temperature and light (lux) were monitored in each container throughout the duration of the experiment (EMS 3, 4) using a HOBO pendant temperature and light data logger (Onset, USA). Two fragments from each colony (total of  $n = 8$ ) were sampled at the beginning of the experiment (day 1) and 18 fragments ( $n \geq 2$  for each colony) at the end of the experiment (day 13). All sampled fragments were dipped in liquid nitrogen and kept at –80 °C for zooxanthellae density and chlorophyll a concentration analysis. During the experiments, the position of the containers was rotated clockwise in order to avoid any differences resulting from potential variation in light intensity (EMS 4).

## Fluorescence imaging

Roth and Deheyn (2013) found a strong correlation between fluorescence intensity measured as pixel intensity and GFP concentration quantified by western blots. In order to ensure an adequate sample size, we chose to measure fluorescence intensity in a noninvasive approach using imaging. At each sampling point (days 1, 5, 7, 9, 11, and 13 or days 1, 5, 7, and 13 for summer and winter, respectively), each fragment was imaged using a custom-made imaging system. The system comprised a dark chamber, a Sony RX100 IV camera mounted with a Y12, 57-mm barrier filter (Tiffen, USA), and two blue (450 nm) light bulbs operated by a 12 V rechargeable battery. Corals were placed on a black sheet next to a fluorescent scale bar (NightSea, USA) in the same position relative to one another and to the system, in order to ensure the same level of illumination for all images. Fluorescent images were analyzed using ImageJ software for fragment size, green layer pixel intensity of the fragment, and green layer pixel intensity of the fluorescent scale bar. The coral fluorescence intensity (i.e., green layer pixel intensity) was divided by the scale bar fluorescence for normalization.

## Symbiodiniaceae density and chlorophyll concentration

Frozen samples ( $n = 4$ –10 fragments for each treatment) were thawed, and the coral tissue was blasted into 15-ml tubes using an artist’s air brush, in the presence of cold BPS buffer. The tissue slurry was homogenized using a motorized homogenizer and centrifuged at low speed (2000 rpm for 5 min) to separate host fraction from symbiotic algae fraction. The supernatant containing the coral

host fraction was discarded, the algal pellet was resuspended in BPS, and a subsample was taken for algal cell density determination and Symbiodiniaceae genetic identification. The pellet was centrifuged again, and the supernatant discarded. The pellet was incubated in 100% cold acetone for photosynthetic pigment extraction for 12 h at 4 °C, followed by spectrometric determination of chlorophyll a content (normalized to the Symbiodiniaceae density) according to Jeffery and Humphrey (1975). Algal cells were counted using a Neubauer hemocytometer in triplicates and normalized to coral surface area (determined from the images taken) for the determination of Symbiodiniaceae density.

### Chlorophyll fluorescence-based measurements

Maximal quantum yield of photosystem II (PSII;  $F_v/F_m$ ) was determined for each fragment prior to the temperature ramping (on day 1) and for the remaining fragments at each sampling point (days 5, 7, 9, 11, and 13 for the summer experiment; and days 5, 7, and 13 for the winter experiment).  $F_v/F_m$  was measured using a diving-pulse amplitude modulated fluorometer (Diving-PAM; Walz GmbH, Germany). Measurements were taken 1 h after sunset in order to allow the corals to fully acclimate to dark and to ensure all reaction centers were vacant.

### Symbiodiniaceae genetic identification

DNA was extracted from the preserved algae subsamples using DNeasy blood & tissue kit (Qiagen, MD, USA) according to the manufacturer's protocol for tissue samples. A ~ 700 bp fragment of Symbiodiniaceae ITS2 sequence region was amplified using the primers SYM\_VAR\_FWD and SYM\_VAR\_REV following Hume et al. (2013). PCR products were checked for size, yield, and purity on 1.5% TBE agarose gel precast with ethidium bromide. PCR products were cleaned prior to sequencing using ExoSAP-IT<sup>TM</sup> PCR product cleanup reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Samples were bidirectionally sequenced using ABI 3730XL sequencers (MCLAB, USA). Reverse and forward sequences were aligned using ClustalW (BioEdit software version 7.01). A consensus sequence was obtained for each sample, and Symbiodiniaceae species/types were determined using a local BLAST against the GeoSymbio database (Franklin et al. 2012).

### Statistical analyses

All statistical analyses were performed using R software version 3.5.2 (Team 2013). Data were analyzed using permutational multivariate analysis of variance (PERMANOVA) with 5000 permutations and Rd\_keradPajouh\_renaud method using “permuco” package (Frossard and Renaud 2019) with treatment, time, and morph as between factors, and time nested within colony as within factor. Results were considered statistically significant if  $p < 0.05$ .

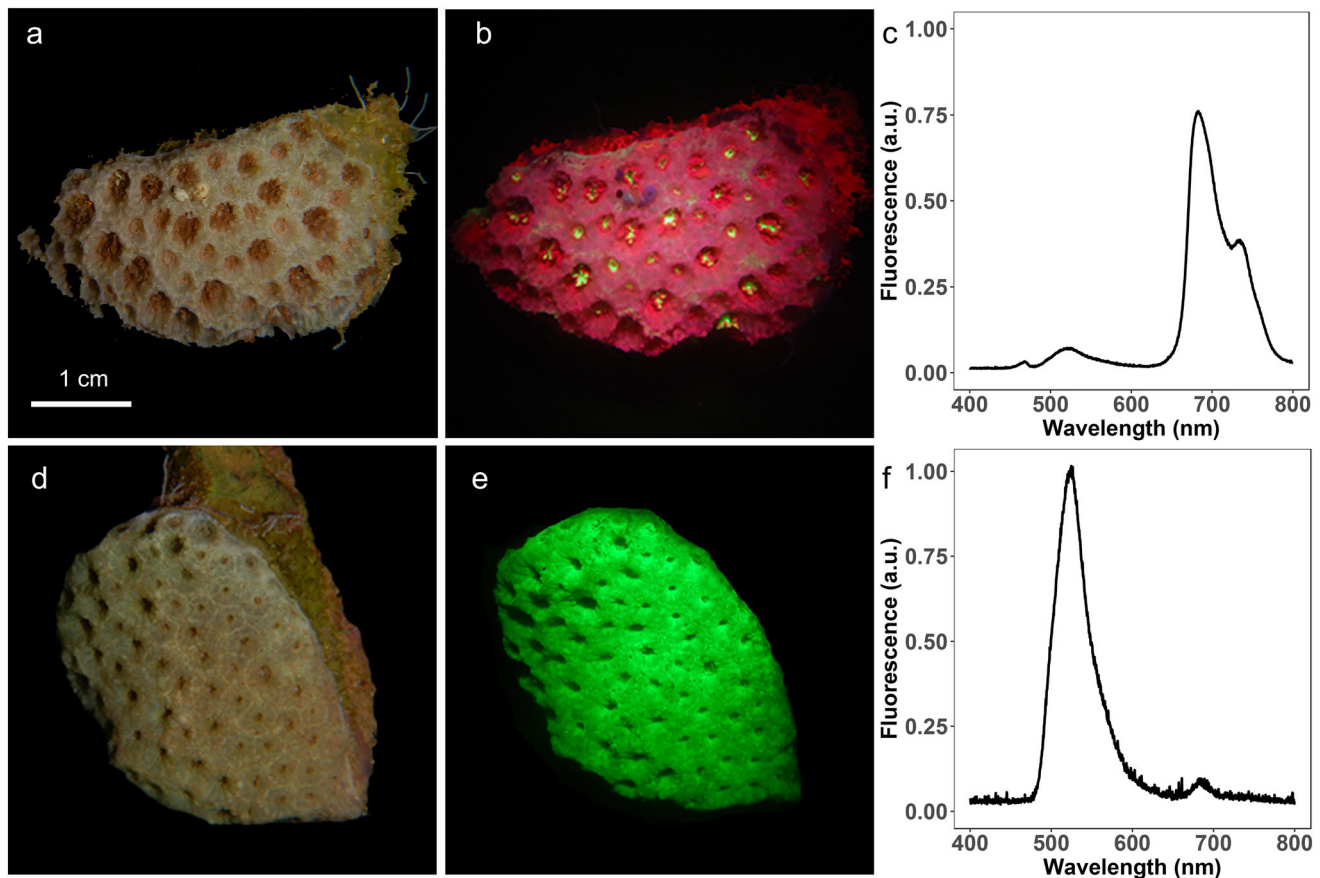
### Results

*A. ocellata* had been previously documented as demonstrating a green fluorescence peak at 516 nm (Eyal et al. 2015). Here, we report two morphs for this species (Fig. 1): a reddish looking, both under ambient and under fluorescence exciting illumination (Fig. 1a, b), low-fluorescence morph (LF) that presents a low, wide emission peak at 520 nm (Fig. 1c); and a highly fluorescent (HF) morph which appears green under ambient and blue light (Fig. 1d, e) and presents a high, narrow emission peak at 520 nm (Fig. 1f). Red fluorescence emission was only found at 680 nm and 720 nm (i.e., chlorophyll fluorescence). Consequently, any red appearance of the LF morph can be attributed to the symbiotic algae and photosynthetic pigments and not to red fluorescent proteins. While the two morphs can be found side-by-side at the same sites and depth, the red, low-fluorescence morph is more abundant as indicated by our field survey ( $84 \pm 17.4\%$  vs.  $15.15 \pm 17.43\%$ , mean  $\pm$  SD for the LF and HF morphs, respectively).

### Photobiology

The genetic analysis of Symbiodiniaceae revealed that all corals harbored symbionts from the genus *Cladocopium* (formerly known as clade C) regardless of their fluorescence morph. Based on the ITS2 sequences, we found that *A. ocellata* harbored *Cladocopium* types C3.10, C101, C3, or C66b. During the summer experiment (Fig. 2a), under the high-temperature (HT) treatment, corals showed a significant loss of their algal symbionts (Fig. 2a; PERMANOVA,  $F = 5.35$ ,  $p = 0.03$ ), while no significant decrease was observed under the ambient temperature (AT) treatment (Fig. 2a; PERMANOVA,  $F = 0.72$ ,  $p = 0.4$ ). Throughout the experiment, the high-fluorescence morph (HF) featured lower zooxanthellae density compared to the low-fluorescence (LF) morph (Fig. 2a; PERMANOVA,  $F = 18.21$ ,  $p < 0.001$ ). However, the LF corals appeared to





**Fig. 1** Fluorescence morphs of the mesophotic coral *Alveopora ocellata*. Representative images under ambient light (a, d), blue light (b, e), and fluorescence spectra (c, f) of low-fluorescence (LF; a–c) and high-fluorescence (HF; d–f) morphs of the mesophotic (45 m) coral *A. ocellata*

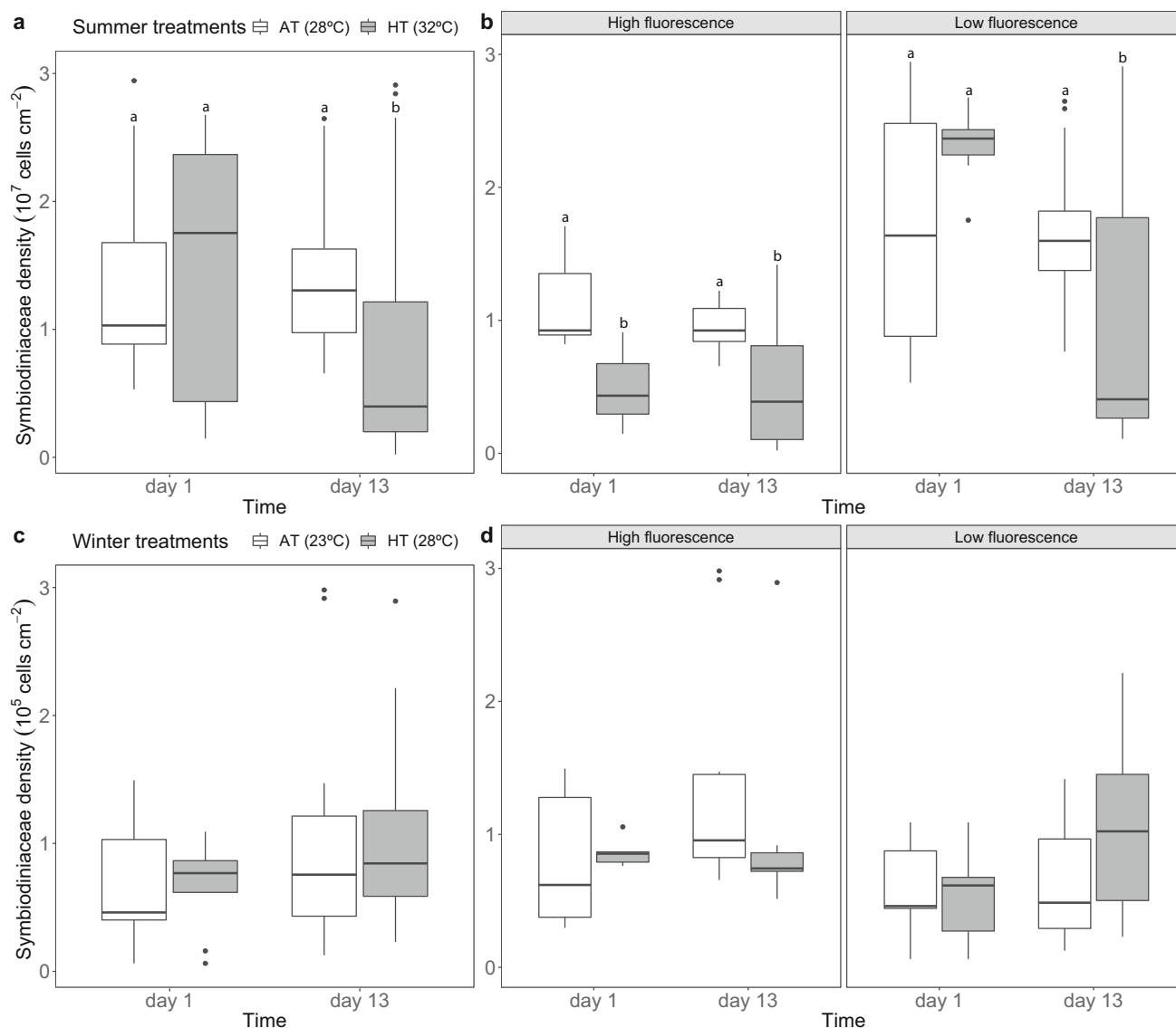
be more affected by high-temperature than the HF corals (−55% and −3% loss of algal cells for LF and HF, respectively; PERMANOVA,  $F = 5.57$ ,  $p = 0.02$ ). In the winter experiment (Fig. 2b), no significant difference was observed between treatments over time (PERMANOVA,  $F = 0.49$ ,  $p = 0.54$ ). A comparison of the average ( $\pm$  SD) number of zooxanthellae between summer and winter under ambient temperature revealed that corals in the summer harbored threefold more cells per  $\text{cm}^2$  than in the winter ( $1.4 \times 10^7 \pm 8.6 \times 10^5$  and  $6.9 \times 10^4 \pm 3.8 \times 10^4$ , for summer and winter, respectively).

In the summer experiment, while the HF corals showed a significant reduction in cellular chlorophyll a under the HT treatment, this remained unchanged under the AT treatment (Fig. 3a; PERMANOVA,  $F = 16.67$ ,  $p = 0.003$  and  $F = 0.22$ ,  $p = 0.66$ , respectively). We also found a significant difference between morphs under the HT treatment, with HF corals presenting a greater loss (−50% compared to −36% in the LF morph) of chlorophyll a (Fig. 3a; PERMANOVA,  $F = 18.44$ ,  $p = 0.002$ ). A similar, but not significant response was also observed in the winter experiment: i.e., a decrease in chlorophyll a under the HT

treatment (Fig. 3b; PERMANOVA,  $F = 3.76$ ,  $p = 0.08$ ), with the two morphs differing under the HT treatment (Fig. 3b; PERMANOVA,  $F = 5.14$ ,  $p = 0.04$ ), and no change under the AT treatment (PERMANOVA,  $F = 1.22$ ,  $p = 0.32$ ).

By the end of the summer experiment, both the high-fluorescence (Fig. 4a) and low-fluorescence (Fig. 4b) corals under high temperature appeared pale (i.e., bleached), while the corals under ambient sea temperatures had retained their original, natural coloration.

During the summer experiment, the maximal PSII quantum yield ( $F_v/F_m$ ) of corals, measured with a Diving-PAM, showed a significant decrease over time under the HT treatment (Fig. 5a; PERMANOVA,  $F = 4.66$ ,  $p = 0.03$ ), with the decrease starting with temperature ramping. Throughout the experiment (excluding the last sampling point on day 13), all LF corals presented higher values compared to the HF corals (PERMANOVA,  $F = 29.51$ ,  $p < 0.001$ ), indicating that they were utilizing light more efficiently for photochemistry. In the winter experiment, an opposite response to elevated temperature was observed (Fig. 5b). Both morphs were exhibiting higher  $F_v/F_m$  values by the end of the experiment under



**Fig. 2** *Alveopora ocellata*: Symbiodiniaceae density of two fluorescence morphs during thermal stress. Fragments of *A. ocellata* (left panels; i.e., the overall response) from a high-fluorescence morph (middle panels) and low-fluorescence morph (right panels) were subjected during both summer (**a**) and winter (**b**) to either ambient sea temperature (AT; white boxes) or + 4/ + 5 °C (summer and winter, respectively) above ambient sea temperature (HT; gray boxes). Symbiodiniaceae densities (cells cm<sup>-2</sup>) were determined at the

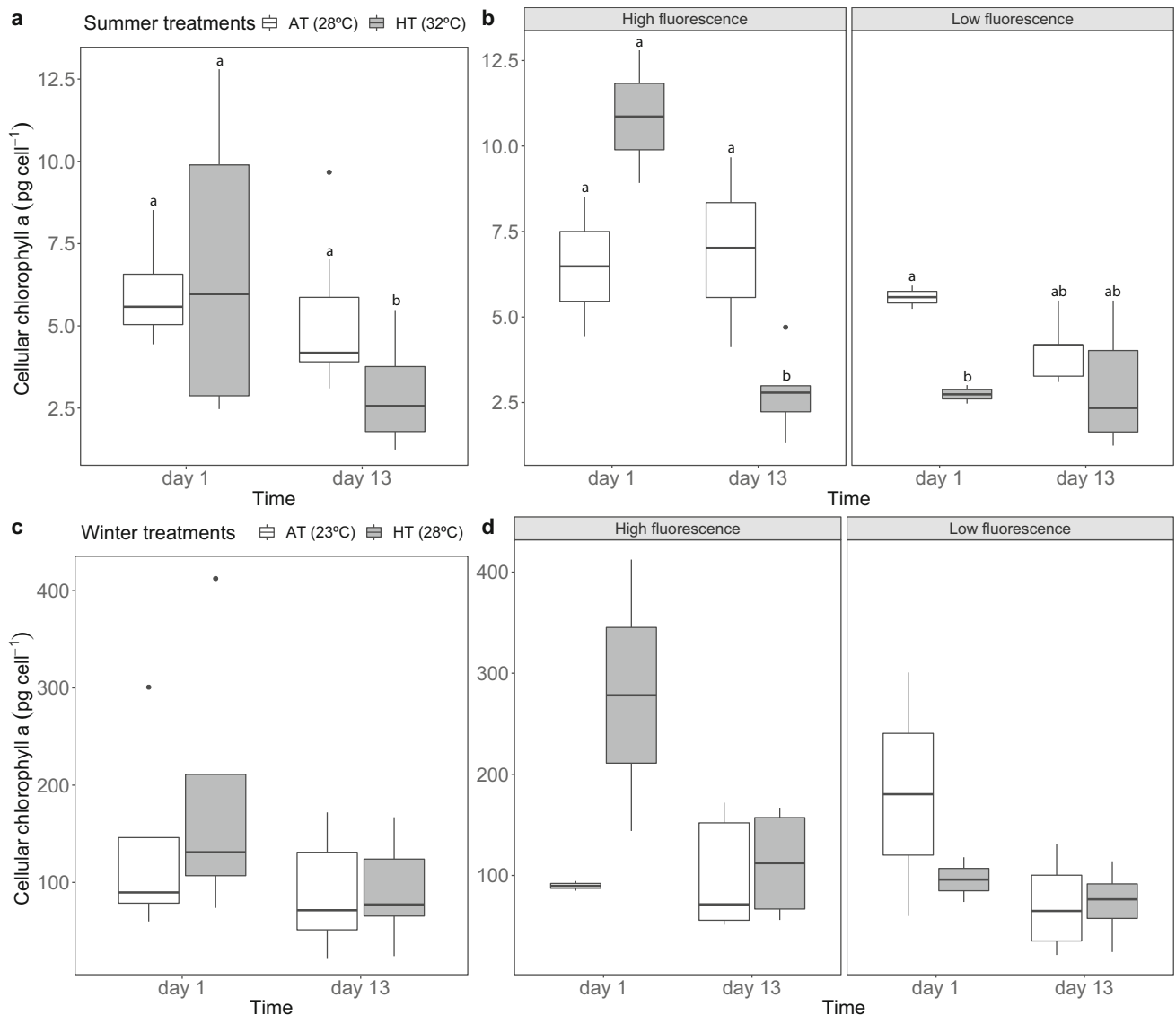
beginning (day 1) and end (day 13) of the experiment. Boxes represent the upper and lower quartile, center lines represent medians, and whiskers extend to data measurements that are less than 1.5\*IQR away from first/third quartiles. Outliers are represented by dots. Different letters above the boxes represent the statistical significance ( $p < 0.05$ ) between treatments and/or morphs. During the winter experiment, such significant differences were not found; therefore, there are no letters indicating significance

higher temperature (PERMANOVA,  $F = 8.12$ ,  $p = 0.008$ ), while no significant change could be observed under ambient sea temperature (PERMANOVA,  $F = 0.4$ ,  $p = 0.53$ ). Complementary to summer experiment measurements, during the winter experiment, the LF corals presented higher, but not significant,  $F_v/F_m$  values. Additionally, at the end of the winter experiment, corals under the HT treatment (i.e., 28 °C) presented a mean ( $\pm$  SD)  $F_v/F_m$  of  $0.637 \pm 0.0291$ , which was the mean value of the corals at the beginning of the summer experiment (i.e.,

ambient sea temperature of 28 °C;  $0.638 \pm 0.08$ ), thus presenting similar values under the same temperature.

### Host fluorescence

Fluorescence intensity changed significantly during the summer experiment under the HT treatment (Fig. 6a; PERMANOVA,  $F = 32.48$ ,  $p < 0.001$ ), while remaining constant under the ambient temperature (PERMANOVA,

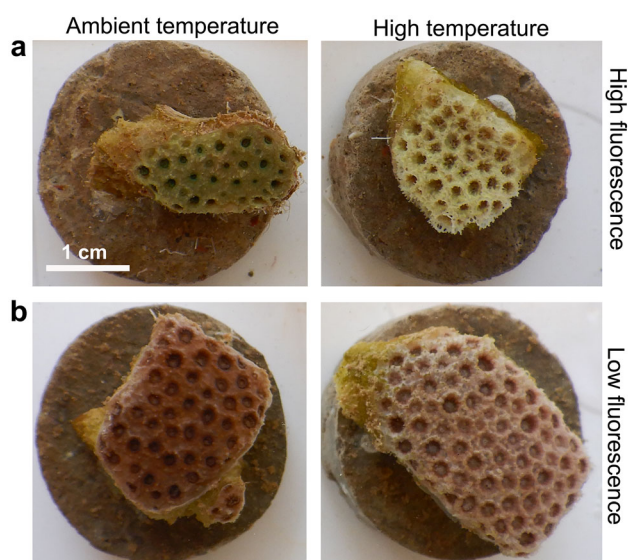


**Fig. 3** *Alveopora ocellata*: Chlorophyll a concentration of two fluorescence during thermal stress. Fragments of *A. ocellata* (left panels; i.e., the overall response) from a high-fluorescence morph (middle panels) and low-fluorescence morph (right panels) were subjected during both summer (**a**) and winter (**b**) to either ambient sea temperature (AT; white boxes) or + 4/ + 5 °C (summer and winter, respectively) above ambient sea temperature (HT; gray boxes). Chlorophyll a concentration (pg chlorophyll cell<sup>-1</sup>) was determined

$F = 0.71$ ,  $p = 0.41$ ). There was also a significant difference between the HF and LF morphs under both treatments (PERMANOVA,  $F = 5.98$ ,  $p = 0.01$ ); whereas the HF morph showed a decrease in fluorescence intensity between day 1 and day 13, the LF morph hardly changed. Following day 9 of the experiment, the HF corals started to recover their fluorescence but did not reach the initial intensity recorded at the beginning of the experiment. In the winter experiment, while fluorescence increased with elevated temperature (Fig. 6b; PERMANOVA,  $F = 20.06$ ,

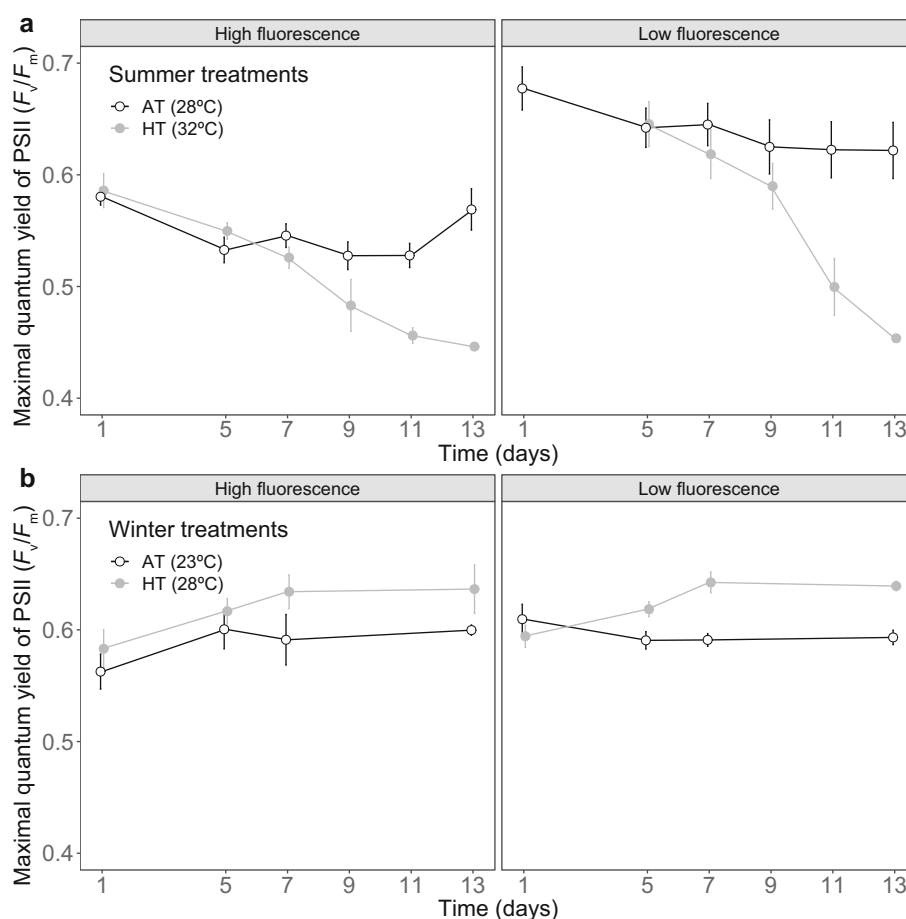
at the beginning (day 1) and end (day 13) of the experiment. Boxes represent the upper and lower quartile, center lines represent medians, and whiskers extend to data measurements that are less than 1.5\*IQR away from first/third quartiles. Outliers are represented by dots. Different letters above the boxes represent the statistical significance ( $p < 0.05$ ) between treatments and/or morphs. During the winter experiment, such significant differences were not found; therefore, there are no letters indicating significance

$p < 0.001$ ), it did not change under ambient sea temperatures (PERMANOVA,  $F = 0.006$ ,  $p = 0.94$ ). As in the summer experiment, HF corals demonstrated greater fluorescence during the experiment compared to the LF corals (PERMANOVA;  $F = 7.55$ ,  $p < 0.001$ ).



**Fig. 4** *Alveopora ocellata*: representative images of the appearance of two fluorescence morphs after 13 days in the summer experiment. High-fluorescence (a) and low-fluorescence (b) fragments after 13 days under ambient sea temperature (images on the left) and high temperature (+ 4 °C; images on the right)

**Fig. 5** *Alveopora ocellata*: Photosystem II maximal quantum yield ( $F_v/F_m$ ) of two fluorescence morphs during thermal stress. Fragments of high-fluorescence morph and low-fluorescence morph were subjected during both summer (a) and winter (b) to either ambient sea temperature (AT; white lines) or + 4/ + 5 °C (summer and winter, respectively) above ambient sea temperature (HT; gray lines). Temperature was ramped on days 1–4 and kept at peak temperature during days 5–13.  $F_v/F_m$  values are represented as means (circles) and SE (bars) at each sampling point

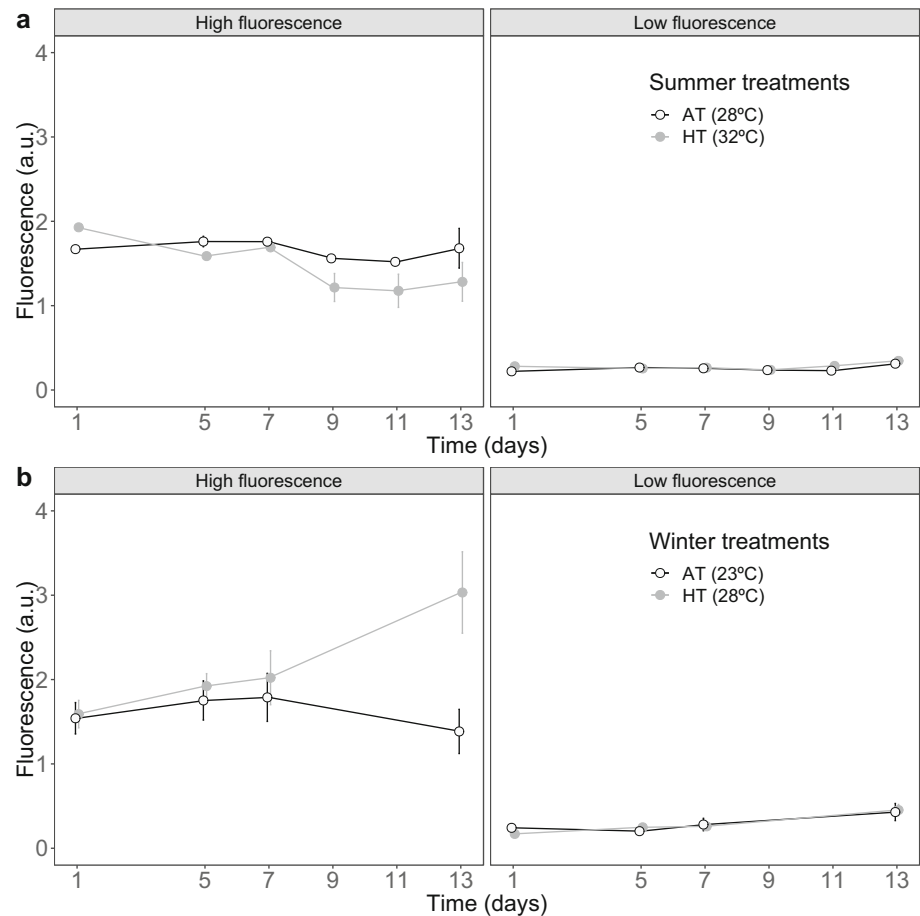


## Discussion

This study provides a novel example of controlled thermal-induced bleaching of a mesophotic coral. Corals from the northern tip of the Red Sea (i.e., GoE/A) are considered to be extremely thermal tolerant due to the selection of resistant genotypes around the warm straits of Bab-el-Mandeb resulting in corals that live below their thermal threshold at the GoE/A (Fine et al. 2013). Here, we have demonstrated that mesophotic corals from this area may be more susceptible to an increase in temperature than previously considered. In the GoE/A, seasonal bleaching and partial recovery of *S. pistillata* in mesophotic depth has been recorded (Nir et al. 2014), and Eyal et al. (2019) reported on bleaching in a few other mesophotic species that led to mortality. MCEs have been suggested to serve as a temporary refuge for corals during short-term disturbances or as refugia in the case of long-term disturbances, due to their relative remoteness and more stable conditions (reviewed by Lesser et al. 2009). In the GoE/A, MCEs are in very close proximity to the shallow reef (within 150 m)



**Fig. 6** *Alveopora ocellata*: Fluorescence intensity of two fluorescence morphs during thermal stress. Fragments of high-fluorescence and low-fluorescence morphs were subjected during both summer (a) and winter (b) to either ambient sea temperature (AT; white lines) or + 4/ + 5 °C (summer and winter, respectively) above ambient sea temperature (HT; gray lines). Temperature was ramped on days 1–4 and kept at peak temperature during days 5–13. Fluorescence intensity (a.u.) mean values (circles) and SE (bars) were calculated from analyzing pixel intensity of fluorescent images



and while they experience almost the same water temperatures as their shallow counterparts (Eyal et al. 2019) they, by definition, experience reduced light and a blueshifted, narrow spectrum compared to the shallow light environment (Eyal et al. 2016; Tamir et al. 2019). As bleaching is suggested to be caused, among other stressors (i.e., sedimentation, diseases, and reduced salinity), by high temperature, high light, or the synergistic effect of both (reviewed by Brown 1997; Courtial et al. 2017), in a reduced light environment (i.e., MCEs) corals may be less affected in the case of elevated temperature (Glynn 1996).

The present experiment was performed twice: first during the summer when ambient sea temperature averaged around 28 °C; and again during the winter with an average sea temperature of 23 °C. In the summer experiment, temperature was artificially elevated by 4 °C and in the winter experiment by 5 °C, thereby covering almost the entire range of temperatures to which corals in the GoE/A might be exposed. We recorded dramatic differences between the response of the corals between the experiments. While all corals under the HT treatment during the summer presented with a classic bleaching response, manifested in reduced zooxanthellae density, loss of

chlorophyll a, and reduced maximal quantum yield of PSII ( $F_v/F_m$ ; Figs. 2a, 3a, 5a), during the winter, the corals under the HT treatment displayed the opposite response, an increase in most photobiological parameters (i.e., higher zooxanthellae density and  $F_v/F_m$  values; Figs. 2b, 5b). Combining the findings from the two experiments results in an optimal temperature at which corals will perform best photosynthetically, as photosynthesis is an enzyme-based process, and it is temperature-dependent (Falkowski and Raven 2007). The comparison between summer and winter experiments revealed bleaching to be highly dependent on the peak temperatures reached during the experiment and the accumulated heat experienced by coral prior to the experiment. This coincides with the degree heating weeks (DHW) index (Liu et al. 2003) used by Coral Reef Watch of the United States National Oceanic and Atmospheric Administration (NOAA) to alert on potential bleaching hot spots. This index projects the cumulative thermal stress on the corals. Additionally, in agreement with the notion that bleaching is a potential risk when sea temperatures exceed the annual maximum temperature by 1–2 °C (Coles et al. 1976), corals in our experiments only bleached after reaching this threshold, indicating a potential value for the

thermal threshold of mesophotic corals at the GoE/A. The timing of bleaching experiments is critical, since zooxanthellae densities are known to fluctuate between seasons, are dynamic, and can vary due to changes in temperature, solar irradiance, or in correlation with nutrient supply (Marubini and Davies 1996; Fagoonee et al. 1999). In most of the studies investigating the annual cycle of zooxanthellae densities, lower densities were recorded during the warmer months (Stimson 1997; Fagoonee et al. 1999; Winters et al. 2009; Cohen and Dubinsky 2015). Winters et al. (2009) examined the annual fluctuations in zooxanthellae densities in *Stylophora pistillata* from the GoE/A and found that following the minimum sea temperature there was a gradual increase in the number of zooxanthellae, peaking a month later, with the peak in nitrite and nitrate concentration. However, in our winter experiment, while zooxanthellae density rose with the increase in temperature, no additional nutrients had been supplied, and therefore, elevated nutrients concentrations cannot explain our results. The higher chlorophyll a concentration found in our winter experiment (Fig. 3b), when zooxanthellae density was lower than during the summer (Fig. 2b), coincides with the trend found in several studies that observed a correlation between lower zooxanthellae densities and higher cellular chlorophyll a concentration (Costa et al. 2005; Winters et al. 2009). Taking into account that, in our experiments, only temperature was manipulated while light intensity and quality, as well as nutrient supply, were not, we cautiously conclude that mesophotic corals may be sensitive to temperature alone which may cause bleaching even without any synergistic or combined effect involving other stressors. Consequently, this would make them even more susceptible to bleaching compared to shallow corals.

In both experiments (i.e., summer and winter), we found significant differences between the two fluorescence morphs in their initial  $F_v/F_m$  values (Fig. 5) and host fluorescence intensity (Fig. 6). The HF corals were less efficient in utilizing light for photosynthesis prior to initiation of the temperature ramping (Fig. 5) and also presented up to sixfold higher host fluorescence (Fig. 6). In regard to the bleaching response (i.e., the summer experiment results), although the HF corals did not experience a significant decrease in their zooxanthellae (Fig. 2a) they did lose a significant amount of chlorophyll (Fig. 3a). In contrast to the HF morph, the LF morph presented a significant decrease in zooxanthellae density (Fig. 2a), but without a decrease in cellular chlorophyll (Fig. 3a). These two different pathogenies may imply that they are caused by different stressors. Bleaching by means of pigment loss rather than cell loss, as in the case of the HF corals, is associated with high radiation (Hoegh-Guldberg and Smith 1989; Gleason and Wellington 1995). Even if we take into

account that MCEs experience minimal ultraviolet radiation, if any at all (Tamir et al. 2019), it is known that photosynthetically active radiation can itself facilitate bleaching, even in the absence of the ultraviolet radiation that is considered to be the more harmful region of the light spectrum (Lesser and Shick 1989; Lesser et al. 1990). Temperature-induced bleaching is associated with a reduction in zooxanthellae cells (Hoegh-Guldberg and Smith 1989; Glynn and D'Croz 1990), reflecting the pathogeny presented by the LF corals in our experiment. Hence, the HF corals displayed light-induced bleaching symptoms, while the LF corals presented with a response that is more associated with temperature-induced bleaching. We suggest that the high levels of fluorescence in the HF morph created an enhanced light environment that imposed light stress on the top of the temperature stress. This is supported by studies showing that FPs enhance the scalar irradiance by scattering, peaking under blue light illumination (Lyndby et al. 2016). This enhancement of scalar irradiance will be even more pronounced during bleaching due to the optical feedback loop created by the reduced shading of symbiont cells and photosynthetic pigments (Enríquez et al. 2005; Wangpraseurt et al. 2017). Consequently, we would expect HF corals to be more light-damaged than LF ones. However, it seems that the latter were more damaged as they presented a 35% reduction in  $F_v/F_m$ , 54% in zooxanthellae density, and a 36% reduction in cellular chlorophyll a concentration (compared to 23%, 3%, and 50% decrease in the HF corals, respectively). This result may be explained by the fact that, according to the zooxanthellae counts (Fig. 2) and Diving-PAM measurement (Fig. 5), LF corals were more photosynthetically efficient and harbored more zooxanthellae and therefore may have produced more oxygen, that under temperature stress will transform to reactive oxygen species due to damage to the carboxylation site in the Calvin cycle (Jones et al. 1998) or an impaired PSII (Lesser et al. 1990). This was also supported by Cuning and Baker (2013), who showed that a higher algal ratio within the same coral species will increase the sensitivity of an individual to thermal stress and bleaching. Additionally, FPs have been suggested to be beneficial to corals, specifically under thermal stress, by means of their antioxidant activity (Bou-Abdallah et al. 2006; Palmer et al. 2009). Despite their considerable low turnover rate (Leutenegger et al. 2007), a rapid decrease in fluorescence intensity was recorded in our summer experiment, suggesting that the GFP was perhaps used, as a means of defense against the free radicals produced following the thermal stress. This was suggested previously by Roth and Deheyn (2013) and is further supported by the results of the present study. Another suggested mechanism that involves FPs during bleaching was proposed by Bollati et al. (2020) in which, following

the loss of zooxanthellae, FPs are upregulated by the enhanced light within the coral tissue, and this accumulation of FPs helps in the mediation of access light, assisting the recolonization of zooxanthellae after the relief of the thermal stress.

The current findings reinforce the results of previous studies on the regulation and role of FPs under thermal stress (DeSalvo et al. 2008; Smith-Keune and Dove 2008; Roth and Deheyn 2013). Since fluorescence is a visual phenomenon, it has been proposed as a useful tool for detecting very early stages of coral settlement (Zweifler et al. 2017), onset of diseases (Ramesh et al. 2019), and as a general tool for assessing coral health (Treibitz et al. 2015). Together with the significant decrease in fluorescence during the onset of bleaching in corals under the HT treatment, we further support the suggestion that fluorescence monitoring could be used as a noninvasive and inexpensive technique for the detection of bleaching, although this technique might be relevant only for fluorescent species or morphs.

In this study, we sought to explore the thermal-induced bleaching process in a mesophotic coral, as well as to examine the relationship between fluorescence and the bleaching at a high sampling resolution. Although many questions regarding the mechanisms involved in the relationship between fluorescence and bleaching remain unclear, we present further support for the use of fluorescence as a monitoring tool and emphasize the huge effect of different fluorescence morphs on the physiology of corals. With the exception of studies focusing on color or fluorescence morphs (Gleason 1993; Klueter et al. 2006; Gittins et al. 2015; Nakaema and Hidaka 2015; Quick et al. 2018), this issue of fluorescence morphs or other host pigmentation differences has been mostly ignored in the published literature involving coral physiology, though it may have profound impact on the results. Since we did not investigate the possible recovery of mesophotic corals from bleaching, we cannot conclude that this is a fatal condition. While the thermal threshold of mesophotic corals is yet to be determined together with the risk assessment of reaching that threshold, we wish to emphasize the vulnerability of MCEs, despite the fact that they are considered more stable and protected than the shallow reefs. We call for further studies in the field of mesophotic coral physiology and on the impact of global climate changes on deeper reefs, in order to improve environmental management in the future.

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