Photosynthetic responses of corals *Mussismilia harttii* (Verrill, 1867) from turbid waters to changes in temperature and presence/absence of light

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**Abstract**

Responses of corals to climate change stresses are species and locality specific. As light is an important component of temperature-induced stresses, we experimentally tested the responses of a turbid water coral, *Mussismilia harttii*, to changes in temperature in the presence and absence of light. Chlorophyll fluorescence parameters were measured using a diving-PAM. Experiments were carried out at distinct temperatures. Polyps were kept in the dark or were continuously exposed to 300 µmol photons m⁻².s⁻¹ irradiance. No visible bleaching of coral samples was seen in temperatures between 26.5 and 35.0 °C, but most polyps at higher temperatures showed signs of tissue necrosis. There was a reduction of $F_v/F_m$ as temperature increased, with a further drop in the presence of light, indicating a synergistic effect of these factors. We suggest that the photodamage to *M. harttii* endosymbionts triggered by temperatures of 33.0 °C and 35.0 °C results from a decline of the repair process, as well as the effect of light on the PSII. Recovery data for polyps kept in 31.0 °C showed that this temperature (depending on time of exposure) seems to be borderline; temperatures higher than 31.0 °C lead to long-term damage or death of *M. harttii*.

**Descriptors:** Climate changes, Coral, Photosynthesis, Temperature, Chlorophyll fluorescence, Diving-PAM.

**Resumo**

Respostas de corais a estresses de mudanças de temperatura são específicas e dependem também da localidade. Como a luz é um componente importante dos estresses induzidos pela temperatura, testamos experimentalmente as respostas de *Mussismilia harttii*, coral originário de águas turvas, a mudanças de temperatura, na presença e ausência de luz. Parâmetros de fluorescência da clorofila foram medidos com Diving-PAM. Os experimentos foram mantidos a temperaturas distintas. Os pólipos permaneceram no escuro ou foram continuamente expostos a 300 µmol photons m⁻².s⁻¹ de irradiância. Nenhum branqueamento visível foi observado entre as temperaturas de 26,5 a 35,0 °C, mas a maioria dos pólipos expostos a temperaturas elevadas mostraram sinais de necrose do tecido. Houve uma redução de $F_v/F_m$ em resposta ao aumento da temperatura, que foi exacerbada na presença de luz, indicando um efeito sinergético desses fatores. Sugerimos que o fotodano sofrido pelos endossimbiontes de *M. harttii*, desencadeados nas temperaturas de 33,0 e 35,0 °C, resultou de uma diminuição do processo de reparação, assim como o efeito da luz sobre o PSII. A recuperação de pólipos mantidos a 31,0 °C indicou que essa temperatura parece ser limítrofe (dependendo do tempo de exposição); temperaturas acima de 31,0 °C levaram a danos irreversíveis ou morte de *M. harttii*.

**Descritores:** Mudanças climáticas, Coral, FOTOSÍNTETE, Temperatura, Fluorescência da clorofila, Diving-PAM.
INTRODUCTION

Climate change is already affecting coral reefs around the globe (BUDDEMEIER et al., 2004). Shallow-water reef-building corals host single-celled microalgae (zooxanthellae) that live in their body tissues. Stresses can interfere in this symbiotic relationship. “Bleaching” describes the loss of symbiotic algae or their pigments by the coral. Bleaching is a stress response to several oceanographic parameters, such as high or low temperatures, intense light, changes in salinity, or other physical or chemical stresses like cyanide and herbicides. High seawater temperature is one of the main concerns associated with climate change and reef corals (HOEGH-GULDBERG, 1999; SAXBY et al., 2003). However, the temperature leading to bleaching is not an absolute value; it is associated with other variables, especially light (MUMBY et al., 2001). The difference between “normal” local temperatures and stress temperatures seems to be more important than the latter itself. However, localities where temperatures are usually higher seem to be more vulnerable to bleaching (FITT et al., 2001). According to SAXBY et al. (2003), apparently, coral reefs exposed to low temperatures respond in the same way as corals exposed to high temperatures, including coral reefs exposed to low temperatures. However, localities where temperatures are usually higher seem to be more important than the latter itself. Therefore, responses of corals to temperature change are species and locality specific.

Southwestern Atlantic reefs are unique, with a relatively low coral diversity (only 18 Scleractinia species), but with high endemism (CASTRO; PIRES, 2001; NUNES et al., 2008). Among the six endemic species in Brazil is Mussismilia harttii (VERRILL, 1868), distributed from the State of Rio Grande do Norte to the state of Espirito Santo, in addition to the islands of Fernando de Noronha and Atol das Rocs (LEÃO et al., 2008). The reef-builder corals of Brazil coexist with a highly terrigenous environment. Excessive sedimentation is one of the main threats to coral reefs. There is a variation in tolerance to sedimentation among corals species (PHILIPP; FABRICIUS, 2003; ROGERS, 1990). M. harttii presents large polyps suggesting greater resistance to environmental stresses, having developed a more efficient mechanism for cleaning themselves of sediment (ROGERS, 1990; LEÃO et al., 2003). Almost all reefs located on the continental shelf are immersed in turbid waters (LEÃO; DOMINGUEZ, 2000). Therefore, studies from other oceans do not provide adequate support to understanding ecological processes and responses to climate change in this remarkable environment.

Large-scale bleaching has been reported in turbid reefs of the Southwestern Atlantic since the 1990s. Here, we use the categories of bleaching indicated in OLIVER et al., 2009. In the summer of 1994, severe bleaching was reported in reef (Abrolhos, 18° S – CASTRO; PIRES, 1999) and non-reef areas (Canal de São Sebastião, 24° S - MIGOTTO, 1997). Both reports associated the events with temperature anomalies and, in the reef area, to higher temperatures occurring in a longer daylight period (summer solstice). In the 1998 summer-autumn season, temperature-related severe bleaching events were reported in eastern Brazil (12-13° S – LEÃO et al., 2003; 2008). Mild to moderate bleaching was reported in 2002, 2003, and 2005 in eastern (12-18° S - LEÃO et al., 2008) and northeastern Brazil (9° S – MINISTÉRIO DO MEIO AMBIENTE, 2006). Although there are discrepancies in data acquisition (for instance, see types of registered bleaching [weak and strong] grouped together by LEÃO et al. (2008): Figure 6, and for assessment of the extent of coral bleaching, see SEIBECK et al. (2006), it is evident that bleaching phenomena are frequent in these reefs. However, unlike reefs in most other oceans, no mass mortality of scleractinian corals has been associated so far with bleaching events in the Southwestern Atlantic.

Turbidity in Southwestern coral reefs may be affecting responses of their corals to temperature changes. Corals in these environments are adapted to low-light conditions. As light is an important component of temperature-induced stresses, we experimentally tested the responses of an endemic coral from a turbid water area, M. harttii through measurements of variation in photochemical efficiency (Fv/Fm), to changes in temperature in the presence and absence of light, as well as the synergistic effects between these parameters.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

About 120 coral polyps of the scleractinian coral Mussismilia harttii were collected from 2.5-4.0 m depth, with a branch collected from each colony, near “Recife de Fora” (16°25’06.67” S, 039°00’05.58” W), State of Bahia,
Brazil, in November 2008. Corals were transported in seawater at circa 26.5 °C (ambient seawater temperature). Subsequently, they were transferred to a semi-open seawater system consisting of a 1,000 L tank under natural daylight, which varied between 50 and 180 µmol.m⁻².s⁻¹. Due to logistic constraints, all polyps were collected in a single day and kept under these conditions for 5-12 days for acclimation (PHILIPP; FABRICIUS, 2003; SAXBAY et al., 2003; WINTERS et al., 2009; KUGURO et al., 2010; PUTNAM; EDMUNDS, 2011) prior to the experiments. They were monitored visually once a day and the $F_v/F_m$ verified prior to the start of the experiment in order to demonstrate the health of the samples.

**Chlorophyll-a Fluorescence Measurements**

Chlorophyll fluorescence parameters were measured using a pulse amplitude modulated fluorometer (diving-PAM, Walz, Effeldricht, Germany) and an 8mm standard glass-fiber optic probe (Walz, Effeldricht, Germany), which was positioned above the oral disk of polyps. After dark adaptation for 20 min, initial fluorescence ($F'_0$) was measured using a weak probe of modulated light (<1 µmol photons.m⁻².s⁻¹) and maximum fluorescence ($F'_m$) was estimated with a saturating pulse (800 ms pulse of 6,000 µmol photons.m⁻².s⁻¹). Variable fluorescence ($F_v$) was calculated from $F'_m - F'_0$, and the maximum quantum efficiency of Photosystem II (PSII) photochemistry was obtained from the ratio $F'_m/F'_n$. Samples were acclimated to an irradiance of 187 µmol.m⁻².s⁻¹ for 5 min during measurements of a light induction curve. At the end of this 5-min curve, rETR (relative electron transport rate) measurements were obtained through the application of a series of saturation pulses under increasing irradiance (121, 187, 262, 393, 535, 817, 1205, and 1820 µmol photons.m⁻².s⁻¹). Variable fluorescence ($F_v$) was calculated from $F'_m - F'_0$, and the maximum quantum efficiency of Photosystem II (PSII) photochemistry was obtained from the ratio $F'_m/F'_n$. Samples were acclimated to an irradiance of 187 µmol.m⁻².s⁻¹ for 5 min during measurements of a light induction curve. At the end of this 5-min curve, rETR (relative electron transport rate) measurements were obtained through the application of a series of saturation pulses under increasing irradiance (121, 187, 262, 393, 535, 817, 1205, and 1820 µmol photons.m⁻².s⁻¹) in the form of a rapid light curve (RALPH et al., 1999; RALPH; GADEMAN, 2005). Light-adapted fluorescence yield ($F$) and maximum light-adapted fluorescence yield ($F'_m$) were determined at the end of a 10 sec period at each irradiance level, and the effective quantum yield of PSII as a function of irradiance was calculated from $F'_m/F'_n = (F'_m - F'_0)/F'_n$. The proportion of open PSII reaction centers under each actinic light was determined by the photochemical quenching coefficient, $qP = (F'_m - F'_0)/(F'_m - F'_n)$, and the non-photochemical Stern-Volmer coefficient (NPQ) calculated from $(F'_m - F'_n)/F'_n$. rETR was calculated as $F'_n$ PAR 0.5 Ar (GENTY et al., 1989), where the fraction of absorbed light (Ar) was presumed to be 1, due to the technical difficulty of measuring this parameter, and the figure 0.5 is used as an invariable number (assuming all light energy is absorbed by chlorophyll and that the incident light quantum of PSI and PSII is equal). The Diving-PAM settings were: measuring light 2, saturating intensity 8, saturating width 0.8s, gain 2 and damping 2.

**Experimental Protocol**

Given the elapsed time for fluorescence measurements at each temperature, the polyps remained in the dark at 26.5 °C from 20 to 60 min prior to the beginning of the experiments. This incubation time was sufficient to promote a dark adaptation of the samples and did not influence the physiological response, since distinct times used for dark adaptation are found in the literature. For instance, 20 and 120 min were used for Pocillopora damicornis (JONES et al., 1998; JONES et al., 2000; FRISCH et al., 2007), and a dark adaptation of from 20 to 60 min was used for calculating $F_v/F'_n$ in other coral species (BROWN et al., 1999; WARNER et al., 1999; JONES et al., 2000; JONES; HOEGH-GULDBERG, 2001). Experiments were carried out in open-topped glass aquaria kept at distinct temperatures (26.5 [control], 29.0, 31.0, 33.0, and 35.0 °C). Temperatures were stabilized by immersion heaters (Sarlo Pet 200-300 W) monitored and adjusted using automatic controllers (Full Gauge MT543Ri Plus). Additionally, each aquarium was immersed in a water bath, which received cold water whenever necessary for cooling the system. Temperatures were recorded in a laptop through an USB interface (Sitrad Conv 32), and varied, at most, 0.1 °C throughout the entire experiment. Due to logistic constraints, only two polyps from different colonies were placed in each aquarium, fixed in sand: one remained in the dark (covered by an aluminum cup) and the other was continuously exposed to 300 µmol photons.m⁻².s⁻¹ irradiance, provided by four fluorescent bulbs (Philips T5 HO Activiva Active 54 W) suspended above the tanks. Photosynthetically active radiation (PAR) was measured with a Fiber Quantum Sensor connected to the Diving-PAM. Chlorophyll-a fluorescence measurements were taken every 95 min for 285 min. After measuring $F'_0$ and $F'_m$, samples were light activated (187 µmol.m⁻².s⁻¹) for 3-5 min and rETR measurements were obtained from rapid light curves, as described above. Six independent runs (with new polyps and new seawater) of the experiment were carried out, each being considered a replicate. At the end of each run, polyps were moved to larger aquaria (circa 26.5 °C and circa 50 µmol.m⁻³.s⁻¹). Twenty-four hours after the experiment, the maximum quantum efficiency of PSII ($F_v/F'_n$) was estimated to evaluate recovery.
STATISTICAL ANALYSIS

Data were tested for normality using the Kolmogorov-Smirnov’s test (K–S) and for homoscedasticity of variances using Levene’s test. Repeated measures analyses of variance (ANOVA) were used to test if the maximum quantum yield of PSII ($F_v/F_m$), the photosynthetic efficiencies ($\alpha$), the maximum relative electron transport rate ($rETR_{max}$), and the minimum saturating irradiance ($E_k$) were affected by exposure to different temperatures, light conditions (dark/light), and time span of exposure to these different conditions. ANOVA was used even when samples were heteroscedastic because, if the size of the sample is equal for all treatments, the analysis is robust even with considerable heterogeneity (GLASS et al., 1972 apud ZAR, 1999). Tukey’s (HSD) post hoc test was applied for ANOVAs that indicated a significant probability of rejection of the null hypothesis ($p<0.05$). Bifactorial ANOVA was used to test recovery 24 h after the experiment. Repeated measures ANOVAs were carried out in R, version 2.10 (http://www.r-project.org/); all other tests were carried out with Statistica 7.0 (StatSoft). Graphs were prepared with SigmaPlot, version 10.0 or Microsoft Excel 2007.

All data for $F_m$, $F/F_m$, $rETR_{max}$, $\alpha$ and $E_k$ were normal (Kolmogorov-Smirnov, $p>0.20$). Levene’s test showed heteroscedastic variances for $F_m$ ($F_{3,30}=2.423$), $F/F_m$ ($F_{3,30}=2.235$), $rETR_{max}$ ($F_{3,30}=1.551$) and $E_k$ ($F_{3,30}=1.314$; $p=0.142$) except for $\alpha$ that showed homoscedastic variances ($F_{3,30}=1.289$; $p=0.134$). Sample size was equal.

RESULTS

Repeated measures ANOVA with $F_m$ showed that most variables tested (time, temperature, polyps, irradiance (dark/light), time+temperature and time+irradiance) presented highly significant differences ($p<0.001$; Table 1). The results for $F_o$ were more stable in all treatments, so the $F_o$ variation is related to changes in $F_m$ (Figure 1).

MAXIMUM QUANTUM YIELD OF PHOTOSYSTEM II ($F_v/F_m$) FOR DARK-ADAPTED SAMPLES

In polyps kept in the dark, there was a decrease in $F_v/F_m$ of approximately 10-21% in the initial 95 min of the experiment at temperatures between 26.5 and 31.0 °C, with a subsequent stabilization (Figure 2A). At 33.0 °C, there was a continuous drop in $F_v/F_m$ values. Polyps maintained at 35.0 °C showed a strong initial decrease (circa 57% after 95 min), which continued throughout the experiment and reached a 67% decrease at the end of the experiment (285 min). Comparatively, polyps exposed to light showed an even stronger effect of increased temperatures on the maximum quantum yield (Figure 2B), suggesting again a synergy of temperature and light. Furthermore, polyps maintained at 33.0 °C differed from those kept at 31.0 °C only at the end of the experiment (with light), suggesting an added effect due to time of exposure.

In spite of a gradational decrease in photochemical efficiency, no bleaching was observed in M. harttii kept at temperatures between 26.5 and 35.0 °C throughout the experiment (circa 5 h), either in the dark or the light. However, polyps seemed to present an initial tissue necrosis as soon as 95 min after exposure to light and temperatures of 35.0 °C (Figure 3), a condition also observed in the dark treatment at this temperature (Figure 4).

Repeated measures ANOVA with $F_v/F_m$ showed again that most variables tested (time, temperature, polyps, irradiance (dark/light) and time+irradiance showed highly significant differences ($p=0.001$; Table 1).

FLUORESCENCE YIELD ($F$) AND MAXIMUM FLUORESCENCE YIELD OF LIGHT-ADAPTED SAMPLES ($F_{m}^{*}$)

Rapid light curves (RLC) at the beginning of the experiments (polyps of all treatments kept in the dark at 26.5 °C) showed that $F$ increased with irradiance intensity until the sixth step of the RLC (seventh light pulse), nearly stabilizing afterwards (Figure 5A). A similar, but opposite, behavior was seen with $F_{m}^{*}$, with its values decreasing and coming closer those of $F$ (Figure 5A).

Polyps incubated in the dark at different temperatures showed a decrease in the difference between $F$ and $F_{m}^{*}$ especially with increasing temperatures (rows in Figure 5). Subsequent (time) measurements showed little variation (columns in Figure 5). Polyps incubated in light behaved similarly, but $F$ values at different temperatures were essentially the same, while $F_{m}^{*}$ values presented an increasing drop (except at 35.0 °C, when they became equal to $F$) (Figure 6).

RELATIVE ELECTRON TRANSPORT RATES ($rETR$), MAXIMUM RELATIVE ELECTRON TRANSPORT RATES ($rETR_{max}$), PHOTOSYNTHETIC EFFICIENCIES ($\alpha$ - PHOTOSYNTHETIC RATE IN LIGHT-LIMITED REGION OF RAPID LIGHT CURVES), AND MINIMUM SATURATING IRRADIANCES ($E_k$)

Rapid Light Curves (RLCs) at the beginning of the experiments (t0; all treatments in the dark and at 26.5
Table 1. Repeated measures ANOVA with $F_m$, $F_v/F_m$, rETR$_{max}$, $\alpha$ and $E_k$ established that most variables tested (time, temperature, polyps, irradiance, time+temperature, time+irradiance) showed highly significant differences ($p < 0.001$), where $p$ = significance level, $F$ = $F$-value, $df$ = degrees of freedom.

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Figure 1. Minimum ($F_0$) and maximum ($F_m$) fluorescence values of *Mussismilia harttii* polyps maintained at different temperatures (26.5 [control], 29, 31, 33, and 35 °C) and different experimental periods (zero, 95, 190, and 285 min). (A) kept in the dark; (B) exposed to light (300 mmol photons m$^{-2}$.s$^{-1}$). $F_0$ presented as an average for all temperatures; $n$ = 30; $n$ for $F_m$ = 6. Bars show standard errors.

Figure 2. Maximum quantum yield ($F_v/F_m$) of *Mussismilia harttii* polyps maintained at different temperatures (26.5 [control], 29, 31, 33, and 35 °C) and different experimental periods (zero, 95, 190, and 285 min). (A) kept in the dark; (B) exposed to light (300 mmol photons m$^{-2}$.s$^{-1}$). Bars show standard errors; $n$ = 6.

°C) show an increase in rETR as light intensity increased and a small decline under irradiances higher than 817 μmol photons.m$^{-2}$.s$^{-1}$ (Figures 7-8). In the dark-adapted samples, RLCs of polyps incubated at temperatures up to 33.0 °C did not show large departures from this baseline pre-experiment curve, with a slight increase in rETR$_{max}$ (except at 33.0 °C after 235 min). Polyps incubated at 35.0 °C, on the other hand, showed a large departure from the baseline curve at all times, with significant decreases in rETR$_{max}$ and $\alpha$ (initial slope of the rapid light curve before the onset of saturation) (Figure 7). In light-adapted samples, large departures from the baseline curve can be seen at temperatures equal to or higher than 31.0 °C (Figure 8). In the light-adapted samples, there was a decrease of rETR$_{max}$ and $\alpha$ after incubation started at 31.0 °C, which remained constant during the entire experiment.
Figure 3. Polyp of *Mussismilia harttii* kept at 35.0 °C with light (300 mmol of photons.m⁻².s⁻¹): A = before exposure; B = after 95 min; C = after 190 min; and D = after 285 min.

Figure 4. Polyp of *Mussismilia harttii* after 285 min maintained at 35.0 °C in the dark.

This decrease was even more pronounced at higher temperatures, with an added time effect, which resulted in significantly different values of rETRₘₐₓ and α. Dark-adapted polyps incubated at 35.0 °C presented a severe decrease in photosynthetic activity (*p*<0.03), with *circa* 63% reduction after 285 min of experiment (Figure 7). Light-adapted polyps incubated at this temperature resulted in an absence of photosynthetic activities after 95 min (Figure 8). Light-adapted polyps presented smaller α than similar dark-adapted polyps in all treatments.

Repeated measures ANOVA with rETRₘₐₓ showed that most variables tested (time, temperature, polyps, irradiance [dark/light], time+temperature) showed highly significant differences (*p*<0.001; Table 1).

Repeated measures ANOVA with α showed that most variables tested (time, temperature, polyps, irradiance (dark/light), time+temperature, and time+irradiance) showed highly significant differences (*p*<0.001; Table 1).

Minimum saturating irradiances (Eₛ) were larger in light-than in dark-adapted polyps throughout the experiment. Control polyps (maintained at 26.5 °C) did not show significant differences along the experiment (Tukey’s HSD). However, Tukey’s HSD post-Hoc tests showed differences between the beginning of the experiment (all polyps dark-adapted and at 26.5 °C) and light-adapted polyps after 285 min of maintenance in different temperatures: 29.0 °C (*p*<0.029), 31.0 °C (*p*<0.015), and 33.0 °C (*p*<0.008). Repeated measures ANOVA with Eₛ showed that most variables tested (time, temperature, polyps, irradiance (dark/light), time+temperature, and time+irradiance) showed highly significant differences (*p*<0.001; Table 1).
Figure 5. Stationary ($F$) and maximum ($F^\prime_m$) fluorescence of *Mussismilia harttii* polyps kept in the dark at different temperatures and experimental periods: 26.5 °C (B-D), 29.0 °C (E-G), 31.0 °C (H-J), 33.0 °C (K-M), and 35.0 °C (N-P); 95 min (B, E, H, K, and N), 190 min (C, F, I, L, and O), and 285 min (D, G, J, M, and P). Bars show standard errors; n=6.

EFFECTIVE QUANTUM YIELD OF PSII ($\Phi_{PSII}$ OR $\Delta F/F^\prime_m$) AND NON-PHOTOCHEMICAL QUENCHING (NPQ)

As expected, there was an inverse relationship between $\Phi_{PSII}$ and NPQ. The former decreased with higher irradiances, while the latter increased. At the beginning of the experiment (all polyps dark-adapted and at 26.5 °C), $\Phi_{PSII}$ started at circa 0.5 at the first pulse of saturating light. Subsequently this value progressively decreased at higher light intensities. NPQ values increased from zero at the first pulse to almost 0.3 at the last pulse of the RLC (Figures 7 and 8).

Dark-adapted polyps incubated at temperatures between 26.5 °C and 33.0 °C showed initial $\Phi_{PSII}$ values slowly decreasing to 0.4 as temperatures increased (Figure 7). Dark-adapted polyps incubated at 35.0 °C showed a strong decrease of the initial $\Phi_{PSII}$ value after 95 min (58% less than control dark-adapted polyps).

Comparatively, control light-adapted polyps (26.5 °C) showed smaller $\Phi_{PSII}$ values than dark-adapted polyps (Figure 7 x Fig. 8). Even so, light-adapted polyps showed a stronger progressive decrease of the initial value of $\Phi_{PSII}$ as temperatures increased (Figure 8). Decrease of the initial $\Phi_{PSII}$ values in light-adapted polyps incubated at 35.0 °C reached values 75% less than control light-adapted polyps after 95 min.

At control temperatures (26.5 °C), NPQ progress along the experiment was slightly smaller in dark-adapted (Figure...
7) than in light-adapted polyps (Figure 8). At 29.0 °C, NPQ of dark- and light-adapted polyps evolved with quite similar values. Dark-adapted polyps showed an increase in NPQ values at 31.0 °C, followed by a slight and heavy decrease at 33.0 °C and 35.0 °C, respectively (Figure 7). In light-adapted polyps, there was a diverse tendency; NPQ values dropped almost continuously as temperatures were elevated, reaching near zero values at 35.0 °C (Figure 8).

**Post-Experiment Recovery**

Twenty-four hours after returning to normal conditions (aquarium at 26.5 °C), \( F_v/F_m \) showed that all polyps incubated at 26.5 (control) and 29.0 °C fully recovered, with values close to the average for all polyps before the experiment (0.659). At higher temperatures, there was an increasing tendency towards non-recovery. At 31.0 °C, one (out of six) dark-adapted and two (out of six) light-adapted polyps presented \( F_v/F_m < 0.390 \). At 33.0 °C, only two (out of 12 and both dark-adapted) presented \( F_v/F_m > 0.600 \), suggesting a full recovery. All polyps incubated at 35.0 °C died.

Bifactorial ANOVA (light and temperature) showed differences only among temperatures \( (F = 70.5104, df = 4, p<0.001) \). Tukey’s (HSD) post-Hoc test showed significant differences between the highest (33.0 and 35.0 °C) and lowest temperatures (26.5, 29.0, and 31.0 °C) \( (p<0.001) \). All data were normal (Kolmogorov-Smirnov, \( p>0.20) \). Levene’s test indicated heterocedastic variances \( (F_{4,60} = 15.300; p<0.001) \).
DISCUSSION

This study has explored how higher temperatures combined with light will impact a coral-dinoflagellate symbiosis in *Mussismilia harttii* in Porto Seguro, State of Bahia, Brazil. No visible bleaching of coral samples was seen during nearly 5 h of maintenance at temperatures between 26.5 and 35.0 °C, either in the dark or in the light. Such a result is compatible with those of other studies. Bleaching was reported in dark-adapted *Stylophora pistillata* only 24 h after exposure to excess irradiance (2000 µmol photons.m⁻².s⁻¹) at temperatures between 21.0 and 26.5 °C (JONES; HOEGH-GULDBERG, 2001). JONES et al. (1998) did not see bleaching in *S. pistillata* after 4 h at temperatures up to 34.0 °C. However, they observed a significant loss (cell count) of endosymbionts under natural light (but not under artificial light). Solar radiation is an environmental factor that significantly affects the intensity of bleaching in heat-stressed corals (LESSER; FARRELL, 2004). However, most of the change in photosynthetic activity of *Mussismilia harttii* endosymbionts occurred in the first 95 min of the experiment. Subsequent times showed stability (31.0 °C or less) or a continuing drop (33.0 °C and 35.0 °C) in photosynthetic activity. PUTNAM and EDMUNDS (2011) demonstrated that temperature fluctuations going beyond the limits of high or low temperatures could...
cause potentially harmful responses equivalent to that occurring in corals exposed to constant high temperatures. Their experiments showed significantly different results depending on the time period over which they were carried out: especially were the density of *Symbiodinium* and $F_v/F_m$ responses significantly different only in their Trial 1 – when natural seasonal *Symbiodinium* densities were higher (PUTNAM; EDMUNDS, 2011). Corals growing in environments with large daily temperature variability (up to 7.0 °C) and temperature extremes of up to 37.0 °C were highly susceptible to coral bleaching when exposed to heat stress (SCHOEPF et al., 2015). Corals were highly sensitive to daily average temperatures exceeding their maximum monthly mean by 1 °C for only a few days (SCHOEPF et al., 2015).

High sea surface temperature combined with high levels of solar irradiance has been found to be responsible for the induction of oxidative stress that triggers disruption of the symbiosis between cnidarians and *Symbiodinium* (ROBERTY et al., 2015). There was a gradual reduction of $F_v/F_m$ as temperature increased, with a further drop in the presence of light, indicating a synergistic effect of these factors - as seen in other studies (FITT; WARNER, 1995; DUNNE; BROWN, 1996, 2001; GLYNN, 1996; WARNER et al., 1996; JONES et al., 1998; HOEGH-GULDBERG, 1999; FITT et al., 2001; LESSER; FARRELL, 2004).
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Similar physiological responses were also observed at low temperatures for Montipora digitata (SAXBY et al., 2003) under high intensity light, clearly demonstrating the exacerbated effect caused by light. In the present study, especially at high temperatures (32.0 °C and 35.0 °C), light aggravated the effect of temperature, resulting in a rapid reduction in $F_v/F_m \Phi_{psii}$ rETR$_{max}$, and $\alpha$.

$F_v/F_m$ significantly decreased only when the coral Pocillopora damicornis was incubated at high temperatures (32.0 °C) and high irradiance (475 mmol photons.m$^{-2}$.s$^{-1}$), while at the same temperature and 225 mmol photons.m$^{-2}$.s$^{-1}$ PSII activity was not significantly reduced (HILL et al., 2005). High irradiances alone diminished $F_v/F_m$ values in endosymbionts of the corals Stylophora pistillata (JONES; HOEGH-GULDBERG, 2001). Response appears to be species-specific because other studies have shown that temperature alone could decrease $F_v/F_m$ (WARNER et al., 1996), even at subsaturating irradiances (40 μmol photons.m$^{-2}$.s$^{-1}$) (IGLESIAS-PRIETO, 1995).

Chlorophyll fluorescence emission under ambient temperature is almost entirely related to the PSII and the ratio $F_v/F_m$, which describes the maximum quantum yield of the PSII (BJÖRKMAN; DEMMIG, 1987), has been widely used as diagnostic of environmental stresses on the physiology of photosynthetic organisms (SCHREIBER et al., 1994; CAMPBELL et al., 1998). Therefore, the ratio $F_v/F_m$ adequately reflects the influence of temperature and light on the photosynthetic activity of the endosymbionts of M. harttii. As mentioned, there is a decline of photosynthetic activity when the rate of absorption of light energy exceeds its utilization, i. e., photoinhibition (OSMOND, 1994). Photosynthetic organisms developed a fast and efficient process to repair photodamage to the PSII (Aro et al., 1993; 2005; TAKAHASHI; MURATA, 2008). Therefore, the extent of photoinhibition depends on the balance between photodamage to the PSII and the repair process. Elevated temperatures do not accelerate the damage to the PSII, but can inhibit its repair (MURATA et al., 2007; TAKAHASHI; MURATA, 2008; TAKAHASHI et al., 2009). These authors showed that the sensitivity to photobleaching in Symbiodinium under thermal stresses differs between Clade A Symbiodinium strains. The repair process was strongly inhibited by temperatures exceeding 31.0 °C in one strain but only weakly in other. Therefore, we suggest that the photodamage to M. harttii endosymbionts triggered by temperatures of 33.0 °C and 35.0 °C results from a decline of the repair process, as well as the effect of light on the PSII. This would explain the enhanced effect when polyps were incubated under both factors: high temperatures and light. Temperature alone also caused an effect on the PSII, as there was a severe decline of $F_v/F_m$ under 35.0 °C even in the absence of light.

Rapid light curves (RLCs) at the beginning of the experiments (dark-adapted polyps kept at 26.5 °C for all treatments) showed a gradual increase of the stationary fluorescence (circa 54%), indicating a gradual closing of the reaction centers, which impair their photochemical processes (RALPH; Gademann, 2005). Concomitantly, there was a decrease of the maximum fluorescence of light-adapted samples, suggesting the onset of non-photochemical quenching mechanisms (NPQ). These mechanisms became predominant under irradiances higher than the minimum saturating irradiances (E$_k$). In fact, there was a gradual increase in NPQ, especially at irradiances over 300 mmol photons.m$^{-2}$.s$^{-1}$, which correspond to E$_k$ under the initial conditions of the experiment. The relative electron transport rate (rETR) showed a slight decline at the end of the RLCs, probably associated with an increase in energy dissipation as heat (down-regulation of the PSII) as there was not enough time of light exposure to damage the photosystems (RALPH; Gademann, 2005). The effect of temperature on the maximum relative electron transport rates (rETR$_{max}$) seems to result from a balance between a protection and an inhibition of the photosynthetic activity (OSMOND, 1994). Light-adapted polyps showed dynamic photoinhibition (photoprotection) at temperatures between 26.5 °C and 31.0 °C, as suggested by the decline in α, the rise in $E_{k}$, and the continuation of rETR$_{max}$. The gradual declines of α and rETR$_{max}$ in samples incubated at 33.0 °C suggest that the onset of chronic photoinhibition (photodamage) occurs in light-adapted polyps at temperatures above 31.0 °C. ROBERTY et al. (2015) demonstrated for the first time that combined heat and light stress inactivate antioxidant capacities of the water-water cycle, WWC. In corals under high light and at 33.0 °C, O$_2$-dependent electron transport had significantly increased relative to total electron transport. This increase was concomitant with a twofold increase in ROS generation compared with the treatment at 26.0 °C, while enzymes involved in the WWC were largely inactivated. Photosynthetic organisms developed mechanisms to dissipate excessive light-energy as heat (non-photochemical quenching – NPQ), which can be quantified through the decline of the fluorescence of the chlorophyll (WARNER; BERRY-LOWE, 2006). Among protection mechanisms, the xanthophyll cycle stands out
in *Symbiodinium* sp. and other microalgae (Lavaud et al., 2004) and can be inferred by the NPQ (Brown et al., 1999; Schreiber, 2004). NPQ plays an important role during bleaching (Hill et al., 2004a; Hill et al., 2005). Therefore, the fast development of NPQ in endosymbionts found in *M. harttii* is probably due to the xanthophyll cycle acting to protect against factors as temperature and irradiance. Recovery studies showed different results. Ten hours after removal of stress conditions, at high temperatures, were enough for PSI recovery in *Acropora nobilis* (Hill et al., 2004b). *Acropora digitifera* incubated at 28.0 °C showed complete recovery only 3h after decreasing light from 500 to 20 mmol photons m\(^{-2}\) s\(^{-1}\). A similar experiment with *A. digitifera* incubated at 32.0 °C resulted in *F*\(\text{v}/F\text{m}\) significantly smaller than original values even 7 h after decreasing light intensity (Takahashi et al., 2004). Despite the considerable bleaching observed at low temperatures, there was no recovery of *F*\(\text{v}/F\text{m}\) for *M. digitata* exposed to 12.0 °C for more than 12h (Saxby et al., 2003).

While bleaching was not observed in *M. harttii*, this coral showed incomplete recovery (estimated 24 h after ceasing the stress) of three out of 12 polyps incubated at 31.0 °C, with light-adapted polyps showing a slightly inferior recovery. The full recovery of only two (out of 12) polyps incubated at 33.0 °C may be due to the dominance of different thermal tolerant zooxanthellae clades. Clades A, B, and C have been recorded on the Southeastern Brazilian coast (Baker, 2003), but only clade C on the Northeastern coast (closer to the Equator), including areas to the North and to the South of our specimens (Baker, 2003; Costa et al., 2008). However, this hypothesis needs to be tested. Positive thermal anomalies of up to 0.75 °C were recorded during the summer, between 1998 and 2005, in Bahia State (including the Porto Seguro area), resulting in different degrees of mild to severe bleaching (Leão et al., 2008). The presence of thermal-tolerant endosymbionts may be fostered by such anomalies (see Baker et al., 2004).

Although light enhanced the harmful effects of increased temperatures, there was a complete recovery of all polyps maintained at 26.5 °C and 29.0 °C, despite the presence of light. Recovery data for polyps kept at 31.0 °C showed that this temperature (depending on time of exposure) seems to mark the threshold before more serious damage occurs. There are no species or genotypes of corals capable of surviving a water temperature rise between 3.0 °C and 6.0 °C for more than a month (Hoegh-Guldberg, 2009), or reef builders that have the ability to tolerate projected changes in sea temperature (see several general circulation models in Hoegh-Guldberg, 1999), as predicted for the end of this century (IPCC, 2007). Therefore, unless the presence of thermotolerant endosymbionts is confirmed and corals are able to establish a population with such clades, continued exposure to temperatures higher than 31.0 °C can lead to mass bleaching of *M. harttii* in the Porto Seguro area.

Coral reefs have experienced several large-scale bleaching events due to climate change and these events are expected to increase in frequency and severity in the future jeopardizing the survival of coral reefs. The geographically large-scale bleaching observed in the South Atlantic in 2010 reported only minor bleaching events. In Porto Seguro (locality of our experiments), seawater temperatures reached 31.0 °C during several days in late summer/early autumn (PROJETO CORAL VIVO, unp. data). Under these conditions, our experiments showed a few affected colonies, suggesting that our experimental conditions are correctly reflecting responses observed in the field in this area.

**ACKNOWLEDGEMENTS**

We wish to thank Nuno Seabra, Leones Lopes for their laboratory assistance; Renata Arantes, Antonio Santos and Sandro Santana for field assistance; Paulo Paiva and Carla Zilberberg for help with statistical analyses. Funding was provided in part by Projeto Coral Vivo, sponsored by Petrobras Socioenvironmental Program and Arraial d’Ajuda Eco Parque. APMW is grateful for a graduate fellowship from CENPES/Petrobras, and CBC for a research fellowship from the Brazilian Research Council (CNPq).

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