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# Synergistic Effect of Elevated Temperature and Light Stresses on Physiology of *Pocillopora acuta* from Different Environments

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**Abstract:** Increasing levels of greenhouse gases lead to ocean warming, which affects a range of marine organisms. Corals live in a narrow temperature range and become stressed when the temperatures change. Bleaching occurs when the temperature exceeds the coral's threshold, and can be severe when this is combined with other stressors such as light. In order to understand how temperature and light affect corals in their physiological responses and photosynthetic performance, *Pocillopora acuta* from Maiton Island (MT) and Panwa Cape (PW), representing different environments, were investigated. The results show that light and temperature had by regime different effects on Symbiodiniaceae photosynthesis and the coral growth rate. There was a synergistic effect of elevated temperature and light on photosynthesis, as observed in the photochemical efficiency and pigment contents, suggesting photo-damage. A higher growth rate in Panwa corals was observed in control, and while elevated temperature reduced coral growth. Elevated temperature affected the Panwa coral less, suggesting that corals from this regime might be able to recover when the temperature returns to normal. This information is important for predicting the coral responses to elevated temperature especially in the summer, as regards the possibility of coral bleaching.

**Keywords:** coral bleaching; resilience; PAM fluorometry; climate change; ecophysiology

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## 1. Introduction

Human activities have increased the concentrations of greenhouse gases, such as carbon dioxide and methane, in the atmosphere, leading to global warming due to the greenhouse effect [1,2]. This has led to ocean warming, which subsequently affects many physical and chemical parameters, inducing damage to the productive habitats, especially for corals that live in a narrow temperature range [3].

Temperature is an important factor influencing coral growth and photosynthesis [4], and elevated temperature induces oxidative stress and coral bleaching [5,6]. Coral is typically sensitive to temperature changes, resulting in coral stress at the cellular level. This has led to bleaching and imbalance in the mutualistic relationship, with a loss of Symbiodiniaceae in coral tissue [6–8]. A reduction in photosynthetic performance might occur and lead to coral mortality. Temperature elevation anomalies reduce coral growth [9]. Furthermore, Kuanui et al. [10] showed that temperature affects both coral growth and survival. Tropical coral reefs are currently under pressure because corals are at their upper thermal limits [11].

The effects of temperature on corals can be exacerbated by other stressors, such as irradiance [12]. Responses of the coral holobiont to elevated temperature and light have been observed, e.g., a reduction in Symbiodiniaceae density [13], changes in photosynthetic performance [12,14], changes in reactive oxygen species (ROS) [15,16] and enzymatic antioxidant activity [17], and bleaching responses [18]. Higher irradiance increases the susceptibility to elevated-temperature stress [17]. However, some corals might be able to adapt to and resist elevated temperatures [19]. A high temperature (32–34 °C) reduces the light threshold for photoinhibition in *Stylophora pistillata* and *Platygyra ryukyuensis* [20]. Rosic et al. [21] found that *S. pistillata* was negatively influenced by a combination of high temperature (32 °C) and high irradiance (250 µmol photons $m^{-2}$ $s^{-1}$), which caused bleaching after 24h of stress. Combined responses to light and temperature were also investigated by Weston et al. [22], showing that 2 days of exposure to severe conditions of elevated temperature and low light or high light and low temperature led to coral bleaching. When the light or temperature exceeds corals' thresholds, some cases that are resistant do not bleach, and some that have tolerance can recover and survive. The ability of coral to recover from temperature stress might depend on its thermal history [23] and light history [18,24]. Corals under different regimes face different environmental parameters, such as temperature, irradiance, turbidity, and pollution, contributing to differences in thermal and light exposure history. In addition, the gene expression (an internal factor) and protein expression in the coral host and in Symbiodiniaceae can affect the coral's susceptibility or responses to stressors such as light or temperature [25–28].

Mass coral bleaching and mortality can be attributed to global climate-induced ocean warming [29]. Phuket Island is a marine attraction in Thailand with its fringing reef exposed to mass tourism. The multiple stressors as well as mass coral bleaching have been issues in Phuket, but the physiological responses of corals and Symbiodiniaceae from this area have not been investigated [30]. Phongsuwan and Chansang [31] reported that coral bleaching in the Andaman Sea, Thailand, was first documented in 1991. Then, it occurred in 1995, 1998, 2003, 2010, and 2016 due to the rising sea surface temperature (SST). In Phuket, bleaching events occurred in 1991, 1995, 2010, and 2016, and Putchim et al. [23] observed that some formerly bleaching-susceptible fast-growing branching coral taxa (e.g., *Acropora, Montipora, Echinopora*, and *Pocillopora damicornis*) were more tolerant to elevated temperature than they had been in previous years, while some of the formerly bleaching-resistant slow-growing coral taxa (e.g., *Porites, Goniastrea, Dipsastraea*, and *Favites*) became more susceptible to bleaching over repeated thermal stress events [23]. This resulted in losses of living corals, and changes in coral diversity and abundance when combined with direct and indirect artificial disturbances [23,32].

An inshore area of Phuket, Panwa Cape (PW), with turbid water and varied environmental conditions due to higher sedimentation and water runoff, exhibits different physical and chemical environments in the local reef when compared with an offshore reef at Maiton Island (MT), for coral, as regards the ranges of light, temperature, and water flow. Environmental background can determine the physiological responses of Symbiodiniaceae and coral holobionts, and the experienced local conditions may drive different coral tolerances [33,34]. In this study, we examined physiological parameters of corals from those habitats to address the differences in coral responses to the temperature and light stresses, including combined effects. We hypothesized that corals from PW adjusted to extreme experiences would be able to acclimate better than the MT corals. This information is also important for predicting coral responses and thresholds to elevated temperature, especially in the summer, and is useful for coral bleaching management plans.

## 2. Materials and Methods

### 2.1. Coral Sampling

*Pocillopora acuta* were selected from MT (7°45′43.94″ N; 98°28′35.37″ E) and PW (7°48′6.26″ N; 98°24′23.75″ E), Phuket, Thailand (Figure 1a). The MT coral reef is 8 km from the mainland, and the collected samples were in very healthy condition. On the

other hand, the PW reef located near shore represents a poor reef condition. A prior report revealed 75.51 ± 19.76% and 12.76 ± 0.52% of live and dead coral coverages, respectively, in the MT reef, whereas the proportions were 35.34 ± 1.02% and 63.65 ± 6.21% in the PW reef [35]. In July 2018, four biological samples of *P. acuta* colonies, 25–30 cm in diameter, were collected from a shallow reef slope (at 5–7 m depth) at both study sites using a stainless steel bone cutter. The healthy tissues of the selected corals were carefully investigated, displaying no visible signs of stress, bleaching, or disease, and rechecked for photosynthetic efficiency using Diving-PAM (Walz, Effeltrich, Germany). All the samples were maintained in natural seawater and transferred to an indoor aquarium within 12 h. Supplemental environment data of the two study sites are provided to document the local conditions (Table 1). The temperature, salinity, pH, dissolved oxygen, light intensity, total dissolved solids, total suspended solids, and chlorophyll were measured using an AAQ-RINKO 176 water quality profiler (JFE Advantech Co. Ltd., Hyogo, Japan). A Secchi disk was used for transparency determination, and seawater samples were collected and preserved for further chemical analysis of $NO_2^-$, $NO_3^-$, $NH_3$, and $PO_4^{3-}$. All seawater parameters and samples ($n$ = 3) were collected around midday, at the same time as coral sampling, which might favor observing extreme values of light intensity.

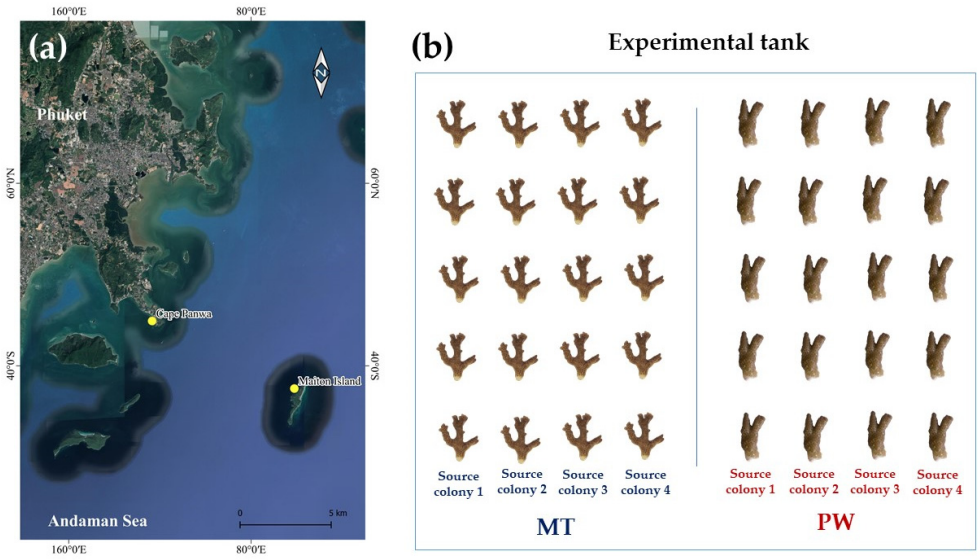


**Figure 1.** Sampling site locations (**a**), and (**b**) coral nubbins in each experimental tank (20 nubbins/study site/tank) originally from Maiton Island (MT) and Panwa Cape (PW).

**Table 1.** Environmental parameters of the reef sampled from 5–6 July 2018. Data are given as mean ±SE.

| Environmental Parameter | Maiton Island | Panwa Cape |
|---|---|---|
| Temperature (°C) | 28.09 ± 0.10 | 28.17 ± 0.09 |
| Salinity (PSU) | 32.73 ± 0.01 | 32.69 ± 0.05 |
| pH | 8.79 ± 0.10 | 7.96 ± 0.05 |
| Dissolved oxygen (mg $L^{-1}$) | 5.62 ± 0.05 | 5.61 ± 0.13 |
| Transparency (m) | 6.25 ± 0.25 | 3.20 ± 0.70 |
| Light intensity (µmol photons $m^{-2}$ $s^{-1}$) | 483.25 ± 16.73 | 149.50 ± 4.75 |
| Total dissolved solids (mg $L^{-1}$) | 31,634.33 ± 8.65 | 32,597.67 ± 36.86 |
| Total suspended solids (mg $L^{-1}$) | 28.93 ± 1.29 | 33.55 ± 0.68 |
| Chlorophyll (µg $L^{-1}$) | 0.13 ± 0.06 | 0.25 ± 0.03 |
| $NO_2^-$ (µg−atm N-$NO_2$ $L^{-1}$) | 0.07 ± 0.01 | 0.04 ± 0.01 |
| $NO_3^-$ (µg−atm N-$NO_3$ $L^{-1}$) | 0.48 ± 0.11 | 0.41 ± 0.05 |
| $NH_3$ (µg−atm N-$NH_3$ $L^{-1}$) | 1.08 ± 0.13 | 2.48 ± 0.09 |
| $PO_4^{3-}$ (µg–atm P-$PO_4$ $L^{-1}$) | 0.23 ± 0.14 | 0.49 ± 0.02 |

### 2.2. Experimental Design

Coral colonies were acclimated for 7 days in 600 L holding tanks with flowing seawater pumped directly from the natural seawater stock (filtered with a Nomex Filter Bag, and treated with 50 ppm of chlorine), under a light intensity, temperature, salinity, and pH of 150 µmol photons $m^{-2}$ $s^{-1}$, 28 °C, 33 PSU, and pH 8.2, respectively. LEDs were set at a 12:12 h light:dark cycle and turned on and off at 6 a.m. and 6 p.m. A heater–chiller (JMC-02, JBA, Zhongshan, China), COB light (TS-A600, Aquarium lamp, Zhongshan, China), and LEDs (A601, Chihiros, NingBo, China) were used to control the water temperature and light intensity in the aquarium tank. Seawater was changed for 20% of the tank volume weekly, and the water quality parameters (phosphate, ammonia, nitrate, magnesium, calcium, and alkalinity) were measured weekly with a test kit (Salifert, Netherlands) in order to maintain the nitrate and phosphate concentrations at 0 mg $L^{-1}$ and below 2 mg $L^{-1}$, respectively.

After the above acclimation, the 8 coral colonies (4 colonies from MT and 4 colonies from PW) were cut into nubbins of 3–5 cm using a bone cutter. Each colony was divided into 80 nubbins and allocated to 4 experimental tanks (62 L) as 20 nubbins/study site/tank (Figure 1b): (1) control (ambient temperature, ambient light intensity; ATAL; 27 °C; 150 µmol photons $m^{-2}$ $s^{-1}$); (2) ambient temperature, high light intensity (ATHL; 27 °C; 300 µmol photons $m^{-2}$ $s^{-1}$); (3) high temperature, ambient light intensity (HTAL; 33 °C; 150 µmol photons $m^{-2}$ $s^{-1}$); and (4) high temperature, high light intensity (HTHL; 33 °C; 300 µmol photons $m^{-2}$ $s^{-1}$). All coral nubbins were acclimated again in the experimental tanks for 7 days as per the above settings of the holding tanks, some of which were equipped with temperature chillers (JMC-02, JBA, China). The stress temperature in this study was in the range of the average sea surface temperature recorded at Phuket [6,23] and the extreme temperature reported in shallow Thailand reefs [36]. The light intensity was determined by non-photoinhibitory irradiance during the sampling period (Table 1).

To investigate the effects of temperature and light stress (Figure 2), the experiment was performed for a total of 14 days by gradually increasing temperature (Days 1 to 7; 1 °C per day from 27 to 33 °C) and then decreasing temperature (Days 8 to 14; 1 °C per day from 33 to 27 °C) for the high-temperature treatments (HTAL and HTHL). The high light was set at 300 µmol photons $m^{-2}$ $s^{-1}$ over 14 days for the high-light treatments (ATHL and HTHL). As shown in Figure 2, coral nubbins from Row 1 (4 nubbins from MT and 4 nubbins from PW of each treatment) were measured for photosynthetic performance at the initial time (Day 1), beginning of stress (Day 4), threshold (Day 8), and end of experiment (Day 14). Destructive coral sampling (4 nubbins from MT and 4 nubbins from PW of each treatment) was performed on Days 1, 4, 8, and 14 using coral nubbins from Rows 2, 3, 4, and 5, respectively, for analyses of Symbiodiniaceae density and pigments. The bleaching and mortality assessment and determinations of growth-related attributes were done with coral nubbins from Row 1 (same nubbins as used in non-destructive photosynthesis measurements) initially and at the end of the experiment.

#### 2.2.1. Photosynthetic Efficiency

The photosynthetic activity of the coral Symbiodiniaceae was determined through the measurements of the chlorophyll (Chl) *a* fluorescence, Symbiodiniaceae density, and photosynthetic pigment concentration. After dark adaptation, the basal ($F_0$) and maximal fluorescence ($F_m$) were measured, and the maximum quantum yield of Photosystem II (PSII) ($F_v/F_m$) was calculated as ($F_m$–$F_0$)/$F_m$, measured at 5 a.m. using a JUNIOR-PAM fluorometer (Walz, Germany). The light-dependent photosynthetic performance was investigated at 10:30 a.m. by determining rapid light curves (RLCs) using the JUNIOR-PAM fluorometer with WinControl software version 3.26 (PAM settings: measuring intensity <0.15 µmol photons $m^{-2}$ $s^{-1}$, saturating intensity >4500 µmol photons $m^{-2}$ $s^{-1}$, saturating width = 0.8 s, gain = 2, damping = 2). RLCs with nine increasing actinic light intensity levels (0, 66, 90, 125, 190, 285, 420, 625, and 820 µmol photons $m^{-2}$ $s^{-1}$) were applied with 0.8 s saturating pulses (>4500 µmol photons $m^{-2}$ $s^{-1}$) between each actinic light intensity, every 10 s. The effective quantum yield of PSII ($\Delta F/F_{m'}$; Schreiber [37]), maximum

relative electron transport rate ($rETR_{max}$), minimum saturating irradiance ($I_k$), and initial slope ($\alpha$) of the RLCs were calculated using curve-fitting protocols following Ralph and Gademann [38].

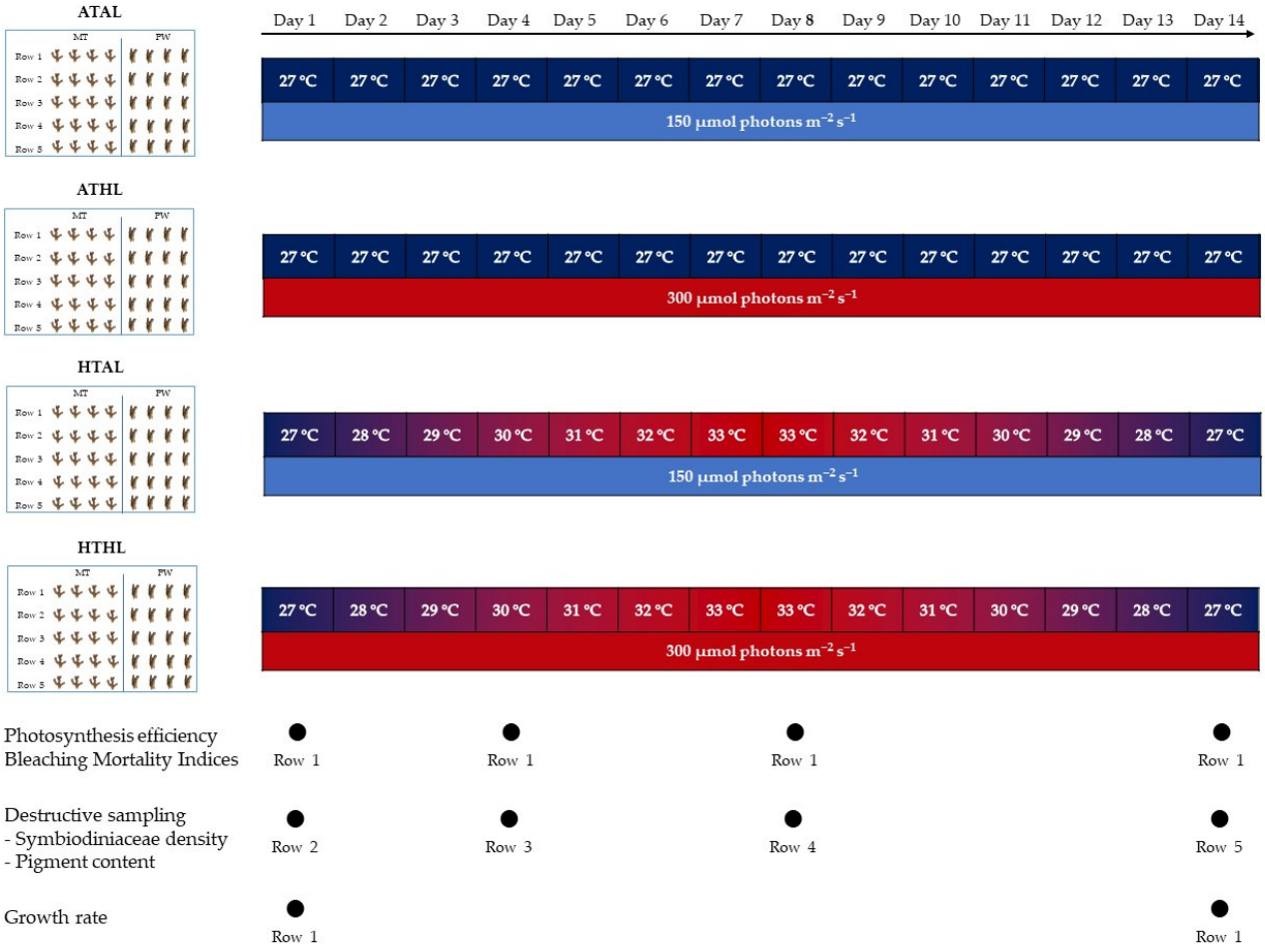


**Figure 2.** Experimental design applied to the coral nubbins from MT and PW in each treatment tank. The nubbins in Row 1 were used for non-destructive measurements of photosynthetic efficiency, Bleaching Mortality Indices, and growth rate, whereas the nubbins in Rows 2–5 were used for destructive sampling of Symbiodiniaceae density and pigment at Days 1, 4, 8, and 14, respectively. The destructive coral samples were removed from experimental tanks on the day previously described.

### 2.2.2. Symbiodiniaceae Density

Coral samples (n = 4 nubbins) were collected at each stage (Days 1, 4, 8, and 14) and airbrushed into 10 mL of 0.2 µm filtered seawater to remove the tissue from the skeleton. The slurry was centrifuged (MPW-260, MPW MED. Instruments, Warszawa, Poland) at 4000 rpm for 4 min to separate Symbiodiniaceae cells from the animal tissue [39]. The supernatant containing animal tissue was discarded; the Symbiodiniaceae pellet was resuspended in 10 mL of 0.2 µm filtered seawater, homogenized for 10 s at 15,000 rpm, and centrifuged again. The pellets were resuspended in 1 mL of filtered seawater for cell counts and chlorophyll analyses. For Symbiodiniaceae density analysis, four subreplicate cell counts were performed using a hemocytometer under a light microscope (Leica DM500, Leica Microsystem, Germany). The cell density was determined per $cm^2$ following coral surface area calculations using the paraffin wax technique [39].

2.2.3. Pigment Concentration

For the analysis of the photosynthetic pigment concentration (chlorophyll (Chl) *a* and $c_2$), algal pellets were resuspended in 90% acetone and stored in darkness overnight at 4 °C. After centrifugation, chlorophyll *a* and $c_2$ (μg $cm^{-2}$) were determined using the standard spectrophotometric method of Ritchie [40], with the absorbance measured at 630, 664, and 750 nm as follows [41]:

$$\text{Chlorophyll } a = (-0.4574 \times A_{630\ nm}) + (11.4754 \times A_{664\ nm}), \quad (1)$$

$$\text{Chlorophyll } c_2 = (23.3900 \times A_{630\ nm}) + (-3.5322 \times A_{664\ nm}). \quad (2)$$

2.2.4. Bleaching Mortality Indices (BMI)

Bleaching and mortality were used to evaluate the responses of corals to thermal and light stresses. The Bleaching Mortality Indices (BMIs) were calculated following Putchim et al. [23] as

$$BMI = (0c_1 + 1c_2 + 2c_3 + 3c_4) \times 3^{-1} \quad (3)$$

where:
$c_1$ = the numbers of non-bleached corals;
$c_2$ = the numbers of pale corals;
$c_3$ = the numbers of fully bleached corals;
$c_4$ = the numbers of recently dead corals.

2.2.5. Growth-Related Attributes

The coral growth rates were measured with the buoyant weight technique [42] and calculated as the percentage (%) increase in coral weight per day. This rate refers to the increase in the combined skeletal and tissue weight of the coral [43].

The coral nubbin for each treatment was weighed in seawater; both the seawater temperature and salinity were recorded for calculating the density of the sea water, and a glass reference was weighed in both sea water and air; then, the density of *P. acuta*, taken as 2.01 g $cc^{-1}$ [44,45], was used to calculate coral dry weight in Equation (4). The determined weights were used in Equation (5):

$$DW = WW/(1-(a/(1000 \times b))) \quad (4)$$

where:
DW = dry weight (g);
WW = wet weight (g);
a = seawater density (g $cc^{-1}$);
b = coral density (g $cc^{-1}$);

$$G = ((a/b)^{(1/c)} - 1) \times 100 \quad (5)$$

where:
G = growth (% per day);
a = the final dry/buoyant weight (g);
b = the initial dry weight (g);
c = the number of days between measuring a and b.

*2.3. Statistical Analysis*

All the data met the assumptions of normality (Kolmogorov–Smirnov test) and equal variance (Levene's test). Changes in dependent variables such as maximum quantum yield, photosynthetic efficiency, RLC-derived parameters, and pigment contents according to a fixed factor such as time, temperature, light, and site of origin were determined using four-way ANOVA with a significance level of 95%. To determine any significant differences among temperature, light, and sites in growth rate, three-way ANOVA was performed with a significance level of 95%. Tukey's HSD post hoc test was used to confirm statistically

significant differences. Statistical analysis was performed using SPSS software (Version 23.0, IBM Corp, Armonk, NY, USA).

## 3. Results

### 3.1. Photosynthetic Efficiency

The maximum quantum yields ($F_v/F_m$) of the MT and PW corals on Day 1 were 0.51 ± 0.01, n = 4, and 0.58 ± 0.02 (mean ±SE), n = 4, respectively (Figure 3a,b). There were no significant differences by time × site × temperature × site, time × temperature × light, and site × temperature × light ($p$ = 0.803, 0.303, and 0.994, respectively). However, there were significant differences by time × temperature and time × light ($p < 0.001$ and $p = 0.010$, respectively). These results suggested that temperature and light affected the corals at different times, regardless of colony location (Table 2). There were significant differences in $F_v/F_m$ by site × temperature, suggesting that corals from MT responded differently to temperature than corals from PW. In addition, the results showed that the PW coral had higher $F_v/F_m$ than MT in all the treatments and at almost all the times of measurement. On Day 8, the $F_v/F_m$ of the MT corals in ATHL, HTAL, and HTHL treatments were significantly lower by 12.78%, 35.96%, and 40.80% than the initial value, respectively ($p < 0.001$), while that for the PW coral had significantly decreased by 13.00%, 26.40%, and 26.33% from the initial value, respectively ($p < 0.001$). There was sign of recovery for both PW and MT corals only in the HTAL treatment, as shown by the $F_v/F_m$ (Figure 3a; Table 2), whereas high light intensity (ATHL and HTHL) presented a continuous decrease in $F_v/F_m$. This implied a light effect that was related to high temperature, particularly in combination factors (HTHL).

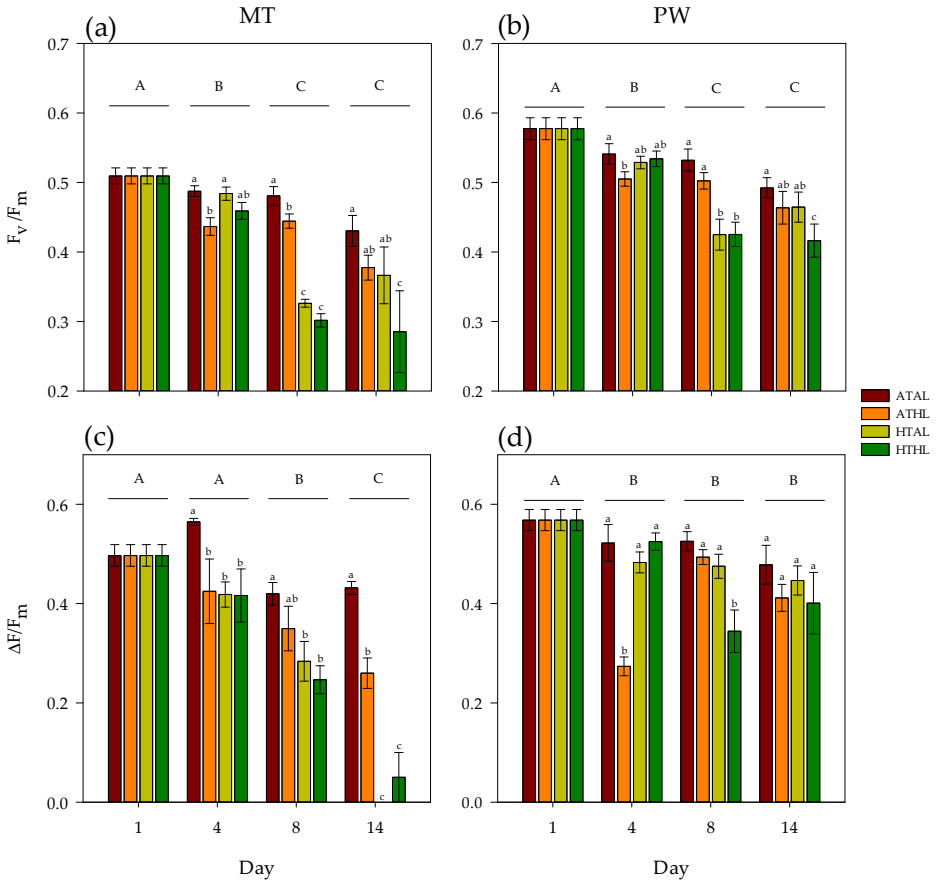


**Figure 3.** Maximum quantum yield ($F_v/F_m$; **a**,**b**) and effective quantum yield ($\Delta F/F_m$; **c**,**d**) of *P. acuta* from MT and PW at each sampling time. Data are presented as mean ± SE (n = 4). A, B, C, D and a, b, c, d indicate significant differences by time and by treatment, respectively.

**Table 2.** Statistical indices of maximum quantum yield ($F_v/F_m$), photosynthetic efficiency ($\Delta F/F_m$), RLC-derived parameters (alpha, $rETR_{max}$, and $I_k$), cell density, and pigment contents (Chl *a* and Chl $c_2$) (four-way ANOVA).

| Factors | | $F_v/F_m$ | $\Delta F/F_m$ | Alpha | $rETR_{max}$ | $I_k$ | Cell density | Chl *a* | Chl $c_2$ |
|---|---|---|---|---|---|---|---|---|---|
| **time** | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 69.001 | 70.614 | 61.512 | 74.123 | 37.293 | 20.905 | 23.808 | 22.340 |
| | *p* | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * |
| time × site | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 1.074 | 23.980 | 7.529 | 4.434 | 18.917 | 1.145 | 3.291 | 3.777 |
| | *p* | 0.348 | <0.001 * | <0.001 * | 0.018 * | <0.001 * | 0.312 | 0.059 | 0.028* |
| time × temperature | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 16.040 | 15.245 | 4.795 | 5.196 | 14.196 | 4.539 | 2.493 | 5.654 |
| | *p* | <0.001 * | <0.001 * | 0.004 * | 0.009 * | <0.001 * | 0.030 * | 0.107 | 0.006 * |
| time × light | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 4.537 | 2.139 | 2.638 | 0.850 | 2.107 | 1.016 | 1.535 | 1.424 |
| | *p* | 0.010* | 0.123 | 0.056 | 0.432 | 0.107 | 0.346 | 0.229 | 0.250 |
| time × site × temperature | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 1.052 | 14.823 | 11.789 | 0.995 | 2.234 | 0.789 | 0.143 | 0.845 |
| | *p* | 0.356 | <0.001* | <0.001* | 0.376 | 0.092 | 0.418 | 0.819 | 0.440 |
| time × site × light | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 0.057 | 2.224 | 1.586 | 0.928 | 4.371 | 0.726 | 0.843 | 1.025 |
| | *p* | 0.955 | 0.113 | 0.200 | 0.401 | 0.007 * | 0.442 | 0.414 | 0.369 |
| time × temperature × light | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 1.208 | 5.268 | 0.965 | 1.195 | 0.673 | 0.686 | 1.238 | 1.784 |
| | *p* | 0.303 | 0.007 * | 0.414 | 0.311 | 0.572 | 0.457 | 0.294 | 0.177 |
| time × site × temperature × light | df | 2.187 | 2.200 | 3 | 1.966 | 3 | 1.367 | 1.582 | 2.085 |
| | F | 0.244 | 2.269 | 0.639 | 1.299 | 1.447 | 0.942 | 0.387 | 1.981 |
| | *p* | 0.803 | 0.109 | 0.592 | 0.282 | 0.236 | 0.368 | 0.633 | 0.147 |
| error (time) | df | 192.490 | 52.798 | 72 | 47.179 | 72 | 32.808 | 37.965 | 50.041 |
| | F | - | - | - | - | - | - | - | - |
| | *p* | - | - | - | - | - | - | - | - |
| site | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 89.582 | 73.075 | 12.346 | 0.393 | 0.066 | 0.579 | 54.067 | 54.449 |
| | *p* | <0.001 * | <0.001 * | 0.002 * | 0.537 | 0.799 | 0.454 | <0.001 * | <0.001 * |
| temperature | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 29.143 | 30.026 | 8.895 | 20.863 | 22.402 | 17.040 | 18.358 | 21.324 |
| | *p* | <0.001 * | <0.001 * | 0.006 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * | <0.001 * |
| light | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 6.049 | 18.992 | 13.911 | 6.050 | 0.801 | 8.185 | 15.705 | 9.288 |
| | *p* | 0.016 * | <0.001 * | 0.001 * | 0.021 * | 0.380 | 0.009 * | 0.001 * | 0.006 * |
| site × temperature | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 6.417 | 17.356 | 3.668 | 7.086 | 8.705 | 0.751 | 0.001 | 0.141 |
| | *p* | 0.013 * | <0.001 * | 0.067 | 0.014 * | 0.007 * | 0.395 | 0.974 | 0.710 |
| site × light | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 2.331 | 0.620 | 1.178 | 3.375 | 0.566 | 0.426 | 0.556 | 1.837 |
| | *p* | 0.130 | 0.439 | 0.289 | 0.079 | 0.459 | 0.520 | 0.463 | 0.188 |
| temperature × light | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 0.000 | 8.071 | 0.407 | 0.923 | 0.000 | 8.111 | 7.514 | 7.718 |
| | *p* | 0.994 | 0.009 * | 0.529 | 0.346 | 0.988 | 0.009 * | 0.011 * | 0.010 * |
| site × temperature × light | df | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | F | 0.000 | 0.635 | 0.201 | 5.905 | 4.202 | 0.682 | 1.294 | 4.420 |
| | *p* | 0.994 | 0.433 | 0.658 | 0.023 * | 0.051 | 0.417 | 0.267 | 0.046 * |
| error | df | 88 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| | F | - | - | - | - | - | - | - | - |
| | *p* | - | - | - | - | - | - | - | - |

*, significant difference; -, absent.

The effective quantum yields ($\Delta F/F_{m'}$) of the MT and PW corals on Day 1 were 0.50 ± 0.04, n = 4, and 0.57 ± 0.02, n = 4, respectively (Figure 3c,d). There were no significant

differences in $\Delta F/F_{m'}$ by time × site × temperature × light ($p = 0.109$); however, there were significant differences by time × temperature × light ($p = 0.007$), suggesting that corals from both PW and MT responded differently to different time, temperature, and light levels (Table 2). The $\Delta F/F_{m'}$ in the ATAL and ATHL treatments of PW coral were higher than those of MT corals in the decreasing temperature phases (Day 14), and PW corals' HTAL and HTHL were higher than those of MT corals in almost all phases. On Day 8, the $\Delta F/F_{m'}$ of MT corals in the ATHL, HTAL, and HTHL treatments were significantly decreased by 29.65%, 42.89%, and 50.38% from the initial value, respectively ($p < 0.001$; Table 2). That for the PW coral was significantly decreased by 13.13%, 16.38%, and 39.40% from the initial, respectively ($p < 0.001$; Table 2).

The alpha ($\alpha$) values of the MT and PW corals on Day 1 were 0.17 ± 0.01, n = 4, and 0.18 ± 0.01, n = 4, respectively (Figure 4a,b). There were no significant differences by time × site × temperature × site, time × temperature × light, and site × temperature × light ($p = 0.592$, 0.414, and 0.658, respectively). However, there were significant differences by time × temperature ($p = 0.004$), suggesting temperature affects alpha at different times, regardless of colony location and light level (Table 2). There were significant differences by site ($p = 0.002$), temperature and light ($p = 0.006$ and $p = 0.001$, respectively), and by time ($p < 0.001$) (Table 2). The alpha ($\alpha$) values of the PW coral in the ATAL and ATHL treatments were higher than of those of the MT coral on Day 8. A decrease in $\alpha$ occurred in both the MT and PW corals. On Day 8, the $\alpha$ of the MT coral in ATAL, ATHL, HTAL, and HTHL treatments was significantly decreased by 22.22%, 47.45%, 17.73%, and 50.75% from the initial value, respectively ($p < 0.001$; Table 2); the $\alpha$ of the PW corals significantly decreased by 10.74%, 24.30%, 29.30%, and 51.83% from the initial value, respectively ($p < 0.001$; Table 2). Comparing the end of the experiment (Day 14) with the last day of increasing the temperature (Day 8) showed that only the PW coral in the HTHL treatment had an increase in $\alpha$ (18% from the end of the stress) (Figure 4a,b).

Table 1 of the MT and PW corals was 196.76 ± 8.45 µmol photons $m^{-2}$ $s^{-1}$, n = 4, and 184.84 ± 9.34 µmol photons $m^{-2}$ $s^{-1}$, n = 4, respectively (Figure 4c,d). There were no significant differences by time × site × temperature × site, time × temperature × light, and site × temperature × light (p = 0.236, 0.572, and 0.051, respectively). However, there were significant differences by time × temperature and site × temperature ($p < 0.001$ and $p = 0.014$, respectively). These results suggested that temperature affected the corals from PW and MT differently at different times (Table 2). The $I_k$ of MT coral was higher than that of PW coral on Day 8 in the ATAL, ATHL, and HTHL treatments and the opposite was true for HTAL. At the end of the experiment, $I_k$ of the PW coral was higher than that of the MT coral in all the treatments except for ATAL. On Day 8, there were decreases in $I_k$ in both MT and PW corals. The MT coral in the HTAL treatment had decreased by 58.44% from the initial value, while the PW corals in the ATAL, ATHL, HTAL, and HTHL treatments had decreased by 48.10%, 27.98%, 39.19%, and 57.07% from the initial value, respectively ($p < 0.001$; Table 2). On comparing the end of the experiment (Day 14) with the last day of increasing temperature (Day 8), it was observed that the MT coral in all the treatments had a decreasing $I_k$ except for that in the ATAL treatment, while the PW coral in ATAL, ATHL, and HTHL treatments showed increases of 49.99%, 48.85%, and 83.48% from Day 8, respectively ($p < 0.001$) (Figure 4c,d).

The maximum relative electron transport rates ($rETR_{max}$) of the MT and PW corals on Day 1 were 33.39 ± 0.01 µmol electrons $m^{-2}$ $s^{-1}$, n = 4, and 34.03 ± 1.87 µmol electrons $m^{-2}$ $s^{-1}$, n = 4, respectively (Figure 4e,f). There were no significant differences by time × site × temperature × site, time × temperature × light, and time × light ($p = 0.282$, 0.311, and 0.432, respectively). However, there were significant differences by time × temperature and site × temperature ($p < 0.009$ and 0.014, respectively). These results suggested that temperature affected the corals from PW and MT differently at different times (Table 2). On Day 8, a decrease in $rETR_{max}$ occurred in both the MT and PW corals in all the treatments. The $rETR_{max}$ of the MT corals significantly decreased by 24.88%, 48.63%, 78.42%, and 60.92% from the initial value in the ATAL, ATHL, HTAL, and HTHL treatments, respectively ($p < 0.001$), while the $rETR_{max}$ of the PW corals significantly decreased by 53.68%, 43.23%, 57.19%, and

81.65% from the initial value in the ATAL, ATHL, HTAL, and HTHL treatments, respectively ($p < 0.001$; Table 2). There was no sign of recovery on Day 18 when compared with Day 8 (Figure 4e,f).

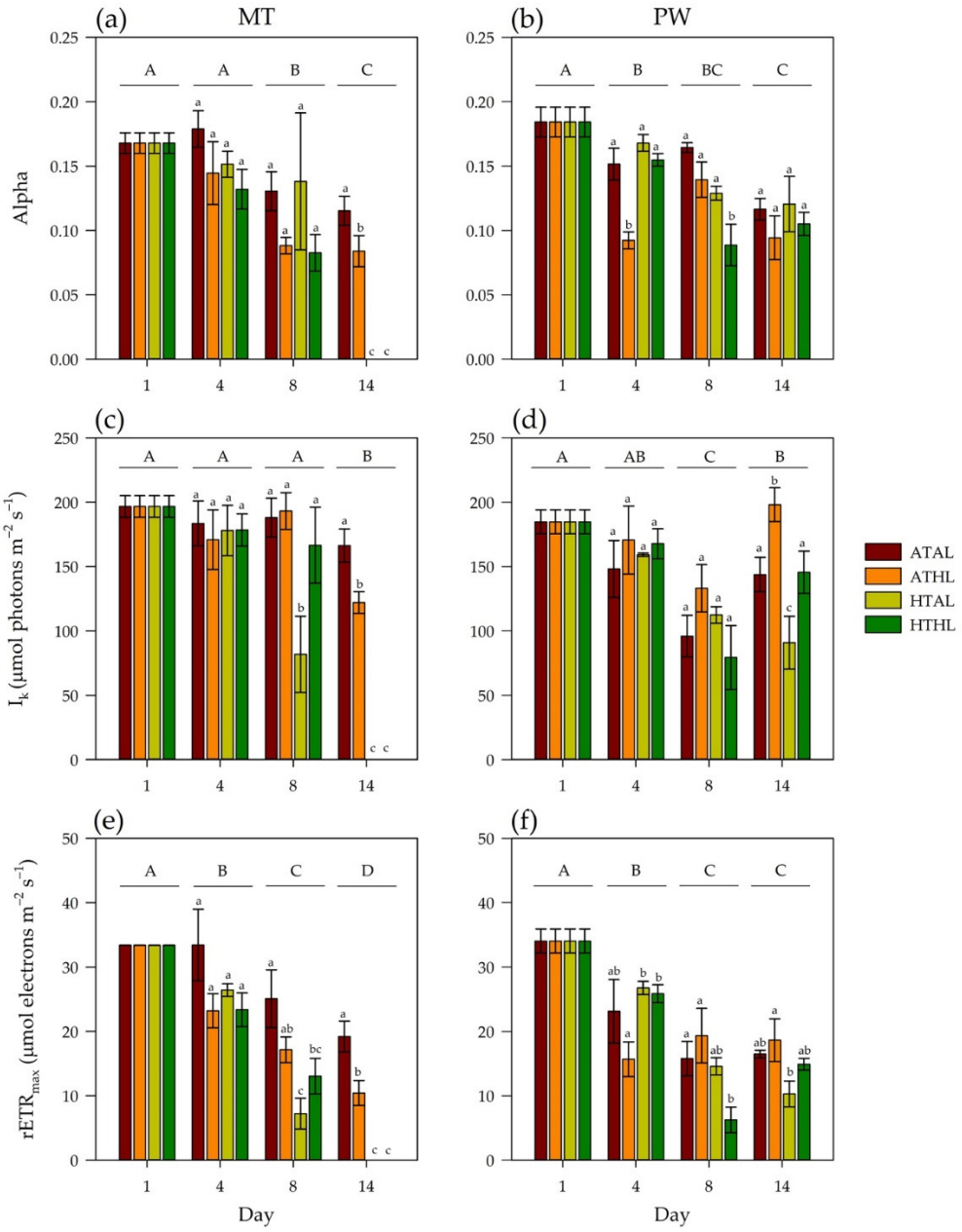


**Figure 4.** Alpha (**a**,**b**), minimum saturating irradiance (**c**,**d**), and maximum relative electron transport rate (**e**,**f**) of *P. acuta* from MT and PW at each sampling time. Data are presented as mean ±SE (n = 4). A, B, C, D and a, b, c, d indicate significant differences by time and by treatment, respectively.

### 3.2. Symbiodiniaceae Density and Pigment Contents

Symbiodiniaceae densities of the MT and PW corals on Day 1 were 1.46 ± 0.43 ($\times 10^6$) cells $cm^{-2}$, n = 4, and 1.24 ± 0.43 ($\times 10^6$) cells $cm^{-2}$, n = 4, respectively (Figure 5a,b). There were no significant differences by time × site × temperature × light, time × temperature × light, and site × temperature × light ($p = 0.368$, 0.457, and 0.417, respectively (Table 2). However, there were significant differences by time × temperature ($p < 0.001$), regardless of where the corals were from (Table 2). The progressive decrease with time in Symbiodiniaceae was found in both PW and MT corals, but it was more severe in MT corals. On Day 8, Symbiodiniaceae density of the MT coral in the HTAL and HTHL treatments had decreased

by 48.25% and 81.41% from the initial value (Day 1), respectively, and the Symbiodiniaceae density of the PW coral had decreased only in the HTAL cases by 30.11% from the initial value (Day 1). At the end of the experiment (Day 14), the Symbiodiniaceae density for all treatments of the MT and PW corals was significantly lower than the initial value.

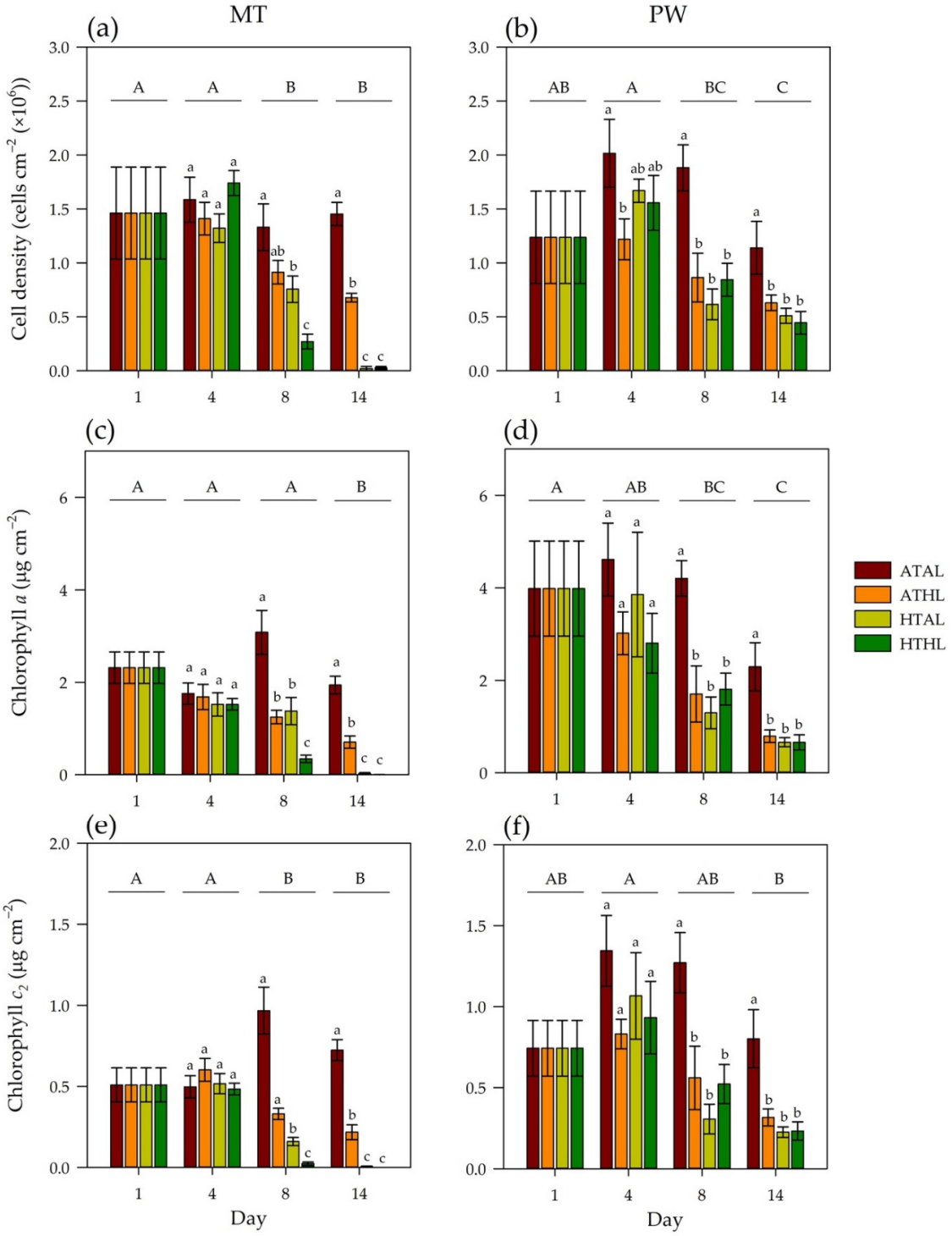


**Figure 5.** Symbiodiniaceae cell density (**a**,**b**), chlorophyll *a* (**c**,**d**), and $c_2$ (**e**,**f**) of *P. acuta* from MT and PW at each sampling time. Data are presented as mean ±SE (n = 4). A, B, C, D and a, b, c, d indicate significant differences by time and by treatment, respectively.

There were significant differences by temperature × light in Symbiodiniaceae density where we observed lower density in corals kept in high light intensity than those in ambient light. In addition, at the end of the experiment (Day 14), the Symbiodiniaceae density of the MT coral in ATHL, HTAL, and HTHL was significantly lower than that on Day 8, while this only occurred in the HTHL case with the PW coral. From the start until the end of the experiment, the MT coral in HTAL and HTHL had the greatest decrease, while the PW coral in all the treatments except for ATAL had a similar decrease in density by the end of the experiment (49.10%, 58.71%, and 63.98% for ATHL, HTAL, and HTHL, respectively) (Figure 5a,b).

The chlorophyll *a* concentration at the initial time was 2.32 ± 0.34 μg $cm^{-2}$, n = 4, and 3.98 ± 1.03 μg $cm^{-2}$, n = 4, for the MT and PW corals, respectively (Figure 5c,d). There were no significant differences in chlorophyll *a* concentration by time × site × temperature × light, time × temperature × light, site × temperature × light, time × temperature, and time × light ($p$ = 0.633, 0.294, 0.267, 0.107, and 0.229, respectively) (Table 2). However, there was a significant difference by temperature × light in chlorophyll *a* concentration ($p$ = 0.011), suggesting that both PW and MT corals responded to temperature and light differently, where high light intensity had more effect on the chlorophyll *a* concentration than temperature, regardless of the time of sampling.

On Day 8, the chlorophyll *a* in ATHL, HTAL, and HTHL had declined from the initial value by 46.14%, 40.69%, and 85.16% for the MT coral, and by 57.22%, 67.53%, and 54.63% for the PW coral, respectively ($p$ < 0.001; Table 2). On Day 14, the Chl *a* of the MT and PW corals had significantly decreased from Day 8 in all the treatments. The HTAL and HTHL treatments of the MT coral resulted in the lowest Chl *a* concentration, while the PW coral in the ATHL, HTAL, and HTHL treatments had similar Chl *a* concentrations, lower than the concentration for ATAL (Figure 5c,d).

The chlorophyll $c_2$ concentrations at the initial time were 0.51 ± 0.11 μg $cm^{-2}$, n = 4, and 0.74 ± 0.17 μg $cm^{-2}$, n = 4, for the MT and PW corals, respectively (Figure 5e,f). There were no significant differences in chlorophyll $c_2$ concentration by time × site × temperature × light, time × temperature × light, and time × light ($p$ = 0.147, 0.177, and 0.250, respectively) (Table 2). However, there were significant differences by time × temperature, temperature × light, and site × temperature × light ($p$ = 0.006, 0.010, and 0.046), suggesting that the chlorophyll $c_2$ concentration was more severely affected by light than temperature, especially in MT corals.

### 3.3. Bleaching Mortality Indices (BMIs)

The BMI of the MT coral in all the treatments was higher than for the corresponding treatment of the PW coral, except for the ATAL treatment. The highest BMI was observed with the HTHL treatment, followed by HTAL, for MT coral, which started to respond on Days 3 and 5 of the experiment, respectively, while the PW corals in HTHL and HTAL started to respond on Day 5 (Figures 6 and 7). The combination of temperature and light induced MT corals to bleach and die in all the treatments. On the other hand, the PW coral was bleached and had a 60% death rate (Figures 6 and 7).

### 3.4. Growth Rates

The coral growth rates were measured for the MT and PW corals as percentages per day. There were significant differences by site ($p$ < 0.001) and temperature ($p$ < 0.001) (Table 3). A significant decrease in growth rate was found in the HTAL and HTHL treatments for both the MT and PW corals. Comparing the sites showed that the PW coral had significantly higher growth rates than the MT coral in all the treatments (Figure 8).

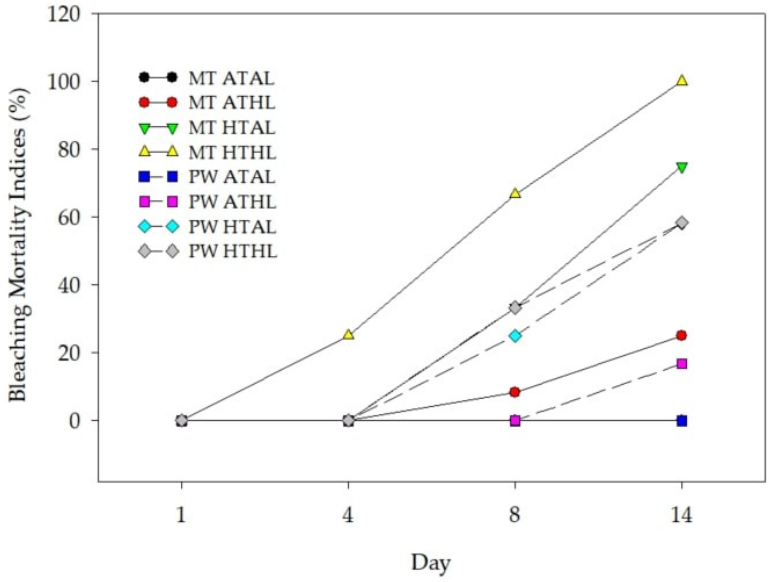


**Figure 6.** Bleaching Mortality Indices (BMIs; %) of *P. acuta* from MT and PW at each sampling time (n = 4).

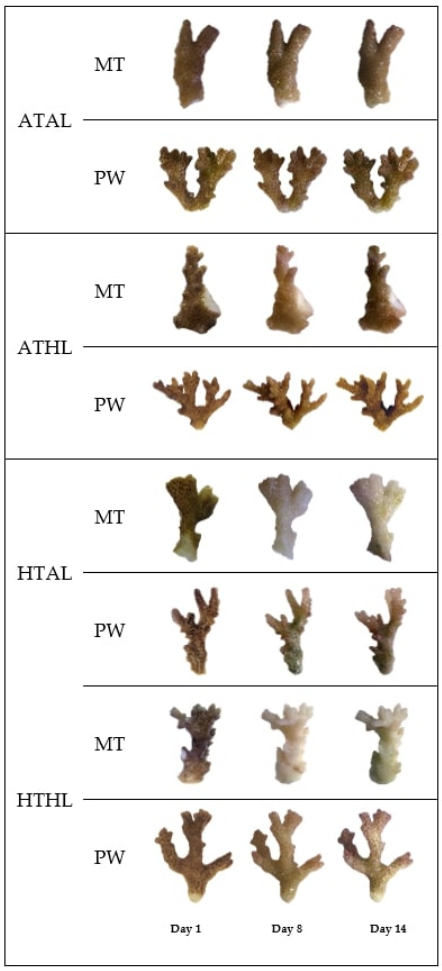


**Figure 7.** Coral nubbins in ATAL, ATHL, HTAL, and HTHL treatments from MT and PW at initial time (Day 1), last day of increasing temperature (Day 8), and end of the experiment (Day 14).

**Table 3.** Statistical indices of growth rate (three-way ANOVA).

| Factor | Growth Rate | | |
|---|---|---|---|
| | df | F | *p* |
| site | 1 | 20.325 | <0.001 * |
| temperature | 1 | 23.628 | <0.001 * |
| light | 1 | 0.032 | 0.859 |
| site × temperature | 1 | 2.388 | 0.135 |
| site × light | 1 | 0.038 | 0.847 |
| temperature × light | 1 | 0.388 | 0.539 |
| site × temperature ×light | 1 | 0.719 | 0.405 |
| error | 24 | - | - |

*, significant difference; -, absent.

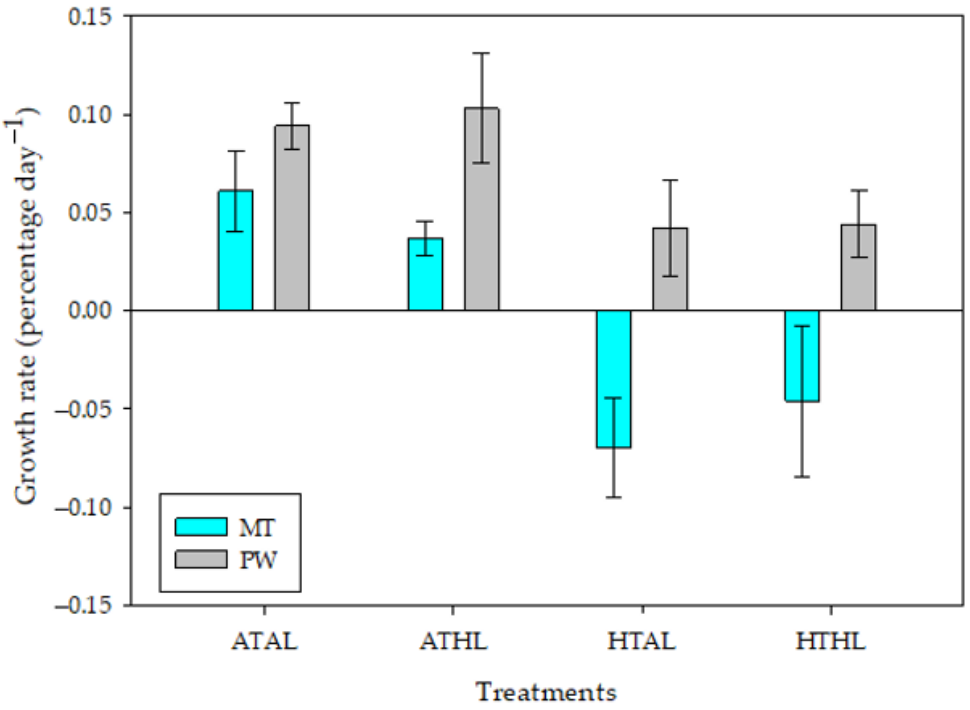


**Figure 8.** Growth of *P. acuta* from MT and PW. Data are presented as mean ±SE (n = 4).

## 4. Discussion

*P. acuta* corals from an offshore (MT) and an inshore reef (PW) were collected and maintained in an indoor aquarium system implementing four treatments, namely ATAL, ATHL, HTAL, and HTHL, to investigate the combined effects of elevated temperature and light intensity on photosynthetic capacity and growth. The results showed that the combination of elevated temperatures and a high level of light had the greatest effect on photosynthesis and growth, followed by elevated temperatures only and then by high light intensity only.

The photosynthetic performance ($\Delta F/F_{m'}$, $rETR_{max}$, $I_k$, and $F_v/F_m$) of *P. acuta* showed differences among the four treatments on Day 8 (the last day of increasing temperature). Elevated temperature induced the downregulation of photosynthesis, as shown by the decreased $F_v/F_m$. Both the MT and PW corals in the elevated temperature treatments presented greater decreases in $F_v/F_m$ than those in other treatments. This result is consistent with Yucharoen et al. [6] in which the $F_v/F_m$ of *P. acuta* and *P. lutea* was reduced by elevated-temperature treatments (32 °C and 34.5 °C). When comparing the ATHL and HTAL treatments on Day 8 (the last day of increasing temperature), it was observed that a greater decline in photosynthetic efficiency occurred with an elevated-temperature treatment when combined with a high light intensity treatment. At the end of the experiment,

after decreasing the temperature from Day 9 to Day 14, both the MT and PW corals showed signs of recovery with no decrease in $F_v/F_m$. These results revealed that high light intensity did not affect coral health as much as high temperature did, and when the temperature returned to normal, the photosynthesis of the symbiont could recover to harvest light and maintain a positive carbon balance. However, high light intensity played a role in the recovery of these corals as we observed that corals kept at high light showed a progressive decrease in $F_v/F_m$ and $\Delta F/F_m$ (Figure 3). This is consistent with Gustafsson et al. [46], who presented a model for the rate of bleaching that depended on the temperature, light intensity, and rate of heterotrophic feeding, and found a clear decrease in maximum quantum yield ($F_v/F_m$) and cell numbers when the coral was exposed to elevated temperature. On the other hand, heat stress might increase the metabolic energy demand of the coral host, leading to energy limitation, altering symbiotic nutrient cycling, and inducing breakdown of coral–algal symbiosis [47].

Regarding the Symbiodiniaceae density and pigment contents, there were similar trends of a dramatic decrease in photosynthetic efficiency, consistent with the Bleaching Mortality Indices (BMIs). Upon comparing the last day of increasing temperature (Day 8) with the initial time, we found that the MT coral had the largest decrease in Symbiodiniacea density and pigment contents, and the largest increases in BMI in the HTHL treatments, followed by HTAL and ATHL. This indicates the synergistic effects of elevated temperature and light on MT coral, followed in magnitude by temperature only and light only, respectively. This led to greater bleaching susceptibility in MT corals, indicating dependence on habitat and irradiance [17]. The synergistic effects of temperature and light are locality- and species-specific [48]. Rosic et al. [21] found that *S. pistillata* was negatively affected by a synergistic effect of temperature and high irradiance, while *Acropora millepora* was more thermally sensitive at a severely low light intensity. Upon comparing a single factor, Gustafsson et al. [46] found a decreased cell number in the corals at the Great Barrier Reef when the coral had been exposed to elevated temperature in a model in which the rate of bleaching is dependent on the temperature, light intensity, and rate of heterotrophic feeding. Furthermore, Rodolfo-Metalpa et al. [49], upon comparing *Cladocora caespitosa* and *Oculina patagonica* under normal and elevated temperatures, found that the growth rate, photosynthetic efficiency ($F_v/F_m$), relative electron transport rate (ETR), Symbiodiniaceae, and chlorophyll (Chl) contents were severely decreased at elevated temperatures. On the other hand, there was no significant difference among ATHL, HTAL, and HTHL in PW coral, suggesting that temperature and light had minor effects on the Symbiodiniaceae density of the PW coral. There was no sign of recovery in the MT coral in the HTAL and HTHL treatments when the temperature was decreased from Day 9 to Day 14, suggesting that these corals lack the ability to recover in the short term (6 days), and the recovery of corals might depend on their light and temperature history and local environment, e.g., habitat, disturbances, and irradiance [28]. Different responses to and recovery from heat stress were also observed among sites (closer to and further away from mainland Singapore) [28]. Nakamura et al. [50] found that the recovery of cell density and chlorophyll *a* concentration of *S. pistillata* increased rapidly in moderate flow treatments (of 20 cm $s^{-1}$), after an initial 3 weeks of stasis. Moreover, Thomas and Palumbi [51] found that in *A. hyacinthus* after a natural bleaching event, the transcriptome remained largely perturbed for at least six months after the temperatures had cooled, and for four months after the Symbiodiniaceae populations had recovered.

The coral growth rates differed by temperature treatment (contrasting ATAL and ATHL, and HTAL and HTHL), and there was no difference between ambient and high light intensity treatments. This indicates that temperature was the main factor determining coral growth in this experiment. Coral metabolism is related to ambient temperature. Elevated temperatures induced the downregulation of photosynthesis in symbiotic dinoflagellates [52,53], increased the coral respiration rate, and caused excessive dissolved $CO_2$, which led to a decreased pH in the microenvironment. These processes indirectly affected coral growth by inducing Symbiodiniaceae dysfunctions and by reducing the

alkalinity, which affected coral calcification [54,55]. Thus, there are different experiences for the corals in each reef and this induced them to have different adaptive capacities. It has also been found that skeletal growth of the coral *P. lutea* in Phuket was reduced due to a gradual increase in temperature from 1984 to 1986 and from 2003 to 2005 [56]. Our study supports the concept that future warming will lead to a reduction in coral growth.

The adaptive capacity was assessed via $\alpha$ and $I_k$ for both the MT and PW corals. The decline in $\alpha$ in the MT and PW corals might suggest coral's symbiont adaptation. In the high-light regime, Symbiodiniaceae responded by the expulsion of their symbiont or by reducing their chlorophyll concentrations to prevent photodamage [57] which might affect $\alpha$. At the end of the experiment (Day 14), $\alpha$ showed significant differences by site and treatment, indicating that corals from different regimes (MT and PW) might have different abilities to adapt to light [58]. The maximum saturating irradiance ($I_k$) showed a higher adaptive capacity for PW coral with changes in $I_k$ for all the treatments, which decreased on Day 8 and increased at the end of the experiment (Day 14). This reveals that PW corals can adapt to live in a high-light regime, although PW coral was familiar with high turbidity [59] and inshore extreme conditions [60] at Panwa Cape and a high sediment accumulation rate in the rainy season. On the other hand, the MT corals could not adapt in this experiment, and they were susceptible to anomalous light and temperature exposure, because of the low turbidity in their environment and fairly stable temperature experiences, which led to the MT corals being more susceptible to heat stress. It has been shown that temperature threshold and resilience for coral bleaching vary with local environmental conditions and background climate conditions [61]. Moreover, the adaptive capacity and thermal tolerance in the PW coral might also have arisen from the gene regulation in endosymbionts, which might be upregulated in the inshore extreme conditions [62]. In an urbanized reef system, gene regulation by the endosymbionts plays a key role in maintaining the health and function of the coral host, and leads to the persistence of *P. acuta* in Singapore's urbanized reef [62]. Gene regulation in MT and PW corals should be studied in the future.

Consequently, our findings indicate that the synergistic effects of elevated temperature and high light intensity had the largest impact on the physiological responses and photosynthetic performance of corals *P. acuta*, followed by temperature alone and light alone. Furthermore, PW coral facing an extreme environment in the long term might be able to adapt, and be more tolerant to heat stress and less susceptible to bleaching [59,60]. This can lead to changes in biodiversity and community structure in the reefs as *P. acuta* is the dominant reef-building species and is a common species in Thai waters [23,63], and negative effects on this species might affect Maiton Reef and related fauna and flora. While *P. acuta* at PW was more tolerant to anomalous conditions, the effects from climate change with elevated temperature and high light intensity might reduce the primary production in Panwa Reef.

## 5. Conclusions

This study showed that light and temperature have different effects on Symbiodiniacea photosynthesis and coral growth rates, with differences between corals from different regimes. This study provides an improved understanding of synergistic effects and how corals in different regimes respond to the main stressors, which should benefit bleaching responses and coral reef management plans.

## 6. Future Work

Due to the frequent decolorization of coral reefs, the responses of corals from different regimes to the main stressors of light and temperature are of importance. This study revealed that an elevated temperature had a stronger effect than high light intensity. Moreover, the combination of light and temperature synergistically affected the growth of corals and their endosymbiont's photosynthesis, which led to the destruction of coral reefs. Rapid and non-destructive assessment is important for evaluating the statuses of coral and coral reef. Symbiotic algae in the "Symbiodiniaceae" family provide about 90%

of a coral's energy. Hence, the health and status of coral and coral reefs can be estimated from the photosynthetic efficiency of photosystem II of Symbiodineaceae, which can be assessed by the chlorophyll *a* fluorescence measured using a pulsed amplitude modulated (PAM) fluorometer [38,64].

The results of this study can be used for marine and coastal management planning in a restricted area, in which elevated temperature impacts coral growth and survival more than high light intensity does. Reducing a coral's stress and increasing its chances of recovery when the temperature returns to normal should be pursued. MT should receive priority as a restricted area due to the coral in this area being more susceptible to stressors than the Panwa coral.

Diverse coral genetics can induce various responses to stressors, so four replicates might not be sufficient to represent the responses of MT and PW coral reefs. Hence, we suggest that, in studies on responses to stressors, there should be at least six replicates, to provide greater accuracy.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data are available upon request to the corresponding author of this article.

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