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Article

Differing Responses of Three Scleractinian Corals from Phuket Coast in the Andaman Sea to Experimental Warming and Hypoxia

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Abstract: An unprecedented decline in the diversity and health of coral reefs is occurring around the world as they are threatened by multiple global and local stressors. Rising seawater temperature and low dissolved oxygen (DO) conditions are expected to intensify as a result of climate change. Understanding the responses of corals to these stressors is necessary for making predictions and devising mitigation strategies. The three coral species—*Porites lutea*, *Montipora tuberculosa*, and *Pocillopora verrucosa*—were sampled from Patong Bay, Phuket, Thailand, as representatives of different coral morphologies. Coral nubbins were subjected to experimental investigation under ambient conditions (29 °C, DO > 6 mgL^{-1}), heat stress (32 °C), hypoxia (DO < 2 mgL^{-1}), and heat stress + hypoxia treatments. Photosynthetic performance indicators Fv/Fm and Fv/F0 and physiological parameters *Symbiodiniaceae* density, pigment concentration, and growth rate were quantified. We found *P. verrucosa* (branching) to be the most sensitive and severely affected by heat stress or hypoxia, more so than *P. lutea* (massive) and *M. tuberculosa* (tabular). The combination of these stressors had less impact on these species, except for a decline in growth rate of *M. tuberculosa*. This study also suggests that the corals respond differently to high temperature and low oxygen, with their sensitivity depending on species. These responses, however, may differ according to the lighting, especially in hypoxic conditions. The results fill a research gap to help predict the vulnerability of these three coral species in shallow reef habitats under climate change scenarios.

Keywords: climate change; heat stress; hypoxia; coral photosynthesis; coral ecophysiology

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1. Introduction

The ocean absorbs over 90% of the total heat accumulation in the Earth's climate system, which has increased rapidly over the past several decades due to greenhouse gas emissions, leading to ocean warming [1–5]. An estimated 1–3 °C increase in sea surface temperature (SST) by the year 2100 is now a likely scenario [6]. The occurrence and extent of marine heatwaves and temperature anomalies are also changing as a result of global warming [7–10]. This increase in ocean temperatures can affect the habitat, population, migration, and breeding patterns of marine plants, animals, and microbes as well as threaten the building blocks of ocean life such as corals [7,11]. Heat stress (as well as ocean warming and heat waves) has been correlated with the increase in frequency and scale of mass coral bleaching events since the late 1990s, when the first evidence of mass bleaching was recorded after a series of El Niño events [12]. At the mechanism level, heat stress raises the metabolic energy requirement of the coral host, altering the nutrition cycle, and this has been shown to be the key cause for functional breakdown of symbiotic algae in corals [1], leading to a decline in calcification processes [13,14]. The response of corals to heat stress

also depends on their morphology, as small encrusting colonies are more likely than big branching colonies to continue mass transfer and hence to endure temperature stress. The volume of space between colony branches also predicts their reaction to temperature stress, and as the frequency and intensity of heat stress rise, huge branching coral colonies may give way to tiny colonies and flat-massive colonies with low aspect ratios [15]. There is growing evidence to also suggest that a recent increase in coral disease outbreaks can be attributed primarily to heat stress, among other local factors [16].

Corals reefs are facing an unprecedented threat from heat stress, hypoxia, acidification, and other scenarios as a result of anthropogenic activities and climate change, leading to stress and decline of several reef-building corals [2,17–20]. Some simulations and downsized climate models applied on eutrophic and seasonally hypoxic estuaries or coastal areas predict a reduction of dissolved oxygen (DO) by 1–2 mgL^{-1} and a 10–30% increase in hypoxic and anoxic volumes by the mid-21st century. These reductions are primarily attributed to the decreasing solubility as a result of ocean warming [21,22]. An overall decrease of 2–4% in oceanic oxygen reserves is also projected based on the range of different climate change scenarios [6,23]. In recent years, several coral bleaching-induced mass mortality events have been observed in large areas with low oxygen (hypoxia/anoxia) and indicate that oxygen level can also be a critical factor impacting the survival of coral reefs. Nevertheless, the exact effects of periodic or persistent hypoxia on coral reefs are currently unpredictable [24,25]. Tropical reefs in particular are estimated to be at higher risk from localized hypoxia [24], as reefs with inherently restricted water movement, such as atolls, tidal pools, semi-enclosed lagoons, and fringing reef flats, have regular occurrences of low DO concentration (2–3 mgL^{-1}), and this condition is compounded by the increase in metabolic rates and episodic nutrient flushing due to tidal and seasonal warming [26–30].

These stressors can promote micro- and macroalgal blooms [31,32], further deteriorating the situation as the capacity to buffer deoxygenation through photosynthetic oxygen production by coral's *Symbiodiniaceae*, macroalgae, and phytoplankton is also limited due to reduced light levels caused by turbidity [33]. Corals have been noted as being more susceptible to low oxygen than other marine organisms [6] as hypoxia can lead to reduced photosynthesis and calcification, inducing bleaching mortality in extreme cases [24,25]. It can also promote diseases such as black band disease (BBD), which creates a high concentration of sulfides that kill the underlying coral tissue [24,34–36].

Global warming and local eutrophication also lower the DO (by reducing the solubility and saturation) and increase the biological oxygen demand of most marine organisms, worsening the effect. This stress caused by low DO concentrations and relatively high temperatures is an energy challenge for most marine organisms' metabolisms as they face frequent exposure to oxygen below their optimum functioning thresholds [32,37–40]. In isolation, hypoxia may be more dangerous to corals than other stressors, and there are also some studies showing that the combined effects of hypoxia and other stressors can be more damaging than their individual effects [41,42]. However, most of the research and studies on corals affected by hypoxia have reported field observations as a response to bleaching or mass mortality events [43], and the exact effects of multiple stressors in relation to hypoxia on coral physiology are currently unknown.

The coral reefs at our study site of Patong Bay, located on the west coast of Phuket, Thailand, have been subjected to excessive nutrient enrichment due to the rapid increase in tourism activities since the 1980s [44,45]. As a result, the reefs are not only vulnerable to heat stress, with a reported annual seawater temperature range of 29–32 °C [14,46], but may also be at risk from co-occurring episodes of hypoxia as algal blooms have been reported periodically in this area [44]. The aim of this study was to contribute to understanding the individual and combined (additive, synergistic, or antagonistic) effects of heat stress and hypoxia on three reef-building corals, *Porites lutea*, *Montipora tuberculosa*, and *Pocillopora verrucosa*. The results obtained in this study provide insights into the impacts of ocean warming and deoxygenation, to support projections of reef biodiversity under a changing climate.

2. Material and Methods

2.1. Sample Collection and Acclimation

In April 2021, three coral species representing different growth forms, namely *P. lutea*, *M. tuberculosa* and *P. verrucosa*, were selected for sampling based on their abundance in the reef edge at Patong Bay, Phuket, Thailand, 7°53′31.3″ N 98°15′56.8″ E (Figure 1A). The healthy coral colonies (no stress, bleaching, compromised tissues, or diseases) were investigated. Four source colonies of each coral species were selectively sampled using a hammer and chisel, from a depth of 5–6 m. All coral colony samples were transferred with natural seawater to the aquarium facility of the Coastal Oceanography and Climate Change Research Center (COCC), Prince of Songkla University, within 12 h of collection.

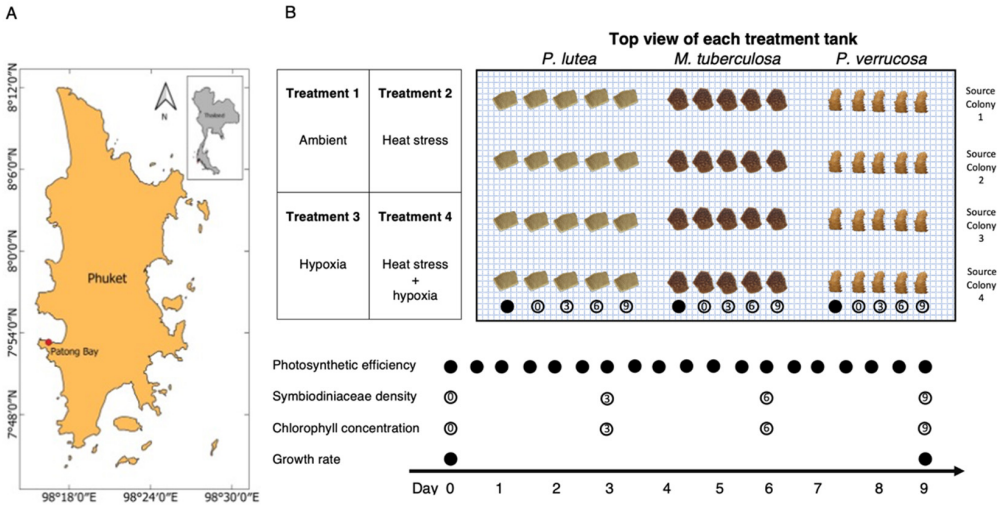

Figure 1. (**A**) Sampling site at Patong Bay, Phuket, Thailand. (**B**) Design of experiments and placement of coral nubbins (20 nubbins/species/tank) in the four treatments. The nubbins in the first row (black dots) were used for photosynthetic and growth rate measurements, while the next rows were used for destructive sampling of *Symbiodiniaceae* and chlorophyll analysis on days 0, 3, 6, and 9.

All the coral colonies were allowed to acclimate in a 600 L holding tank fitted with recirculating pumps (WP-300M, SOBO, Zhongshan, China and AT-107, Atman, Zhongshan, China), a heater chiller (CS-160CIRV1, Atman, Zhongshan, China), COB light (TS-A600, aquarium lamp, Shandong, China), and LED light (A601, Chihiros, Ningbo, China) for a week at 29 °C temperature, 32 ppt salinity, pH 8.2, 12:12 h light:dark cycle at 150 µmol photons $m^{-2}s^{-1}$, and 6.45 mgL^{-1}> DO > 6.04 mgL^{-1}, similar to the conditions recorded at the sampling site (Supplementary Table S1). After one week, the coral colonies were cut into nubbins of 3–5 cm diameter and transferred into 62 L treatment tanks (20 nubbins/species/tank) with the same conditions and equipment as that of the holding tank, except for the models of water pumps (AP-1200, SONIC, Zhongshan, China) and heater chiller (JMC-02, JBA, Zhongshan, China). Each replicate was allocated from each coral colony and assigned for a specific sampling (Figure 1B). The coral nubbins were then allowed to acclimate for an additional week in the experiment tanks. Temperature, DO, pH, and salinity of the seawater were monitored every day throughout the experiment. Partial seawater changes (20%) were conducted every 3 days. Prior to seawater replacement, nitrate and ammonia concentrations of the seawater in each tank were measured using an aquarium test kit (Salifert Profi-Test®, Duiven, The Netherlands).

2.2. Experimental Design

After acclimation in treatment tanks, temperature and DO concentration were modified as follows:

- Treatment 1 (ambient conditions), 6.45 mgL^{-1}> DO > 6.04 mgL^{-1} and temperature 29 °C.

- Treatment 2 (heat stress), 6.45 mgL^{-1}> DO > 6.04 mgL^{-1} and temperature 32 °C.
- Treatment 3 (hypoxia), 2 mgL^{-1} > DO > 1.75 mgL^{-1} and temperature 29 °C.
- Treatment 4 (heat stress + hypoxia), 2 mgL^{-1}> DO > 1.75 mgL^{-1} and temperature 32 °C.

The 2 mgL^{-1} DO concentration is generally defined as hypoxia in marine ecosystems [6] and therefore was set to represent hypoxic condition. Similarly, the annual seawater temperature range of 29–32 °C has been reported in Phuket [14,46]. As a maximum of 32.45 °C sea surface temperature was recorded in the central part of the Bay of Bengal for over a month in 2010 [47], and a warmer scenario is predicted for the future [48,49], 32 °C was chosen for heat stress. The ambient DO and temperature were determined from field measurements (Supplementary Table S1) and the long-term sea surface temperature of Patong Bay, Phuket (Supplementary Figure S1).

Constant temperature was maintained by the heater chiller (JMC-02, JBA, Zhongshan, China) while DO concentration was maintained by carefully controlling the flow of air (from air pumps) and nitrogen gas (from N_2 tanks) using multiple flow controllers. The high pressure from N_2 tanks was reduced to a pressure slightly above ambient in 2 steps by connecting the nitrogen high-pressure regulator (IM-TECH, Zhejiang, China) to an air compressor pressure regulator and a switch control valve (Xcpc, Zhejiang, China). Aquarium flow control valves with air stones were also used to further control the amount of air and N_2 being introduced into each treatment tank. A moderate water flow within the tank was generated with recirculating pumps (AP-1200, SONIC, Zhongshan, China). DO was measured at frequent intervals using the YSI ProDSS Multiparameter (Xylem Inc., Ohio, USA). The parameters were mostly stable except for a slightly elevated pH in hypoxia and heat stress + hypoxia treatment tanks as a result of N_2 bubbling [50,51] (Supplementary Table S2).

The experiment was run for nine days, and the photosynthetic efficiency was measured every day at 08:45 and 21:30 for dark-adapted coral nubbins. The light was set on a schedule to switch on and off every day at 09:00 and 21:00, respectively. Destructive sampling was conducted on days 0, 3, 6, and 9 (refer to Figure 1B) at 10:00 in the morning. Each nubbin was snap-frozen using liquid nitrogen and stored at −80 °C for *Symbiodiniaceae* density and chlorophyll concentration analysis. Buoyant weight measurements were taken on days 0 and 9 to determine coral growth rate.

2.3. Measurement Protocols

2.3.1. Photosynthetic Efficiency

Coral–*Symbiodiniaceae* photosynthetic performance was assessed by measuring the maximum quantum yield (F_v/F_m) and the photochemical efficiency (F_v/F_0) of Photosystem II (PSII), where F_0 represents minimum fluorescence, F_m represents maximum fluorescence of dark-adapted sample after a saturating pulse is applied, and F_v ($F_m - F_0$) is the variable fluorescence. The measurements were taken at three random points on the coral tissue surface for each replicate using a Diving-PAM fluorometer (Walz GmbH, Effeltrich, Germany) connected to a 6 mm diameter fiber-optic probe. The PAM intensity of measuring light (MEAS-INT) was set at 5, electronic signal gain (GAIN) at 2, saturation pulse intensity (SAT-INT) at 8, and width of saturating light pulse (SATWIDTH) at 0.6 s. The measurements were taken twice a day, before the light was turned on (08:45) and after the light was turned off (21:30).

2.3.2. Symbiodiniaceae and Chlorophyll Content

The coral nubbins (n = 4) were collected for destructive testing on days 0, 3, 6, and 9, for *Symbiodiniaceae* density and chlorophyll analysis. The frozen nubbins were blasted with 50 mL of artificial seawater (35% NaCl solution) to remove the coral tissue. The slurry was homogenized for 10 min at 1500 rpm and 4 °C temperature to obtain a well-mixed sample [52]. Then, 1 mL of each suspension was taken for counting *Symbiodiniaceae* cells with a hemocytometer, under a light microscope with 40× magnification. The remaining sample from *Symbiodiniaceae* counting was centrifuged at 4,000 rpm for 5 min to separate

Symbiodiniaceae cells from the coral tissue. The supernatant containing coral tissue was discarded, and the *Symbiodiniaceae* pellet was collected, 3 mL of 90% acetone was added to it, and it was kept at 4 °C for 24 h. The algal pellet was centrifuged again, and the chlorophyll *a* and c_2 concentrations were determined using the standard spectrophotometric method [53] with absorbance measured at 630, 664, and 750 nm [54].

Each coral skeleton was bleached in 10% sodium hypochlorite and washed several times before measuring the surface area using a modified paraffin wax dipping method [55,56]. The *Symbiodiniaceae* cell density and chlorophyll concentration were normalized as number of individual cells per cm^2 and μg per cm^2, respectively.

2.3.3. Growth Rate

The buoyant weight technique was used to determine coral growth rate [57,58] in a nondestructive manner. The same coral nubbin was weighed initially and at the end of experiment using a 4-digit precision balance (Ohaus, New Jersey, USA). During each measurement, both temperature and salinity were recorded for the calculation of seawater density, and a glass reference was weighed in both sea water and air [58]. The skeleton bulk densities used for *P. lutea, M. tuberculosa, and P. verrucosa* were 1.41, 1.58, and 2.93 g cm^{-3}, respectively [59–61]. Coral growth rates were expressed as net increases in biomass (%) per day.

2.3.4. Statistical Analysis

The data for all parameters were tested for normality using Shapiro–Wilk test. Sphericity was tested for F_v/F_m and F_v/F_0 data while homoscedasticity was tested for other parameters. Repeated measures ANOVA was used to determine significant differences between temperature, DO, and time of sampling for F_v/F_m and F_v/F_0 of each coral species.

Three-way ANOVA was used to determine statistically significant differences between temperature, DO, and time for *Symbiodiniaceae* density and chlorophyll concentration of each species. Two-way ANOVA was used to test significant differences by temperature and DO in growth rate of each species. All tests used 95% confidence level threshold, and post hoc Tukey HSD test was performed to determine statistically significant differences among the distinct groups. All analyses were performed using R Studio version 1.4.1717.

3. Results

Exposure to high temperature and low DO were found to induce physiological changes in corals as well as in the symbiotic algae. The temperature and DO affect the photosynthetic performance, *Symbiodiniaceae* density, chlorophyll concentration, and coral growth (see Supplementary Tables S3–S5). Each coral species revealed a different response, and the combined stresses had a significantly worse effect only on the growth rate of *M. tuberculosa*.

3.1. Photosynthetic Efficiency

The F_v/F_m and F_v/F_0 of *P. lutea* measured before the light period under ambient conditions on day 0 were 0.639 ± 0.023 and 1.781 ± 0.177, respectively. The treatment with heat stress + hypoxia showed a significant decrease compared to ambient on day 1 ($p = 0.003$ for F_v/F_m and $p = 0.003$ for F_v/F_0), but the performance subsequently increased, and samples could maintain effective photosynthesis until day 9, with no significant differences between the treatments (Figure 2A,B). For the measurements taken after the light period, the F_v/F_m and F_v/F_0 of *P. lutea* under heat stress significantly decreased starting on day 1 ($p = 0.008$ and 0.009, respectively).

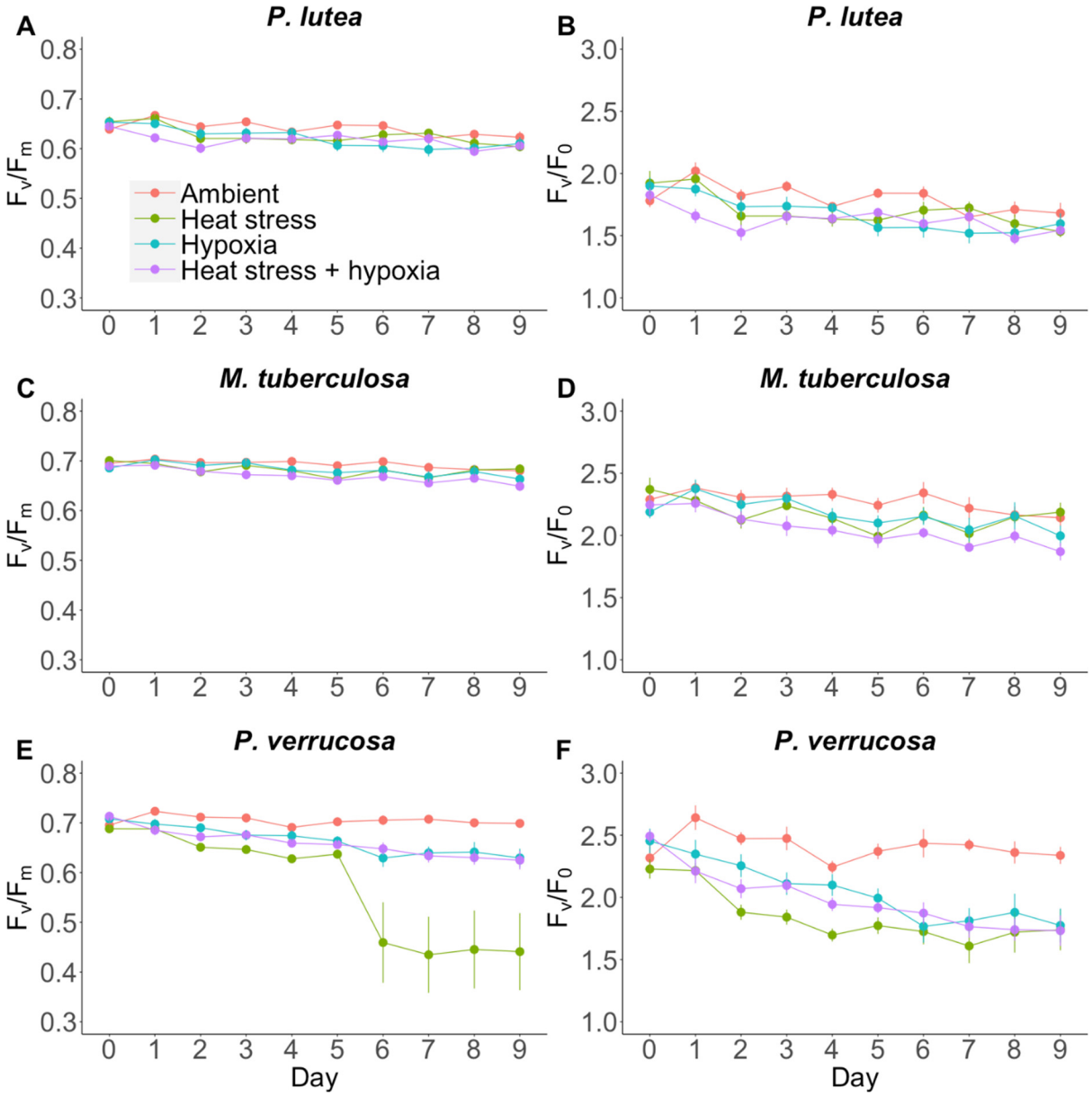

Figure 2. Maximum quantum yield of PSII (F_v/F_m; left) and photochemical efficiency of PSII (F_v/F_0; right) in *P. lutea* (**A**,**B**), *M. tuberculosa* (**C**,**D**), and *P. verrucosa* (**E**,**F**) measured before the light period. Data are shown as mean ± SE (n = 4).

In contrast, significant increases were recorded under hypoxia (p = 0.049 for F_v/F_m, p = 0.038 for F_v/F_0) and heat stress + hypoxia (p = 0.004 for F_v/F_m, p = 0.002 for F_v/F_0) on day 3 (Figure 3A,B). There was no significant interaction between the factors for measurements taken before the light period whereas the measurements taken after light period showed significance for all interactions (Supplementary Table S3).

M. tuberculosa had similar results to *P. lutea* and seemed to express a tolerance to stress conditions. During the experiment period, F_v/F_m and F_v/F_0 showed no significant differences between the stress treatments when compared to ambient conditions in measurements taken before the light period (Figure 2C,D). For measurements taken after light period, coral nubbins under heat stress + hypoxia showed significant decrease only on day 5 for both F_v/F_m and F_v/F_0 (p = 0.019 and 0.023, respectively) compared to ambient condition treatment (Figure 3C,D). Subsequently, there was no significant difference between the treatments. We found no significant interaction between the factors (in F_v/F_m and F_v/F_0)

in measurements taken before or after the light period; however, the temperature and DO factors individually affected the coral nubbins (Supplementary Table S4).

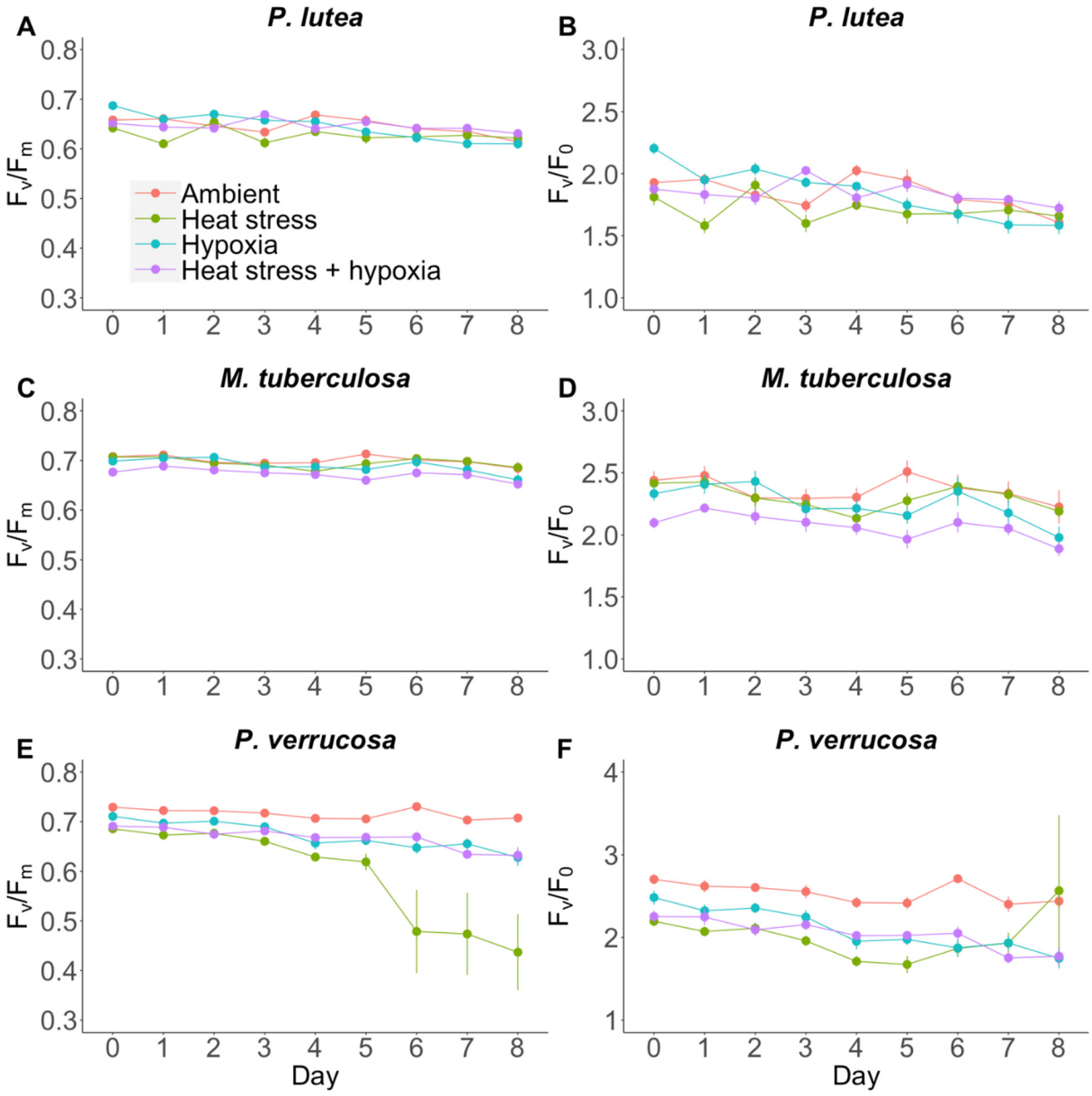

Figure 3. Maximum quantum yield of PSII (F_v/F_m; left) and photochemical efficiency of PSII (F_v/F_0; right) in *P. lutea* (**A**,**B**), *M. tuberculosa* (**C**,**D**), and *P. verrucosa* (**E**,**F**) measured after the light period. Data are shown as mean ± SE (n = 4).

P. verrucosa measurements taken before the light period presented a significant decline on day 2 for heat stress (p = 0.003 for F_v/F_m, p = 0.003 for F_v/F_0) and for heat stress + hypoxia (p = 0.045 for F_v/F_m, p = 0.038 for F_v/F_0) compared to ambient condition (Figure 2E,F). Progressive decreases by 33.3% for F_v/F_m and by 22.6% for F_v/F_0 were detected under heat stress by day 6. Coral nubbins experienced bleaching and death afterwards. In measurements taken after the light period, the photosynthetic performance of coral nubbins (Figure 3E,F) significantly declined after day 1 under heat stress (p = 0.031 for F_v/F_m, p = 0.029 for F_v/F_0) and continued to decline, especially on day 6, with decreases by 30.2% and 15% from day 0 in F_v/F_m and F_v/F_0, respectively. In hypoxia condition, the corals showed a slight decrease on day 6 ($p < 0.001$ for F_v/F_m, $p < 0.001$ for F_v/F_0) while heat stress + hypoxia significantly differed from day 2 when com-

pared to ambient condition ($p < 0.001$ for F_v/F_m, $p < 0.001$ for F_v/F_0). The measurements taken before the light period showed significant differences by time ($p = 0.004$ for F_v/F_m, $p < 0.001$ for F_v/F_0), by temperature ($p < 0.001$ for F_v/F_m, $p < 0.001$ for F_v/F_0), and an interaction effect of time × DO ($p < 0.001$ for F_v/F_m, $p < 0.001$ for F_v/F_0). Measurements taken after the light period were significant for the interaction time × DO ($p < 0.001$ for F_v/F_m, $p < 0.002$ for F_v/F_0) and for the individual factors time ($p = 0.012$) and temperature ($p < 0.001$) in F_v/F_m and for temperature ($p < 0.001$) and DO ($p = 0.006$) in F_v/F_0, as shown in Supplementary Table S5.

3.2. Symbiodiniaceae Density

On day 9, *Symbiodiniaceae* densities in *P. lutea* in all actual treatments were significantly lower than in the baseline ambient condition (heat stress $p < 0.001$, hypoxia $p = 0.019$, heat stress + hypoxia $p = 0.001$) (Figure 4A). There were significant effects of time ($p = 0.036$), temperature ($p < 0.001$), DO ($p = 0.015$), and their interaction time x temperature × DO ($p = 0.024$) (Supplementary Table S3). Interestingly, *M. tuberculosa* presented a significant increase in *Symbiodiniaceae* density (Figure 4B) during the experimental period ($p = 0.012$, <0.001, <0.001 for days 3, 6, and 9, respectively, compared to initial value). Otherwise, the factors had no significance except for time ($p < 0.001$) and interaction temperature × DO ($p = 0.030$), as shown in Supplementary Table S4. *P. verrucosa* revealed significant difference in *Symbiodiniaceae* density by treatment (Figure 4C), especially on day 9. We identified a drastic decline under heat stress ($p = 0.003$) and hypoxia ($p = 0.028$) compared to ambient condition. The temperature and DO factors were significant ($p < 0.001$ and $p = 0.010$, respectively), including influences in the interactions time × temperature ($p = 0.049$), temperature × DO ($p < 0.001$), and time x temperature × DO ($p = 0.042$) (Supplementary Table S5).

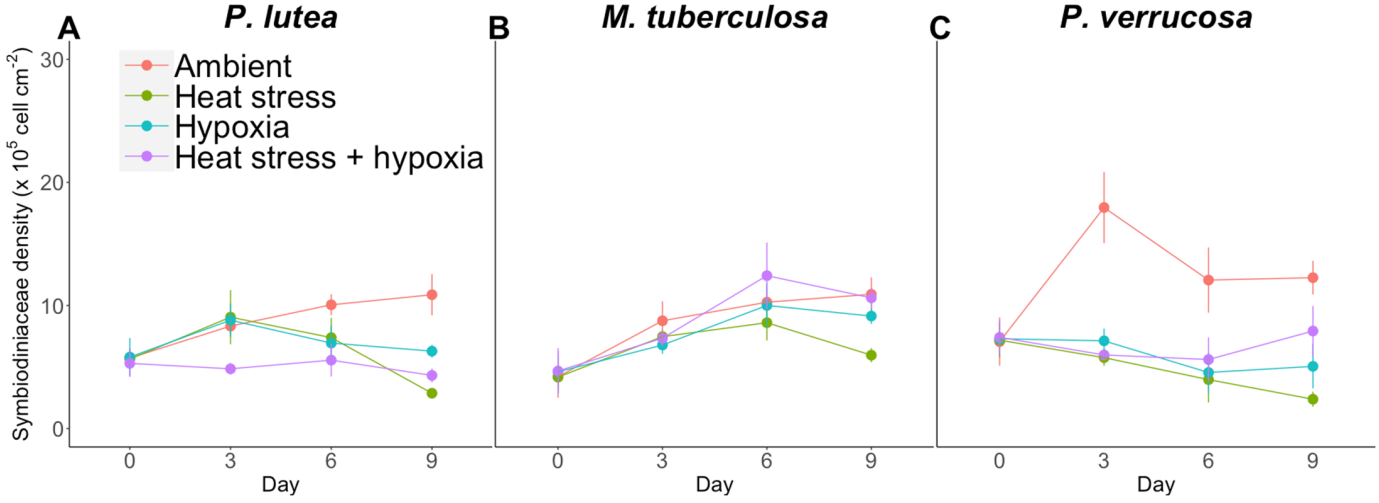

Figure 4. *Symbiodiniaceae* density (× 10^5 cells cm^{-2}) of *P. lutea* (**A**), *M. tuberculosa* (**B**), and *P. verrucosa* (**C**) under ambient baseline conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean ± SE ($n = 4$).

3.3. Chlorophyll Content

In *P. lutea*, we found consistent chlorophyll *a* and c_2 concentrations throughout the experiment period in all treatments (Figures 5A and 6A), and the interaction time × temperature × DO was the only significant factor for chlorophyll *a* ($p = 0.047$; Supplementary Table S3) whereas chlorophyll c_2 was significantly influenced by the individual factor temperature ($p = 0.014$; Supplementary Table S3). On the other hand, *M. tuberculosa* revealed a significant increase on day 6 in chlorophyll *a* ($p < 0.001$) and on days 3, 6, and 9 in chlorophyll c_2 ($p < 0.001$), although the coral nubbins were under stress (Figures 5B and 6B). Time was a significant factor for both chlorophyll *a* and c_2 in *M. tuberculosa* ($p < 0.001$; Supplementary Table S4).

In *P. verrucosa*, we found an obvious significant difference between the treatments, as a reduction in chlorophyll *a* was recorded from day 3 for all stress exposures compared to ambient condition ($p < 0.001$), but a subsequent increase was found on day 9 under heat stress + hypoxia (Figure 5C). Chlorophyll c_2 in *P. verrucosa* presented a similar trend (Figure 6C), with significant decreases under heat stress ($p < 0.001$) and hypoxia ($p < 0.001$) on day 3. The significant interactions of factors for both chlorophyll *a* and c_2 were temperature × DO and time × temperature × DO ($p < 0.001$; Supplementary Table S5).

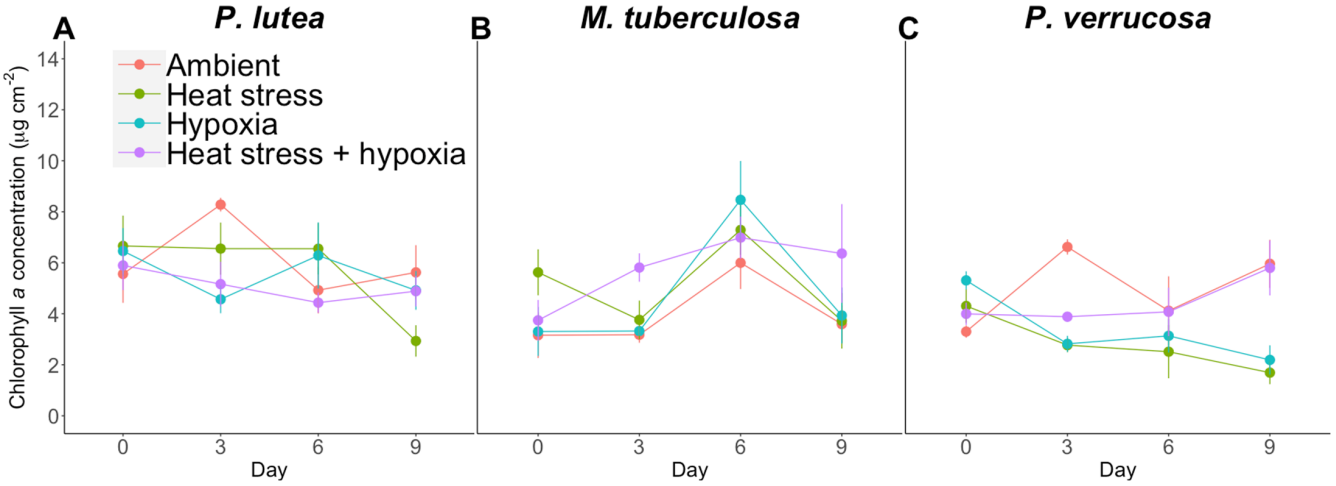

Figure 5. Chlorophyll *a* and *c*2 concentration (µg cm^{-2}) of *P. lutea* (**A**), *M. tuberculosa* (**B**), and *P. verrucosa* (**C**) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean ± SE ($n = 4$).

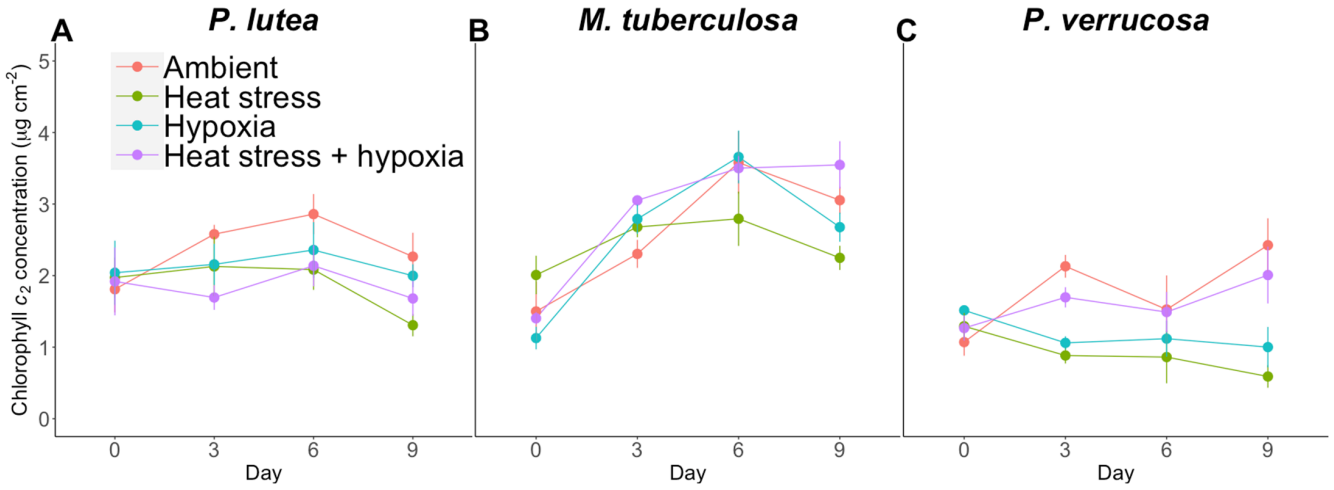

Figure 6. Chlorophyll *c*2 concentration (µg cm^{-2}) of *P. lutea* (**A**), *M. tuberculosa* (**B**), and *P. verrucosa* (**C**) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean ± SE ($n = 4$).

3.4. Growth Rate

There was no significant difference in growth rate of *P. lutea* between the treatments (Figure 7A), while a significant decrease in the growth of *M. tuberculosa* was recorded under heat stress + hypoxia (46.9% reduction, $p = 0.041$) compared to ambient condition (Figure 7B). The growth rate of *M. tuberculosa* under hypoxia showed a slight increase but had no significant difference from ambient condition. On the other hand, some tissue loss was detected in *P. verrucosa* under heat stress and hypoxia. The growth rates were significantly lower compared to ambient (Figure 7C) for both heat stress ($p = 0.044$) and

hypoxia (p = 0.042). However, no significant difference was observed in the heat stress + hypoxia treatment compared to ambient condition.

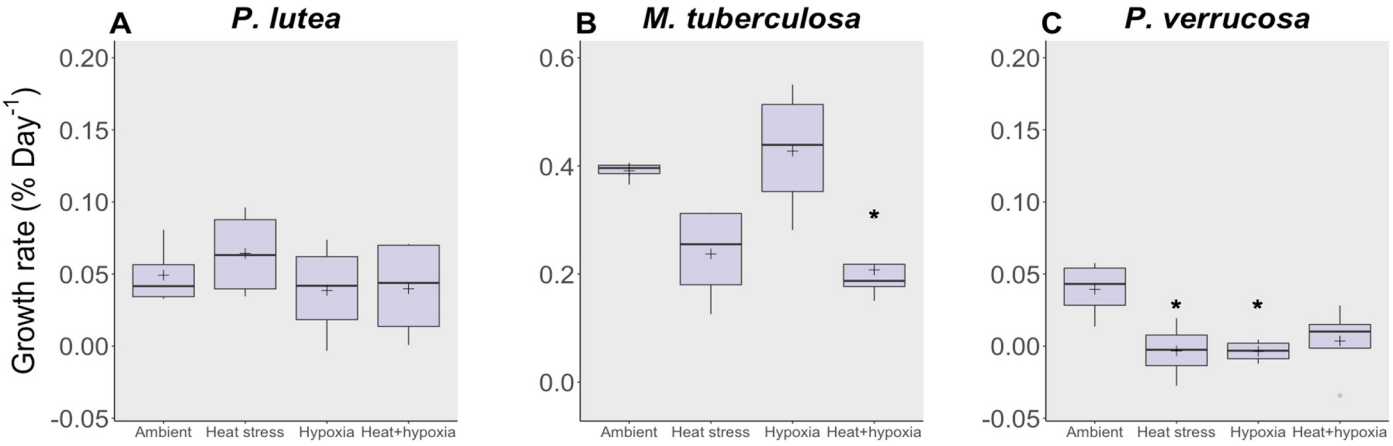

Figure 7. Growth rates of *P. lutea* (**A**), *M. tuberculosa* (**B**), and *P. verrucosa* (**C**) under ambient, heat stress, hypoxia, and heat stress + hypoxia. Data are shown as mean (+), median, and range (n = 4) in a boxplot, where an asterisk indicates statistical significance of treatment when compared to ambient.

4. Discussion

The 32 °C temperature had significant adverse effects on all three species. Nevertheless, the severity of effects and responses vary by coral species. *P. verrucosa* was the most sensitive and experienced complete bleaching by the end of our experiment, unlike *P. lutea* and *M. tuberculosa* (refer to Supplementary Figures S2–S4). Under heat stress, a significant decline in *P. verrucosa*'s growth rate, *Symbiodiniaceae* density, and chlorophyll concentration on day 9 were detected. The photochemical efficiency showed a gradual decline starting on day 1, but it decreased sharply on day 6. This suggests that 5–6 days might be the limit for *P. verrucosa* to handle direct heat stress of 32 °C. Previous experiments have found that tropical *Pocillopora* is sensitive to moderate heat (32–33 °C) whereas *Porites* can maintain the symbiont population and their performance to keep up with the metabolic energy requirements of the coral host [14,62]. *P. lutea* and *M. tuberculosa* in this study did not reveal drastic effects from heat stress. A slight increase in *Symbiodiniaceae* was observed in *P. lutea* after facing the elevated temperature, with a subsequent decline on day 9. This case can occur when a coral lacks in carbon translocation, and the nutrition is balanced by *Symbiodiniaceae* growth for a short period [1]. Our results also support previous findings that the response of corals to heat stress is related to coral morphology, with branched coral species being the most susceptible, i.e., the first to bleach and die in mass bleaching events [63–68]. The difference in bleaching vulnerability of branching corals has been attributed to several physiological and morphological properties: tissue thickness [69], skeletal fragility and within tissue light scattering [70,71], genetic constitution of symbiotic algae [72], *Symbiodiniaceae* density per coral cell [73], and mass transfer rate [15,74].

Symbiotic cnidarians are subjected to broad, fast, and daily changes in oxygen content because of the presence of intracellular dinoflagellates. The oxygen levels in coral tissue can be hyperoxic during daylight and hypoxic during darkness as they become net consumers of oxygen in light-limiting conditions [6,75,76]. The fast shift between hyperoxia and hypoxia sometimes induces microenvironment acclimation in coral tissues [77]. Our results indicate that light availability plays a role in the response of some coral species when hypoxia is involved. The F_v/F_m and F_v/F_0 trends were different after light/dark in measurements for *P. lutea* and *P. verrucosa*, while *M. tuberculosa* only presented slight fluctuations. During the initial days, *P. lutea* occasionally presented significantly higher photosynthetic performance under hypoxia during light availability compared to ambient condition. This might be a cellular mechanism of photosynthetic translocation for energy saving and photo acclimation

contributing to this species' tolerance of stress [78,79]. Photosynthesis (CO_2 consumption and O_2 production) could partially reduce the stress of deoxygenation, but increased respiration by the same photosynthetic organisms during the night could have the opposite effect, particularly with rising temperatures [80]. Thermal stress and coral bleaching usually occur in conjunction with high summer temperatures and calm breezes when water-flow rates are low. Anoxia can occur when there is little or no water flow, resulting in limited diffusion in many branching coral colonies [15,66,81].

The respiration rate of corals is generally believed to be directly proportional to ambient DO concentration based on their anatomy. Some evidence has suggested that corals can moderately sustain respiration rates during declining oxygen concentration, for example, deep-sea corals (thriving in less than 1 mgL^{-1} DO concentration in the Red Sea [82]), and some corals appear to oxy-regulate using epidermal cilia movements during stagnant flow [83]. Furthermore, previous studies have revealed polyp expansion process of cold-water coral, *Lophelia pertusa*, and other cnidarians, such as sea anemones, to increase tissue surface area, thereby increasing the diffusive gas exchange [84–86], while some soft corals use tentacle pulsation to increase oxygen levels in their tissue [87]. The hypoxia (~2 mgL^{-1}) in our case showed severe adverse effects on *P. verrucosa* in terms of physiological and visual expressions, bleaching, and tissue loss (refer to Supplementary Figure S4). The growth rate of *Symbiodiniaceae* and the chlorophyll concentration had significantly declined by the end of experiment, while a reduction in photosynthetic parameters was detected earlier. Further supported by experimental studies, the branching corals have been shown to be highly susceptible to low oxygen, with some species expressing rapid tissue loss even when subjected to a 4 mgL^{-1} oxygen concentration [32,88]. On the other hand, massive or submassive corals seem to be the survivors of severe hypoxic events [89,90], even when exposed to 0.5 mgL^{-1} oxygen levels [24]. However, hypoxia stress can also lead to changes in microbial populations, altering the nutrient cycle, for example, from nitrification towards nitrogen fixation and thereby increasing the host's susceptibility to pathogens. It can also lead to the production of toxic compounds, such as H_2S, NO, and H_2O_2, that further compromise the host's immunity [6,91,92].

The growth rates of *M. tuberculosa* in the combined heat and hypoxia treatment were lower than in control and hypoxia treatments, suggesting that heat aggravated the hypoxic stress effects in this coral species. Energy deficit could be an explanation for such an effect on growth, as hypoxia limits ATP production [39] while heat stress increases metabolic energy demand [1]. Nevertheless, the photosynthetic performance and chlorophyll content of *M. tuberculosa* and *P. lutea* in the combined stress treatment were generally higher than in the only heat treatment, suggesting that hypoxia may alleviate heat stress at the physiological level. Heat stress is known to lead to an overproduction of reactive oxygen species (ROS) [93,94]. In contrast, reduced activity of mitochondrial electron transport chain as a result of hypoxia could decrease the formation of other ROS and relieve the oxidative stress. Lower ROS has been reported in *Acropora* species facing hypoxia [95]. Although more replicates and experiments are needed to confirm this speculation, our results suggest that responses at the physiological level may be decoupled from the more integrative response in growth rate, depending on the mechanisms of the stressors.

The ocean is warming at an alarming rate, with an estimated 1–3 °C increase in sea surface temperature (SST) being a likely scenario by the end of this century [96–98]. The frequency and scale of mass coral bleaching events associated with thermal stress have been increasing. An anomaly with high sea water temperature between 30 and 34 °C was reported in the Andaman Sea from April–June 2010 that led to a decline in *Acropora* and *Pocillopora* cover [99,100] and reduction in *P. lutea* growth [101]. Unfortunately, thermal stress does not occur in isolation, but is compounded by coastal runoff, nutrient enrichment-induced eutrophication, etc., contributing to progressive decrease in oceanic oxygen reserves over the last 50 years [17,26,102]. Almost 15% of tropical coral reefs are estimated to be at high risk from local hypoxia due to their exposure to anthropogenic activities and geomorphology [19,24,103]. Coral reefs at our study site of Phuket are

also vulnerable to heat stress [14,46] and excessive nutrient enrichment, and co-occurring episodes of hypoxia with harmful algal blooms (HAB) are periodically reported in the area [44,45]. The frequency of extreme temperatures and hypoxic events in Thai waters is increasing at an alarming rate [9], and the stress conditions used in our experiment might soon become a likely scenario. Recent observational studies show that corals exposed to sublethal doses of thermal stress have potential to acclimatize, increasing the baseline of thermal limit for subsequent stress events [90,104,105]. Most species also have an inherent tolerance to naturally occurring hypoxia, but its coexistence with other stressors can lead to a breakdown of mutualisms and feedback systems pushing them beyond limits [106]. Ongoing global and local threats may cause a shift toward tolerant local species (for example, *P. lutea* and *M. tuberculosa* from our study). Other studies also point towards a lower physiological threshold with the concurrence of high temperatures and low dissolved oxygen leading to a loss and change in reef marine biodiversity [37,107,108].

While the baseline information from our results can be integrated into a guideline for evaluating coral sensitivity to future scenarios where warming and hypoxic events are expected to occur with greater intensity and increased frequency, extrapolation of the results obtained from experiments conducted in a closed system should be interpreted with caution. Limitations in the setup such as a lack of flow may result in a drift of certain physicochemical characteristics of the seawater. In addition, more replicates and a longer experimental period would improve future experiments. We also propose that warming and hypoxic episodes should be closely monitored in order to protect the coral ecosystem.

5. Conclusions

Heat stress was dominant as a stressor compared to hypoxia. Among the three coral species, *P. verrucosa* seems to be the most vulnerable to both heat stress and hypoxia when these are present individually. The short-term combined stress showed a stronger negative effect compared to individual stresses only on the growth rate of *M. tuberculosa*. Under hypoxic condition, coral responses varied according to light availability. The photosynthetic efficiency, especially F_v/F_0, is a sensitive parameter for early detection, whereas growth-related attributes may serve well as indicators of the long-term effects under a future scenario. Further studies are needed to dissect the combined effects of heat stress and hypoxia and to improve mechanistic understanding of synergistic and antagonistic responses in these three scleractinian corals.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jmse11020403/s1. Table S1: environmental parameters recorded at the study area, Patong Bay, Phuket, Thailand, during April–October 2021. Table S2: Water quality parameters in the treatment tanks measured during the experiment. Data are shown as mean ± SE. Table S3: Summary of different ANOVAs for photosynthesis, *Symbiodiniaceae* density, chlorophyll content, and growth rate of *P. lutea*. Significant values ($p < 0.05$) are shown in bold. Table S4: Summary of different ANOVAs for photosynthesis, *Symbiodiniaceae* density, chlorophyll content, and growth rate of *M. tuberculosa*. Significant values ($p < 0.05$) are shown in bold. Table S5: Summary of different ANOVAs for photosynthesis, *Symbiodiniaceae* density, chlorophyll content, and growth rate of *P. verrucosa*. Significant values ($p < 0.05$) are shown in bold. Figure S1: Sea surface temperature data for Patong Bay, Phuket, from 2016 to 2021. Data source—Multi-scale Ultra-high Resolution (MUR) SST Analysis fv04.1, Global, 0.01°, 2002–present, daily. These data were provided by JPL under support from NASA MEaSUREs program. Figure S2: *P. lutea* samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments. The white lines represent 1 cm scale bar. Figure S3: *M. tuberculosa* samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments. The white lines represent 1 cm scale bar. Figure S4: *P. verrucosa* samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments. The white lines represent 1 cm scale bar.

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References

1. Rädecker, N.; Pogoreutz, C.; Gegner, H.M.; Cárdenas, A.; Roth, F.; Bougoure, J.; Guagliardo, P.; Wild, C.; Pernice, M.; Raina, J.B.; et al. Heat stress destabilizes symbiotic nutrient cycling in corals. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2022653118. [CrossRef]
2. Levitus, S.; Antonov, J.I.; Boyer, T.P.; Stephens, C. Warming of the world ocean. *Science* **2000**, *287*, 2225–2229. [CrossRef]
3. Lyman, J.; Good, S.; Gouretski, V. Robust warming of the global upper ocean. *Nature* **2010**, *465*, 334–337. [CrossRef]
4. Roxy, M.K.; Ritika, K.; Terray, P.; Masson, S. The curious case of Indian Ocean warming. *J. Clim.* **2014**, *27*, 8501–8509. [CrossRef]
5. Church, J.A.; White, N.J.; Konikow, L.F.; Domingues, C.M.; Cogley, J.G.; Rignot, E.; Gregory, J.M.; Broeke, M.R.; Van Den Broeke, M.R.; Monaghan, A.J.; et al. Revisiting the Earth's sea-level and energy budgets from 1961 to 2008. *Geophys. Res. Lett.* **2011**, *38*, L18601. [CrossRef]
6. Hughes, D.J.; Alderdice, R.; Cooney, C.; Kühl, M.; Pernice, M.; Voolstra, C.R.; Suggett, D.J. Coral reef survival under accelerating ocean deoxygenation. *Nat. Clim. Change* **2020**, *10*, 296–307. [CrossRef]
7. Smale, D.A.; Wernberg, T.; Oliver, E.C.J. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Change* **2019**, *9*, 306–312. [CrossRef]
8. Kingsolver, J.G.; Buckley, L.B. Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Philos. Trans. R. Soc. B Biol. Sci.* **2017**, *372*, 20160147. [CrossRef] [PubMed]
9. Limsakul, A.; Sangchan, L.; Thavivongse, S.; Suttamanuswong, B. Trends in temperature and its extremes in Thailand. *Thai Environ. Eng. J.* **2011**, *25*, 9–16.
10. Frölicher, T.L.; Laufkötter, C. Emerging risks from marine heat waves. *Nat. Commun.* **2018**, *9*, 650. [CrossRef]
11. Poloczanska, E.S.; Burrows, M.T.; Brown, C.J.; Molinos, J.G.; Halpern, B.S.; Hoegh-Guldberg, O.; Kappel, C.V.; Moore, P.J.; Richardson, A.J.; Schoeman, D.S.; et al. Responses of marine organisms to climate change across oceans. *Front. Mar. Sci.* **2016**, *3*, 1–21. [CrossRef]
12. Cziesielski, M.J.; Schmidt-Roach, S.; Aranda, M. The past, present, and future of coral heat stress studies. *Ecol. Evol.* **2019**, *9*, 10055–10066. [CrossRef] [PubMed]
13. Schoepf, V.; Olivo, J.P.; Rigal, C.; Jung, E.M.U.; Mcculloch, M.T. Heat stress differentially impacts key calcification mechanisms in reef-building corals. *Coral Reefs* **2021**, *40*, 459–471. [CrossRef]
14. Yucharoen, M.; Sinutok, S.; Chotikarn, P.; Buapet, P. Experimental assessment of vulnerability to warming in tropical shallow-water marine organisms. *Front. Mar. Sci.* **2021**, *8*, 767628. [CrossRef]
15. Woesik, R.V.; Irikawa, A.; Anzai, R.; Nakamura, T. Effects of coral colony morphologies on mass transfer and susceptibility to thermal stress. *Coral Reefs* **2012**, *31*, 633–639. [CrossRef]
16. Boyett, H.V.; Bourne, D.G.; Willis, B.L. Elevated temperature and light enhance progression and spread of black band disease on staghorn corals of the Great Barrier Reef. *Mar. Biol.* **2007**, *151*, 1711–1720. [CrossRef]
17. Keeling, R.F.; Körtzinger, A.; Gruber, N. Ocean deoxygenation in a warming world. *Annu. Rev. Mar. Sci.* **2010**, *2*, 199–229. [CrossRef]
18. Blakeway, D. Hypoxia shapes coral reefs. *PeerJ Prepr.* **2018**, *6*, e26794v1.

19. Kealoha, A.K.; Doyle, S.M.; Shamberger, K.E.F.; Sylvan, J.B.; Hetland, R.D.; Dimarco, S.F. Localized hypoxia may have caused coral reef mortality at the Flower Garden Banks. *Coral Reefs* **2020**, *39*, 119–132. [CrossRef]
20. Falkowski, P.G.; Algeo, T.J.; Codispoti, L.A.; Deutsch, C.A.; Emerson, S.R.; Hales, B.; Huey, R.B.; Jenkins, W.J.; Kump, L.R.; Levin, L.A.; et al. Ocean deoxygenation: Past, present, and future. *Eos, Trans. Am. Geophys. Union* **2011**, *92*, 409–410. [CrossRef]
21. Blumberg, A.F.; Toro, D.M.D. Effects of climate warming on dissolved oxygen concentrations in Lake Erie. *Trans. Am. Fish. Soc.* **1990**, *119*, 210–223. [CrossRef]
22. Ni, W.; Li, M.; Ross, A.C.; Najjar, R.G. Large projected decline in dissolved oxygen in a eutrophic estuary due to climate change. *J. Geophys. Res. Ocean* **2019**, *124*, 8271–8289. [CrossRef]
23. Cocco, V.; Joos, F.; Steinacher, M.; Frölicher, T.L.; Bopp, L.; Dunne, J.; Gehlen, M.; Heinze, C.; Orr, J.; Oschlies, A.; et al. Oxygen and indicators of stress for marine life in multi-model global warming projections. *Biogeosciences* **2013**, *10*, 1849–1868. [CrossRef]
24. Altieri, A.H.; Harrison, S.B.; Seemann, J.; Collin, R.; Diaz, R.J.; Knowlton, N. Tropical dead zones and mass mortalities on coral reefs. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 3660–3665. [CrossRef]
25. Nelson, H.R.; Altieri, A.H. Oxygen: The universal currency on coral reefs. *Coral Reefs* **2019**, *38*, 177–198. [CrossRef]
26. Breitburg, D.; Levin, L.A.; Oschlies, A.; Grégoire, M.; Chavez, F.P.; Conley, D.J.; Garçon, V.; Gilbert, D.; Gutiérrez, D.; Isensee, K.; et al. Declining oxygen in the global ocean and coastal waters. *Science* **2018**, *359*, eaam7240. [CrossRef]
27. Osinga, R.; Derksen-Hooijberg, M.; Wijgerde, T.; Verreth, J.A.J. Interactive effects of oxygen, carbon dioxide and flow on photosynthesis and respiration in the Scleractinia coral *Galaxea fascicularis*. *J. Exp. Biol.* **2017**, *220*, 2236–2242. [PubMed]
28. Sinutok, S.; Hill, R.; Doblin, M.A.; Wuhrer, R.; Ralph, P.J. Warmer more acidic conditions cause decreased productivity and calcification in subtropical coral reef sediment-dwelling calcifiers. *Limnol. Oceanogr.* **2011**, *56*, 1200–1212. [CrossRef]
29. Gooday, A.J.; Levin, L.A.; Silva, A.A.D.; Bett, B.J.; Cowie, G.L.; Dissard, D.; Gage, J.D.; Hughes, D.J.; Jeffreys, R.; Lamont, P.A.; et al. Faunal responses to oxygen gradients on the Pakistan margin: A comparison of foraminiferans, macrofauna and megafauna. *Deep.-Sea Res. Part II: Top. Stud. Oceanogr.* **2009**, *56*, 488–502. [CrossRef]
30. Heron, S.F.; Eakin, C.M.; Douvere, F.; Anderson, K.; Day, J.C.; Geiger, E.; Hoegh-Guldberg, O.; Van Hooidonk, R.; Hughes, T.; Marshall, P.; et al. *Impacts of Climate Change on World Heritage Coral Reefs: A First Global Scientific Assessment*; UNESCO World Heritage Centre: Paris, France, 2017.
31. Haas, A.F.; Fairoz, M.F.M.; Kelly, L.W.; Nelson, C.E.; Dinsdale, E.A.; Edwards, R.A.; Giles, S.; Hatay, M.; Hisakawa, N.; Knowles, B.; et al. Global microbialization of coral reefs. *Nat. Microbiol.* **2016**, *1*, 16042. [CrossRef] [PubMed]
32. Haas, A.F.; Smith, J.E.; Thompson, M.; Deheyn, D.D. Effects of reduced dissolved oxygen concentrations on physiology and fluorescence of hermatypic corals and benthic algae. *PeerJ* **2014**, *2*, e235. [CrossRef] [PubMed]
33. Haas, A.F.; Jantzen, C.; Naumann, M.S.; Iglesias-Prieto, R.; Wild, C. Organic matter release by the dominant primary producers in a Caribbean Reef Lagoon: Implication for in situ O_2 availability. *Mar. Ecol. Prog. Ser.* **2010**, *409*, 27–39. [CrossRef]
34. Voss, J.D.; Richardson, L.L. Nutrient enrichment enhances black band disease progression in corals. *Coral Reefs* **2006**, *25*, 569–576. [CrossRef]
35. Kuta, K.; Richardson, L. Ecological aspects of black band disease of corals: Relationships between disease incidence and environmental factors. *Coral Reefs* **2002**, *21*, 393–398. [CrossRef]
36. Richardson, L.L. Black Band Disease. In *Coral Health and Disease*; Rosenberg, E., Loya, Y., Eds.; Springer: Berlin/Heidelberg, Germany, 2004.
37. Hobbs, J.P.A.; Mcdonald, C.A. Increased seawater temperature and decreased dissolved oxygen triggers fish kill at the Cocos (Keeling) Islands, Indian Ocean. *J. Fish Biol.* **2010**, *77*, 1219–1229. [CrossRef]
38. Murphy, J.W.A.; Richmond, R.H. Changes to coral health and metabolic activity under oxygen deprivation. *PeerJ* **2016**, *4*, e1956. [CrossRef]
39. Campanati, C.; Yip, S.; Lane, A.; Thiyagarajan, V. Combined effects of low pH and low oxygen on the early-life stages of the barnacle *Balanus amphitrite*. *ICES J. Mar. Sci.* **2015**, *73*, 791–802. [CrossRef]
40. Muñiz-Castillo, A.I.; Rivera-Sosa, A.; Chollett, I.; Eakin, C.M.; Andrade-Gómez, L.; Mcfield, M. Three decades of heat stress exposure in Caribbean Coral Reefs: A new regional delineation to enhance conservation. *Sci. Rep.* **2019**, *9*, 11013. [CrossRef]
41. Gobler, C.J.; Baumann, H. Hypoxia and acidification in ocean ecosystems: Coupled dynamics and effects on marine life. *Biol. Lett.* **2016**, *12*, 20150976. [CrossRef]
42. Gravinese, P.M.; Douwes, A.; Eaton, K.R.; Muller, E.M. Ephemeral hypoxia reduces oxygen consumption in the Caribbean coral *Orbicella faveolata*. *Coral Reefs* **2021**, *41*, 13–18. [CrossRef]
43. Altieri, A.H. Dead zones enhance key fisheries species by providing predation refuge. *Ecology* **2008**, *89*, 2808–2818. [CrossRef] [PubMed]
44. Charoenvattanaporn, J. Eutrophication status and red tide phenomena along the west coast of Phuket Island, the Andaman Sea. In Proceedings of the 10th WESTPAC International Scientific Conference, Qingdao, China, 17–20 April 2017.
45. Reopanichkul, P.; Carter, R.W.; Worachananant, S.; Crossland, C.J. Wastewater discharge degrades coastal waters and reef communities in southern Thailand. *Mar. Environ. Res.* **2010**, *69*, 287–296. [CrossRef] [PubMed]
46. Brown, B.E.; Phongsuwan, N.; Thongtham, N.; Putchim, L.; Hawkridge, J.M. The recent history of coral reefs and their environment in Tang Khem Bay, Phuket, Thailand—An example of corals living in a potential climate change refuge? *Phuket Mar. Biol. Cent. Res. Bull.* **2019**, *76*, 25–39.

47. Khokiattiwong, S.; Yu, W. Note on the occurrence of high sea surface temperatures in the Andaman Sea, in 2010. *Phuket Mar. Biol. Cent. Res. Bull.* **2012**, *71*, 1–9.
48. Hughes, T.P.; Kerry, J.T.; Baird, A.H. Global warming transforms coral reef assemblages. *Nature* **2018**, *556*, 492–496. [CrossRef]
49. Bindoff, N.; Cheung, W.W.L.; Kairo, J.G.; Arístegui, J.; Guinder, V.A.; Hallberg, R.; Hilmi, N.; Jiao, N.; Karim, M.; Levin, L.; et al. Changing ocean, marine ecosystems, and dependent communities. In *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2022; pp. 447–587.
50. Fricke, G.; Carpenter, R.; Battino, R. Effect of various gases on the pH of water. *J. Phys. Chem.* **1973**, *77*, 826–827. [CrossRef]
51. Cohen, Y.; Kirchmann, H. Increasing the pH of wastewater to high levels with different gases-CO_2 stripping. *Water Air Soil Pollut.* **2004**, *159*, 265–275. [CrossRef]
52. Hill, R.; Ralph, P.J. Post-bleaching viability of expelled zooxanthellae from the scleractinian coral *Pocillopora damicornis*. *Mar. Ecol. Prog. Ser.* **2007**, *352*, 137–144. [CrossRef]
53. Ritchie, R.J. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth. Res.* **2006**, *89*, 27–41. [CrossRef]
54. Winters, G.; Holzman, R.; Blekhman, A.; Beer, S.; Loya, Y. Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring. *J. Exp. Mar. Biol. Ecol.* **2009**, *380*, 25–35. [CrossRef]
55. Holmes, G. Estimating three-dimensional surface areas on coral reefs. *J. Exp. Mar. Biol. Ecol.* **2008**, *365*, 67–73. [CrossRef]
56. Stimson, J.; Kinzie, R.A. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Biol. Ecol.* **1991**, *153*, 63–74. [CrossRef]
57. Jokiel, P.L.; Maragos, J.E.; Franzisket, L. Coral growth buoyant weight technique. In *Coral Reefs: Research Methods*; Stoddart, D.R., Johannes, R.E., Eds.; Catalog Number 0000029306; UNESCO: Paris, France, 1978; pp. 529–542.
58. Davies, P.S. Short-term growth measurements of corals using an accurate buoyant weighing technique. *Mar. Biololy* **1989**, *101*, 389–395. [CrossRef]
59. Tanzil, J.T.I.; Brown, B.E.; Tudhope, A.W.; Dunne, R.P. Decline in skeletal growth of the coral *Porites lutea* from the Andaman Sea, South Thailand between 1984 and 2005. *Coral Reefs* **2009**, *28*, 519–528. [CrossRef]
60. Ng, C.S.L.; Lim, J.; Sam, S.Q.; Kikuzawa, Y.; Toh, T.C.; Wee, T. Variability in skeletal bulk densities of common hard corals in Southeast Asia. *Coral Reefs* **2019**, *38*, 1133–1143. [CrossRef]
61. Hughes, T.P. Skeletal density and growth form of corals. *Mar. Ecol. Prog. Ser.* **1987**, *35*, 259–266. [CrossRef]
62. Sinutok, S.; Chotikarn, P.; Pattaratumrong, M.S.; Moungkeaw, P.; Pramneechote, P.; Yucharoen, M. Synergistic effect of elevated temperature and light stresses on physiology of *Pocillopora acuta* from different environments. *J. Mar. Sci. Eng.* **2022**, *10*, 790. [CrossRef]
63. Brown, B. Coral bleaching: Causes and consequences. *Coral Reefs* **1997**, *16*, 129–138. [CrossRef]
64. Brown, B.E.; Suharsono. Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. *Coral Reefs* **1990**, *8*, 163–170. [CrossRef]
65. Fisk, D.; Done, T.J. Taxonomic and bathymetric patterns of bleaching in corals, Myrmidon Reef (Queensland). *Proceeding 5th Int. Coral Reef Congr.* **1985**, *6*, 149–154.
66. Nakamura, T.; Woesik, R.V. Water-flow rates and passive diffusion partially explain differential survival of corals during the 1998 bleaching event. *Mar. Ecol. Prog. Ser.* **2001**, *212*, 301–304. [CrossRef]
67. Kayanne, H.; Harii, S.; Yamano, H.; Tamura, M.; Ide, Y.; Akimoto, F. Changes in living coral coverage before and after the 1998 bleaching event on coral reef flats of Ishigaki Island, Ryukyu Islands. *J. Jpn. Coral Reef Soc.* **1999**, *1*, 73–82. [CrossRef]
68. Jackson, J.; Donovan, M.; Cramer, K.; Lam, V. Status and trends of Caribbean Coral Reefs. In *Global Coral Reef Monitoring Network*; IUCN: Gland, Switzerland, 2012.
69. Loya, Y.; Sakai, K.; Yamazato, K.; Nakano, Y.; Sambali, H.; Van Woesik, R. Coral bleaching: The winners and losers. *Ecol. Lett.* **2001**, *4*, 122–131. [CrossRef]
70. Marcelino, L.A.; Westneat, M.W.; Stoyneva, V.; Henss, J.; Rogers, J.D.; Radosevich, A. Modulation of light-enhancement to symbiotic algae by light-scattering in corals and evolutionary trends in bleaching. *PLoS ONE* **2013**, *8*, e61492. [CrossRef] [PubMed]
71. Swain, T.D.; Dubois, E.; Gomes, A. Skeletal light-scattering accelerates bleaching response in reef-building corals. *BMC Ecol.* **2016**, *16*, 10. [CrossRef] [PubMed]
72. Rowan, R.; Knowlton, N.; Baker, A.; Jara, J. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* **1997**, *388*, 265–269. [CrossRef]
73. Cunning, R.; Baker, A.C. Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nat. Clim. Change* **2013**, *3*, 259–262. [CrossRef]
74. Wooldridge, S.A. Differential thermal bleaching susceptibilities amongst coral taxa: Re-posing the role of the host. *Coral Reefs* **2014**, *33*, 15–27. [CrossRef]
75. Mcclanahan, T.R. Coral responses to climate change exposure. *Environ. Res. Lett.* **2022**, *17*, 073001. [CrossRef]
76. Wangpraseurt, D.; Polerecky, L.; Larkum, A.W.; Ralph, P.J.; Nielsen, D.A.; Pernice, M.; Kühl, M. The in situ light microenvironment of corals. *Limnol. Oceanogr.* **2014**, *59*, 917–926. [CrossRef]
77. Zoccola, D.; Morain, J.; Pagès, G.; Caminiti-Segonds, N.; Giuliano, S.; Tambutté, S.; Allemand, D. Structural and functional analysis of coral hypoxia inducible factor. *PLoS ONE* **2017**, *12*, 1–13. [CrossRef]

78. Edmunds, P.J.; Davies, P.S. An energy budget for *Porites porites* (Scleractinia), growing in a stressed environment. *Coral Reefs.* **1989**, *8*, 37–43. [CrossRef]
79. Mubin, N.A.A.A.; Jonik, M.G.G.; Kamphol, N.; Juhi, Z.S.; Mohammad, M.; Salleh, S. In situ photosynthetic performance of *Porites lutea* inhabiting contrasting habitats of the Northern Straits of Malacca (NSoM), Malaysia. *Mar. Freshw. Behav. Physiol.* **2021**, *54*, 149–167. [CrossRef]
80. Laffoley, D.; Baxter, J.M. *Ocean Deoxygenation: Everyone's Problem. Causes, Impacts, Consequences and Solutions*; IUCN, Global Marine and Polar Programme: Gland, Switzerland, 2019.
81. Lesser, M.P. Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annu. Rev.* **2006**, *68*, 253–278. [CrossRef]
82. Yum, L.K.; Baumgarten, S.; Röthig, T.; Roder, C.; Roik, A.; Michell, C.; Voolstra, C.R. Transcriptomes and expression profiling of deep-sea corals from the Red Sea provide insight into the biology of azooxanthellate corals. *Sci. Rep.* **2017**, *7*, 6442. [CrossRef]
83. Shapiro, O.H.; Fernandez, V.I.; Garren, M.; Guasto, J.S.; Debaillon-Vesque, F.P.; Kramarsky-Winter, E.; Vardi, A.; Stocker, R. Vortical ciliary flows actively enhance mass transport in reef corals. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 13391–13396. [CrossRef] [PubMed]
84. Hebbeln, D.; Wienberg, C.; Dullo, W.C.; Freiwald, A.; Mienis, F.; Orejas, C.; Titschack, J. Cold-water coral reefs thriving under hypoxia. *Coral Reefs* **2020**, *39*, 853–859. [CrossRef]
85. Shick, J.M. Diffusion limitation and hyperoxic enhancement of oxygen consumption in zooxanthellate sea anemones, zoanthids, and corals. *Biol. Bull.* **1990**, *179*, 148–158. [CrossRef] [PubMed]
86. Dodds, L.A.; Roberts, J.M.; Taylor, A.C.; Marubini, F. Metabolic tolerance of the cold-water coral *Lophelia pertusa* (Scleractinia) to temperature and dissolved oxygen change. *J. Exp. Mar. Biol. Ecol.* **2007**, *349*, 205–214. [CrossRef]
87. Kremien, M.; Shavit, U.; Mass, T.; Genin, A. Benefit of pulsation in soft corals. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 8978–8983. [CrossRef] [PubMed]
88. Alderdice, R.; Suggett, D.J.; Cárdenas, A.; Hughes, D.J.; Kühl, M.; Pernice, M.; Voolstra, C.R. Divergent expression of hypoxia response systems under deoxygenation in reef-forming corals aligns with bleaching susceptibility. *Glob. Change Biol.* **2021**, *27*, 312–326. [CrossRef] [PubMed]
89. Andréfouët, S.; Dutheil, C.; Menkes, C.E.; Bador, M.; Lengaigne, M. Mass mortality events in atoll lagoons: Environmental control and increased future vulnerability. *Glob. Change Biol.* **2015**, *21*, 195–205. [CrossRef]
90. Raj, K.D.; Mathews, G.; Obura, D.O.; Laju, R.L.; Bharath, M.S.; Kumar, P.D.; Arasamuthu, A.; Kumar, T.K.A.; Edward, J.K.P. Low oxygen levels caused by *Noctiluca scintillans* bloom kills corals in Gulf of Mannar, India. *Sci. Rep.* **2020**, *10*, 1–7. [CrossRef] [PubMed]
91. Lesser, M.P. Coral reef bleaching and global climate change: Can corals survive the next century. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5259–5260. [CrossRef]
92. Jokiel, P.L.; Guinther, E.B. Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*. *Bull. Mar. Sci.* **1978**, *28*, 786–789.
93. Safuan, C.D.M.; Samshuri, M.A.; Jaafar, S.N.; Tan, C.H.; Bachok, Z. Physiological response of shallow-water hard coral *Acropora digitifera* to heat stress via fatty acid composition. *Front. Mar. Sci.* **2021**, *8*, 1187. [CrossRef]
94. Deleja, M.; Paula, J.R.; Repolho, T.; Franzitta, M.; Baptista, M.; Lopes, V.; Simão, S.; Fonseca, V.F.; Duarte, B.; Rosa, R. Effects of hypoxia on coral photobiology and oxidative stress. *Biology* **2022**, *11*, 1068. [CrossRef]
95. Johnson, M.D.; Swaminathan, S.D.; Nixon, E.N. Differential susceptibility of reef-building corals to deoxygenation reveals remarkable hypoxia tolerance. *Sci. Rep.* **2021**, *11*, 23168. [CrossRef]
96. Wang, Y.; Yu, Y.; Zhang, Y.; Zhang, H.R.; Chai, F. Distribution and variability of sea surface temperature fronts in the south China sea. *Estuar. Coast. Shelf Sci.* **2020**, *240*, 106793. [CrossRef]
97. Xiao, C.; Chen, N.; Hu, C.; Wang, K.; Gong, J.; Chen, Z. Short and mid-term sea surface temperature prediction using time-series satellite data and LSTM-AdaBoost combination approach. *Remote Sens. Environ.* **2019**, *233*, 111358. [CrossRef]
98. Pastor, F.; Valiente, J.A.; Palau, J.L. Sea surface temperature in the Mediterranean: Trends and spatial patterns (1982–2016). *Pure Appl. Geophys.* **2017**, 297–309. [CrossRef]
99. Chavanich, S.; Voranop, V.; Adams, P.; Klammer, J.; Cook, N. Reef communities after the 2010 mass coral bleaching at Racha Yai Island in the Andaman Sea and Koh Tao in the Gulf of Thailand. *Phuket Mar. Biol. Cent. Res. Bull.* **2012**, *71*, 103–110.
100. Yeemin, H.; Pengsakun, S.; Yucharoen, M.; Klinthong, W.; Sangmanee, K.; Sutthacheep, M. Long-term decline in *Acropora* species at Kut Island, Thailand, in relation to coral bleaching events. *Mar. Biodivers.* **2013**, *43*, 23–29. [CrossRef]
101. Tanzil, J.T.I. Bleaching susceptibility and growth characteristics of *Porites lutea* from the Andaman Sea, South Thailand. *Phuket Mar. Biol. Cent. Res. Bull.* **2012**, *70*, 49–56.
102. Bruno, J.F.; Bates, A.E.; Cacciapaglia, C.; Pike, E.P.; Amstrup, S.C.; Van Hooidonk, R.; Henson, S.A.; Aronson, R.B. Climate change threatens the world's marine protected areas. *Nat. Clim. Change* **2018**, *8*, 499–503. [CrossRef]
103. Altieri, A.H.; Gedan, K.B. Climate change and dead zones. *Glob. Change Biol.* **2015**, *21*, 1395–1406. [CrossRef]
104. Ainsworth, T.D.; Heron, S.F.; Ortiz, J.C.; Mumby, P.J.; Grech, A.; Ogawa, D.; Eakin, C.M.; Leggat, W. Climate change disables coral bleaching protection on the Great Barrier Reef. *Science* **2016**, *352*, 338–342. [CrossRef]
105. Chavanich, S.; Viyakarn, V.; Loyjiw, T.; Pattaratamrong, P.; Chankong, A. Mass bleaching of soft coral, *Sarcophyton* spp. In Thailand and the role of temperature and salinity stress. *ICES J. Mar. Sci.* **2009**, *66*, 1515–1519. [CrossRef]

106. Altieri, A.H.; Johnson, M.D.; Swaminathan, S.D.; Nelson, H.R.; Gedan, K.B. Resilience of tropical ecosystems to ocean deoxygenation. *Trends Ecol. Evol.* **2021**, *36*, 227–238. [CrossRef] [PubMed]
107. Vaquer-Sunyer, R.; Duarte, C.M. Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. *Glob. Change Biol.* **2011**, *17*, 1788–1797. [CrossRef]
108. Deutsch, C.; Ferrel, A.; Seibel, B.; Pörtner, H.O.; Huey, R.B. Climate change tightens a metabolic constraint on marine habitats. *Science* **2015**, *348*, 1132–1135. [CrossRef] [PubMed]