



**Coral Physiology and Growth Response to Heat Stress and Hypoxia:  
a Case of Patong Reef, Phuket**

**Tarun Jain**

**A Thesis Submitted in Fulfillment of the Requirements for the  
Degree of Master of Science in Marine and Coastal Resources  
Management**

**Prince of Songkla University**

**2023**

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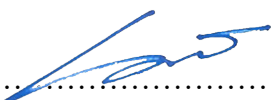
**Thesis Title** Coral physiology and growth response to heat stress and hypoxia: a case of Patong reef, Phuket

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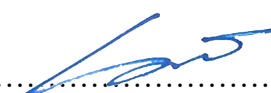
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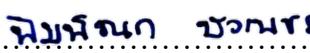
  
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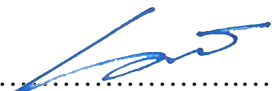
  
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

  
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Candidate

|                      |  |
|----------------------|--|
| <b>Thesis Title</b>  | Coral physiology and growth response to heat stress and hypoxia: a case of Patong reef, Phuket |
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### ABSTRACT

Coral reefs around the world are under threat from multiple global and local stressors. Understanding the responses of different coral species to these stressors is essential to develop effective management and conservation strategies and mitigate the impacts of climate change on coral reefs.

This study examines the responses of three coral species - *Porites lutea* (massive), *Montipora tuberculosa* (tabular), and *Pocillopora verrucosa* (branching) - to rising seawater temperature and low dissolved oxygen (DO) levels, which are expected to intensify as a result of climate change. Coral nubbins were collected from Patong Bay, Phuket, Thailand, and subjected to experimental investigations under ambient conditions (29 °C, DO > 6 mgL<sup>-1</sup>), heat stress (32 °C), hypoxia (DO < 2 mgL<sup>-1</sup>) and heat stress + hypoxia treatments. Photosynthetic performance indicators  $F_v/F_m$  and  $F_v/F_0$ , and physiological parameters - Symbiodiniaceae density, pigment concentration, and growth rate were quantified.

Results indicate that *P. verrucosa* is the most sensitive and severely affected by heat stress and hypoxia, more so than *P. lutea* and *M. tuberculosa*. The combination of heat stress and hypoxia had less impact on these species, except for a decline in growth rate of *M. tuberculosa*. Moreover, the coral species respond differently to high temperature and low oxygen, with their sensitivity depending on the species. These responses may differ according to the lighting, especially in hypoxic conditions. Additional research is required to better understand the combined impacts of heat stress and hypoxia, and to enhance the mechanistic comprehension of synergistic and antagonistic responses in the three scleractinian corals.

The study fills a research gap and provides useful information to predict the vulnerability of these three coral species in shallow reef habitats under climate change scenarios, helping to devise effective mitigation strategies for the preservation of coral reefs globally.

## ACKNOWLEDGEMENT

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**Tarun Jain**

**CONTENTS**

|     | <b>Title</b>                      | <b>Page</b> |
|-----|-----------------------------------|-------------|
| 1.  | ABSTRACT                          | v           |
| 2.  | ACKNOWLEDGEMENT                   | vi          |
| 3.  | LIST OF TABLES                    | viii        |
| 4.  | LIST OF FIGURES                   | ix          |
| 5.  | LIST OF ABBREVIATIONS AND SYMBOLS | x           |
| 6.  | LIST OF PAPERS AND CONFERENCES    | xii         |
| 7.  | INTRODUCTION                      | 1           |
| 8.  | OBJECTIVES                        | 2           |
| 9.  | RESULTS                           | 3           |
| 10. | DISCUSSION                        | 11          |
| 11. | REPEAT EXPERIMENT                 | 16          |
| 12. | CONCLUDING REMARKS                | 19          |
| 13. | REFERENCES                        | 19          |
| 14. | APPENDIX 1                        | 26          |
| 15. | APPENDIX 2                        | 43          |
| 16. | APPENDIX 3                        | 44          |
| 17. | VITAE                             | 46          |



**LIST OF TABLES**

|    | <b>Table</b>   | <b>Page</b> |
|----|--|-------------|
| 1. | Summary of different ANOVAs for photosynthesis, <i>Symbiodiniaceae</i> density, chlorophyll content and growth rate of <i>P. lutea</i> . Significant values ( $p < 0.05$ ) are shown in bold.        | 3           |
| 2. | Summary of different ANOVAs for photosynthesis, <i>Symbiodiniaceae</i> density, chlorophyll content and growth rate of <i>M. tuberculosis</i> . Significant values ( $p < 0.05$ ) are shown in bold. | 4           |
| 3. | Summary of different ANOVAs for photosynthesis, <i>Symbiodiniaceae</i> density, chlorophyll content and growth rate of <i>P. verrucosa</i> . Significant values ( $p < 0.05$ ) are shown in bold.    | 5           |

## LIST OF FIGURES

|     | <b>Figure</b>   | <b>Page</b> |
|-----|---|-------------|
| 1.  | Maximum quantum yield of PSII (Fv/Fm; left) and photochemical efficiency of PSII (Fv/F0; right) in <i>P. lutea</i> (A,B), <i>M. tuberculosis</i> (C,D), and <i>P. verrucosa</i> (E,F) measured before the light period.                                     | 6           |
| 2.  | Maximum quantum yield of PSII (Fv/Fm; left) and photochemical efficiency of PSII (Fv/F0; right) in <i>P. lutea</i> (A,B), <i>M. tuberculosis</i> (C,D), and <i>P. verrucosa</i> (E,F) measured after the light period.                                      | 7           |
| 3.  | Symbiodiniaceae density ( $\times 10^5$ cells $\text{cm}^{-2}$ ) of <i>P. lutea</i> (A), <i>M. tuberculosis</i> (B), and <i>P. verrucosa</i> (C) under ambient baseline conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. | 9           |
| 4.  | Chlorophyll a concentration ( $\mu\text{g cm}^{-2}$ ) of <i>P. lutea</i> (A), <i>M. tuberculosis</i> (B), and <i>P. verrucosa</i> (C) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9.                     | 9           |
| 5.  | Chlorophyll c2 concentration ( $\mu\text{g cm}^{-2}$ ) of <i>P. lutea</i> (A), <i>M. tuberculosis</i> (B), and <i>P. verrucosa</i> (C) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9.                    | 10          |
| 6.  | Growth rates of <i>P. lutea</i> (A), <i>M. tuberculosis</i> (B), and <i>P. verrucosa</i> (C) under ambient, heat stress, hypoxia, and heat stress + hypoxia.  | 10          |
| 7.  | <i>P. lutea</i> samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments.  | 12          |
| 8.  | <i>M. tuberculosis</i> samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments.   | 13          |
| 9.  | <i>P. verrucosa</i> samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments.  | 14          |
| 10. | Maximum quantum yield of PSII (Fv/Fm; left) and photochemical efficiency of PSII (Fv/F0; right) in <i>P. lutea</i> (A,B), <i>M. tuberculosis</i> (C,D), and <i>P. verrucosa</i> (E,F) measured before the light period.                                     | 17          |
| 11. | Maximum quantum yield of PSII (Fv/Fm; left) and photochemical efficiency of PSII (Fv/F0; right) in <i>P. lutea</i> (A,B), <i>M. tuberculosis</i> (C,D), and <i>P. verrucosa</i> (E,F) measured after the light period.                                      | 18          |

## LIST OF ABBREVIATIONS AND SYMBOLS

\* = Significance when  $p < 0.05$

°C = Temperature in Celsius

ANOVA = Analysis of Variance

ATP = Adenosine triphosphate

BBD = Black Band Disease

cell  $\text{cm}^{-2}$  = cell per centimeter squared

chl *a* = Chlorophyll *a* concentration

chl *c*<sub>2</sub> = Chlorophyll *c*<sub>2</sub> concentration

cm = centimeter

CO<sub>2</sub> = Carbon di-oxide

df = degree of freedoms

DO = Dissolved Oxygen

F = F-value ( ratio of two variances in statistics )

$F_v/F_0$  = Photochemical efficiency

$F_v/F_m$  = Maximum Quantum Yield

H<sub>2</sub>O<sub>2</sub> = Hydrogen Peroxide

H<sub>2</sub>S = Hydrogen Sulphide

HAB = Harmful algal blooms

*M. tuberculosa* = *Montipora Tuberculosa*

$\text{mgL}^{-1}$  = Milligram per Litre

n = Number of replicates

NO = Nitric Oxide

O<sub>2</sub> = Oxygen

**LIST OF ABBREVIATIONS AND SYMBOLS (cont.)**

p = p-value (statistics)

*P. lutea* = *Porites lutea*

*P. verrucosa* = *Pocillopora Verrucosa*

PSII = Photosystem II

ROS = Reactive oxygen species

SE = Standard error

SST = Sea Surface Temperature

$\mu\text{g cm}^{-2}$  = microgram per centimeter squared

## LIST OF PAPERS AND CONFERENCES

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<https://doi.org/10.3390/jmse11020403>
2. Jain, T., Yucharoen, M. & Buapet, P. (2021). Coral response to heat stress and hypoxia. Presented in the 1<sup>st</sup> International Postgraduate Conference on Science and Marine Environment (IPCoSME 2021)
3. Jain, T., Ying, L., Buapet, P., & Yucharoen, M. (2022). Effects of heat stress and hypoxia on the photosynthetic efficiency and growth rate of three scleractinian corals. Presented in the 15<sup>th</sup> International Coral Reef Symposium (ICRS 2022)

## 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<sup>-1</sup> 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<sup>-1</sup>), 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.

## OBJECTIVES

This study aimed to demonstrate the combined effect of heat stress and hypoxia on the physiological response of three sclerectanian corals.

Specific Objectives:

1. Examine the physiological response of corals to heat stress
2. Examine the physiological response of corals to hypoxia
3. Investigate the combined effect of heat stress and hypoxia on corals

## 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 Tables 1–3). Each coral species revealed a different response, and the combined stresses had a significantly worse effect only on the growth rate of *M. tuberculosa*.

**Table 1.** 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.

| <i>P. lutea</i>         |    |                                      |                                       |                                      |                                       |                                   |               |                           |                |
|-------------------------|----|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|-----------------------------------|---------------|---------------------------|----------------|
| Factor or interaction   |    | $F_w/F_m$<br>after dark<br>condition | $F_w/F_m$<br>after light<br>condition | $F_w/F_0$<br>after dark<br>condition | $F_w/F_0$<br>after light<br>condition | <i>Symbiodiniaceae</i><br>density | Chl <i>a</i>  | Chl <i>c</i> <sub>2</sub> | Growth<br>rate |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3             | 3                         |                |
| Time                    | F  | 5.674                                | 7.506                                 | 6.403                                | 7.960                                 | 3.075                             | 2.696         | 2.427                     |                |
|                         | p  | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>0.036*</b>                     | 0.056         | 0.077                     |                |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1             | 1                         | 1              |
| Temperature             | F  | 6.814                                | 7.917                                 | 8.327                                | 9.315                                 | 14.012                            | 0.982         | 6.502                     | 0.273          |
|                         | p  | <b>0.010*</b>                        | <b>0.006*</b>                         | <b>0.005*</b>                        | <b>0.003*</b>                         | <b>&lt;0.001*</b>                 | 0.327         | <b>0.014*</b>             | 0.611          |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1             | 1                         | 1              |
| DO                      | F  | 14.720                               | 8.190                                 | 15.723                               | 8.903                                 | 6.395                             | 1.571         | 0.685                     | 1.260          |
|                         | p  | <b>&lt;0.001*</b>                    | <b>0.005*</b>                         | <b>&lt;0.001*</b>                    | <b>0.004*</b>                         | <b>0.015*</b>                     | 0.216         | 0.412                     | 0.284          |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3             | 3                         |                |
| Time x temperature      | F  | 1.153                                | 3.193                                 | 1.227                                | 3.269                                 | 2.756                             | 0.615         | 0.866                     |                |
|                         | p  | 0.331                                | <b>0.003*</b>                         | 0.285                                | <b>0.002*</b>                         | 0.052                             | 0.609         | 0.465                     |                |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3             | 3                         |                |
| Time x DO               | F  | 0.841                                | 2.456                                 | 0.925                                | 2.692                                 | 0.691                             | 2.421         | 0.628                     |                |
|                         | p  | 0.579                                | <b>0.018*</b>                         | 0.506                                | <b>0.010*</b>                         | 0.562                             | 0.077         | 0.601                     |                |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1             | 1                         | 1              |
| Temperature x DO        | F  | 1.572                                | 9.755                                 | 0.940                                | 8.136                                 | 0.213                             | 0.002         | 0.532                     | 0.196          |
|                         | p  | 0.212                                | <b>0.002*</b>                         | 0.334                                | <b>0.005*</b>                         | 0.647                             | 0.964         | 0.469                     | 0.666          |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3             | 3                         |                |
| Time x temperature x DO | F  | 0.976                                | 2.579                                 | 1.018                                | 3.110                                 | 3.452                             | 2.852         | 0.526                     |                |
|                         | p  | 0.463                                | <b>0.013*</b>                         | 0.429                                | <b>0.003*</b>                         | <b>0.024*</b>                     | <b>0.047*</b> | 0.667                     |                |



**Table 2.** 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.

| <i>M. tuberculosa</i>   |    |                                      |                                       |                                      |                                       |                                   |                   |                     |                   |
|-------------------------|----|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|-----------------------------------|-------------------|---------------------|-------------------|
| Factor or interaction   |    | $F_w/F_m$<br>after dark<br>condition | $F_w/F_m$<br>after light<br>condition | $F_w/F_0$<br>after dark<br>condition | $F_w/F_0$<br>after light<br>condition | <i>Symbiodiniaceae</i><br>density | Chl. a            | Chl. c <sub>2</sub> | Growth<br>rate    |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                   |                   |
| Time                    | F  | 3.872                                | 2.424                                 | 3.861                                | 2.238                                 | 13.467                            | 9.593             | 36.852              |                   |
|                         | p  | <b>&lt;0.001*</b>                    | <b>0.019*</b>                         | <b>&lt;0.001*</b>                    | <b>0.03*</b>                          | <b>&lt;0.001*</b>                 | <b>&lt;0.001*</b> | <b>&lt;0.001*</b>   |                   |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                   | 1                 |
| Temperature             | F  | 12.895                               | 7.323                                 | 13.936                               | 8.832                                 | 0.373                             | 4.395             | 0.271               | 19.825            |
|                         | p  | <b>&lt;0.001*</b>                    | <b>0.008*</b>                         | <b>&lt;0.001*</b>                    | <b>0.004*</b>                         | 0.544                             | <b>0.041*</b>     | 0.605               | <b>&lt;0.001*</b> |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                   | 1                 |
| DO                      | F  | 12.578                               | 17.652                                | 12.545                               | 17.472                                | 0.880                             | 1.983             | 2.316               | 0.007             |
|                         | p  | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | 0.353                             | 0.166             | 0.135               | 0.936             |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                   |                   |
| Time x temperature      | F  | 0.532                                | 0.205                                 | 0.651                                | 0.155                                 | 0.438                             | 0.594             | 2.239               |                   |
|                         | p  | 0.849                                | 0.989                                 | 0.751                                | 0.996                                 | 0.726                             | 0.622             | 0.096               |                   |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                   |                   |
| Time x DO               | F  | 0.464                                | 0.670                                 | 0.447                                | 0.654                                 | 0.827                             | 1.140             | 3.085               |                   |
|                         | p  | 0.896                                | 0.717                                 | 0.907                                | 0.731                                 | 0.486                             | 0.343             | <b>0.036*</b>       |                   |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                   | 1                 |
| Temperature x DO        | F  | 0.041                                | 2.405                                 | 0.045                                | 2.092                                 | 4.975                             | 0.022             | 3.495               | 0.623             |
|                         | p  | 0.840                                | 0.124                                 | 0.832                                | 0.151                                 | <b>0.030*</b>                     | 0.883             | 0.068               | 0.445             |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                   |                   |
| Time x temperature x DO | F  | 0.308                                | 0.158                                 | 0.304                                | 0.223                                 | 0.968                             | 1.733             | 2.818               |                   |
|                         | p  | 0.971                                | 0.996                                 | 0.972                                | 0.986                                 | 0.416                             | 0.173             | <b>0.049*</b>       |                   |

**Table 3.** 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.

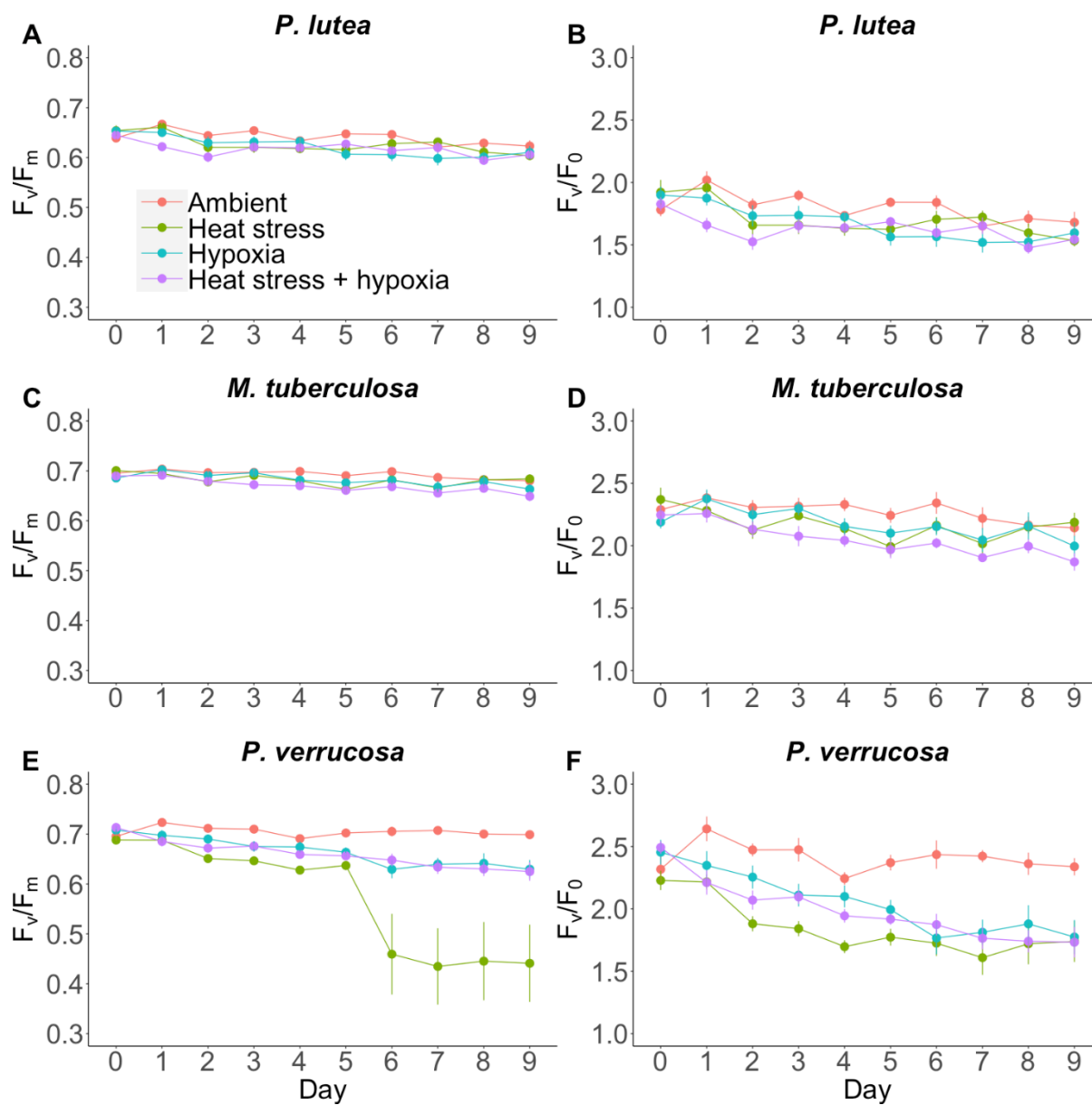
| <i>P. verrucosa</i>     |    |                                      |                                       |                                      |                                       |                                   |                   |                   |                |
|-------------------------|----|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|-----------------------------------|-------------------|-------------------|----------------|
| Factor or interaction   |    | $F_v/F_m$<br>after dark<br>condition | $F_v/F_m$<br>after light<br>condition | $F_v/F_0$<br>after dark<br>condition | $F_v/F_0$<br>after light<br>condition | <i>Symbiodiniaceae</i><br>density | Chl.a             | Chl.c2            | Growth<br>rate |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                 |                |
| Time                    | F  | 2.873                                | 2.620                                 | 7.077                                | 1.726                                 | 2.060                             | 0.853             | 0.900             |                |
|                         | p  | <b>0.004*</b>                        | <b>0.012*</b>                         | <b>&lt;0.001*</b>                    | 0.100                                 | 0.118                             | 0.472             | 0.448             |                |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                 | 1              |
| Temperature             | F  | 19.782                               | 17.202                                | 54.731                               | 15.946                                | 16.810                            | 2.463             | 2.874             | 3.232          |
|                         | p  | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>&lt;0.001*</b>                 | 0.123             | 0.096             | 0.097          |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                 | 1              |
| DO                      | F  | 2.437                                | 1.010                                 | 6.482                                | 7.720                                 | 7.134                             | 0.001             | 0.132             | 3.319          |
|                         | p  | 0.121                                | 0.317                                 | <b>0.012*</b>                        | <b>0.006*</b>                         | <b>0.010*</b>                     | 0.982             | 0.718             | 0.093          |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                 |                |
| Time x temperature      | F  | 1.200                                | 0.975                                 | 0.684                                | 0.404                                 | 2.807                             | 0.648             | 0.462             |                |
|                         | p  | 0.301                                | 0.460                                 | 0.722                                | 0.916                                 | <b>0.049*</b>                     | 0.588             | 0.710             |                |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                 |                |
| Time x DO               | F  | 0.445                                | 0.438                                 | 1.335                                | 1.079                                 | 2.186                             | 1.778             | 0.317             |                |
|                         | p  | 0.908                                | 0.896                                 | 0.226                                | 0.384                                 | 0.102                             | 0.164             | 0.813             |                |
|                         | df | 1                                    | 1                                     | 1                                    | 1                                     | 1                                 | 1                 | 1                 | 1              |
| Temperature x DO        | F  | 17.016                               | 14.885                                | 34.425                               | 10.299                                | 24.739                            | 21.413            | 26.080            | 6.312          |
|                         | p  | <b>&lt;0.001*</b>                    | <b>&lt;0.001*</b>                     | <b>&lt;0.001*</b>                    | <b>0.002*</b>                         | <b>&lt;0.001*</b>                 | <b>&lt;0.001*</b> | <b>&lt;0.001*</b> | <b>0.027*</b>  |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                 |                |
| Time x temperature x DO | F  | 1.322                                | 1.282                                 | 0.602                                | 0.609                                 | 2.949                             | 9.368             | 7.344             |                |
|                         | p  | 0.233                                | 0.260                                 | 0.794                                | 0.768                                 | <b>0.042*</b>                     | <b>&lt;0.001*</b> | <b>&lt;0.001*</b> |                |
|                         | df | 9                                    | 8                                     | 9                                    | 8                                     | 3                                 | 3                 | 3                 |                |

## 1. Photosynthetic Efficiency

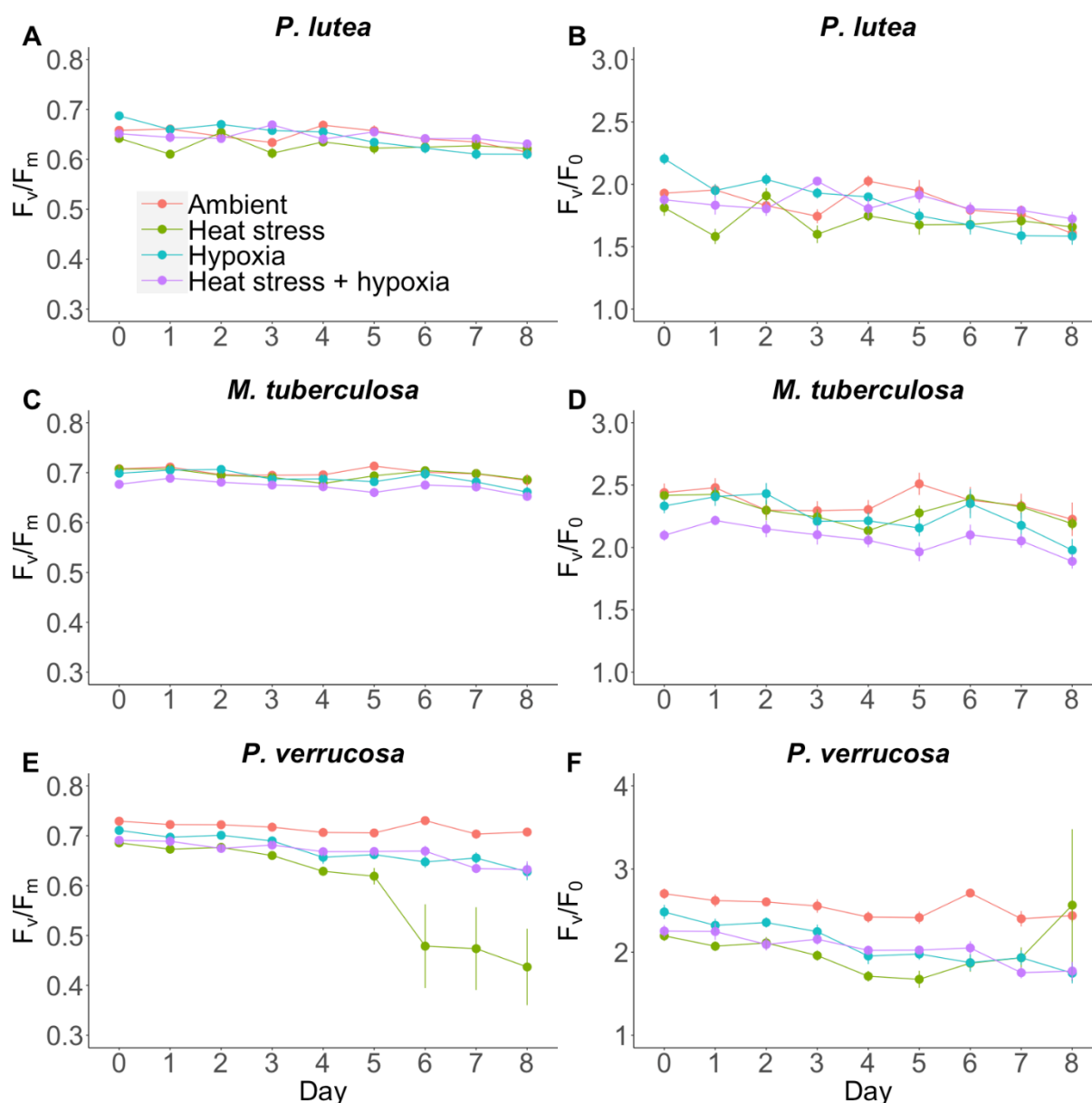
The  $F_v/F_m$  and  $F_v/F_0$  of *P. lutea* measured before the light period under ambient conditions on day 0 were  $0.639 \pm 0.023$  and  $1.781 \pm 0.177$ , respectively. The treatment with heat stress + hypoxia showed a significant decrease compared to ambient on day 1 ( $p = 0.003$  for  $F_v/F_m$  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 1A,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).

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 2A,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 (Table 1).



**Figure 1.** 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  $\pm$  SE ( $n = 4$ ).



**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 after the light period. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

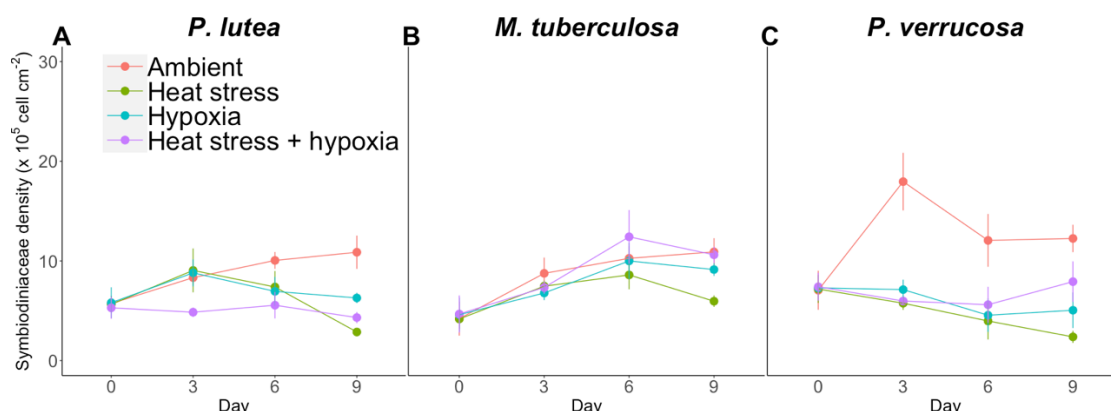
*M. tuberculosa* had similar results to *P. lutea* and seemed to express a tolerance to stress conditions. During the experiment period,  $F_v/F_m$  and  $F_v/F_0$  showed no significant differences between the stress treatments when compared to ambient conditions in measurements taken before the light 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 2C,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 (Table 2).

*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 1E,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 2E,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 compared 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  $\times$  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  $\times$  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 Table 3.

## 2. Symbiodiniaceae Density

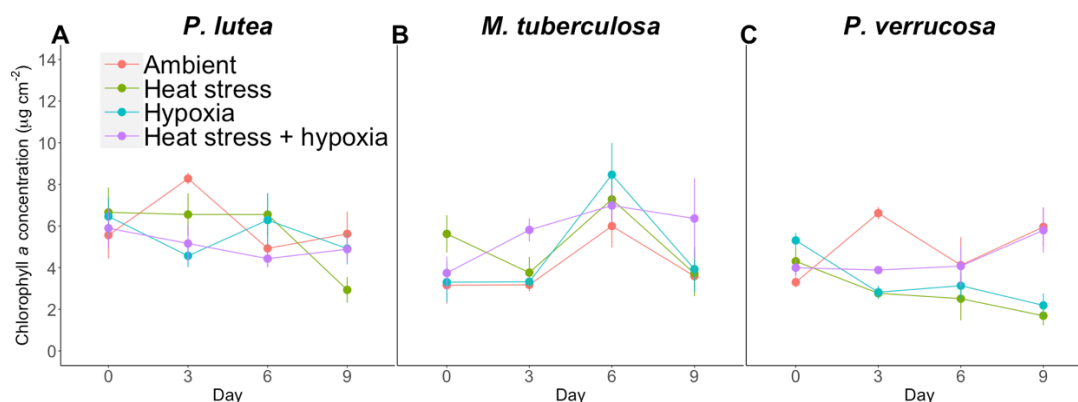
On day 9, *Symbiodiniaceae* densities in *P. lutea* in all actual treatments were significantly lower than in the baseline ambient condition (heat stress  $p < 0.001$ , hypoxia  $p = 0.019$ , heat stress + hypoxia  $p = 0.001$ ) (Figure 3A). There were significant effects of time ( $p = 0.036$ ), temperature ( $p < 0.001$ ), DO ( $p = 0.015$ ), and their interaction time  $\times$  temperature  $\times$  DO ( $p = 0.024$ ) (Table 1). Interestingly, *M. tuberculosa* presented a significant increase in *Symbiodiniaceae* density (Figure 3B) 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  $\times$  DO ( $p = 0.030$ ), as shown in Table 2. *P. verrucosa* revealed significant difference in *Symbiodiniaceae* density by treatment (Figure 3C), 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  $\times$  temperature ( $p = 0.049$ ), temperature  $\times$  DO ( $p < 0.001$ ), and time  $\times$  temperature  $\times$  DO ( $p = 0.042$ ) (Table 3).



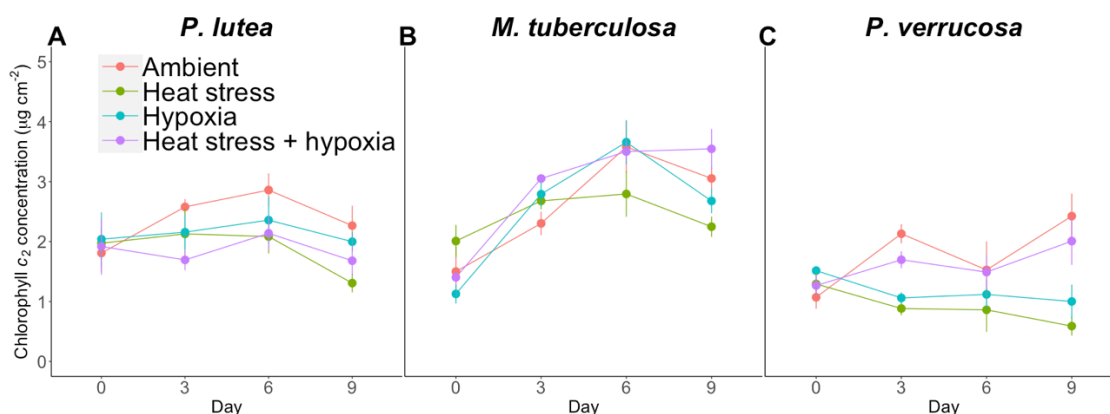
**Figure 3.** Symbiodiniaceae density ( $\times 10^5$  cells  $\text{cm}^{-2}$ ) of *P. lutea* (A), *M. tuberculosis* (B), and *P. verrucosa* (C) under ambient baseline conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

### 3. Chlorophyll Content

In *P. lutea*, we found consistent chlorophyll *a* and *c*<sub>2</sub> concentrations throughout the experiment period in all treatments (Figures 4A and 5A), and the interaction time  $\times$  temperature  $\times$  DO was the only significant factor for chlorophyll *a* ( $p = 0.047$ ; Table 1) whereas chlorophyll *c*<sub>2</sub> was significantly influenced by the individual factor temperature ( $p = 0.014$ ; Table 1). On the other hand, *M. tuberculosis* revealed a significant increase on day 6 in chlorophyll *a* ( $p < 0.001$ ) and on days 3, 6, and 9 in chlorophyll *c*<sub>2</sub> ( $p < 0.001$ ), although the coral nubbins were under stress (Figures 4B and 6B). Time was a significant factor for both chlorophyll *a* and *c*<sub>2</sub> in *M. tuberculosis* ( $p < 0.001$ ; Table 2). 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 4C). Chlorophyll *c*<sub>2</sub> in *P. verrucosa* presented a similar trend (Figure 5C), 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*<sub>2</sub> were temperature  $\times$  DO and time  $\times$  temperature  $\times$  DO ( $p < 0.001$ ; Table 3).



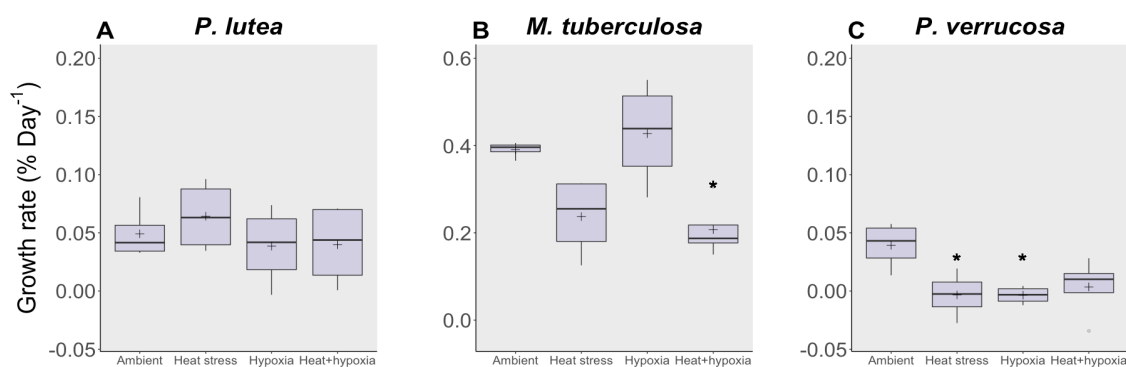
**Figure 4.** Chlorophyll *a* and *c*<sub>2</sub> concentration ( $\mu\text{g cm}^{-2}$ ) of *P. lutea* (A), *M. tuberculosis* (B), and *P. verrucosa* (C) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean  $\pm$  SE ( $n = 4$ ).



**Figure 5.** Chlorophyll *c*<sub>2</sub> concentration (µg cm<sup>-2</sup>) of *P. lutea* (A), *M. tuberculosis* (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).

#### 4. Growth Rate

There was no significant difference in growth rate of *P. lutea* between the treatments (Figure 6A), while a significant decrease in the growth of *M. tuberculosis* was recorded under heat stress + hypoxia (46.9% reduction, *p* = 0.041) compared to ambient condition (Figure 6B). The growth rate of *M. tuberculosis* 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 6C) 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 6.** Growth rates of *P. lutea* (A), *M. tuberculosis* (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.

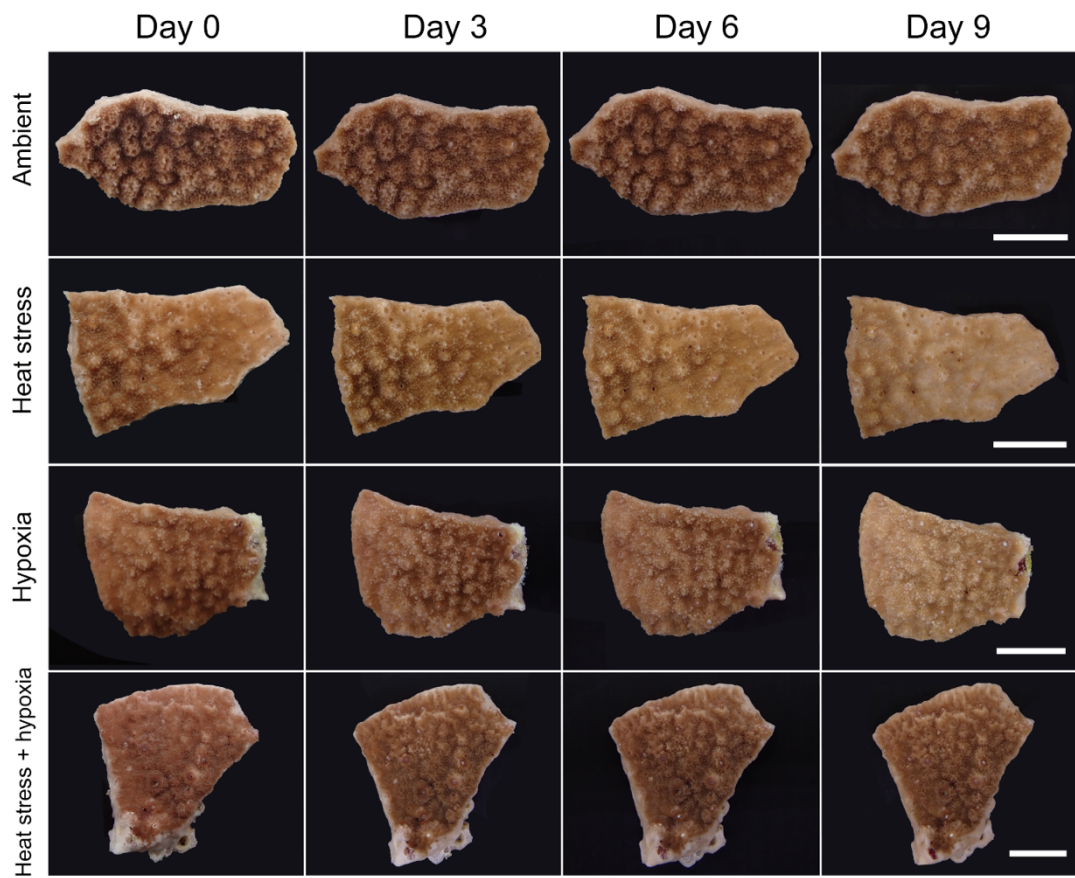
## DISCUSSION

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 Figures 7–9). 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 [14,47]. *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 [48–53]. The difference in bleaching vulnerability of branching corals has been attributed to several physiological and morphological properties: tissue thickness [54], skeletal fragility and within tissue light scattering [55,56], genetic constitution of symbiotic algae [57], *Symbiodiniaceae* density per coral cell [58], and mass transfer rate [15,59].

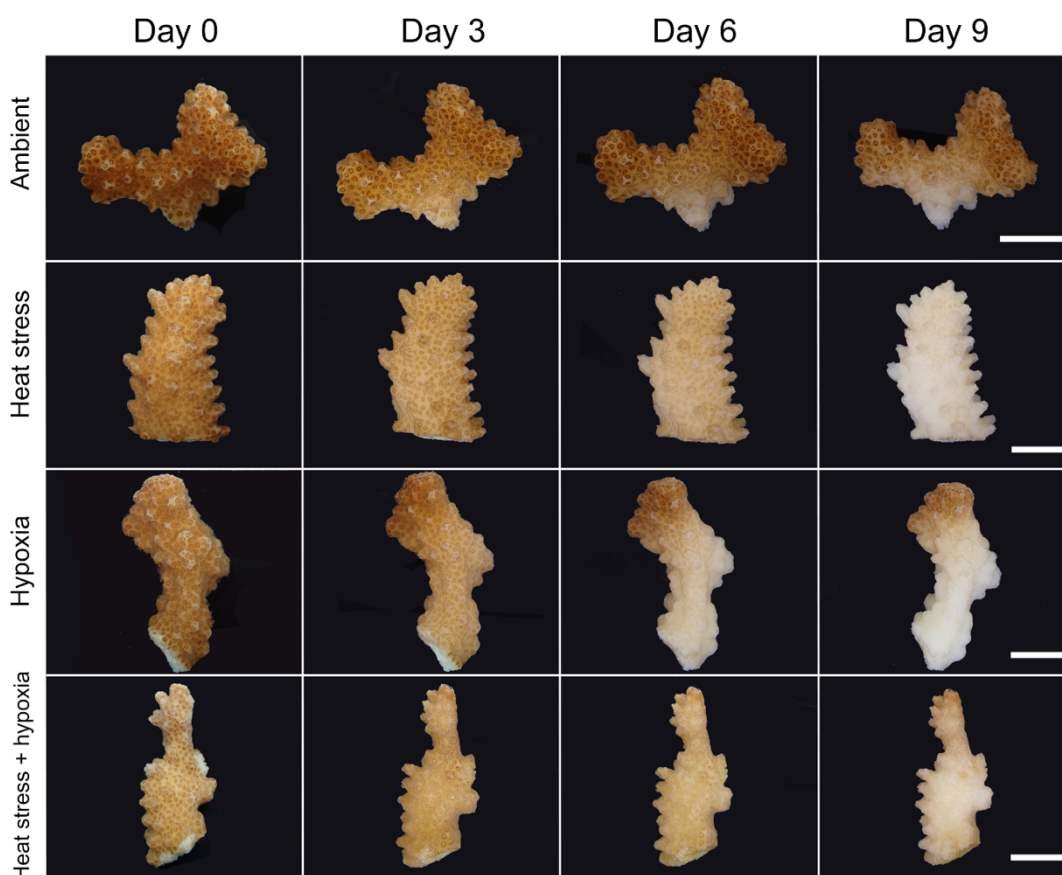




**Figure 7.** *P. lutea* samples from day 0 to day 9 under ambient, heat stress, hypoxia, and heat stress + hypoxia treatments. The white lines represent 1 cm scale bar.



**Figure 8.** *M. tuberculosis* 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 9.** *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.

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 [6,60,61]. The fast shift between hyperoxia and hypoxia sometimes induces microenvironment acclimation in coral tissues [62]. 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 [63,64]. Photosynthesis ( $\text{CO}_2$  consumption and  $\text{O}_2$  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 [65]. 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 [15,51,66].

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  $1 \text{ mgL}^{-1}$  DO concentration in the Red Sea [67]), and some corals appear to oxy-regulate using epidermal cilia movements during stagnant flow [68]. 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 [69–71], while some soft corals use tentacle pulsation to increase oxygen levels in their tissue [72]. The hypoxia ( $\sim 2 \text{ mgL}^{-1}$ ) in our case showed severe adverse effects on *P. verrucosa* in terms of physiological and visual expressions, bleaching, and tissue loss (refer to Figure 9). 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  $4 \text{ mgL}^{-1}$  oxygen concentration [32,73]. On the other hand, massive or submassive corals seem to be the survivors of severe hypoxic events [74,75], even when exposed to  $0.5 \text{ mgL}^{-1}$  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  $\text{H}_2\text{S}$ ,  $\text{NO}$ , and  $\text{H}_2\text{O}_2$ , that further compromise the host's immunity [6,76,77].

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) [78,79]. 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 [80]. 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  $1\text{--}3 \text{ }^\circ\text{C}$  increase in sea surface temperature (SST) being a likely scenario by the end of this century [81–83]. 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  $34 \text{ }^\circ\text{C}$  was reported in the Andaman Sea from April–June 2010 that led to a decline in *Acropora* and *Pocillopora* cover [84,85] and reduction in *P. lutea* growth [86]. 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 [17,26,87]. 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 [19,24,88]. 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 [75,89,90]. 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 [91]. Ongoing global and local threats may cause a shift toward tolerant local species (for example, *P. lutea* and *M. tuberculosis* 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 [37,92,93].

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.

### REPEAT EXPERIMENT

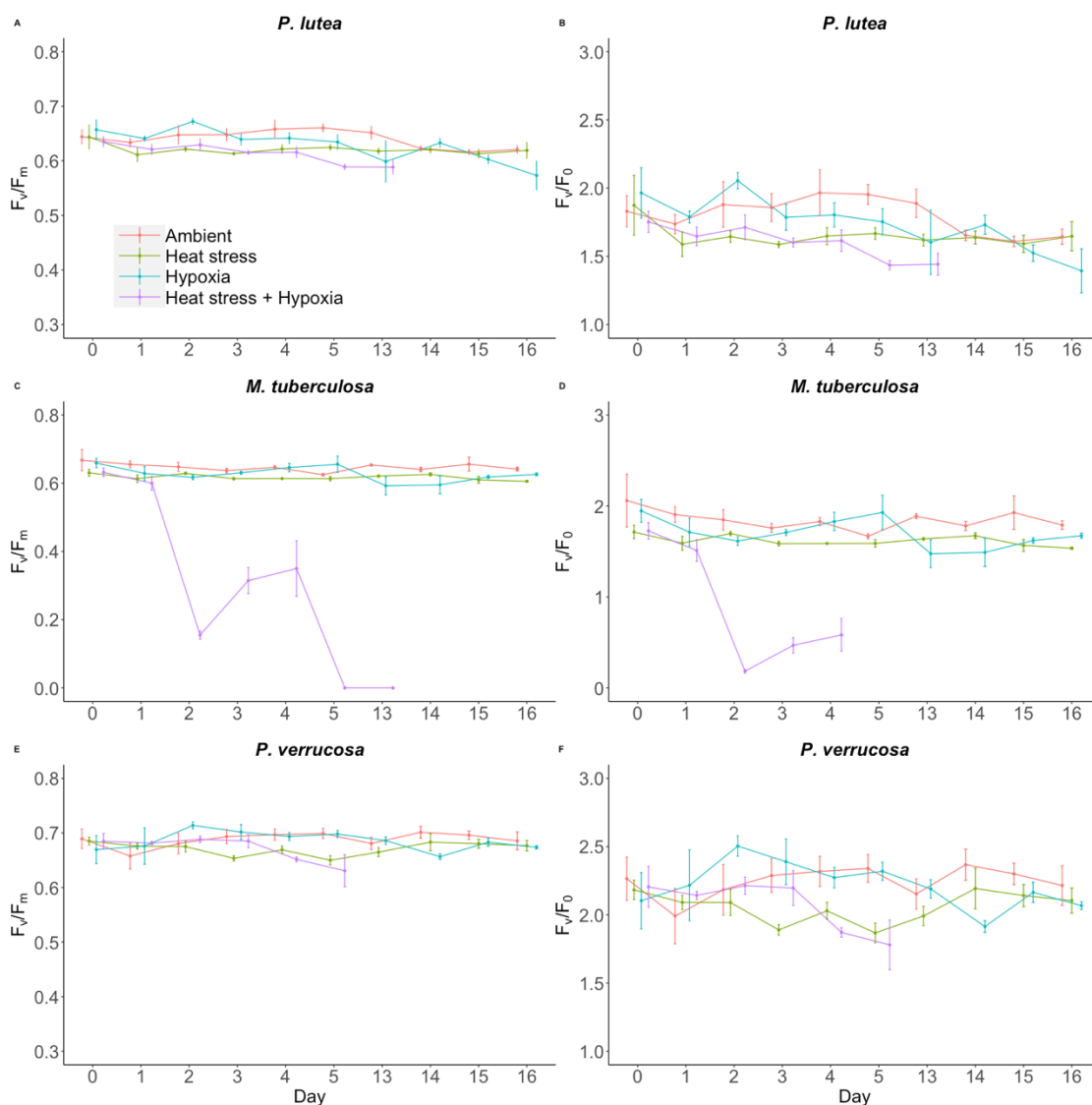
In a follow-up to our initial study, a partial repeat of the experiment was conducted in May 2023 with 3 sample colonies of *P. lutea*, *M. tuberculosis* and *P. verrucosa* collected from Patong Bay, Phuket in April 2023.

The samples were acclimated in a holding tank for a week, before being broken down into smaller nubbins and placed in treatment tanks just like the previous experiment except for following modifications to the experimental setup.

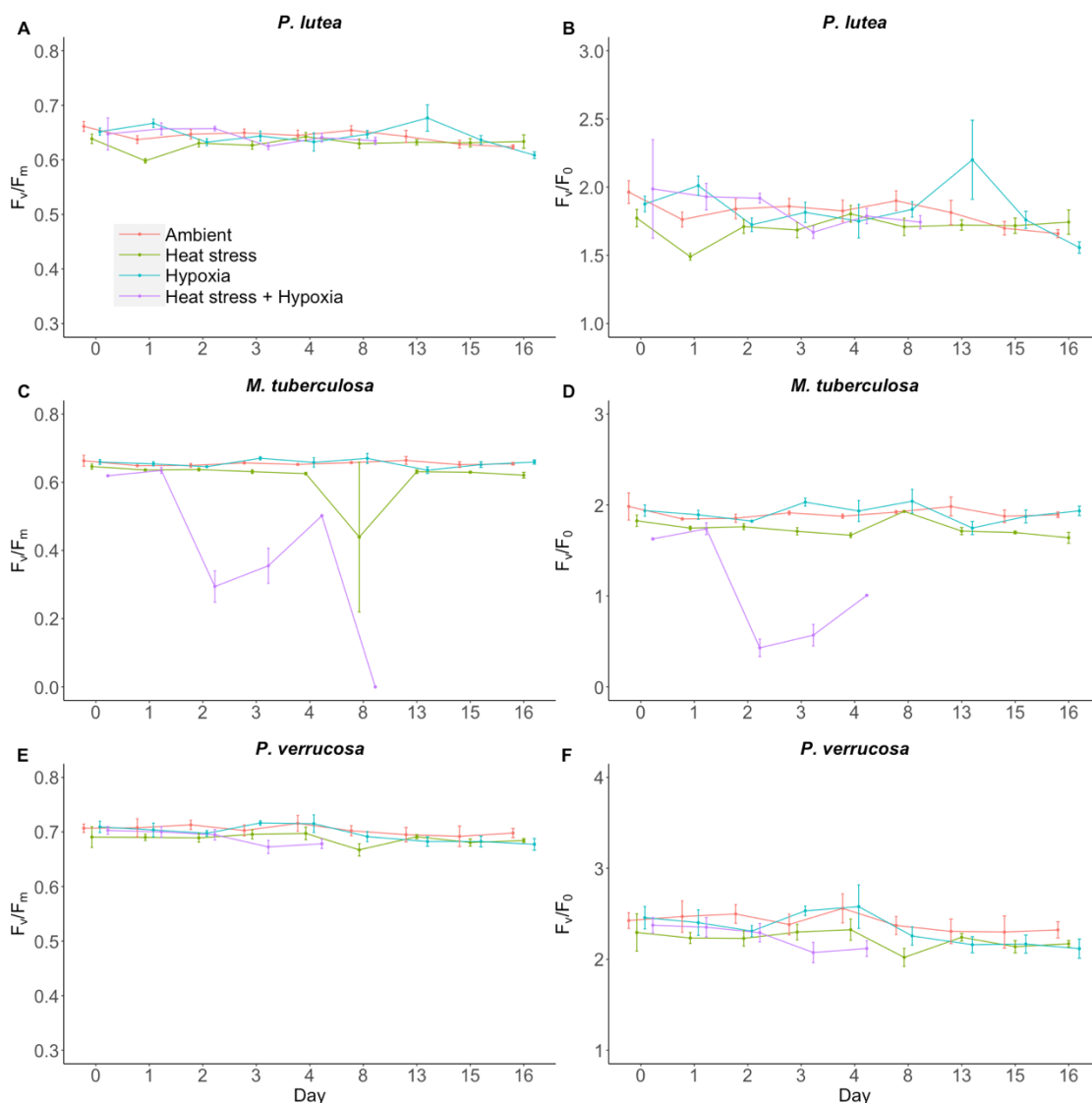
1. The treatment tanks were upgraded to include a sump system, providing continuous flow and filtration of water.
2. The focus of the measurements was narrowed to photosynthetic efficiency, collected daily.
3. The experiment duration was set at 16 days.

We observed trends in photosynthetic efficiency that echoed findings from our preliminary experiment (Figure 10 and Figure 11). The influence of light availability

was consistently impactful across all three species, *P. lutea*, *M. tuberculosa*, and *P. verrucosa*.



**Figure 10.** 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  $\pm$  SE ( $n = 4$ ).



**Figure 11.** 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  $\pm$  SE ( $n = 4$ ).

However, we noted some divergences from our initial findings:

1. Heat Stress + Hypoxia: All three species exhibited poorer performance under the combined effects of heat stress and hypoxia compared to our initial findings.
2. Heat Stress: Conversely, the species fared significantly better when subjected solely to heat stress, again differing from our earlier results.

We posit that the corals' relative resilience to heat stress may be due to a recent acclimation effect. The sampling area of Patong Bay, Phuket, experienced a series of heatwaves just prior to sample collection [94]. Previous studies on *P. verrucosa* have similarly documented this acclimation and adaptation response [95].

The Sump-fitted tanks might have contributed to the corals' resilience by enhancing mass transfer processes [15,59] , thereby improving water quality and potentially alleviating some stress conditions.

These variances between our initial and follow-up experiments underscore the complexities of coral responses to stressors and the potential for localized environmental factors to significantly influence outcomes. They also emphasize the urgent need for more comprehensive research into how heat stress and hypoxia interact to affect coral health, particularly under varying environmental conditions.

### CONCLUDING REMARKS

This research investigated the effects of heat stress and hypoxia on three species of coral, finding that the severity of these effects and responses varied by coral species. *P. verrucosa* was the most vulnerable to both heat stress and hypoxia when present individually, while *P. lutea* and *M. tuberculosa* did not reveal drastic effects. Under hypoxic conditions, coral responses varied according to light availability, and 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.

The study has some limitations, as the experiments were conducted in a closed system, which may not fully replicate the variability and complexity of natural coral reef ecosystems. In this study, we only examined the effects of short-term stressors on three species of coral, and longer-term stressors may have different effects. Additionally, the study only examined the effects of two stressors, heat stress, and hypoxia, while coral reefs face multiple stressors, such as ocean acidification and pollution. Despite these limitations, the study provides valuable baseline information for evaluating coral sensitivity to future scenarios, and the research emphasizes the need for ongoing monitoring and protection of coral ecosystems. This study's findings can help inform conservation and management strategies to protect coral reefs and the many species that rely on them for survival. However, further research is needed to deepen our understanding of the mechanisms underlying synergistic and antagonistic responses in coral reefs facing multiple stressors and to explore the adaptation and acclimatization potential of these ecosystems.



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## APPENDIX 1

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<sup>-1</sup>), heat stress (32 °C), hypoxia (DO < 2 mgL<sup>-1</sup>), 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<sup>-1</sup> 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<sup>-1</sup>), 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.

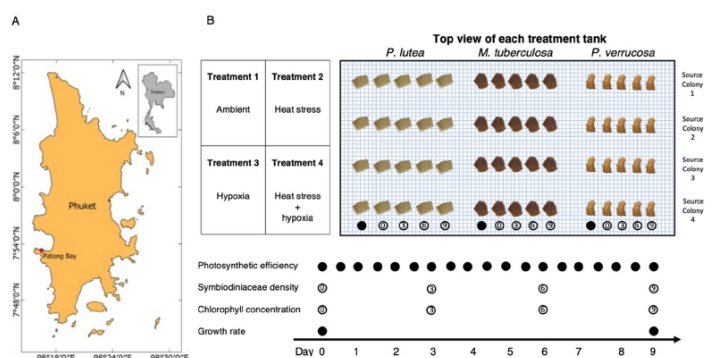
The coral reefs at our study site of Patong Bay, located on the west coast of Phuket, Thailand, have been subjected to excessive nutrient enrichment due to the rapid increase in tourism activities since the 1980s [44,45]. As a result, the reefs are not only vulnerable to heat stress, with a reported 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  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ , and  $6.45 \text{ mgL}^{-1} > \text{DO} > 6.04 \text{ mgL}^{-1}$ , 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),  $6.45 \text{ mgL}^{-1} > \text{DO} > 6.04 \text{ mgL}^{-1}$  and temperature 29 °C.

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

The  $2 \text{ mgL}^{-1}$  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  $29\text{--}32 \text{ }^\circ\text{C}$  has been reported in Phuket [14,46]. As a maximum of  $32.45 \text{ }^\circ\text{C}$  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],  $32 \text{ }^\circ\text{C}$  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  $\text{N}_2$  tanks) using multiple flow controllers. The high pressure from  $\text{N}_2$  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  $\text{N}_2$  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  $\text{N}_2$  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  $-80 \text{ }^\circ\text{C}$  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  $4 \text{ }^\circ\text{C}$  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  $40\times$  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*<sub>2</sub> 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<sup>2</sup> and µg per cm<sup>2</sup>, 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<sup>-3</sup>, 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.

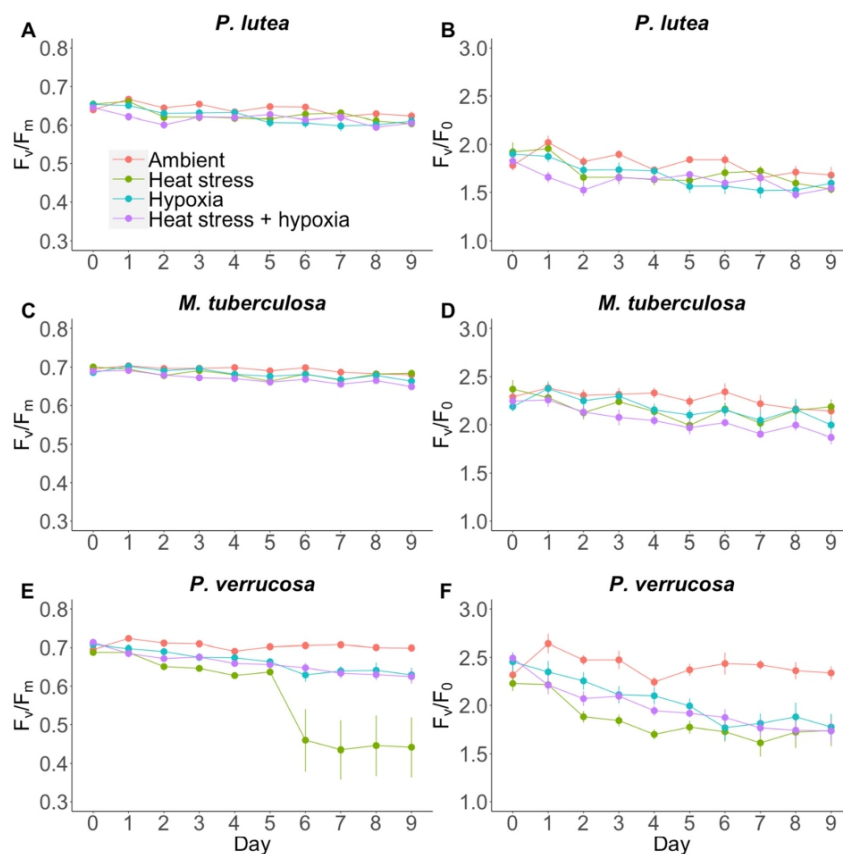
Three-way ANOVA was used to determine statistically significant differences between temperature, DO, and time for *Symbiodiniaceae* density and chlorophyll concentration of each species. Two-way ANOVA was used to test significant differences by temperature and DO in growth rate of each species. All tests 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  $0.639 \pm 0.023$  and  $1.781 \pm 0.177$ , 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).

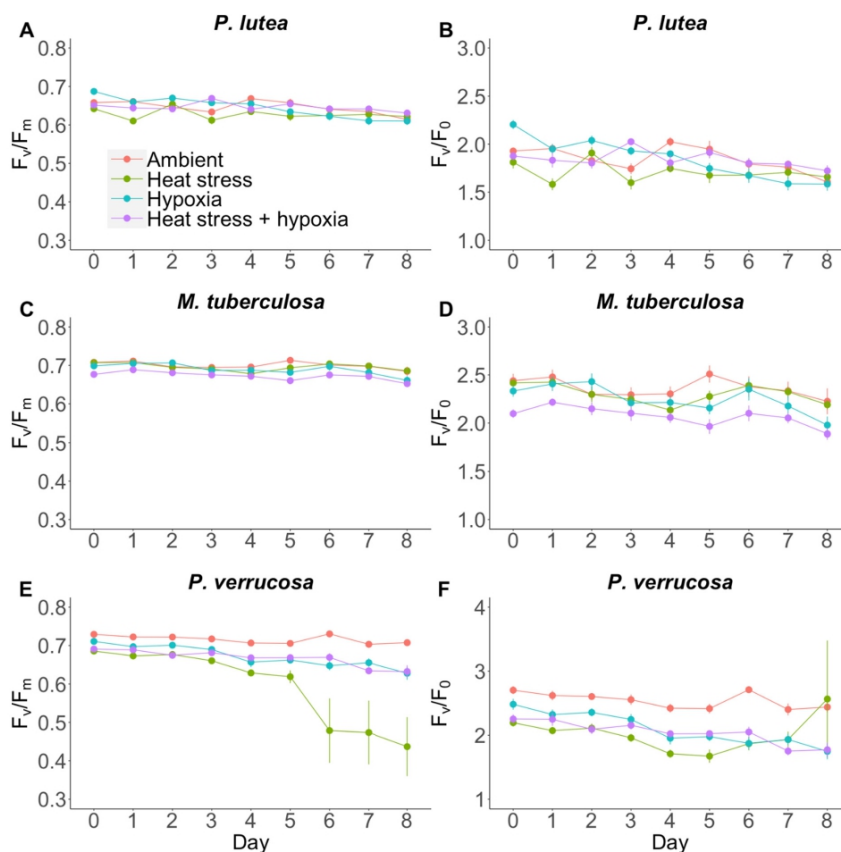


**Figure 2.** Maximum quantum yield of PSII ( $F_v/F_m$ ; left) and photochemical efficiency of PSII ( $F_v/F_0$ ; right) in *P. lutea* (A,B), *M. tuberculosa* (C,D), and *P. verrucosa* (E,F) measured before the light period. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

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).



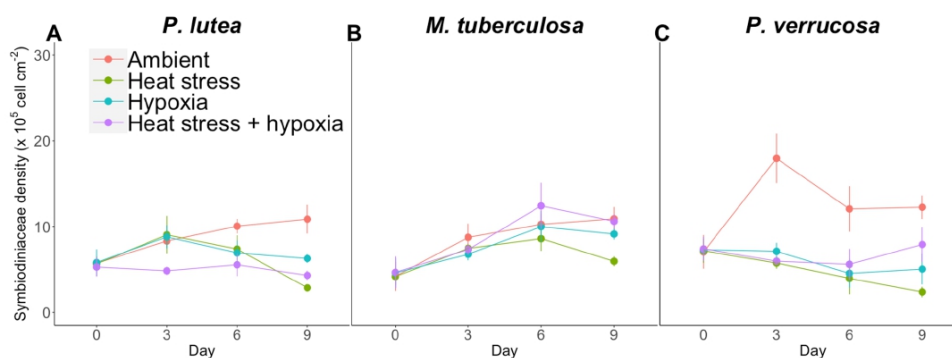
**Figure 3.** Maximum quantum yield of PSII ( $F_v/F_m$ ; left) and photochemical efficiency of PSII ( $F_v/F_0$ ; right) in *P. lutea* (A,B), *M. tuberculosa* (C,D), and *P. verrucosa* (E,F) measured after the light period. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

*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  $\times$  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  $\times$  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  $\times$  temperature  $\times$  DO ( $p = 0.024$ ) (Supplementary Table S3). Interestingly, *M. tuberculosis* 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  $\times$  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  $\times$  temperature ( $p = 0.049$ ), temperature  $\times$  DO ( $p < 0.001$ ), and time  $\times$  temperature  $\times$  DO ( $p = 0.042$ ) (Supplementary Table S5).

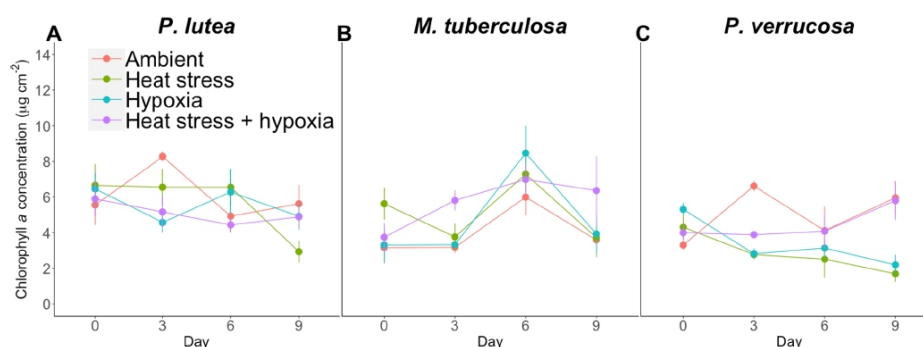


**Figure 4.** Symbiodiniaceae density ( $\times 10^5$  cells  $\text{cm}^{-2}$ ) of *P. lutea* (A), *M. tuberculosis* (B), and *P. verrucosa* (C) under ambient baseline conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

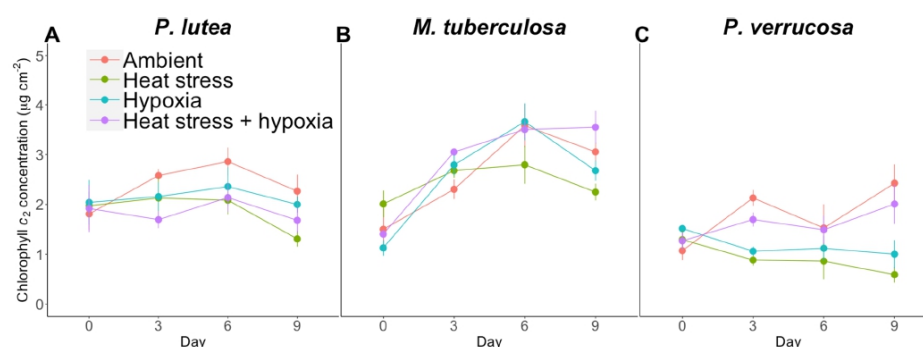
### 3.3. Chlorophyll Content

In *P. lutea*, we found consistent chlorophyll *a* and *c*<sub>2</sub> concentrations throughout the experiment period in all treatments (Figures 5A and 6A), and the interaction time  $\times$  temperature  $\times$  DO was the only significant factor for chlorophyll *a* ( $p = 0.047$ ; Supplementary Table S3) whereas chlorophyll *c*<sub>2</sub> was significantly influenced by the individual factor temperature ( $p = 0.014$ ; Supplementary Table S3). On the other hand, *M. tuberculosis* revealed a significant increase on day 6 in chlorophyll *a* ( $p < 0.001$ ) and on days 3, 6, and 9 in chlorophyll *c*<sub>2</sub> ( $p < 0.001$ ), although the coral nubbins were under stress (Figures 5B and 6B). Time was a significant factor for both chlorophyll *a* and *c*<sub>2</sub> in *M. tuberculosis* ( $p < 0.001$ ; 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*<sub>2</sub> 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*<sub>2</sub> were temperature  $\times$  DO and time  $\times$  temperature  $\times$  DO ( $p < 0.001$ ; Supplementary Table S5).



**Figure 5.** Chlorophyll *a* and *c*<sub>2</sub> concentration ( $\mu\text{g cm}^{-2}$ ) 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  $\pm$  SE ( $n = 4$ ).

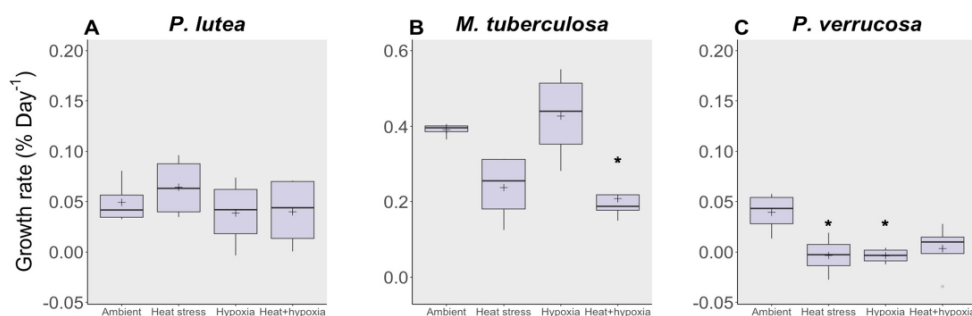


**Figure 6.** Chlorophyll *c*<sub>2</sub> concentration ( $\mu\text{g cm}^{-2}$ ) of *P. lutea* (A), *M. tuberculosa* (B), and *P. verrucosa* (C) under ambient conditions, heat stress, hypoxia, and heat stress + hypoxia on days 0, 3, 6, and 9. Data are shown as mean  $\pm$  SE ( $n = 4$ ).

### 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<sub>2</sub> consumption and O<sub>2</sub> 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<sup>-1</sup> 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<sup>-1</sup>) 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<sup>-1</sup> 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<sup>-1</sup> 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<sub>2</sub>S, NO, and H<sub>2</sub>O<sub>2</sub>, that further compromise the host's immunity [6,91,92].

The growth rates of *M. tuberculosis* 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. tuberculosis* 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. tuberculosis* 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. tuberculosis*. 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  $\pm$  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. tuberculosis*. 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. tuberculosis* 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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**Conflicts of Interest:** The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## APPENDIX 2



1<sup>st</sup> INTERNATIONAL POSTGRADUATE CONFERENCE ON SCIENCE AND MARINE ENVIRONMENT 2021  
(IPCoSME 2021)  
“Environmental Sustainability Enhancement Through the Collaboration of Sciences”

RD-03

## CORAL RESPONSE TO HEAT STRESS AND HYPOXIA

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**Abstract:** In recent decades, coral reefs have been suffering an unprecedented decline due to environmental stress, especially climate change caused by natural fluctuations and anthropogenic drivers. There is a lack of research in heat-stressed & hypoxic coral reefs and it is urgent to figure out the extent of this problem and its potential consequences. This research is required to investigate the physiological response of corals to dissolved oxygen concentration changes and assess heat stress's synergistic or antagonistic effects with low dissolved oxygen. Results from this research can help local management bodies predict the consequences of warming and hypoxia, locate sensitive coral species, define measurable goals, and timely deploy management strategies to mitigate local stressors and better plan restoration activities. We measured the effects of hypoxia on maximum quantum yield of *Porites lutea*, *Acropora millepora* and *Pavona decussata* during our preliminary experiment and found that *A. millepora* was most effected by hypoxia compared to other two species with mortality observed in 36 hours of treatment. The results from preliminary experiment and future experiment designs are discussed for this conference.

Keywords: Climate change, corals, deoxygenation, heat stress, hypoxia



APPENDIX 3

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# ICRS 2022

## Book of Abstracts



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10A - Open Session: Organismal physiology, adaptation and acclimation

**Virtual  
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**Effects of heat stress and hypoxia on the photosynthetic efficiency and growth rate of three scleractinian corals**

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**Abstract**

In recent decades, coral reefs have been suffering an unprecedented decline due to environmental stress, especially climate change caused by natural fluctuations and anthropogenic drivers. There is a lack of research in heat-stressed & hypoxic coral reefs. It is urgent to figure out the extent of this problem and its potential consequences. This study investigated the physiological response of corals to low dissolved oxygen concentration and assessed heat stress's synergistic or antagonistic effects with low dissolved oxygen. We collected three coral species representative of different growth forms, *Porites lutea*, *Montipora tuberculosa* and *Pocillopora verrucosa* from Phuket, Thailand and subjected them to nine days of heat stress (32 °C), hypoxia (DO < 2 mg l<sup>-1</sup>) and heat stress + hypoxia treatments. Photosynthetic parameters ( $F_v/F_m$  and  $F_v/F_0$ ) were measured daily while the growth rate was quantified at the end of experiment. We found that the initial response of *P. lutea* to hypoxia was an increase in  $F_v/F_m$  and  $F_v/F_0$  during light availability which started declining from Day 5. *P. verrucosa* (branching coral) was found to be most sensitive and severely affected by these stressors in comparison to *P. lutea* (massive) and *M. tuberculosa* (tabular).

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### **Scholarship Awards during Enrolment**

- Graduate School Research Grant for Thesis in Fiscal Year 2022
- Graduate School Study Grant

### **List of Publication and Proceeding**

1. Jain, T.; Buapet, P.; Ying, L.; Yucharoen, M. Differing Responses of Three Scleractinian Corals from Phuket Coast in the Andaman Sea to Experimental Warming and Hypoxia. *J. Mar. Sci. Eng.* **2023**, *11*, 403.  
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