PRIMING-MEDIATED RESPONSES OF TROPICAL SEAGRASSES TO EXTREME WARMING STRESS: A PRELIMINARY ANALYSIS

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Abstract. This study investigates the effects of priming and extreme warming on the physiological responses, focusing on photosynthesis and oxidative stress, in three seagrass species: *Thalassia hemprichii*, *Cymodocea rotundata*, and *Halophila ovalis*. Results reveal significant interactions among priming treatment, extreme warming treatment, and duration of warming, with varying impacts across species. While *C. rotundata* shows minor stress responses to priming and extreme warming, *T. hemprichii* and *H. ovalis* exhibit distinct patterns. Primed *T. hemprichii* maintains higher ϕPSII compared to non-primed counterparts under extreme warming, whereas primed *H. ovalis* displays exacerbated inhibition of ϕPSII in both control and extreme warming conditions. Additionally, primed *T. hemprichii* shows significant differences in total antioxidant capacity, with elevated levels observed across both control and extreme warming scenarios. Although lipid peroxidation increases with warming in both *T. hemprichii* and *H. ovalis*, priming attenuates this effect in *T. hemprichii* while intensifying it in *H. ovalis*. Combining all three physiological parameters, the biomarker response index revealed a slight response in primed *T. hemprichii* and a severe response in non-primed *T. hemprichii* under extreme warming. Both non-primed and primed *H. ovalis* exhibited severe responses, while *C. rotundata* responses were negligible. These findings highlight species-specific responses to thermal stress and provide initial insights into the potential of priming to modulate thermotolerance. Understanding these dynamics is crucial for accurately predicting climate change challenges and ensuring effective seagrass conservation and management.
Keywords: *marine plant, hardening, heat stress, photosynthetic activity, thermotolerance*

Introduction

Rising global temperature presents a severe threat to marine ecosystems. Seagrasses undergo thermal stress when exposed to prolonged periods of warmer waters, resulting in inhibited growth and an increased risk of potential die-offs (Arias-Ortiz et al., 2018; Strydom et al., 2020; Aoki et al., 2021; Serrano et al., 2021). These repercussions extend to the complex web of life dependent on seagrass habitats and their associated ecosystem services (Serrano et al., 2021; Zhang et al., 2023a). Thus, addressing the potential impacts of rising temperatures is crucial for effectively managing and enhancing the resilience of seagrass ecosystems.

In recent years, intensive experimental studies have explored the impacts of heat stress on seagrass performance (Costa et al., 2021; Yucharoen et al., 2021; Deguette et al., 2022; Saewong et al., 2022; Soonthornkalump et al., 2022; Zhang et al., 2023b). While initial research revealed physiological responses to elevated temperatures, there

is a growing recognition of the need for a nuanced understanding of stress in natural environments, often involving repeated episodes (Fox-Kemper, 2021). The consequences of persistent or recurrent warming events on seagrasses are relatively understudied but hold significance in elucidating their resilience mechanisms (DuBois et al., 2020; Saha et al., 2020; Vivanco-Bercovich et al., 2024). Such research can offer valuable insights into whether repeated stress induces acclimation, thereby enhancing resilience, or exacerbates negative impacts, leading to a deterioration in seagrass health.

Recent studies have introduced the concept of "priming" to enhance resilience in macrophytes and seagrasses (Nguyen et al., 2020; Jueterbock et al., 2021; Pazzaglia et al., 2022). Thermo-priming, involving brief heat exposure, has been found to boost thermotolerance, thereby improving seagrass performance in various aspects, including photosynthesis, stress-related gene expression, epigenetics, and growth in *Posidonia oceanica*. Thermo-priming has been suggested as a potential explanation for the resilience observed in the same seagrass species during heatwaves in 2012, 2015, and 2017 (Nguyen et al., 2021). For tropical species such as *Thalassia hemprichii* (Purnama et al., 2019) and *Enhalus acoroides* (Zhang et al., 2023b), heat shock has been identified as a trigger for increased expression of antioxidant and heat shock protein genes. This suggests physiological acclimation, potentially altering responses to subsequent exposure to warming.

The current study investigates the impact of repeated warming stress scenarios, incorporating the concept of priming, on three common intertidal seagrasses in Thailand: *Thalassia hemprichii*, *Cymodocea rotundata*, and *Halophila ovalis*. Given the vulnerability of tropical seagrasses to warming, with ambient temperatures approaching their upper threshold, it is crucial to explore their potential responses in repeated warming scenarios (Rasmusson et al., 2020, 2021). Seagrasses underwent exposure to a sub-lethal temperature increase (36°C) as a "priming" phase, followed by extreme heat stress conditions with temperatures rising to 40°C. The investigation included crucial physiological parameters such as photosynthesis, total antioxidant capacity, and lipid peroxidation. By incorporating the concept of priming, the study aims to elucidate whether initial exposure to elevated but sub-lethal temperatures can induce a protective response in seagrasses when confronted with more extreme heat stress conditions. This nuanced understanding provides valuable insights for informing conservation strategies and contributes to the broader knowledge of plant stress physiology in marine environments. It guides future research and management efforts aimed at mitigating the impacts of climate change on critical coastal habitats.

Materials and methods

Seagrass collection and acclimation

Healthy individuals of three common intertidal seagrass species, *Thalassia hemprichii*, *Cymodocea rotundata*, and *Halophila ovalis* were collected from Tung Khen Bay, Phuket Province, Thailand (7° 48' 36.3" N, 98° 24' 16.8" E). The seagrasses were promptly transported to the laboratory at Coastal Oceanography and Climate Change Research Center in Songkhla Province and were acclimated in large seawater tanks under controlled conditions, including a temperature of 28°C, salinity of 32 psu, and a photoperiod of 12/12 with an irradiance of 200 µmol photons m^{-2} s^{-1}. The temperatures were regulated using submersible heater (Alpha A, Lauda, Germany), and

illumination was provided by aquarium LED lights (Chichiros, Shanghai Ogino Biotechnology, China). This acclimation phase spanned one week.

Priming phase

Following acclimation, the seagrasses were divided into two groups. The first group was maintained at the acclimation temperature of 28°C, serving as a non-primed group. The second group underwent a priming phase, where the temperature was increased at a rate of 1°C per hour until reaching 36°C. During the priming phase, the elevated temperature of 36°C was sustained for a duration of 3 h. This specific temperature was chosen based on insights from our prior study (Yucharoen et al., 2021), which demonstrated that exposure to 36°C induced sub-lethal effects in tropical seagrasses. Following this priming exposure, the temperature was systematically decreased back to the acclimation temperature of 28°C at the same gradual rate.

Extreme heat stress exposure

Subsequently, both non-primed and primed seagrasses were subjected to two experimental conditions: the control condition, where seagrasses were maintained at the acclimation temperature of 28°C, and the extreme heat stress condition. In the extreme heat stress condition, the temperature was raised at a rate of 2°C per hour until reaching 40°C. The seagrasses were then held at 40°C for 6 h before a gradual decrease in temperature at the same rate. The selection of 40°C as the extreme heat stress temperature was based on findings from our previous studies (Saewong et al., 2022; Soonthornkalump et al., 2022) and corroborated by other relevant research studies (Collier and Waycott, 2014; George et al., 2018).

Physiological measurements

During the extreme heat exposure phase, physiological measurements took place, covering plant photosynthesis, total antioxidant capacity and lipid peroxidation. The effective quantum yield (ϕPSII) was a key parameter indicating photosynthetic efficiency, and measurements were taken at two critical time points. The first measurement occurred when the temperature in the warming tanks reached 40°C, capturing the immediate impact of the extreme heat stress on the seagrasses. Subsequently, a second measurement was conducted after the seagrasses had been exposed to 40°C for 6 h, allowing for an evaluation of the sustained effects of prolonged extreme heat stress.

Following the determination of the ϕPSII, seagrass leaves were carefully collected for further analyses. The collected leaves were promptly stored at -80°C. This freezing process ensures the preservation of cellular structures and molecular components, allowing for subsequent investigations into the stress responses of seagrasses.

Two specific biochemical analyses were planned for the collected leaves: total antioxidant capacity and lipid peroxidation. These measurements offer insights into the state of oxidative stress, a consequence of extreme environmental conditions. Total antioxidant capacity reflects the overall ability of the seagrasses to neutralize reactive oxygen species, which are commonly produced under heat stress, while lipid peroxidation is indicative of oxidative damage to cellular membranes. The experimental design and timeline of the physiological measurements are summarized in *Figure 1*.
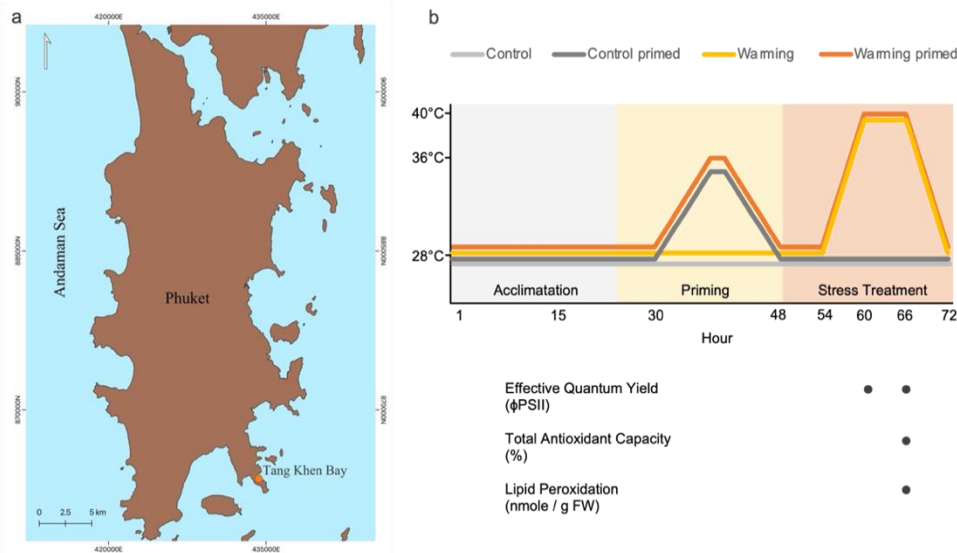
Figure 1. *A. Sampling location B. Temperature profile for each treatment, parameters and time of measurements*

Photosynthesis assessment

The evaluation of photosynthetic performance employed the effective quantum yield as a key marker. For this measurement, a Diving Pulse Amplitude Modulated Fluorometer (Diving-PAM, Walz, Heinz Walz GmbH, Effeltrich, Germany) was employed. This parameter acts as an indicator of the efficiency of photosystem II during light exposure—a reliable gauge of the capacity to convert light energy into chemical energy through the process of photosynthesis. Consequently, it offers valuable insights into the overall photosynthetic activity and health of the plant materials.

The effective quantum yield (ϕPSII = ΔF/Fm') was calculated using the formula: ϕPSII = (Fm' - Ft) / Fm', where Fm' represents the maximum fluorescence in light-adapted conditions, and Ft represents the steady-state fluorescence during illumination (Murchie and Lawson, 2013).

Total antioxidant capacity

The DPPH method, adapted from Choi et al. (2009), was employed to assess the antioxidant capacity of the sample. Initially, 100 mg of plant material was finely powdered using liquid nitrogen. The resulting sample was placed in a 2-ml tube, and 1.5 ml of 95% methanol was added, followed by vortexing for approximately 5 min. After storing the sample in the dark at room temperature for 2 hours, the tube was centrifuged at $12,000 \times g$ for 20 min at 25°C. The clear supernatant was transferred to another 2-ml tube.

For analysis, two sets were prepared: the control set, comprising 100 µl of 95% methanol and 1 ml of 0.2 mM DPPH (prepared in methanol); and the sample set, consisting of 100 µl of the plant extract and 1 ml of 0.2 mM DPPH. After keeping the mixture in the dark for an additional 30 min, absorbance was measured at 517 nanometer using a Spectrophotometer (UV1720, Yoke instrument, China). Each sample included 3 technical replicates. The percentage DPPH scavenging effect of the sample set was calculated in relation to the control set.

Lipid peroxidation

The quantification of Malondialdehyde (MDA), a byproduct of lipid oxidative degradation by reactive oxygen species (ROS), was conducted using the Thiobarbituric acid Reactive Substances (TBARS) assay adapted from Torasa et al. (2019). Leaf tissue (100 mg) was ground in liquid nitrogen, homogenized with 2 ml of 0.1% trichloroacetic acid (TCA), and centrifuged at $10,000 \times g$ for 15 min. The resulting supernatant (0.5 ml) was mixed with 1 ml of 20% TCA and 1 ml of 0.5% TBA, heated at 95°C for 30 min, and promptly cooled on ice. After centrifugation at $10,000 \times g$ for 10 min, the absorbance of the supernatant was read at 532 and 600 nm using a spectrophotometer (UV1720, Yoke instrument, China). Normalization utilized the fresh weight of tissue, and each sample included three technical replicates.

Data analysis

In our statistical analysis, we utilized the STATISTICA academic platform to examine the data derived from the experimental study. To ensure the validity of subsequent analyses, Cochran's Test was employed to validate the homogeneity of variances prior to performing ANOVA.

For the analysis of the effective quantum yield (ϕPSII), a repeated-measures ANOVA was employed. The categorical factors considered in this analysis were the heat treatment (control vs warming) and priming treatment (yes vs no), while the time of measurements served as the within-group factor.

For the assessment of differences in total antioxidant capacity (TAC) and lipid peroxidation (LPO), measured at the conclusion of the experiment, a two-way ANOVA was conducted. The categorical factors included in this analysis were the heat treatment (control vs warming) and priming treatment (yes vs no).

To allow for comparisons between different treatments and time points, we implemented the Tukey Honestly Significant Difference (HSD) test.

To compare the effects of extreme warming on integrated seagrass performance, we calculated the Biomarker Response Index (BRI) (*Eq. 1*) following the methods of Luengluetham et al. (2023). Groups were divided into four: control with non-primed seagrass (baseline), control with primed seagrass, extreme warming with non-primed seagrass, and extreme warming with primed seagrass. The level of alteration in each parameter (ϕPSII, TAC, and LPO) was assessed based on percentage difference compared to the control group of non-primed plants. Scores ranged from 1 to 4: < 20% (4), 20-50% (3), 50-100% (2), and > 100% (1). Biomarker weights were assigned based on biological significance: TAC (1.0), ϕPSII (1.2), and LPO (1.8). Note that the ϕPSII values referenced here were measured after subjecting the treatment samples to an extreme warming condition for a duration of 6 h (at 18:00).

The formula for BRI calculation is:

$$BRI = \frac{\sum(\text{Biomarker score} \times \text{Biomarker weight})}{\sum(\text{Biomarker weight})}$$
(Eq.1)

BRI values ranged from 0 to 4. Values below 2.5 indicated a severe response, 2.50 to 2.75 indicated a major response, 2.76 to 3.0 indicated a moderate response, 3.10 to 3.50 indicated a slight response, and values above 3.5 indicated a negligible response. This categorization was slightly modified to align with the observed value range in this study.

Results

In *Figure 2*, the effective quantum yield (ϕPSII) values, reflecting the photosynthetic activity of three seagrass species, exhibit distinct response patterns to extreme warming treatment. For *Thalassia hemprichii* (*Fig. 2A; Tables A1* and *A2*), a significant interaction between priming, extreme warming treatment, and the duration of warming treatment was identified (Repeated-measures ANOVA, $F = 10.63$, $p < 0.01$). A significant difference between non-primed and primed groups emerged during the extreme warming treatment, particularly after exposure to 40°C for 6 h (Tukey's HSD test, $p < 0.05$). Specifically, primed seagrass displays a higher ϕPSII compared to non-primed plants.
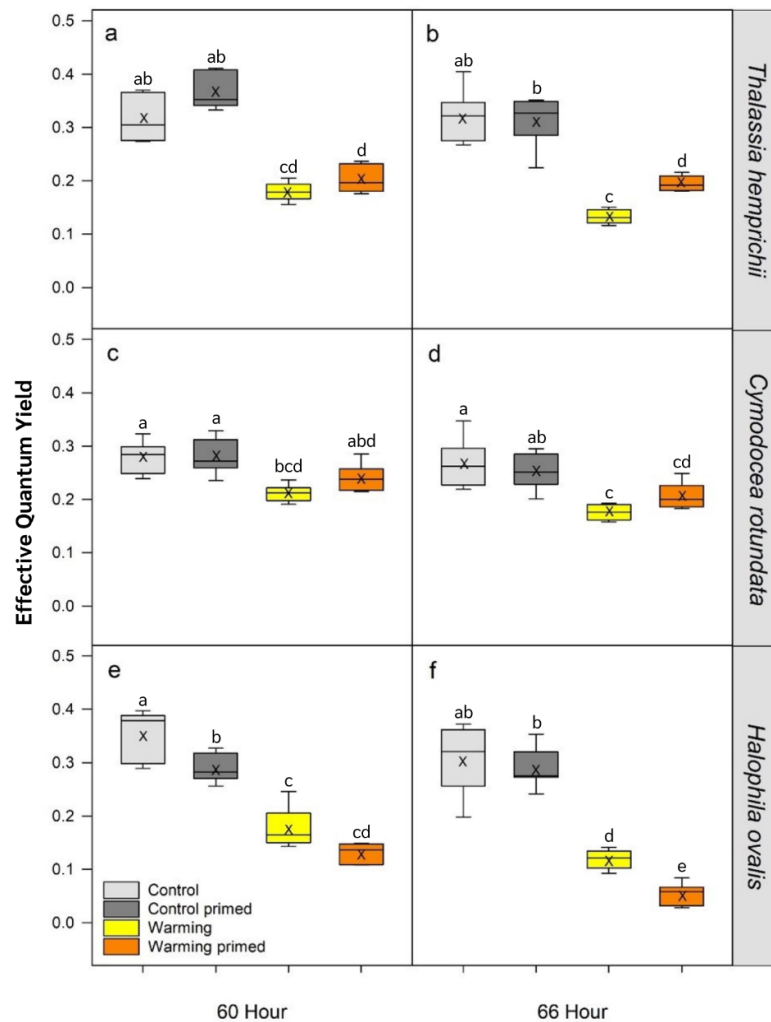
Figure 2. *Box plots showing the responses of effective quantum yield in both non-primed and primed groups of three seagrass species: (A) T. hemprichii, (B) C. rotundata, and (C) H. ovalis under control conditions (28°C) and extreme warming conditions (40°C). Measurements were taken at two distinct time points: 60 h, corresponding to when the temperature in the extreme warming treatment reached 40°C, and 66 h, after the seagrasses had been exposed to 40°C for 6 h. Whiskers indicate minimum and maximum values, the middle line indicates median, and x indicates mean of each box plot. The asterisk indicates a significant difference between primed and non-primed plants*

For *Cymodocea rotundata* (*Fig. 2B; Tables A1* and *A2*), a significant interaction between priming and extreme warming treatment was detected (Repeated-measures ANOVA, F = 7.40, p < 0.01). However, post-hoc analysis (Tukey's HSD test) revealed no substantial difference between non-primed and primed groups in either treatment condition.

For *Halophila ovalis* (*Fig. 2C; Tables A1* and *A2*), a significant interaction between priming, extreme warming treatment, and the duration of warming treatment was observed (Repeated-measures ANOVA, F = 4.30, p < 0.05). Notably, a significant difference between non-primed and primed groups was detected in the control treatment, and during the extreme warming treatment, assessed after exposure to 40°C for 6 h (Tukey's HSD test, p < 0.05). In contrast to *T. hemprichii*, primed *H. ovalis* exhibits a lower ϕPSII compared to non-primed plants during both control and extreme warming conditions.

The total antioxidant capacity (TAC) also exhibited species-specific responses. Specifically, only *T. hemprichii* showed a significant difference influenced by priming (*Fig. 3A; Tables A3* and *A4*, Two-way ANOVA, F = 15.61, p < 0.001), a finding confirmed by a post-hoc test (Tukey's HSD test) revealing differences between non-primed and primed seagrasses in both control and extreme warming treatments. In contrast, neither priming nor extreme warming treatment caused any change in *C. rotundata* and *H. ovalis* (*Fig. 3B, C; Tables A3* and *A4*).
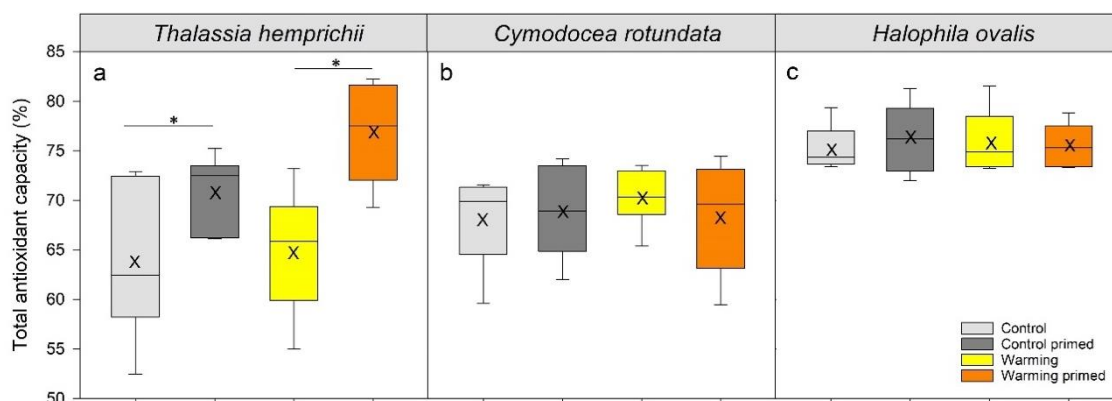

Figure 3. Box plots showing the total antioxidant capacity in both non-primed and primed groups of three seagrass species: (A) T. hemprichii, (B) C. rotundata, and (C) H. ovalis under control conditions (28°C) and extreme warming conditions (40°C). The assessments were conducted after the temperature in the extreme warming treatment reached 40°C for 6 h. Whiskers indicate minimum and maximum values, the middle line indicates median, and x indicates mean of each box plot. The asterisk indicates a significant difference between primed and non-primed plants

Lipid peroxidation, as indicated by the level of MDA in the seagrass leaves, was intensified by warming treatment in *T. hemprichii* and *H. ovalis*. In the case of *T. hemprichii* (*Fig. 4A; Tables A5* and *A6*), a significant interaction between priming and extreme warming treatment was identified (Two-way ANOVA, F = 29.95, p < 0.001). A significant difference between non-primed and primed groups emerged in the extreme warming treatment (Tukey's HSD test, p < 0.05), with non-primed seagrass displaying significantly higher lipid peroxidation compared to primed plants, which maintained

levels similar to the control. Similar to other parameters, *C. rotundata* was not affected by either priming or extreme warming treatment (*Fig. 4B; Tables A5* and *A6*). However, for *H. ovalis*, a significant interaction between priming and extreme warming treatment was identified (*Fig. 4C; Tables A5* and *A6*, Two-way ANOVA, $F = 9.07$, $p < 0.01$). A significant difference between non-primed and primed groups was detected in the extreme warming treatment (Tukey's HSD test, $p < 0.05$). Interestingly, in contrast to the pattern observed in *T. hemprichii*, primed *H. ovalis* displayed significantly higher lipid peroxidation compared to non-primed plants under extreme warming condition.
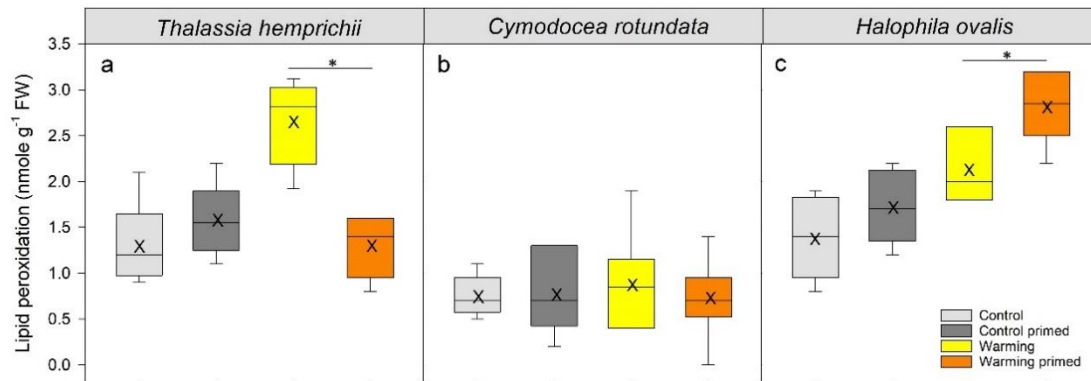

Figure 4. Box plots showing the lipid peroxidation level in both non-primed and primed groups of three seagrass species: (A) T. hemprichii, (B) C. rotundata, and (C) H. ovalis under control conditions (28°C) and extreme warming conditions (40°C). The assessments were conducted after the temperature in the extreme warming treatment reached 40°C for 6 h. Whiskers indicate minimum and maximum values, the middle line indicates median, and x indicates mean of each box plot. The asterisk indicates a significant difference between primed and non-primed plants

Table 1 displays the Biomarker Response Index (BRI) and corresponding stress response categories for three seagrass species. *T. hemprichii* exhibits BRI values ranging from 2.05 to 4.00, with both non-primed and primed plants displaying negligible responses under control temperature (BRI = 4.00 and 3.55, respectively). However, under warming conditions, non-primed *T. hemprichii* shows a severe response (BRI = 2.05), while primed *T. hemprichii* displays a slight response (BRI = 3.45). *C. rotundata* demonstrates BRI values ranging from 3.70 to 4.00, indicating negligible responses across all treatments. Similarly, for *H. ovalis*, both non-primed and primed plants exhibit negligible responses under control temperature (BRI = 4.00 and 3.55, respectively), but under warming conditions, both non-primed and primed *H. ovalis* show severe responses, with the BRI being lower in primed seagrass (BRI = 2.50 and 2.05, respectively).

Discussion

Despite extensive studies on heat priming in terrestrial plant models (Bäurle, 2016; Liu et al., 2021; Nishad and Nandi, 2021; Balazadeh, 2022), investigations into their roles in seagrasses are still at an early stage (see Nguyen et al., 2020; Jueterbock et al., 2021; Pazzaglia et al., 2022). Our study has revealed three distinctive outcomes regarding the impact of pre-exposure to sub-lethal thermal stress in seagrasses: (1) it

acts as a priming mechanism, promoting the development of defense mechanisms and enhancing tolerance of photosynthesis to extreme warming; (2) it functions as a stressor, leading to increased levels of photoinhibition and oxidative damage when later exposed to extreme warming treatment, and (3) pre-exposure to sub-lethal thermal stress shows no observable effect on seagrass health. Importantly, the three seagrass species exhibited diverse response patterns, underscoring the necessity of recognizing species-specific vulnerabilities. This insight is crucial for designing management strategies to mitigate the impacts of extreme warming episodes, emphasizing the need for tailored approaches for seagrass ecosystem management.

Table 1. Biomarker response index (BRI) and corresponding stress response categories in non-primed and primed plants of T. hemprichii, C. rotundata, and H. ovalis after exposure to control temperature (28°C) or extreme warming treatment (40°C). BRI was calculated using three parameters: Photosystem II effective quantum yield (φPSII) measured after the warming treatment reached 40°C for 6 h, total antioxidant capacity (TAC), and lipid peroxidation (LPO)

Species	Treatments	BRI	Stress response category
T. hemprichii	Control/non-primed (baseline)	4.00	Negligible
	Control/primed	3.55	Negligible
	Warming/non-primed	2.05	Severe
	Warming/primed	3.45	Slight
C. rotundata	Control/non-primed (baseline)	4.00	Negligible
	Control/primed	4.00	Negligible
	Warming/non-primed	3.70	Negligible
	Warming/primed	3.70	Negligible
H. ovalis	Control/non-primed (baseline)	4.00	Negligible
	Control/primed	3.55	Negligible
	Warming/non-primed	2.50	Severe
	Warming/primed	2.05	Severe

BRI values: 0-4. Categories: < 2.5 (severe), 2.50-2.75 (major), 2.76-3.0 (moderate), 3.10-3.50 (slight), > 3.5 (negligible)

Our findings corroborate the conclusions of numerous prior studies, emphasizing the significant threat posed by extreme warming to seagrass health, particularly affecting their photosynthetic performance (Costa et al., 2021; Yucharoen et al., 2021; Deguette et al., 2022; Saewong et al., 2022; Soonthornkalump et al., 2022; Zhang et al., 2023b; Vivanco-Bercovich et al., 2024). This susceptibility can be attributed to the sensitivity of photosynthetic machinery to heat stress compounded by oxidative stress induced by high temperatures (Hüve et al., 2011; Mathur et al., 2014; Filaček et al., 2022). While certain seagrasses may strengthen their defense mechanisms to mitigate oxidative damage (Tutar et al., 2017; Zhang et al., 2023b), extreme warming has been demonstrated to inhibit protective mechanisms in some species, rendering them even more vulnerable (Deguette et al., 2022; Saewong et al., 2022).

The diverse vulnerability observed among seagrass species in our present study aligns consistently with our prior investigations into thermal stress (Yucharoen et al., 2021; Saewong et al., 2022; Soonthornkalump et al., 2022) and various stressors such as

high light and desiccation (Wuthirak et al., 2016; Phandee and Buapet, 2018). Our observations indicate that seagrasses characterized by slower growth rates, like *T. hemprichii*, tend to manifest greater resilience to stress. Conversely, smaller seagrasses with rapid growth, like *H. ovalis*, appear to be more sensitive. Studies on multiple traits among seagrass species have suggested that slower-growing species may be more resistant but slower to recover, while faster-growing ones may recover more rapidly (Kilminster et al., 2015; Collier et al., 2021). *Cymodocea* species are often positioned in between these extremes (Kilminster et al., 2015; Collier et al., 2021). However, several studies have provided contrasting results. Viana et al. (2020) examined *T. hemprichii*, *C. serrulata*, and *H. stipulacea* from the western coast of Zanzibar, Tanzania, under heat stress, reporting *H. stipulacea* as the most tolerant. Similarly, Nguyen et al. (2020), reported that the fast-growing – pioneer *Zostera muelleri* to be more heat tolerant than the long-lived – climax *Posidonia australis* and Marín-Guirao et al. (2016, 2018) also showed *C. nodosa* to be more resistant than *P. oceanica*. These contrasting results point to the importance of considering local adaptation, in addition to life traits of seagrass species. Nevertheless, our findings suggest that in scenarios involving repeated warming episodes in our locality, where the initial episode is sub-lethal, *T. hemprichii* and *C. rotundata* are likely to persist, while *H. ovalis* may be significantly impacted.

The tolerance mechanisms of *T. hemprichii* and *C. rotundata* seem to diverge; *T. hemprichii* appears to derive benefits from priming, while *C. rotundata* exhibits heat tolerance independently of priming as supported by the biomarker response index (BRI). Previous reports on model plant species indicate that heat priming can enhance resistance to heat stress and facilitate improved post-stress recovery (Bäurle, 2016; Liu et al., 2021; Nishad and Nandi, 2021; Filaček et al., 2022). Studies on seagrasses also demonstrate that preheated plants exhibit enhanced coping mechanisms during repetitive heatwave episodes. This is evidenced by improvements in photosynthetic capacity, including the effective quantum yield (ϕPSII), similar to the findings of the present study, as well as increases in chlorophyll *a* content and leaf growth (Nguyen et al., 2020; Pazzaglia et al., 2022). Expanding upon these earlier investigations, it can be inferred that the observed mechanistic benefits of priming in *T. hemprichii* in this study may involve an increase in the heat stability of PSII, consistent with findings in wheat and tall fescue (Hu et al., 2015; Fan et al., 2018), and an enhancement in the capacity of defense mechanisms, as seen in wheat seedlings and maize (Wang et al., 2014; Khanzada et al., 2022; Ru et al., 2023). While the former was also observed in seagrasses (Nguyen et al., 2020; Pazzaglia et al., 2022), the latter—enhanced antioxidant capacity in primed plants—was not consistently observed in previous seagrass studies. Despite priming leading to an increase in the expression of genes encoding manganese superoxide dismutase and catalase, studies on primed seagrasses *Z. muelleri* and *P. australis* did not find significant differences from non-primed plants during the second heatwave (Nguyen et al., 2020). Similarly, no significant difference in the expression of genes encoding manganese superoxide dismutase and other genes associated with defense mechanisms, such as heat shock proteins and alternative oxidase, was observed between non-primed and primed *P. oceanica* seedlings (Pazzaglia et al., 2022).

On the contrary, primed *H. ovalis* exhibited aggravated stress responses, as indicated by ϕPSII, lipid peroxidation, and the BRI. This finding is consistent with a recent study demonstrating that the recurrence of marine heatwaves exacerbates thermal stress in the surfgrass *Phyllospadix scouleri*, causing a decrease in photosynthetic performance and

an increase in oxidative damage (Vivanco-Bercovich et al., 2024). The accumulation of heat-induced damage suggested in this previous study may be applicable to our study. Here, the priming temperature may have induced photoinhibition in *H. ovalis*, observed as lower ϕPSII in primed plants under control conditions. Furthermore, warming has been shown to inhibit the photoprotective mechanisms of *H. ovalis* (Saewong et al., 2022), potentially contributing to worsened photoinhibition and higher oxidative stress following subsequent heat treatment.

It is important to note that caution is warranted when interpreting our results, as they do not necessarily signify adaptation or long-term acclimation. Our study serves as an initial exploration into investigating repeated stress in tropical seagrasses. In-depth investigations, delving into longer-term responses and changes at the epigenetic level, are imperative to better comprehend these phenomena. Furthermore, exploring priming effects on cross-tolerance to abiotic stresses (Hossain et al., 2018) will enhance our understanding of seagrass stress physiology. Recognizing that repeated stress closely mirrors natural occurrences (Bäurle, 2016; Nguyen et al., 2020), a comprehensive and nuanced understanding of the nature of stress is essential to predict changes, especially in climate change scenarios.

Conclusion

In conclusion, our study highlights the varied and species-specific responses of seagrasses to pre-exposure to sub-lethal thermal stress. While some species, like *T. hemprichii* and *C. rotundata*, show promising resilience, others, such as *H. ovalis*, exhibits increased vulnerability. These findings emphasize the need for tailored management strategies to mitigate the impacts of extreme warming on seagrass ecosystems. Further long-term research into the epigenetic and cross-tolerance effects of thermal priming is essential for understanding and enhancing seagrass resilience in the face of climate change.

REFERENCES

[1] Aoki, L. R., McGlathery, K. J., Wiberg, P. L., Oreska, M. P. J., Berger, A. C., Berg, P., Orth, R. J. (2021): Seagrass recovery following marine heat wave influences sediment carbon stocks. – Frontiers in Marine Science 7. https://doi.org/10.3389/fmars.2020.576784.

[2] Arias-Ortiz, A., Serrano, O., Masqué, P., Lavery, P. S., Mueller, U., Kendrick, G. A., Rozaimi, M., Esteban, A., Fourqurean, J. W., Marbà, N., Mateo, M. A., Murray, K., Rule, M. J., Duarte, C. M. (2018): A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. – Nature Climate Change 8(4): 338-344. https://doi.org/10.1038/s41558-018-0096-y.

[3] Balazadeh, S. (2022): A 'hot' cocktail: the multiple layers of thermomemory in plants. – Current Opinion in Plant Biology 65: 102147. https://doi.org/10.1016/j.pbi.2021.102147.

[4] Bäurle, I. (2016): Plant heat adaptation: priming in response to heat stress. – F1000Research 5: 694. https://doi.org/10.12688/f1000research.7526.1.

[5]   Choi, H. G., Lee, J. H., Park, H. H., Sayegh, F. A. Q. (2009): Antioxidant and antimicrobial activity of Zostera marina l. extract. – Algae 24(3): 179-184.

[6]   Collier, C. J., Waycott, M. (2014): Temperature extremes reduce seagrass growth and induce mortality. – Marine Pollution Bulletin 83(2): 483-490. https://doi.org/10.1016/j.marpolbul.2014.03.050.

[7]   Collier, C. J., Langlois, L. M., McMahon, K. M., Udy, J., Rasheed, M., Lawrence, E., Carter, A. B., Fraser, M. W., McKenzie, L. J. (2021): What lies beneath: predicting seagrass below-ground biomass from above-ground biomass, environmental conditions and seagrass community composition. – Ecological Indicators 121: 107156. https://doi.org/10.1016/j.ecolind.2020.107156.

[8]   Costa, M. M., Silva, J., Barrote, I., Santos, R. (2021): Heatwave effects on the photosynthesis and antioxidant activity of the seagrass *Cymodocea nodosa* under contrasting light regimes. – Oceans 2(3): 448-460. https://doi.org/10.3390/oceans2030025.

[9]   Deguette, A., Barrote, I., Silva, J. (2022): Physiological and morphological effects of a marine heatwave on the seagrass *Cymodocea nodosa*. – Scientific Reports 12(1): 7950. https://doi.org/10.1038/s41598-022-12102-x.

[10]   DuBois, K., Williams, S. L., Stachowicz, J. J. (2020): Previous exposure mediates the response of eelgrass to future warming via clonal transgenerational plasticity. – Ecology 101(12). https://doi.org/10.1002/ecy.3169.

[11]   Fan, Y., Ma, C., Huang, Z., Abid, M., Jiang, S., Dai, T., Zhang, W., Ma, S., Jiang, D., Han, X. (2018): Heat priming during early reproductive stages enhances thermo-tolerance to post-anthesis heat stress via improving photosynthesis and plant productivity in winter wheat (*Triticum aestivum* l.). – Frontiers in Plant Science 9. https://doi.org/10.3389/fpls.2018.00805.

[12]   Filaček, A., Živčák, M., Ferroni, L., Barboričová, M., Gašparovič, K., Yang, X., Landi, M., Brestič, M. (2022): Pre-acclimation to elevated temperature stabilizes the activity of photosystem i in wheat plants exposed to an episode of severe heat stress. – Plants 11(5): 616. https://doi.org/10.3390/plants11050616.

[13]   Fox-Kemper, B. (2021): Ocean, cryosphere and sea level change. – AGU Fall Meeting 2021 13 December: U13B-09.

[14]   George, R., Gullström, M., Mangora, M. M., Mtolera, M. S. P., Björk, M. (2018): High midday temperature stress has stronger effects on biomass than on photosynthesis: a mesocosm experiment on four tropical seagrass species. – Ecology and Evolution 8(9): 4508-4517. https://doi.org/10.1002/ece3.3952.

[15]   Hossain, M. A., Li, Z.-G., Hoque, T. S., Burritt, D. J., Fujita, M., Munné-Bosch, S. (2018): Heat or cold priming-induced cross-tolerance to abiotic stresses in plants: key regulators and possible mechanisms. – Protoplasma 255(1): 399-412. https://doi.org/10.1007/s00709-017-1150-8.

[16]   Hu, T., Liu, S.-Q., Amombo, E., Fu, J.-M. (2015): Stress memory induced rearrangements of hsp transcription, photosystem ii photochemistry and metabolism of tall fescue (*Festuca arundinacea* Schreb.) in response to high-temperature stress. – Frontiers in Plant Science 6. https://doi.org/10.3389/fpls.2015.00403.

[17]   Hüve, K., Bichele, I., Rasulov, B., Niinemets, Ü. (2011): When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and $h_2 o_2$ formation. – Plant, Cell & Environment 34(1): 113-126. https://doi.org/10.1111/j.1365-3040.2010.02229.x.

[18]   Jueterbock, A., Minne, A. J. P., Cock, J. M., Coleman, M. A., Wernberg, T., Scheschonk, L., Rautenberger, R., Zhang, J., Hu, Z.-M. (2021): Priming of marine macrophytes for enhanced restoration success and food security in future oceans. – Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.658485.

[19]   Khanzada, A., Feng, K., Wang, X., Cai, J., Malko, M. M., Samo, A., Hossain, M. N., Jiang, D. (2022): Comprehensive evaluation of high-temperature tolerance induced by

heat priming at early growth stages in winter wheat. – Physiologia Plantarum 174(4). https://doi.org/10.1111/ppl.13759.

[20] Kilminster, K., McMahon, K., Waycott, M., Kendrick, G. A., Scanes, P., McKenzie, L., O'Brien, K. R., Lyons, M., Ferguson, A., Maxwell, P., Glasby, T., Udy, J. (2015): Unravelling complexity in seagrass systems for management: Australia as a microcosm. – Science of The Total Environment 534: 97-109. https://doi.org/10.1016/j.scitotenv.2015.04.061.

[21] Liu, B., Zhang, L., Rusalepp, L., Kaurilind, E., Sulaiman, H. Y., Püssa, T., Niinemets, Ü. (2021): Heat priming improved heat tolerance of photosynthesis, enhanced terpenoid and benzenoid emission and phenolics accumulation in *Achillea millefolium*. – Plant, Cell & Environment 44(7): 2365-2385. https://doi.org/10.1111/pce.13830.

[22] Luengluetham, P., Chotikarn, P., Nopparat, J., Buapet, P. (2023): Ecotoxicological assessment of copper and zinc in a common aquatic plant *Ceratophyllum demersum*: physiological effects and biomarker responses. – Aquatic Botany 188: 103678. https://doi.org/10.1016/j.aquabot.2023.103678.

[23] Marín-Guirao, L., Ruiz, J. M., Dattolo, E., Garcia-Munoz, R., Procaccini, G. (2016): Physiological and molecular evidence of differential short-term heat tolerance in mediterranean seagrasses. – Scientific Reports 6(1): 28615. https://doi.org/10.1038/srep28615.

[24] Marín-Guirao, L., Bernardeau-Esteller, J., García-Muñoz, R., Ramos, A., Ontoria, Y., Romero, J., Pérez, M., Ruiz, J. M., Procaccini, G. (2018): Carbon economy of mediterranean seagrasses in response to thermal stress. – Marine Pollution Bulletin 135: 617-629. https://doi.org/10.1016/j.marpolbul.2018.07.050.

[25] Mathur, S., Agrawal, D., Jajoo, A. (2014): Photosynthesis: response to high temperature stress. – Journal of Photochemistry and Photobiology B: Biology 137: 116-126. https://doi.org/10.1016/j.jphotobiol.2014.01.010.

[26] Murchie, E. H., Lawson, T. (2013): Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – Journal of Experimental Botany 64(13): 3983-3998. https://doi.org/10.1093/jxb/ert208.

[27] Nguyen, H. M., Kim, M., Ralph, P. J., Marín-Guirao, L., Pernice, M., Procaccini, G. (2020): Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. – Frontiers in Plant Science 11. https://doi.org/10.3389/fpls.2020.00494.

[28] Nguyen, H. M., Ralph, P. J., Marín-Guirao, L., Pernice, M., Procaccini, G. (2021): Seagrasses in an era of ocean warming: a review. – Biological Reviews 96(5): 2009-2030. https://doi.org/10.1111/brv.12736.

[29] Nishad, A., Nandi, A. K. (2021): Recent advances in plant thermomemory. – Plant Cell Reports 40(1): 19-27. https://doi.org/10.1007/s00299-020-02604-1.

[30] Pazzaglia, J., Badalamenti, F., Bernardeau-Esteller, J., Ruiz, J. M., Giacalone, V. M., Procaccini, G., Marín-Guirao, L. (2022): Thermo-priming increases heat-stress tolerance in seedlings of the mediterranean seagrass *Posidonia oceanica*. – Marine Pollution Bulletin 174: 113164. https://doi.org/10.1016/j.marpolbul.2021.113164.

[31] Phandee, S., Buapet, P. (2018): Photosynthetic and antioxidant responses of the tropical intertidal seagrasses *Halophila ovalis* and *Thalassia hemprichii* to moderate and high irradiances. – Botanica Marina 61(3): 247-256. https://doi.org/10.1515/bot-2017-0084.

[32] Purnama, P. R., Hariyanto, S., Manuhara, Y. S. W., Purnobasuki, H. (2019): Gene expression of antioxidant enzymes and heat shock proteins in tropical seagrass *Thalassia hemprichii* under heat stress. – Taiwania: International Journal of Biodiversity 64(2): 117-123.

[33] Rasmusson, L. M., Buapet, P., George, R., Gullström, M., Gunnarsson, P. C. B., Björk, M. (2020): Effects of temperature and hypoxia on respiration, photorespiration, and photosynthesis of seagrass leaves from contrasting temperature regimes. – ICES Journal of Marine Science 77(6): 2056-2065. https://doi.org/10.1093/icesjms/fsaa093.

[34] Rasmusson, L. M., Nualla-ong, A., Wutiruk, T., Björk, M., Gullström, M., Buapet, P. (2021): Sensitivity of photosynthesis to warming in two similar species of the aquatic angiosperm ruppia from tropical and temperate habitats. – Sustainability 13(16): 9433. https://doi.org/10.3390/su13169433.

[35] Ru, C., Hu, X., Chen, D., Wang, W. (2023): Droughts and thermo-priming enhance acclimation to later drought and heat stress in maize seedlings by improving leaf physiological activity. – Agronomy 13(4): 1124. https://doi.org/10.3390/agronomy13041124.

[36] Saewong, C., Soonthornkalump, S., Buapet, P. (2022): Combined effects of high irradiance and temperature on the photosynthetic and antioxidant responses of *Thalassia hemprichii* and *Halophila ovalis*. – Botanica Marina 65(5): 325-335. https://doi.org/10.1515/bot-2022-0014.

[37] Saha, M., Barboza, F. R., Somerfield, P. J., Al-Janabi, B., Beck, M., Brakel, J., Ito, M., Pansch, C., Nascimento-Schulze, J. C., Jakobsson Thor, S., Weinberger, F., Sawall, Y. (2020): Response of foundation macrophytes to near-natural simulated marine heatwaves. – Global Change Biology 26(2): 417-430. https://doi.org/10.1111/gcb.14801.

[38] Serrano, O., Arias-Ortiz, A., Duarte, C. M., Kendrick, G. A., Lavery, P. S. (2021): Impact of marine heatwaves on seagrass ecosystems. – In: 345-364. https://doi.org/10.1007/978-3-030-71330-0_13.

[39] Soonthornkalump, S., Ow, Y. X., Saewong, C., Buapet, P. (2022): Comparative study on anatomical traits and gas exchange responses due to belowground hypoxic stress and thermal stress in three tropical seagrasses. – PeerJ 10: e12899. https://doi.org/10.7717/peerj.12899.

[40] Strydom, S., Murray, K., Wilson, S., Huntley, B., Rule, M., Heithaus, M., Bessey, C., Kendrick, G. A., Burkholder, D., Fraser, M. W., Zdunic, K. (2020): Too hot to handle: unprecedented seagrass death driven by marine heatwave in a world heritage area. – Global Change Biology 26(6): 3525-3538. https://doi.org/10.1111/gcb.15065.

[41] Torasa, S., Boonyarat, P., Phongdara, A., Buapet, P. (2019): Tolerance mechanisms to copper and zinc excess in *Rhizophora mucronata* lam. seedlings involve cell wall sequestration and limited translocation. – Bulletin of Environmental Contamination and Toxicology 102(4): 573-580. https://doi.org/10.1007/s00128-019-02589-y.

[42] Tutar, O., Marín-Guirao, L., Ruiz, J. M., Procaccini, G. (2017): Antioxidant response to heat stress in seagrasses. a gene expression study. – Marine Environmental Research 132: 94-102. https://doi.org/10.1016/j.marenvres.2017.10.011.

[43] Viana, I. G., Moreira-Saporiti, A., Teichberg, M. (2020): Species-specific trait responses of three tropical seagrasses to multiple stressors: the case of increasing temperature and nutrient enrichment. – Frontiers in Plant Science 11. https://doi.org/10.3389/fpls.2020.571363.

[44] Vivanco-Bercovich, M., Sandoval-Gil, J. M., Bonet-Meliá, P., Cabello-Pasini, A., Muñiz-Salazar, R., Montoya, L. R., Schubert, N., Marín-Guirao, L., Procaccini, G., Ferreira-Arrieta, A. (2024): Marine heatwaves recurrence aggravates thermal stress in the surfgrass *Phyllospadix scouleri*. – Marine Pollution Bulletin 199: 115943. https://doi.org/10.1016/j.marpolbul.2023.115943.

[45] Wang, X., Cai, J., Liu, F., Dai, T., Cao, W., Wollenweber, B., Jiang, D. (2014): Multiple heat priming enhances thermo-tolerance to a later high temperature stress via improving subcellular antioxidant activities in wheat seedlings. – Plant Physiology and Biochemistry 74: 185-192. https://doi.org/10.1016/j.plaphy.2013.11.014.

[46] Wuthirak, T., Kongnual, R., Buapet, P. (2016): Desiccation tolerance and underlying mechanisms for the recovery of the photosynthetic efficiency in the tropical intertidal seagrasses *Halophila ovalis* and *thalassia hemprichii*. – Botanica Marina 59(5): 387-396. https://doi.org/10.1515/bot-2016-0052.

[47] Yucharoen, M., Sinutok, S., Chotikarn, P., Buapet, P. (2021): Experimental assessment of vulnerability to warming in tropical shallow-water marine organisms. – Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.767628.

[48] Zhang, J., Yang, Q., Yue, W., Yang, B., Zhou, W., Chen, L., Huang, X., Zhang, W., Dong, J., Ling, J. (2023a): Seagrass *Thalassia hemprichii* and associated bacteria co-response to the synergistic stress of ocean warming and ocean acidification. – Environmental Research 236: 116658. https://doi.org/10.1016/j.envres.2023.116658.

[49] Zhang, M., Li, H., Zhang, L., Liu, J. (2023b): Heat stress, especially when coupled with high light, accelerates the decline of tropical seagrass (*Enhalus acoroides*) meadows. – Marine Pollution Bulletin 192: 115043. https://doi.org/10.1016/j.marpolbul.2023.115043.

APPENDIX

Table A1. *Summary of repeated ANOVAs of effective quantum yield for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments and time (HOUR). Significant values (p < 0.05) are shown in bold*

	Effect	SS	df	MS	F	p
Thalassia hemprichii	Intercept	4.114	1	4.114	3357.731	**0.000000**
	Heat	0.365	1	0.365	298.014	**0.000000**
	Priming	0.017	1	0.017	13.722	**0.000923**
	Heat*Priming	0.002	1	0.002	1.498	0.231184
	Error	0.034	28	0.001		
	HOUR	0.012	1	0.012	14.165	**0.000789**
	HOUR*Heat	0.000	1	0.000	0.000	0.983803
	HOUR*Priming	0.000	1	0.000	0.455	0.505715
	HOUR*Heat*Priming	0.009	1	0.009	10.631	**0.002920**
	Error	0.023	28	0.001		
Cymodocea rotundata	Intercept	3.659	1	3.659	5315.187	**0.000000**
	Heat	0.062	1	0.062	89.611	**0.000000**
	Priming	0.002	1	0.002	2.893	0.100065
	Heat*Priming	0.005	1	0.005	7.400	**0.011080**
	Error	0.019	28	0.001		
	HOUR	0.013	1	0.013	14.019	**0.000831**
	HOUR*Heat	0.001	1	0.001	0.866	0.359903
	HOUR*Priming	0.000	1	0.000	0.215	0.646714
	HOUR*Heat*Priming	0.000	1	0.000	0.264	0.611691
	Error	0.025	28	0.001		
Halophila ovalis	Intercept	2.956	1	2.956	1998.914	**0.000000**
	Heat	0.575	1	0.575	388.593	**0.000000**
	Priming	0.037	1	0.037	25.205	**0.000026**
	Heat*Priming	0.001	1	0.001	0.790	0.381569
	Error	0.041	28	0.001		
	HOUR	0.033	1	0.033	31.523	**0.000005**
	HOUR*Heat	0.008	1	0.008	7.629	**0.010027**
	HOUR*Priming	0.001	1	0.001	0.985	0.329513
	HOUR*Heat*Priming	0.005	1	0.005	4.305	**0.047294**
	Error	0.030	28	0.001		

Table A2. *Summary of Tukey HSD test of effective quantum yield for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments and time (HOUR). Significant values (p < 0.05) are shown in bold*

	Cell No.	Treatment	Time	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
Thalassia hemprichii	1	Control	Hour 12		1.000 000	0.054 083	0.999 979	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**
	2	Control	Hour 18	1.000 000		0.066 981	0.999 899	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**
	3	Control primed	Hour 12	0.054 083	0.066 981		**0.013 088**	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**
	4	Control primed	Hour 18	0.999 979	0.999 899	**0.013 088**		**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**
	5	Warming	Hour 12	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**		0.060 512	0.788 688	0.973 954
	6	Warming	Hour 18	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**	0.060 512		**0.001 396**	**0.007 134**
	7	Warming primed	Hour 12	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**	0.788 688	**0.001 396**		0.998 651
	8	Warming primed	Hour 18	**0.000 134**	**0.000 134**	**0.000 134**	**0.000 134**	0.973 954	**0.007 134**	0.998 651	
Cymodocea rotundata	1	Control	Hour 12		0.981 679	1.000 000	0.509 969	**0.000 329**	**0.000 133**	0.097 424	**0.000 172**
	2	Control	Hour 18	0.981 679		0.969 232	0.973 402	**0.005 519**	**0.000 133**	0.556 662	**0.001 501**
	3	Control primed	Hour 12	1.000 000	0.969 232		0.565 255	**0.000 300**	**0.000 133**	0.087 845	**0.000 166**
	4	Control primed	Hour 18	0.509 969	0.973 402	0.565 255		0.084 240	**0.000 158**	0.984 337	**0.028 426**
	5	Warming	Hour 12	**0.000 329**	**0.005 519**	**0.000 300**	0.084 240		0.297 595	0.469 763	0.999 876
	6	Warming	Hour 18	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 158**	0.297 595		**0.000 857**	0.436 089
	7	Warming primed	Hour 12	0.097 424	0.556 662	0.087 845	0.984 337	0.469 763	**0.000 857**		0.322 200
	8	Warming primed	Hour 18	**0.000 172**	**0.001 501**	**0.000 166**	**0.028 426**	0.999 876	0.436 089	0.322 200	
Halophila ovalis	1	Control	Hour 12		0.098 026	**0.013 588**	0.017 977	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**
	2	Control	Hour 18	0.098 026		0.982 187	0.990 753	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**
	3	Control primed	Hour 12	**0.013 588**	0.982 187		1.000 000	**0.000 134**	**0.000 133**	**0.000 133**	**0.000 133**
	4	Control primed	Hour 18	**0.017 977**	0.990 753	1.000 000		**0.000 134**	**0.000 133**	**0.000 133**	**0.000 133**
	5	Warming	Hour 12	**0.000 133**	**0.000 133**	**0.000 134**	**0.000 134**		**0.020 837**	0.146 516	**0.000 133**
	6	Warming	Hour 18	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**	**0.020 837**		0.998 186	**0.011 668**
	7	Warming primed	Hour 12	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**	0.146 516	0.998 186		**0.001 434**
	8	Warming primed	Hour 18	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**	**0.000 133**	**0.011 668**	**0.001 434**	

Table A3. *Summary of two-way ANOVAs of Total antioxidant capacity for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments. Significant values (p < 0.05) are shown in bold*

	Effect	SS	df	MS	F	p
Thalassia hemprichii	Intercept	114588.226	1	114588.226	3287.375	**0.000000**
	Heat	74.810	1	74.810	2.146	0.158467
	Priming	544.065	1	544.065	15.608	**0.000789**
	Heat*Priming	34.043	1	34.043	0.977	0.334834
	Error	697.141	20	34.857		
Cymodocea rotundata	Intercept	113920.149	1	113920.149	5565.682	**0.000000**
	Heat	4.755	1	4.755	0.232	0.635064
	Priming	2.262	1	2.262	0.110	0.743043
	Heat*Priming	11.343	1	11.343	0.554	0.465271
	Error	409.366	20	20.468		
Halophila ovalis	Intercept	137626.245	1	137626.245	15811.019	**0.000000**
	Heat	0.012	1	0.012	0.001	0.970159
	Priming	0.711	1	0.711	0.082	0.777919
	Heat*Priming	3.049	1	3.049	0.350	0.560612
	Error	174.089	20	8.704		

Table A4. *Summary of HSD test of Total antioxidant capacity for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments. Significant values (p < 0.05) are shown in bold*

	Cell No.	Heat	Prime	{1}	{2}	{3}	{4}
Thalassia hemprichii	1	Control	No		**0.049125**	0.739549	**0.001048**
	2	Control	Yes	**0.049125**		0.094095	0.098179
	3	Warming	No	0.739549	0.094095		**0.002295**
	4	Warming	Yes	**0.001048**	0.098179	**0.002295**	
Cymodocea rotundata	1	Control	No		NS	NS	NS
	2	Control	Yes	NS		NS	NS
	3	Warming	No	NS	NS		NS
	4	Warming	Yes	NS	NS	NS	
Halophila ovalis	1	Control	No		NS	NS	NS
	2	Control	Yes	NS		NS	NS
	3	Warming	No	NS	NS		NS
	4	Warming	Yes	NS	NS	NS	

Table A5. *Summary of two-way ANOVAs of Lipid Peroxidation for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments. Significant values (p < 0.05) are shown in bold*

	Effect	SS	df	MS	F	p
Thalassia hemprichii	Intercept	70.589	1	70.589	425.973	**0.000000**
	Heat	1.685	1	1.685	10.171	**0.004609**
	Priming	1.793	1	1.793	10.820	**0.003664**
	Heat*Priming	3.969	1	3.969	23.951	**0.000088**
	Error	3.314	20	0.166		
Cymodocea rotundata	Intercept	14.727	1	14.727	79.604	**0.000000**
	Heat	0.007	1	0.007	0.036	0.851355
	Priming	0.027	1	0.027	0.144	0.708197
	Heat*Priming	0.060	1	0.060	0.324	0.575362
	Error	3.700	20	0.185		
Halophila ovalis	Intercept	97.204	1	97.204	568.720	**0.000000**
	Heat	5.134	1	5.134	30.037	**0.000023**
	Priming	1.550	1	1.550	9.071	**0.006890**
	Heat*Priming	0.184	1	0.184	1.075	0.312174
	Error	3.418	20	0.171		

Table A6. *Summary of HSD test of Lipid Peroxidation for Thalassia hemprichii, Cymodocea rotundata, and Halophila ovalis in responses to heat and priming treatments. Significant values (p < 0.05) are shown in bold*

	Cell No.	Heat	Prime	{1}	{2}	{3}	{4}
Thalassia hemprichii	1	Control	No		0.269951	**0.000014**	0.944171
	2	Control	Yes	0.269951		**0.000181**	0.242067
	3	Warming	No	**0.000014**	**0.000181**		**0.000012**
	4	Warming	Yes	0.944171	0.242067	**0.000012**	
Cymodocea rotundata	1	Control	No		NS	NS	NS
	2	Control	Yes	NS		NS	NS
	3	Warming	No	NS	NS		NS
	4	Warming	Yes	NS	NS	NS	
Halophila ovalis	1	Control	No		0.177865	**0.005129**	**0.000007**
	2	Control	Yes	0.177865		0.096217	**0.000170**
	3	Warming	No	**0.005129**	0.096217		**0.009618**
	4	Warming	Yes	**0.000007**	**0.000170**	**0.009618**	