Effects of Warming on Carbon Utilization and Photosynthesis of Marine Primary Producers

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ABSTRACT

Climate warming is among the most important threats to the health of shallow-water marine primary producers. In the present study, heat stress responses were investigated in the seagrass *Thalassia hemprichii* and the two macroalgae *Padina boryana* and *Ulva intestinalis* by comparing dissolved inorganic carbon (DIC) utilization, photosynthesis (as maximum quantum yield [F_v/F_m] and effective quantum yield [ϕPSII]), and reactive oxygen species (ROS) production after 4 h of exposure to control temperature (30 °C) or warming (40 °C). At 30 °C, DIC uptake rate of *U. intestinalis* was highest, whereas rates for *T. hemprichii* and *P. boryana* were comparable. Warming significantly reduced DIC use in all species. DIC use of *U. intestinalis* was affected to the greatest extent (from 16.38±0.88 $\mu mol \cdot g^{-1} FW \cdot h^{-1}$ in 30 °C to -1.39±0.36 $\mu mol \cdot g^{-1} FW \cdot h^{-1}$ in 40 °C). Warming significantly reduced the efficiency of photosynthesis in all species. *Thalassia hemprichii* showed the smallest reduction in F_v/F_m (from 0.80±0.03 in 30 °C to 0.66±0.12 in 40 °C). However, down-regulation of ϕPSII by warming in all species was comparable (reaching 0.15-0.18). Warming did not increase ROS accumulation in *T. hemprichii* (45.84±5.32 and 50.30±5.94 fluorescence units at 30 °C and 40 °C, respectively) or *P. boryana* (48.40±6.27 and 35.60±6.27 fluorescence units at 30 °C and 40 °C, respectively) but decreased accumulation of ROS in *U. intestinalis* (from 85.31±4.56 fluorescence units at 30 °C to 46.63±12.37 fluorescence units at 40 °C). Leakage of ROS from damaged algal thalli may contribute to the decrease in ROS observed in our study.

Keywords: Chlorophyll, Dissolved inorganic carbon (DIC), Heat stress, Reactive oxygen species

INTRODUCTION

At present, global climate change is one of the most critical threats to the world's ecosystems, especially the marine ecosystem, because oceans can absorb up to 60 % of the atmosphere's excess heat (Resplandy *et al.*, 2018). Seagrass and macroalgae play roles as primary producers in coastal ecosystems. Their photosynthetic carbon utilization also contributes to the carbon cycle in coastal areas (Laffoley and Grimsditch, 2009).

Seagrass and macroalgae are commonly found in the intertidal areas or in shallow water, where environmental conditions widely fluctuate (Manassa *et al.*, 2017). For example, direct exposure to air during low tides brings about high light intensity and high temperatures. Adverse effects of high temperature on growth and photosynthesis have been reported in many marine primary producers (Eggert, 2012; George *et al.*, 2018; Strydom *et al.*, 2020; Rasmusson *et al.*, 2020). However, tolerance to warming varies among

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species and depends on the ability to maintain carbon balance, photoprotective capacity and defense strategy of each species (Marín-Guirao *et al*., 2016).

In seawater with pH at approximately 8.1, bicarbonate (HCO_3^-) constitutes a major pool of dissolved inorganic carbon (DIC), while dissolved carbon dioxide (CO_2) accounts for only 1 % of DIC (Roleda *et al*., 2012). Many aquatic plants are able to utilize HCO_3^- for photosynthesis (Raven, 1997). Three mechanisms have been described: (i) the extracellular dehydration of HCO_3^- to CO_2 by external carbonic anhydrase, (ii) direct HCO_3^- uptake through anion exchange protein, and (iii) H^+-ATPase pump-assisted acidification of the diffusive boundary layer (Fernández *et al*., 2014). When large amounts of DIC are taken up, seawater pH increases as a result of a shift in DIC equilibrium, making seawater pH an indicator for photosynthetic carbon utilization (Buapet *et al*., 2013a; 2013b).

High temperature affects many cellular processes such as protein folding, membrane fluidity and assembly of cytoskeletons, and thus directly disturbs many physiological functions (Ruelland and Zachowski, 2010). It also reduces RuBP carboxylation rate, a critical step in carbon fixation in the Calvin cycle (Lilley *et al*., 2010), which not only decreases carbohydrate production but also reduces the utilization of ATP and NADPH obtained from light-dependent reactions. This imbalance in the redox state of photosynthetic electron transport can lead to a production of reactive oxygen species (ROS), induction of oxidative stress and photoinhibition due to damaged photosystems (Li *et al*., 2017), and bleaching (Martone *et al*., 2010; Wei *et al*., 2020).

In this experiment, three primary producers commonly found in tropical intertidal habitats are used as study models: (i) the seagrass *Thalassia hemprichii*, (ii) the brown alga *Padina boryana*, and (iii) the green alga *Ulva intestinalis*. The objectives of this experiment are to investigate the effects of warming on (i) the efficiency of carbon utilization estimated by an increase in pH and a decrease in DIC, (ii) the efficiency of photosynthesis measured by chlorophyll fluorescence technique, and (iii) oxidative stress represented by ROS accumulation, and to compare these responses among the species tested. The results obtained in this study facilitate a better understanding of the physiological sensitivity of these key marine primary producers to short-term warming, establishing baseline information for further in-depth study on how the tropical coastal ecosystems will change as a result of global warming.

MATERIALS AND METHODS

Plant material and acclimation

Thalassia hemprichii, Padina boryana and *Ulva intestinalis* were collected from Ban Pa-klok, Thalang District, Phuket Province, Thailand (8°01' 19.3"N 98°24'45.2"E). The samples were cleaned and acclimated in aerated sterilized seawater (30 psu) with an irradiance of 200 µmol photon$\cdot m^{-2}\cdot s^{-1}$ (light-dark cycle 12:12h, measured by LI-250A light meter, LICOR, Germany) and temperature of 30 °C for three days (optimal temperature for photosynthesis determined in a pilot study).

Experimental setup

According to our field records, a temperature of 40 °C is within the range of the highest temperatures recorded during midday at low tide in dry season at the collection site. Therefore, a warming treatment for this experiment was set at 40 °C, while the control treatment was 30 °C. The samples were weighed and 4 g of whole shoots of *Thalassia hemprichii* or thalli of *Padina boryana* or *Ulva intestinalis* were incubated separately in beakers containing 250 mL of sterilized seawater, which were placed inside an aquarium filled with water. Blanks (beakers filled with sterilized seawater without biological materials) were also included in each treatment. The pH of the seawater was adjusted to 8.1 using 0.05 M HCl before commencing the experiment. The temperature of each aquarium was controlled by aquarium heater (Lauda Alpha A, Germany) and variations in temperature did not exceed 2 ºC throughout the experiment. An irradiance of 200 µmol photon$\cdot m^{-2}\cdot s^{-1}$ (measured by LI-250A light meter, LICOR, Germany) was provided by LED lamp (Chihiros A-SERIES, Japan). The experiment lasted 4 h. This duration was selected based on the tidal cycle (Buapet *et al*., 2017) and reflected the duration of low tide, during which the shallow-water organisms are subjected to an increase in temperature. Measurements of seawater pH and total alkalinity as well as photosynthetic parameters took place before starting the experiment and again after 4 h of exposure to the experimental conditions. The biological materials were collected at the end of the experiment, immediately frozen in liquid nitrogen, and kept at -80 °C for the analysis of reactive oxygen species (ROS). The experiments were performed with six replicates (n = 6).

Carbon utilization

The pH level and total alkalinity of seawater were recorded at the beginning and at the end of the experiment. Seawater pH was directly measured using an OHAUS pH meter (STARTER 3100, USA). Total alkalinity was measured using the rapid titration technique following Buapet *et al*. (2013a). A 4-mL sample of seawater was collected using an autopipette (B5000-1, Capp, Denmark) and pH was immediately measured, and then re-measured after adding 1 mL of 0.01 M HCl. The measurement was done with three technical replicates. Total alkalinity and the concentration of DIC was calculated following the formula of Riley (1975) and applying the constants from Smith and Kinsey (1978). The DIC uptake rate was estimated as a reduction in DIC per hour and normalized against the wet weight of the sample.

Photosynthetic parameters

All photosynthetic parameters were measured by Pulse Amplitude Modulated (PAM) fluorometer (Diving-PAM, Walz, Germany). The maximum quantum yield (F_v/F_m) was measured after dark-adaptation for 30 min and was calculated as (F_m-F_0)/F_m, where F_0 is the minimal fluorescence after dark-adaptation and F_m is the maximum fluorescence after saturating light pulse. The effective quantum yield (ϕPSII) was measured under actinic light and was calculated as (F_m'-F')/F_m', where F' is the minimal fluorescence under actinic light, and F_m' is the maximum fluorescence after saturating light pulse.

Reactive oxygen species (ROS) accumulation

ROS level was investigated using the DCFH-DCF fluorescence method following Phandee and Buapet (2018). Seagrass leaves or macroalgal thalli (100 mg) were ground in liquid nitrogen. The powder was added into 1 mL of 10 mM Tris-HCl pH 7.2. The homogenate was centrifuged at 12,000 g at 4 °C for 20 min. Then, 100 µL of the supernatant was diluted 10 times in 10 mM Tris-HCl pH 7.2. To the diluted extract, 10 uL of 1.0 mM DCFDA was added. The fluorescence level was subsequently measured using a spectrofluorometer (FP-8200, Jasco, Japan) using emission wavelength at 524 nm and excitation wavelength at 504 nm.

Statistical analysis

All statistical analyses in this study were performed using Statistica academic (TIBCO Software). Differences in pH, DIC uptake rate, F_v/F_m, ϕPSII, and ROS were analyzed using two-way ANOVA. Fisher's least significant difference (LSD) test was used to compare all parameters across species and treatments. The significance level for all analyses was set at $p<0.05$.

RESULTS

The initial seawater pH was 8.10±0.05 and initial DIC concentration was 1805.07±21.86 $\mu mol \cdot L^{-1}$ SW. The difference in pH (final pH–initial pH, ΔpH), and DIC uptake rate displayed significant differences among the species tested (F = 84.97, $p<0.001$ for ΔpH and F = 500.41, $p<0.001$ for DIC uptake rate) and between temperature levels (F = 1306.54, $p<0.001$ for ΔpH and F = 6556.53, $p<0.001$ for DIC uptake rate). A significant interaction between species and temperature was also detected (F = 181.03, $p<0.001$ for ΔpH and F = 732.56, $p<0.001$ for DIC uptake rate). At 30 °C, seawater pH in all treatments increased from the initial level (Figure 1a), corresponding with uptake rates of dissolved inorganic carbon (DIC) (Figure 1b). The ΔpH of seawater sampled from *Ulva intestinalis* incubation was significantly higher than that from incubation with *Thalassia hemprichii* and *Padina boryana*. Consequently, *U. intestinalis* exhibited highest DIC uptake rate, while *T. hemprichii* and *P. boryana* exhibited comparable DIC uptake rates at 30 °C. Warming significantly reduced carbon utilization of all species tested to varying degrees, depending on species (Figure 1a, 1b). At 40 °C, *P. boryana* and *U. intestinalis* exhibited net release of DIC, whereas *T. hemprichii* maintained approximately 6 % of its DIC uptake (in relation to that at 30 °C). The blank experimental unit (beakers filled with sterilized seawater without biological materials) showed minor change in pH (0.02±0.02 in 30 °C and 0.01±0.03 in 40 °C) and DIC (0.27±0.29 µmole·h^{-1} in 30 °C and 0.23±0.27 µmole·h^{-1} in 40 °C).

The photosynthetic parameters (Figure 2) differed among the species tested (F = 95.57 and p<0.001 for F_v/F_m and F = 10.22 and p<0.001 for

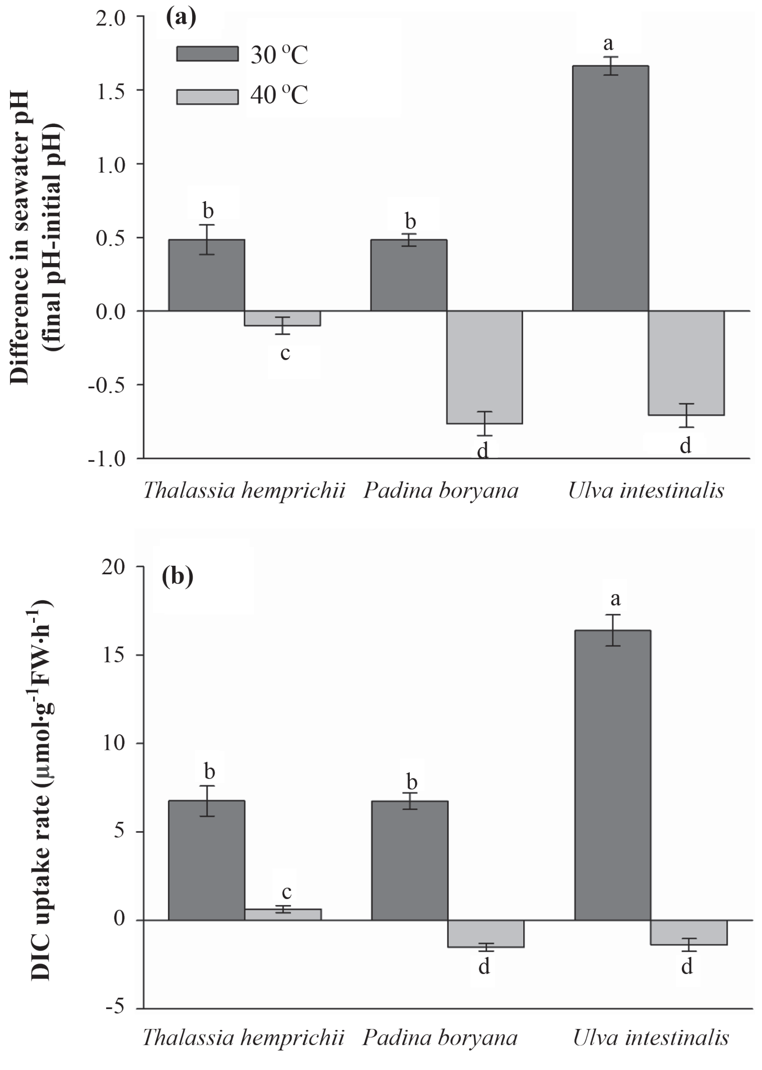

Figure 1. Difference in seawater pH as a result of 4 h incubation at 30 °C or 40 °C (a) and DIC uptake rates of *Thalassia hemprichii, Padina boryana* and *Ulva intestinalis* (b) at 30 °C and 40 ◦C calculated from a reduction of DIC from initial values. Error bars represent standard deviation (n = 6). Different letters above the bars indicate significant difference (p<0.05).

ϕPSII) and between temperature levels (F = 476.92 and $p<0.001$ for Fv/F_m and F = 462.20 and $p<0.001$ for ϕPSII). A significant interaction between species and temperature was also detected in the maximum quantum yield (F_v/F_m) (F = 46.65, $p<0.001$) and the effective quantum yield (ϕPSII) (F = 5.61, $p<0.01$). At 30 °C, the F_v/F_m was highest in *T. hemprichii* (0.80±0.03), whereas F_v/F_m of *P. boryana* (0.72±0.06) and *U. intestinalis* (0.72±0.07) did not differ from one another (Figure 2a). Warming resulted in a significant reduction in F_v/F_m of *U. intestinalis* and *P. boryana*, but had little effect on *T. hemprichii* (Figure 2a). The ϕPSII at 30 °C differed slightly among species (Figure 2b). Warming also resulted in a significant reduction in ϕPSII in all species to a similar degree (Figure 2b).

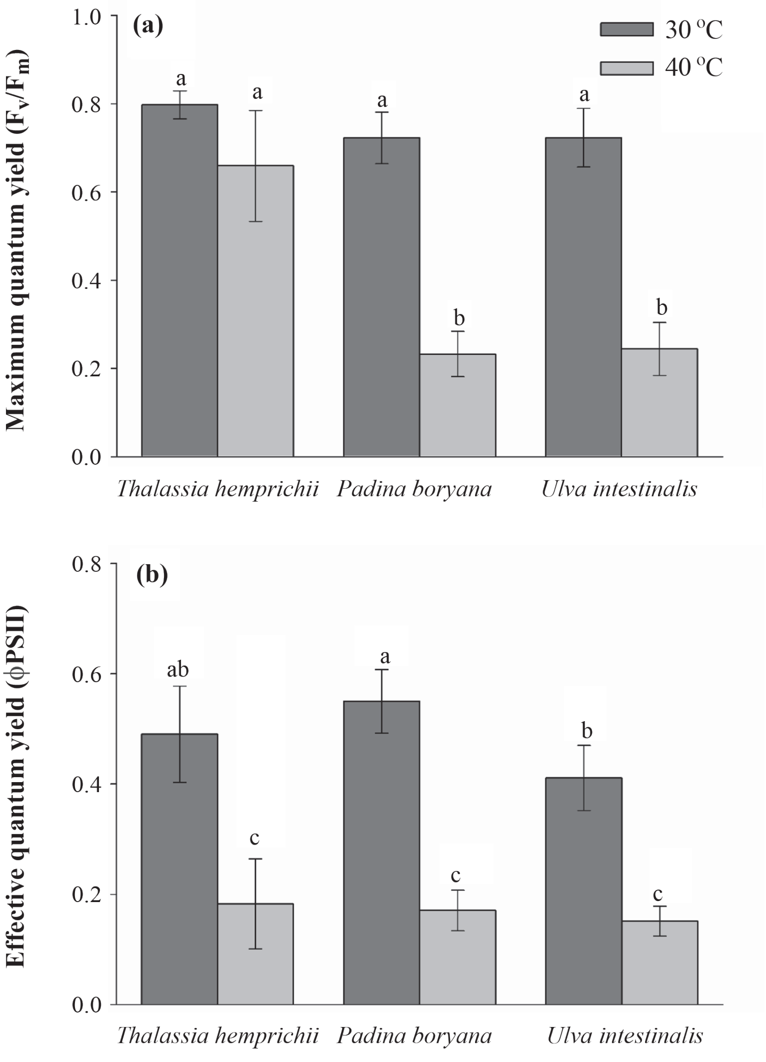

Figure 2. Maximum quantum yield (F_v/F_m) (a) and effective quantum yield (ϕPSII) (b) of *Thalassia hemprichii, Padina boryana* and *Ulva intestinalis* after being subjected to 30 °C or 40 °C for 4 h. Error bars represent standard deviation (n = 6). Different letters above the bars indicate significant difference ($p<0.05$).

Accumulation of reactive oxygen species (ROS, Figure 3) in seagrass leaf or macroalgal thalli differed among species (F = 22.15, $p<0.001$) and was affected by temperature (F = 28.48, $p< 0.001$). A significant interaction between species and temperature was also detected (F = 19.82, $p< 0.001$). At 30 °C, the ROS level of *U. intestinalis* was significantly higher than for *T. hemprichii* and *P. boryana*. At 40 °C, ROS level of *T. hemprichii* remained similar to that at 30 °C, whereas the ROS level of *U. intestinalis* and *P. boryana* was significantly lower than at 30 °C.

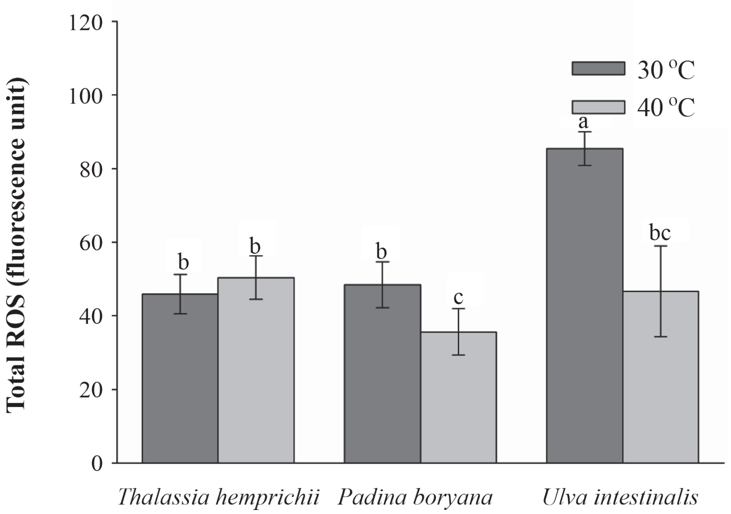

Figure 3. Accumulation of reactive oxygen species (ROS) in *Thalassia hemprichii, Padina boryana* and *Ulva intestinalis* after being subjected to 30 °C or 40 °C for 4 h. Error bars represent standard deviation (n = 6). Different letters above the bars indicate significant difference ($p<0.05$).

DISCUSSION

Our results reveal differences in the capacity for DIC utilization among the three primary producers. At 30 °C, *Ulva intestinalis* was most effective in DIC utilization, as indicated by the final pH of 9.76, whereas *Thalassia hemprichii* and *Padina boryana* showed similar capacity for DIC utilization. Such difference is likely related to their mechanisms for DIC acquisition (Axelsson *et al*., 1995; Larsson and Axelsson, 1999; Hellblom *et al*., 2001; Fernandez *et al*., 2014). It can be assumed that in the condition without heat stress, *U. intestinalis* has a competitive advantage over *T. hemprichii* and *P. boryana*. It has been reported that *U. intestinalis* was capable of depleting the DIC in a rockpool environment, thus inhibiting photosynthesis of co-occurring macroalgae (Björk *et al*., 2004). Similarly, Buapet *et al*. (2013b) reported that carbon limitation as a result of DIC utilization of *U. intestinalis* may reduce the productivity of the seagrasses *Zostera marina* and *Ruppia maritima*, both via lowering photosynthesis and increasing photorespiration.

A decrease in carbon utilization at 40 °C may be a result of a reduction of photosynthesis, owing to the high sensitivity of photosynthesis to heat. It has been reported that high temperature disturbs the functions of various key photosynthetic enzymes including RUBISCO (Allakhverdiev *et al*., 2008). In addition, a decrease in carbon utilization may be due to an increase in photorespiration induced by lower CO_2: O_2 in seawater and lower affinity of RUBISCO for CO_2 (Bowes, 1991). Moreover, we cannot rule out the possibility of an increase in CO_2 released from cellular respiration in the warming treatment, as previously reported in other studies (Pedersen *et al*., 2016; Rasmusson *et al*., 2020). It appears that the primary production of all the species tested may be affected by warming. In addition, *U. intestinalis* may lose the competitive advantage regarding DIC utilization in warming scenarios.

At 30 °C, all three species had comparable maximal photochemical quantum yield (F_v/F_m) and the F_v/F_m was within the range commonly regarded as healthy (0.7-0.8) (Campbell *et al*., 2006), suggesting that the experimental condition of controls did not impose stress on photosynthesis of all species tested. A reduction in the F_v/F_m at 40 °C suggests that warming leads to inactivation or damage of the photosynthetic machinery (Salvucci and Crafts-Brandner, 2004), particularly in *P. boryana* and *U. intestinalis*. Our results also suggest that *T. hemprichii* is the most heat tolerant species, reflected by only a minor decrease in F_v/F_m. This is consistent with the previous studies by Campbell *et al*. (2006) and Rasmusson *et al*. (2020), who reported that photodamage of *T. hemprichii* took place when the temperature reached 45 °C.

Different effective quantum yield of PSII (ϕPSII) among species at 30 °C implies that the three primary producers utilize different proportions of light energy to drive photochemistry (Campbell *et al*., 2006). A reduction in ϕPSII at 40 °C in *P. boryana* and *U. intestinalis* is likely a result of inactivation or photodamage by heat stress, as evidenced by a drastic decrease in F_v/F_m. Photosystem II is regarded as highly sensitive to heat stress (Berry and Bjökman, 1980). Oxygen evolution complex and the D1 protein of the reaction center of photosystem II are among the target sites of heat-induced damage (Allakhverdiev *et al*., 2008; Chan *et al*., 2012; Yamamoto, 2016). Nevertheless, it is also possible that a reduction in ϕPSII at 40 °C is due to an upregulation of non-photochemical quenching (NPQ), a photoprotective mechanism which prevents an imbalance in the redox state of photosynthetic electron transport by dissipating the light energy from the light harvesting antenna, thereby reducing ϕPSII. The ϕPSII, however, can be recovered once the oxidative pressure on the electron transport chain is relieved (Demmig-Adams, 1990). The downregulation of ϕPSII in *T. hemprichii* may be attributed to photoprotection rather than photodamage, as its F_v/F_m exhibited a slight decrease. Recent works on intertidal seagrasses *Halophila beccarii* (Fang *et al*., 2020), *T. hemprichii*, *Cymodocea serrulata* and *H. stipulacea* (Viana *et al*., 2020) suggested that thermal photosynthetic plasticity in seagrasses was partly mediated by regulation of their light use efficiency as evidenced in their rapid light curve (RLC) responses. More detailed quantification of light use characteristics by conducting RLCs could further determine photosynthetic acclimation processes and should be adopted in future studies.

Reactive oxygen species (ROS) accumulation at 30 °C indicates that the three species tested produce different amounts of ROS in the condition without heat stress. While *T. hemprichii* and *P. boryana* had similar ROS levels, *U. intestinalis* accumulated the highest amount of ROS. A previous study reported that *U. intestinalis* produces relatively higher ROS than other macroalgae (Van Hees and Van Alstyne, 2011). The ROS generated is then released outside the cell in H_2O_2 form as a signaling molecule.

Although the cellular damage observed in heat-stressed plants has mainly been attributed to an overproduction of ROS (Sharma *et al*., 2012), we did not observe an increase in ROS in any of the species tested. In the case of *T. hemprichii*, which displayed a minor decrease in F_v/F_m, the relatively unaffected ROS level may imply that it underwent minor or no oxidative stress at high temperature. Our previous study also found that *T. hemprichii* was relatively tolerant, exhibiting slight photoinhibition and unaffected ROS production in the conditions prone to oxidative stress (Wuthirak *et al*., 2016; Phandee and Buapet, 2018). On the other hand, *P. boryana* and *U. intestinalis* showed marked decreases in F_v/F_m, yet their ROS accumulation decreased. One possible explanation is that the photodamage observed in the two species was not related to oxidative stress (Roberfroid and Calderon, 1995). Another possible explanation is that high temperature reduces cellular membrane stability, leading to cellular leakage and injury (Ilík *et al*., 2018). As the thalli of the two macroalgae consist of only a few layers of cells, making it quite fragile compared to *T. hemprichii*, leakage of ROS may contribute to the decrease in ROS accumulation in algal tissue detected in our study. This notion corresponds with the study by Inaba and Grandall (1988) and to our observations that the two macroalgal thalli were bleached, with bleaching being more severe in *U. intestinalis*. Further investigations on the antioxidant activities would shed light on how these organisms mitigate oxidative stress under warming conditions. In addition, it is important to emphasize that the physiological responses measured in dug-up samples should be interpreted with caution, as injury may induce additional stress.

Overall, our results suggest that when the temperature rapidly rises, different species of primary producers may experience different levels of stress, and hence different competitive advantage and chance of survival. Greater temperature anomalies or catastrophic weather events such as heatwave may cause a shift in the species composition and distribution, affecting the balance of coastal ecosystems. Whether these responses translate to long term acclimation under global warming scenarios in which the temperature steadily rises remains to be investigated.

CONCLUSION

Warming led to a reduction in the DIC utilization and the efficiency of photosynthesis in *Thalassia hemprichii*, *Padina boryana* and *Ulva intestinalis. U. intestinalis* was affected by warming to the greatest extent, while *T. hemprichii* was the most effective in maintaining the carbon balance and the functionality of photosystem II.

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LITERATURE CITED

Allakhverdiev, S.I., V.D. Kreslavski, V.V. Klimov, D.A. LOS, R. Carpentier and P. Mohanty. 2008. Heat stress: an overview of molecular responses in photosynthesis. **Photosynthesis Research** 98: 541-550. DOI: 10.1007/s11120-008-9331-0.

Axelsson, L., H. Ryberg and S. Beer. 1995. Two modes of bicarbonate utilization in the marine green macroalgae *Ulva lactuca*. **Plant, Cell and Environment** 18: 439-445. DOI: 10.1111/j.1365-3040.1995.tb00378.x.

Berry, J. and O. Björkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. **Annual Review of Plant Physiology** 31: 491-543. DOI: 10.1146/annurev.pp.31.060180.002423.

Björk, M., L. Axelsson and S. Beer. 2004. Why is *Ulva intestinalis* the only macroalga inhabiting isolated rockpools along the Swedish Atlantic coast? **Marine Ecology Progress Series** 284: 109-116. DOI: 10.3354/meps284109.

Bowes, G. 1991. Growth at elevated CO_2: Photosynthetic responses mediated through Rubisco. **Plant, Cell and Environment** 14(8): 795-806. DOI: 10.1111/j.1365-3040.1991.tb01443.x.

Buapet, P., F. Makkliang and C. Thammakhet-Buranachai. 2017. Photosynthetic activity and photoprotection in green and red leaves of the seagrasses, *Halophila ovalis* and *Cymodocea rotundata*: implications for the photoprotective role of anthocyanin. **Marine Biology** 164: 182. DOI: 10.1007/s00227-017-3215-9.

Buapet, P., M. Gullström and M. Björk. 2013a. Photosynthetic activity of seagrasses and macroalgae in temperate shallow waters can alter seawater pH and total inorganic carbon content at the scale of a coastal embayment. **Marine and Freshwater Research** 64: 1040-1048. DOI: 10.1071/MF12124.

Buapet, P., M.L. Rasmusson, M. Gullström and M. Björk. 2013b. Photorespiration and Carbon Limitation Determine Productivity in Temperate Seagrasses. **PLoS ONE** 8(12): e83804. DOI: 10.1371/journal.pone.0083804.

Campbell, S.J., L.J. McKenzie and S.P. Kerville. 2006. Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. **Journal of Experimental Marine Biology and Ecology** 330: 455-468. DOI: 10.1016/j.jembe.2005.09.017.

Chan T., Y. Shimizu, P. Pospíšil, N. Nijo, A. Fujiwara, Y. Taninaka, T. Ishikawa, H. Hori, D. Nanba, A. Imai, N. Morita, M. Yoshioka-Nishimura, Y. Izumi, Y. Yamamoto, H. Kobayashi, N. Mizusawa, H. Wada and Y. Yamamoto. 2012. Quality control of photosystem II: lipid peroxidation accelerates photoinhibition under excessive illumination. **PLoS ONE** 7: e52100. DOI: 10.1371/journal.pone.0052100.

Demmig-Adams, B. 1990. Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. **Biochimica et Biophysica Acta (BBA)–Bioenergetics** 1020(1): 1-24. DOI: 10.1016/0005-2728(90)90088-l.

Eggert, A. 2012. **Seaweed responses to temperature.** In: Seaweed Biology (eds C. Wiencke and K. Bischof), pp. 47-66. Springer-Verlag, Berlin, Germany.

Fang, Y., Z. Jiang, C. Zhao, L. Li, C.I.P.M. Ranvilage, S. Liu, Y. Wu and X. Huang. 2020. Efficient heat dissipation and cyclic electron flow confer daily air exposure tolerance in the intertidal seagrass *Halophila beccarii* asch. **Frontiers in Plant Science** 11: 571627. DOI: 10.3389/fpls.2020.571627.

Fernández, P.A., C.L. Hurd and M.Y. Roleda. 2014. Bicarbonate uptake via an anion exchange protein is the main mechanism of inorganic carbon acquisition by the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae) under variable pH. **Journal of Phycology** 50: 998-1008. DOI: 10.1111/jpy.12247.

George, R., M. Gullström, M.M. Mangora, M.S.P. Mtolera and M. Björk. 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: 4508-4517. DOI: 10.1002/ece3.3952.

Hellblom, F., S. Beer and M. Björk. 2001. A buffer sensitive inorganic carbon utilization system in *Zostera marina*. **Aquatic botany** 69: 55-62. DOI: 10.1016/S0304-3770(00)00132-7.

Ilík, P., M. Špundová, M. Šicner, H. Melkovičová, Z. Kučerová, P. Krchňák, T. Fürst, K. Večeřová, K. Panzarová, Z. Benediktyová and M. Trtílek. 2018. Estimating heat tolerance of plants by ion leakage: a new method based on gradual heating. **New Phytologist** 218(3): 1278-1287. DOI: 10.1111/nph.15097.

Inaba, M. and P. Grandall. 1988. Electrolyte leakage as an indicator of high-temperature injury to harvested mature green tomatoes. **Journal of The American Society for Horticultural Science** 113: 96-99.

Laffoley, D. and G. Grimsditch. 2009. **The Management of Natural Coastal Carbon Sinks.** International Union for Conservation of Nature, Gland, Switzerland. 53 pp.

Larsson, C. and L. Axelsson. 1999. Bicarbonate uptake and utilization in marine macroalgae. **European Journal of Phycology** 34(1): 79-86. DOI: 10.1080/09670269910001736112.

Li, H., H. Xu, P. Zhang, M. Gao, D. Wang and H. Zhao. 2017. High temperature effects on D1 protein turnover in three wheat varieties with different heat susceptibility. **Plant Growth Regulation** 81: 1-9. DOI: 10.1007/s10725-016-0179-6.

Lilley, R.M.C., P.J. Ralph and A.W.D. Larkum. 2010. The determination of activity of the enzyme Rubisco in cell extractions of the dinoflagellate alga *Symbiodinium* sp. by manganese chemiluminescence and its response to short-term thermal stress of the alga. **Plant, Cell and Environment** 33: 995-1004. DOI: 10.1111/j.1365-3040.2010.02121.x.

Manassa, R.P., T.M. Smith, J. Beardall, M. Keough and P.L.M. Cook. 2017. Capacity of a temperate intertidal seagrass species to tolerate changing environmental conditions: significance of light and tidal exposure. **Ecological Indicators** 81: 578-586. DOI: 10.1016/j.ecolind.2017.04.056.

Marín-Guirao, L., J.M. Ruiz, E. Dattolo, R. Garcia-Munoz and G. Procaccini. 2016. Physiological and molecular evidence of differential short-term heat tolerance in Mediterranean seagrasses. **Scientific Reports** 6: 28615. DOI: 10.1038/srep28615.

Martone, P.T., M. Alyono and S. Stites. 2010. Bleaching of an intertidal coralline alga: untangling the effects of light, temperature, and desiccation. **Marine Ecology Progress Series** 416: 57-67.

Pedersen, O., T.D. Colmer, J. Borum, A. Zavala-Perez and G.A. Kendrick. 2016. Heat stress of two tropical seagrass species during low tides - impact on underwater net phot7 osynthesis, dark respiration and diel in situ internal aeration. **New Phytologist** 210(4): 1207-1218. DOI: 10.1111/nph.13900.

Phandee, S. and P. Buapet. 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. DOI: 10.1515/bot-2017-0084.

Rasmusson, L.M., P. Buapet, R. George, M. Gullström, P.C.B. Gunnarsson and M. Björk. 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. DOI: 10.1093/icesjms/fsaa093.

Raven, J.A. 1997. Inorganic carbon acquisition by marine autotrophs. **Advances in Botanical Research** 27: 85-209. DOI: 10.1016/S0065-2296(08)60281-5.

Resplandy, L., R.F. Keeling, Y. Eddebbar, M.K. Brooks, R. Wang, L. Bopp, M.C. Long, J.P. Dunne, W. Koeve and A. Oschlies. 2018. Quantification of ocean heat uptake from changes in atmospheric O_2 and CO_2 composition. **Nature** 563: 105-108. DOI: 10.1038/s41586-018-0651-8.

Riley, J. P. 1975. **Analytical chemistry of sea water.** In: Chemical Oceanography, 3rd ed. (eds. J.P. Riley and G. Skirrow), pp. 193-514. Academic Press, London, UK.

Roberfroid, M. and P.B. Calderon. 1995. **Free Radicals and Oxidation Phenomena in Biological Systems.** Dekker, New York, USA. 66 pp.

Roleda, M.Y., P.W. Boyd and C.L. Hurd. 2012a. Before ocean acidification: calcifier chemistry lessons. **Journal of Phycology** 48: 840-843. DOI: 10.1111/j.1529-8817.2012.01195.x.

Ruelland, E. and A. Zachowski. 2010. How plants sense temperature. **Environmental and Experimental Botany** 69(3): 225-232. DOI: 10.1016/j.envexpbot.2010.05.011.

Salvucci, M.E. and S.J. Crafts-Brandner. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. **Physiologia Plantarum** 120(2): 179-186. DOI: 10.1111/j.0031-9317.2004.0173.x.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal of Botany** 2012: 1-26. DOI: 10.1155/2012/217037.

Smith, S.V. and W.D. Kinsey. 1978. **Calcification and organic carbon metabolism as indicated by carbon dioxide.** In: Coral Reef: Research Methods (eds. D.R. Stoddart and R.E. Johannes.), pp. 469-484. United Nations Educational, Scientific and Cultural Organization, Paris, France.

Strydom, S., K. Murray, S. Wilson, B. Huntley, M. Rule, M. Heithaus, C. Bessey, G.A. Kendrick, D. Burkholder. M.W. Fraser and K. Zdunic. 2020. Too hot to handle: unprecedented seagrass death driven by marine heatwave in a World Heritage Area. **Global Change Biology** 26(6): 3525-3538. DOI: 10.1111/gcb.15065.

Van Hees, D. and K. van Alstyne. 2013. Effects of emertion, temperature, dopamine, and hypoxia on extracellular oxidant accumulations surrounding the bloom-forming seaweeds *Ulva lactuca* and *Ulvaria obscura*. **Journal of Experimental Marine Biology and Ecology** 448: 207-213. DOI: 10.1016/j.jembe.2013.07.013.

Viana, I.G., A. Moreira-Saporiti and M. Teichberg. 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: 571363. DOI: 10.3389/fpls. 2020.571363.

Wei, Z., J. Mo, R. Huang, Q. Hu, C. Long, D. Ding, F. Yang and L. Long. 2020. Physiological performance of three calcifying green macroalgae *Halimeda* species in response to altered seawater temperatures. **Acta Oceanologica Sinica** 39: 89-100.

Wuthirak, T., R. Kongnual and P. Buapet. 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. DOI: 10.1515/bot-2016-0052.

Yamamoto, Y. 2016. Quality control of Photosystem II: the mechanisms for avoidance and tolerance of light and heat stresses are closely linked to membrane fluidity of the thylakoids. **Frontiers in Plant Science** 7: 1136. DOI: 10.3389/fpls.2016.01136.