Synergistic effects of high temperature and sulfide on tropical seagrass

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Abstract

To examine the synergism of high temperature and sulfide on two dominant tropical seagrass species, a large-scale mesocosm experiment was conducted in which sulfide accumulation rates (SAR) were increased by adding labile carbon (glucose) to intact seagrass sediment cores across a range of temperatures. During the initial 10 d of the 38 d experiment, porewater SAR in cores increased 2- to 3-fold from 44 and 136 μmol L⁻¹ d⁻¹ at 28–29 °C to 80 and 308 μmol L⁻¹ d⁻¹ at 34–35 °C in Halodule wrightii and Thalassia testudinum cores, respectively. Labile C additions to the sediment resulted in SAR of 443 and 601 μmol L⁻¹ d⁻¹ at 28–29 °C and 758 to 1,557 μmol L⁻¹ d⁻¹ at 34–35 °C in H. wrightii and T. testudinum cores, respectively. Both T. testudinum and H. wrightii were highly thermal tolerant, demonstrating their tropical affinities and potential to adapt to high temperatures. While plants survived the 38 d temperature treatments, there was a clear thermal threshold above 33 °C where T. testudinum growth declined and leaf quantum efficiencies (Fv/Fm) fell below 0.7. At this threshold temperature, H. wrightii maintained shoot densities and leaf quantum efficiencies. Although H. wrightii showed a greater tolerance to high temperature, T. testudinum had a greater capacity to sustain biomass and short shoots under thermal stress with labile C enrichment, regardless of the fact that sulfide levels in the T. testudinum cores were 2 times higher than in the H. wrightii cores. Tropical seagrass tolerance to elevated temperatures, predicted in the future with global warming, should be considered in the context of the sediment-plant complex which incorporates the synergism of plant physiological responses and shifts in sulfur biogeochemistry leading to increased plant exposure to sulfides, a known toxin.

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1. Introduction

Sediments in seagrass and other shallow marine soft-bottom communities are characterized as highly organic (Hemminga and Duarte, 2000). The decomposition of organic matter in these coastal marine sediments is microbially mediated through the dissimilatory reduction of sulfate to sulfide (H₂S, HS⁻) (Sørensen et al., 1979; Howarth and Hobbie, 1981; Jørgensen, 1982; Canfield, 1993; Holmer and Kristensen, 1996; Holmer et al., 2003). Consequently, without reoxidation, pore-water sulfides build up in coastal marine sediments and can lead to a chronic exposure of seagrass belowground
tissues to high concentrations of sulfide, a known phytotoxin (Linthurst, 1979; Havill et al., 1985; Koch et al., 1990; Goodman et al., 1995; Raven and Scrimgeour, 1997; Holmer and Bondagarrd, 2001; Barber and Carlson, 1993; Carlson et al., 1994). The degree to which seagrass below ground tissues are exposed to sulfides is determined by multiple factors: microbial respiration rates, the ability of plants to oxidize their internal lacunae tissue (aerenchyma), the extent of the oxidized microzone of sediment surrounding the roots (rhizosphere), and local sediment biogeochemistry (Eldridge et al., 2004).

Temperature and the availability of labile organic substrates are primary factors controlling microbial sulfate reduction rates (SRR) (Holmer and Kristensen, 1996; Blaabjerg et al., 1998; Cotner et al., 2004; Pallud and Van Cappellen, 2006; Marbà et al., 2006). Labile carbon produced by high rates of seagrass photosynthesis provides low molecular weight carbon substrates to fuel the sulfate reducing microbial community (Blaabjerg et al., 1998; Hines et al., 1999; Cotner et al., 2004). Thus high photosynthetic rates of tropical seagrasses, such as *Thalassia testudinum* Banks ex Köing (Four-quean et al., 2001), and warm sub-tropical temperatures (>30 °C) probably account for the high millimolar porewater sulfide levels found in the sediments of Florida Bay (Barber and Carlson, 1993; Carlson et al., 1994), a large semi-enclosed subtropical lagoon at the terminus of the Florida peninsula. The dominant seagrass species in the Bay, *T. testudinum*, appears to be well adapted to reduced sediment conditions. It has extensive aerenchyma (Tomlinson, 1969) and a high capacity to oxidize its rhizosphere (Lee and Dunton, 2000; Borum et al., 2005), characteristics well established in the wetland plant literature as adaptations to sediment hypoxia (Iizumi et al., 1980; Sand-Jensen et al., 1982, Smith et al., 1984, Caffrey and Kemp, 1991; Lee and Dunton, 2000).

While *T. testudinum* and other seagrass species have a high capacity to oxidize their rhizosphere, microbial SRR in the tropics may overwhelm the ability of plants to completely reoxidize reduced sulfur compounds in porewaters. Confounding this problem is the fact that tropical carbonate sediments low in iron are inefficient at binding sulfides into solid-phase forms, contrasting temperate marine sediments dominated by pyrite and iron sulfide compounds (Berner, 1984; Chambers et al., 2001). Seagrass systems in carbonate sediments and with high organic loads, either from high internal organic production and/or undergoing anthropogenic eutrophication, tend to be exposed to high levels of porewater sulfides (Azzoni et al., 2001; Holmer et al., 2003).

Sulfides have been shown to be phytotoxic to a range of aquatic plant species, including seagrasses (Ingold and Havill, 1984; Havill et al., 1985; Koch and Mendelsohn, 1989; Koch et al., 1990; Goodman et al., 1995). In fact, sulfide toxicity has been proposed as an important factor in promoting “die-off” events of *T. testudinum* in Florida Bay (Robblee et al., 1991) and other seagrass species worldwide (Seddon et al., 2000; Azzoni et al., 2001; Holmer and Bondagarrd, 2001; Plus et al., 2003; Holmer et al., 2005). Although these studies implicate sediment reducing conditions, and specifically porewater sulfide, as an agent causing mortality in seagrass, there is conflicting evidence on the direct role of sediment sulfide in causing seagrass mortality (Terrados et al., 1999), and few experimental studies have been conducted to determine the upper threshold levels of sulfide or compare different species tolerances. Further, sulfide accumulation is highest during warm summer months when plants may also experience high thermal stress, particularly in tropical climes, therefore this potential interaction requires evaluation.

Herein we present results of a large-scale mesocosm experiment where sulfide accumulation was stimulated by adding labile carbon (glucose) to intact sediment cores and the seagrass response determined. We also examined the effects of increased temperature on sulfide accumulation rates (SAR) and the synergism of these two stressors (high temperature and sulfide) on plant growth and physiological response in two dominant tropical seagrass species *T. testudinum* and *Halodule wrightii* Aschers. We hypothesized that high temperature may increase sulfide accumulation causing a cumulative impact on tropical seagrasses.

### 2. Materials and methods

#### 2.1. Plant collection and experimental setup

Intact plant cores were collected from Florida Bay, at the southern terminus of the Florida (U.S.A.) peninsula May 21–29th, 2004. Intact cores of *H. wrightii* (15 cm diameter × 20 cm depth) were collected from Porjoe Key (25°13′41″N/80°47′37″W) and cores of *T. testudinum* were collected from Green Mangrove Key (24°55′20″N/80°47′33″W) and transported to the Florida Atlantic University Marine Lab (Boca Raton, FL) in coolers. Upon arrival, intact cores were immediately placed into mesocosm tanks with ambient coastal Atlantic seawater (36 psu), put on a 12:12 hr light–dark cycle and allowed to equilibrate for 4 weeks. The mesocosm setup included sixteen 500 L (3 m diameter × 3 m height)
fiberglass tanks equipped with 2 powersweeps, one for circulation at canopy height, and the other for surface to bottom circulation and continuous aeration (detailed in Koch et al., in press). In summary, each tank had a 1000 W metal halide light delivering PAR light levels of 864±34 μmol photons m$^{-2}$ s$^{-1}$ just below the water surface and 582±56 μmol photons m$^{-2}$ s$^{-1}$ at the canopy. The entire mesocosm experiment was run as a closed system with deionized water amended to the tanks as needed to compensate for evaporation and to maintain ambient salinities (35–37 psu). Coastal seawater from the flow through system on each tank was added weekly to maintain nutrient levels in the tanks.

Temperatures were raised at a rate of 1 °C d$^{-1}$ (June 21, 2004) to allow for slow thermal acclimation. There were four replicate tanks for each temperature treatment (28–29 °C [ambient], 30–31 °C, 32–33 °C and 34–35 °C). The elevated temperatures in the treatment tanks were achieved by using two 300 W aquarium heaters in each tank for the 30–31 °C and 32–33 °C treatments, and one 1000 W titanium heater with digital controls in each of the four tanks for the 34–35 °C treatment. Once tanks reached temperature treatment levels (June 30, 2004) they were maintained for 38 d.

To stimulate sulfide production, glucose was injected through two vertical sippers permanently installed in the intact plant cores. Cores were injected with 5 mL of deoxygenated artificial seawater (Instant Ocean 35 psu) alone (controls) or with 3.2 mol L$^{-1}$ glucose dissolved in artificial seawater calculated to yield a pore water molarity of approximately 10 mmol L$^{-1}$ (Carlson, personal communication). Glucose injections were conducted on July 1 (day 1), 3 (day 3), 5 (day 5), 19 (day 19), and 26 (day 26). Once injections were made, sippers were immediately closed off with three-way valves to prevent porewater exchange. Preliminary experiments showed this method of adding glucose stimulated sulfide production in the porewater to the desired upper mM range found in the field.

2.2. Plant response measurements

Weekly, leaf elongation rates were determined for T. testudinum using the leaf marking technique (Zieman, 1974) and live shoots were counted (number core$^{-1}$) in each core (surface area=201 cm$^2$) for both species. Net shoot change (%) was calculated relative to the initial short shoot number in the cores (initial mean±S. E.; Thalassia: 22.8±0.7 control and 21.8±0.5 glucose; Halodule: 92.3±9.0 control and 89±8.3 glucose) after temperature treatment was attained. Quantum efficiency of photosystem II or chlorophyll fluorescence (Fv/Fm) was measured on dark adapted (5 min) leaves each week using a Diving PAM (Pulse Amplitude Modulation; Walz, Germany). Leaf Fv/Fm ratios have been found to be an excellent indicator of stress in terrestrial and submerged aquatic plants with non-stressed seagrass ratios in the range of 0.7 to 0.8 (Björkman and Demmig, 1987; Ralph, 1999; Durako et al., 2002). In the presentation of our results, we use 0.7 as a threshold value below which the plants are assumed to be stressed.

After 38 d of treatments T. testudinum plant tissue was harvested followed by H. wrightii. Plant tissue was separated (leaf, root and rhizome) and immediately frozen in liquid N$_2$, freeze dried, weighed, ground with liquid N$_2$, and stored in a desiccator for carbohydrate analysis. Total soluble carbohydrates and starch were separated (leaf, root and rhizome) and immediately measured in leaf and rhizome tissue using methods described in Erskine and Koch (2000) modified from Yemm and Willis (1954) according to Zimmerman et al. (1989).

2.3. Physicochemical measurements

Pore water was sampled every 2–5 d by extracting 5 mL from each sippie (10 mL) while simultaneously adding 5 mL of artificial seawater (35 psu) in order to maintain porewater pressure. One subsample (5 mL) was used to measure salinity (refractometer) and pH (Orion 420A pH meter and Orion Triode pH electrode); the second 5 mL subsample was transferred into vials containing 5 mL of a highly alkaline sulfide buffer and immediately measured with a sulfide ion electrode (mV; Orion 420A pH meter and an Orion Model 9616 Sure-Flow Combination Silver/ Sulfide Electrode). The resulting total porewater sulfide pool measured is expressed as ΣTS$_{pw}$ with the H$_2$S (pKa$_1$=7; pKa$_2$=19) and HS$^-$ speciation being defined by pH. The ratio of H$_2$S: HS$^-$ is approximately 50% at pH 7. Tank salinity and temperature were monitored daily (YSI 85) and light (Li-Cor 1400 Data Logger and Li-Cor spherical sensor) and dissolved oxygen (YSI 85) measured weekly.

During the initial 10 d following the first glucose amendment, sulfide rapidly accumulated in the porewaters. We calculated the rate of porewater sulfide accumulation (SAR) in the cores over time (d) using a linear model (n=5) with a high degree of fit for glucose ($R^2=0.92–0.99$) and control ($R^2=0.75–0.99$) treatments. We used SAR as an approximation of a minimum sulfate reduction rate (SRR). The control SAR calculated closely approximated the SRR we measured in
Florida Bay sediments using $^{35}$SO$_4^{2-}$ (Koch and Jensen, unpublished data).

3. Results

3.1. Porewater sulfide and pH

Sediment SAR was significantly influenced by labile C and high temperature treatments and was species and/or sampling location specific. The addition of glucose to *Thalassia testudinum* intact cores from Green Mangrove Key under ambient temperatures (28–29 °C) resulted in average $\Sigma T_{SPw}$ concentrations of 2.3 mM over the 38 d experiment (Fig. 1). With labile C added, the SAR was 5 times higher (502 μmol L$^{-1}$ d$^{-1}$) than controls (114 μmol L$^{-1}$ d$^{-1}$, Table 1) and increased as a function of temperature to levels as high as 1300 μmol L$^{-1}$ d$^{-1}$ at 32–35 °C during the first 10 d of the experiment (Table 1). The high temperatures resulted in maximum SAR and $\Sigma T_{SPw}$ of 14 mM when sulfides peaked after 10 d, but over the 38 d experiment averaged 5.5 mM (Fig. 1). In contrast, without labile C additions, $\Sigma T_{SPw}$ peaked to 3.8 mM at 34–35 °C after 10 d and averaged <2 mM over the 38 d experiment (Fig. 1).

Glucose amendments also stimulated SAR in *H. wrightii* cores, but the rates calculated and resulting $\Sigma T_{SPw}$ concentrations were much lower than those in *T. testudinum* cores with and without glucose amendments (Fig. 1, Table 1). *H. wrightii* cores from Porjoe Key had approximately 50% lower maximum $\Sigma T_{SPw}$ concentrations (6.5 mM) than *T. testudinum* at day 10 following C enrichment. The SAR in *H. wrightii* cores were 33 to 74% of those calculated for *T. testudinum* cores with glucose amendments, and 32 to 51% compared to *T. testudinum* controls (Table 1). The SAR and $\Sigma T_{SPw}$ response to temperature and glucose treatments was also not as pronounced as was found for *T. testudinum*, suggesting a lower saturating rate (Fig. 1, Table 1). Further, $\Sigma T_{SPw}$ concentrations in *H. wrightii* controls never reached 1 mM, even in the highest temperature treatment.

A general lowering of pH in porewaters with labile carbon amendments (Table 2) was coincident with high sulfide accumulation rates (Table 1). This pH shift may indicate sulfide oxidation and/or release of organic acids. If sulfide oxidation occurred, our results in Table 1 probably represent a minimum estimate of sulfide production. A shift from approximately pH 7 to 6 under glucose amendments would have shifted the sulfide speciation ($H_2S:HS^-$) ~10% in favor of $H_2S$.

3.2. Shoot mortality, growth and physiological response

Net shoot loss in *T. testudinum* was less than 5% across all temperature treatments in the control cores, but...
with the combination of high temperature (34–35 °C) and glucose amendments, net shoot loss was 65% over the course of the 38 d experiment (Fig. 2). Growth rates were a sensitive indicator of stress with a highly significant ($P < 0.01$) effect of temperature and glucose treatments on mature leaf elongation rates (2-way ANOVA; Fig. 3). These trends in net production, as indexed by leaf elongation, were also apparent in new leaf allocation. New leaf emergence declined significantly beyond the threshold temperature of 33 °C, in both control and glucose amended cores (Fig. 3), and no new leaf emergence was found in plants at the highest temperatures with glucose. Leaf quantum efficiencies paralleled growth responses in *T. testudinum*, with $F_v/F_m$ ratios significantly different between all temperatures ($P < 0.01$; 2-way ANOVA) with the exception of 28–29 °C and 30–31 °C, indicating a stepwise response to temperature (Fig. 4). Although leaf florescence was not significantly different with glucose amendments, quantum yields tended to decline more steeply in the glucose treatment as a function of increasing temperature relative to controls, particularly beyond 31 °C (Fig. 4).

While *T. testudinum* was sensitive to increasing temperatures, *H. wrightii* was quite remarkable in its thermal tolerance. Across all temperatures, *H. wrightii* sustained shoot numbers greater than initial densities (>100%; Fig. 2) and maintained leaf quantum efficiencies >0.7 in treatments without glucose (Fig. 4). Although *H. wrightii* exhibited a high thermal tolerance, it was apparently highly sensitive to sediment sulfide stimulated by glucose amendments. This was the case even though porewater sulfide levels were lower than in *T. testudinum* cores (Fig 1). Percent short shoot survival was 50% less with glucose amendments (64%) relative to controls (121%). It appears that below ground exposure to sulfides affected individual shoot mortality. This is evidenced by the fact that the short shoots which survived maintained high leaf quantum efficiencies $\geq 0.70$ (Fig. 4), even though the ratio of $F_v/F_m$ significantly declined in the glucose treatment (0.715) relative to controls (0.727; $P < 0.05$, 2-way ANOVA).

### 3.3. Plant biomass allocation

*T. testudinum* final leaf biomass was moderately affected at the highest temperature (0.94 g) relative to lower temperature treatments (1.00–1.19 g), but...
consistent with results on net shoot loss, differences were only significant with glucose amendments (Fig. 5). Root biomass was not affected by temperature, but on average a 50% reduction in root biomass (2.21 to 1.38 g) was observed under glucose amendments. Rhizome biomass did not change significantly under either treatment, but total plant biomass tended ($P=0.08$, 2-way ANOVA) to be lower in the glucose (8.9 g) versus control treatment (10.8), particularly above 29 °C (Fig. 5).

The moderate biomass response observed in *T. testudinum* is sharply contrasted by the highly significant change in *H. wrightii* biomass in response to both temperature and glucose treatments (Fig. 5). *H. wrightii* increased biomass in the leaves and roots up to 33 °C, beyond which leaf, root, and total biomass declined (Fig. 5). Total *H. wrightii* biomass significantly declined with glucose amendments ($P<0.01$, 2-way ANOVA), but the pattern of increasing leaf and root biomass at elevated temperatures was sustained. It is interesting to note however that the threshold beyond which total biomass declined was 2 °C lower under glucose treatments (30 °C) compared to the controls (Fig. 5). At 34–35 °C and glucose amendments, *H. wrightii* biomass was <1 g in the entire core, 5-times lower than in the control treatment at this temperature, indicating the high sensitivity of *H. wrightii* to the interaction of sediment reducing conditions and high temperature.

### 3.4. Leaf and rhizome carbohydrates

Leaf tissue of both *T. testudinum* and *H. wrightii* had significantly higher soluble and insoluble (starch) carbohydrates with increasing temperature (28 to 34 °C, Fig. 6). A general pattern of carbohydrate reduction in the rhizomes paralleled this increase in leaf carbohydrates (Fig. 6), but this reduction was not statistically significant. Although an increase in carbohydrates was observed in *T. testudinum* and *H. wrightii* leaves with increasing temperatures, leaf carbohydrate (soluble and starch) contents were only significantly different ($P<0.01$, 2-way ANOVA) at the highest temperature (34–35 °C), and there was no significant change with the addition of glucose in either species. As a result of high variance, there was no significant reduction found in *T. testudinum* rhizome starch with increasing...
temperatures; however, there was a clear tendency for rhizome starch to decline at 34–35 °C in both species, most prominently under glucose treatments (Fig. 6).

4. Discussion

High temperature can cause direct physiological stress in tropical marine plants living at their thermal tolerance limits (Zieman, 1970, Thorhaug, 1974). In addition, high temperatures stimulate microbial sulfate reduction rates in marine sediments increasing sediment hypoxia and exposure of below-ground tissues to sulfide, a known phytotoxin (Linthurst, 1979; Havill et al., 1985; Koch et al., 1990; Goodman et al., 1995; Raven and Scrimgeour, 1997; Holmer and Bondgaard, 2001; Barber and Carlson, 1993; Carlson et al., 1994; Azzoni et al., 2001). SAR in the porewater of intact seagrass cores from Florida Bay increased 2- to 3-fold with a temperature increase from 28–29 °C to 33–34 °C. Upper temperature treatments in our study simulated maximum temperatures (35–36 °C) recorded in Florida Bay (Boyer et al., 1997; Koch in preparation) which approach the thermal optima for bacterial SRR. Sulfate reduction rates by sulfate reducing bacteria have been shown to have an optimum temperature of approximately 35–40 °C (Wieland and Kühl, 2000; Rabus et al.,

Fig. 6. Leaf and rhizome soluble carbohydrate and starch for *Thalassia testudinum* and *Halodule wrightii* after 38 d of exposure to high temperature treatments with (glucose) and without (control) glucose amendments. Means±S.E. (n=4).
which can be several degrees (10 °C) higher than the growth optima of psychrotolerant species (Rabus et al., 2002); although the upper thermal range for bacterial sulfate reduction is 60 to 80 °C with hyperthermophilic groups found at >110 °C (Machel, 2001). The majority of studies on SRR and sulfide production in the tropics and warm water environments have been conducted on microbial mats where it has been shown that sulfur cycling (SRR and sulfide oxidation) is strongly regulated by temperature (Wieland and Kühl, 2000). Thus, tropical seagrass tolerance to high temperature needs to be considered in the context of sediment-plant interactions in marine sediments. This is principally the case when the stress response promotes the release of plant organic exudates which can be translocated to the sediment microbial community (Rooney-Varga et al., 1997; Blaabjerg et al., 1998; Hines et al., 1999), thereby stimulating SRR (Blaabjerg et al., 1998; Hines et al., 1999; Cotner et al., 2004; Pallud and Van Cappellen, 2006) and plant exposure to porewater sulfides.

By increasing labile C to the sediment of intact cores, we effectively stimulated the potential sulfide production rates 5-fold and shifted the sulfide equilibria slightly in favor of H2S, the most toxic form of the $\Sigma$TS$_{pw}$ pool (Bagarinao 1992). The addition of glucose as well as other labile substrates (lactate) has been shown in freshwater, brackish, and marine sediments to stimulate SRR with greatest stimulation at marine sites (Holmer et al., 2005; Pallud and Van Cappellen, 2006). We observed regional differences in SAR, a proxy for SRR, in Florida Bay sediment in response to labile C amendments, but these differences were not accounted for by salinity, as was found in the aforementioned studies, because in our mesocosm experiment all tanks were maintained at 35 psu. H. wrightii sediments from eastern Florida Bay (Porjoe Key) never attained $\Sigma$TS$_{pw}$ levels of 1 mM, while T. testudinum controls collected from the western Bay site (Green Mangrove Key) frequently had maximum $\Sigma$TS$_{pw}$ levels just below 4 mM. Seagrass species may explain this discrepancy; however, the same pattern was seen with the addition of glucose. Adding labile C resulted in T. testudinum $\Sigma$TS$_{pw}$ levels 2-fold higher than the levels recorded in H. wrightii cores. This difference in $\Sigma$TS$_{pw}$ accumulation could have been influenced by sulfide oxidation or sequestration of sulfide by Fe. Fe gradients in the Bay show 2–3 times lower reactive Fe in western (3–3.5 μmol g$^{-1}$) versus eastern (1–1.5 μmol g$^{-1}$) Bay sediments (Zhang et al., 2004). While sulfide oxidation and sequestration by reactive Fe could contribute to the differences found in $\Sigma$TS$_{pw}$ in our study, we have conducted subsequent experiments examining potential SAR (with and without glucose amendments) across the Bay and find significantly higher potential rates in western versus eastern Bay sites (Koch et al., in preparation) and measured higher SRR in western versus eastern Bay sites using $^{35}$SO$_4^{2-}$ (Koch and Jensen, unpublished data). This in situ east–west SRR gradient, probably accounted for the higher $\Sigma$TS$_{pw}$ levels in T. testudinum cores versus H. wrightii cores in this study.

Remarkably, without glucose amendments, both T. testudinum and H. wrightii were highly thermal tolerant, demonstrating their tropical affinities and potential to thermally adapt to high temperatures. Both species sustained short shoot densities across all temperature treatments with no mass mortality of shoots found in any core with temperature treatments up to 35 °C. While the plants survived temperature treatments, there was a clear thermal threshold above 33 °C where T. testudinum growth, including new allocation of leaf tissue, declined and leaf quantum efficiencies fell below 0.7, indicative of thermal breakdown of photosynthetic function (Campbell et al., 2006). Above this same threshold temperature, H. wrightii maintained shoot densities and high leaf quantum efficiencies (>0.7), but had an overall reduction in biomass. Although few studies have examined tropical seagrass tolerance to long-term high temperature exposure, our thermal thresholds agree quite remarkably with field observations. Zieman (1970) found T. testudinum in South Florida to drop out of the benthic community at thermal effluent sites with temperatures sustained above 36 °C. Further, Zieman (1975) measured maximum growth rates at all sites with temperatures in the range of 28–31 °C and lowest at temperatures of 34–35 °C when salinities were also depressed (13–15 psu), a potential interaction. In another mesocosm experiment (salinity × temperature; Koch et al., unpublished data), we found T. testudinum growth to decline linearly with time (1–39 d) at 36 °C and precipitously at 40 °C, consistent with the temperature threshold for this species established in the present study and reported by Zieman (1970, 1975).

While the thermal threshold for T. testudinum under sustained high temperature exposure is in the region of 33 °C, H. wrightii was shown in this study to increase shoots and sustain relatively high quantum efficiencies at 34–35 °C, albeit with lower overall biomass. McMillan (1984) also determined that H. wrightii was more thermally tolerant than T. testudinum, with all H. wrightii shoots surviving 4 weeks at 36 °C and 2 weeks at 37 °C, while some shoots of T. testudinum did not survive the first treatment and all shoots were lost in the

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**References:**

Rabus et al., 2002; Pallud and Van Cappellen, 2006; Holmer et al., 2005; Rooney-Varga et al., 1997; Blaabjerg et al., 1998; Hines et al., 1999; Cotner et al., 2004; Pallud and Van Cappellen, 2006; Wieland and Kühl, 2000; McMillan (1984).
second treatment, respectively. *Halodule* is also frequently reported to occupy shallow waters in the tropics (Fiji) and subtropics (Florida) that can reach temperatures of 40 °C during some period of the day (see McMillan, 1984).

Tropical seagrass species thermal thresholds for high sustained temperatures (this study; Zieman, 1970) are slightly lower than those established using short-term pulsed (hourly) exposures to high temperature. A recent study by Campbell et al. (2006) confirmed the maintenance of leaf photosynthetic yield in *Thalassia (hemprichii)* and *Halodule (uninervis)* exposed to 1–4 h pulses of 35–40 °C, with *Halodule* exhibiting a greater thermal tolerance than *Thalassia* at 45 °C. This short-term adaptation to temperatures >40 °C may account for the presence of *H. wrightii* and *T. testudinum* in the field with diurnal maximum temperatures above thresholds established under long-term exposure. While short-term intermittent exposures to extreme temperatures appear tolerable, this type of rapid exposure would not lead to significant changes in the sediment biogeochemistry found in this study with sustained elevated temperatures. Under the latter scenario, and increased organic matter loading to estuaries and coastal lagoons with eutrophication (Holmer et al., 2003), the competition among seagrass species in terms of their thermal tolerance might shift.

We found that *T. testudinum* had a greater capacity to sustain biomass and short shoots with labile C enrichment under high thermal stress compared to *H. wrightii*. This was the case regardless of the fact that sulfide exposure to *T. testudinum* was 2 times greater than for *H. wrightii*. Thus, while *H. wrightii* is very thermally tolerant (McMillan, 1984), its low capacity to store oxygen and its very small and delicate root-and rhizome-system, may make it an inferior competitor to *T. testudinum* under extreme hypoxia and high sulfide exposure at moderately high temperatures common in Florida Bay. However, if both species were to succumb to hypoxia, *H. wrightii*, an early successional species, would have the capacity to more rapidly recolonize in the field. It is interesting to note that while *H. wrightii* increased its overall biomass (primarily as leaf biomass) up to 32–33 °C, under glucose treatments biomass was maximum at 30–32 °C. Beyond this temperature a significant amount of below-ground tissue was lost and at 34–35 °C biomass was only 20% of controls. While more tolerant of hypoxia and high temperature, *T. testudinum* had lower leaf growth and quantum efficiencies with increasing temperature in glucose treatments and at the highest temperature had no new leaf production. Even though total mortality of shoots was not observed in either species under high sulfide and temperature interactions, and probably cannot solely account for sudden seagrass die-off events in the Bay, it is evident that these interactive stressors individually and synergistically affect net carbon allocation in seagrass (Touchette and Burkholder, 2000). This was evident in the reduction of total biomass at high temperatures and further with glucose amendments, and is reflected in shifts in tissue carbohydrate levels.

Elevated temperatures increased leaf soluble and insoluble carbohydrate levels in *T. testudinum* and *H. wrightii*. These data suggest that as temperature increased both species were attempting to keep pace with respiratory carbon demands for sucrose, the major form of soluble carbohydrates in seagrass (Touchette and Burkholder, 2000). A stimulation in sucrolysis in response to stress has been observed in seagrass species exposed to elevated temperature and salinity determined by sucrose-P-synthase activity (reviewed in Touchette and Burkholder, 2000). Increases in leaf soluble carbohydrates with temperature did not correspond to a decline in leaf or rhizome starch. These data suggest new carbohydrate synthesis in the leaves or simply accumulation. The latter explanation is supported in *T. testudinum* which had lower leaf elongation rates and quantum efficiencies at high temperatures, but *H. wrightii* sustained leaf quantum efficiencies and increased biomass, at least up to 33 °C, suggesting a stimulation in carbon production. With glucose amendments and at high temperature, *T. testudinum* tended to have lower rhizome carbohydrate levels. A carbon drain may have resulted from anaerobic respiration, metabolic breakdown of belowground tissues, and/or disruption of translocation due to hypoxia (Alcoverro et al., 1999) caused by extremes of high temperature and porewater sulfides. These data indicate that high temperatures and the interactive stressor sulfide have the potential to disrupt carbon metabolism in tropical seagrasses.

Based on this mesocosm study we conclude the following: (1) Sediment sulfides have the potential to accumulate to very high levels in carbonate-dominated seagrass sediments at seasonal high temperature extremes. (2) Porewater sulfide accumulation is related to a sediment biogeochemical response to labile C. (3) Based on the species examined, tropical seagrasses have a high thermal tolerance, but are living very close to their thermal limits of sustained high temperatures, even though short durations at temperature extremes appear tolerable. (4) The competitive dominance of individual seagrass species to high temperature may shift with the added stress of increased sediment hypoxia and/or sulfide accumulation associated with eutrophication. (5)
Tropical seagrass tolerance to elevated temperatures, predicted in the future with global warming, should be considered in the context of the sediment-plant complex which incorporates the synergism between plant physiological response to temperature and changes in sediment biogeochemical sulfur cycling.

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