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Hypersalinity as a trigger of seagrass (*Thalassia testudinum*) die-off events in Florida Bay: Evidence based on shoot meristem O_2 and H_2S dynamics

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ABSTRACT

The mechanisms initiating rapid, large-scale (> 50 km^2) seagrass die-off events globally remain elusive. *Thalassia testudinum*, a dominant habitat-forming tropical seagrass of the Caribbean and tropical Atlantic region, experiences recurrent die-off events in Florida Bay. Thus, *T. testudinum* provides an excellent case study to identify triggers of large-scale seagrass mortality. Presently, seagrass die-off events in Florida Bay are correlated to high total sulfide (H_2S, HS^-, S^{2-}) concentrations in the sediment porewater (≥2000 μM), water column hypoxia, high temperature and hypersalinity (salinity > 45). Because the mortality appears to be initiated at the shoot base within the meristem, we examined the response of shoot meristematic O_2 and H_2S dynamics using microsensors over a 5-hour nighttime simulation. Shoots were held at salinity 35 (ambient) or 65 (hypersaline) using microsensors in intact cores with plants from the field. The rate of H_2S intrusion in the dark and oxidation in the light were similar at 35 and 65 salinity. However, the tissue O_2 consumption rate was significantly higher under hypersaline conditions (−11.07 ± 4.32 kPa pO_2 h^{-1}) compared to ambient (−3.93 ± 0.88 kPa pO_2 h^{-1}) in the dark. Consequently, the meristematic pO_2 threshold (~1.5 kPa O_2) where H_2S intrusion occurred in the meristem was reached more rapidly, increasing the time H_2S accumulated (1.5 to 2.8 h). Longer H_2S accumulation time significantly increased maximum meristem H_2S levels at 65 (536 ± 330 μM H_2S) compared to 35 salinity (121 ± 62 μM H_2S). These results in *T. testudinum* provide evidence for a triggering mechanism that links an enhanced respiratory rate under hypersaline conditions to sulfide toxicity. We propose that hypersalinity in Florida Bay, or any stressor that significantly increases nighttime respiration rates, can subject seagrasses to longer and higher concentrations of sulfide, a known phytotoxin, within the shoot meristem. This mechanism likely explains large-scale mass mortality of *T. testudinum* in Florida Bay during periods of high salinity and elevated porewater sulfide levels, although field experiments are required to further validate this supposition.

1. Introduction

Subtropical/tropical estuaries and coastal lagoons typically support large seagrass meadows that provide multiple ecosystem services, including sediment stabilization, nutrient retention, carbon sequestration, essential fisheries habitat and coastal protection and recreation (reviewed in Barbier et al., 2011). Consequently, loss of seagrass due to mass mortality referred to as “die-off events” diminishes ecosystem services that seagrasses provide. Seagrass mortality that releases live leaf bundles also transfers large quantities of nutrients from the benthos to the water column as leaves decay (Rosch and Koch, 2009), thereby compromises overlying water quality. This can stimulate algal blooms that result in secondary die-off, creating a positive feedback loop of released nutrients into the water column. Large-scale seagrass die-off events have been reported in temperate and subtropical seagrass species world-wide, for example along the Mediterranean coast of France, southern coast of Australia, shallow fjord estuaries of Denmark and in Florida Bay, a large sub-tropical estuary at the terminus of the South Florida Peninsula (Robblee et al., 1991; Seddon et al., 2000; Greve et al., 2003; Plus et al., 2003). Recurrent (decadal), large-scale die-off events (> 50 km^2) have been documented in Florida Bay for *T. testudinum*, the dominant habitat-forming tropical seagrass of the Caribbean and tropical Atlantic region (Robblee et al., 1991; Hall et al., 2016). Although globally seagrass die-off events may be driven by a diversity of processes, *T. testudinum* in Florida Bay presents an excellent case study to investigate potential triggers of sudden and rapid mass seagrass

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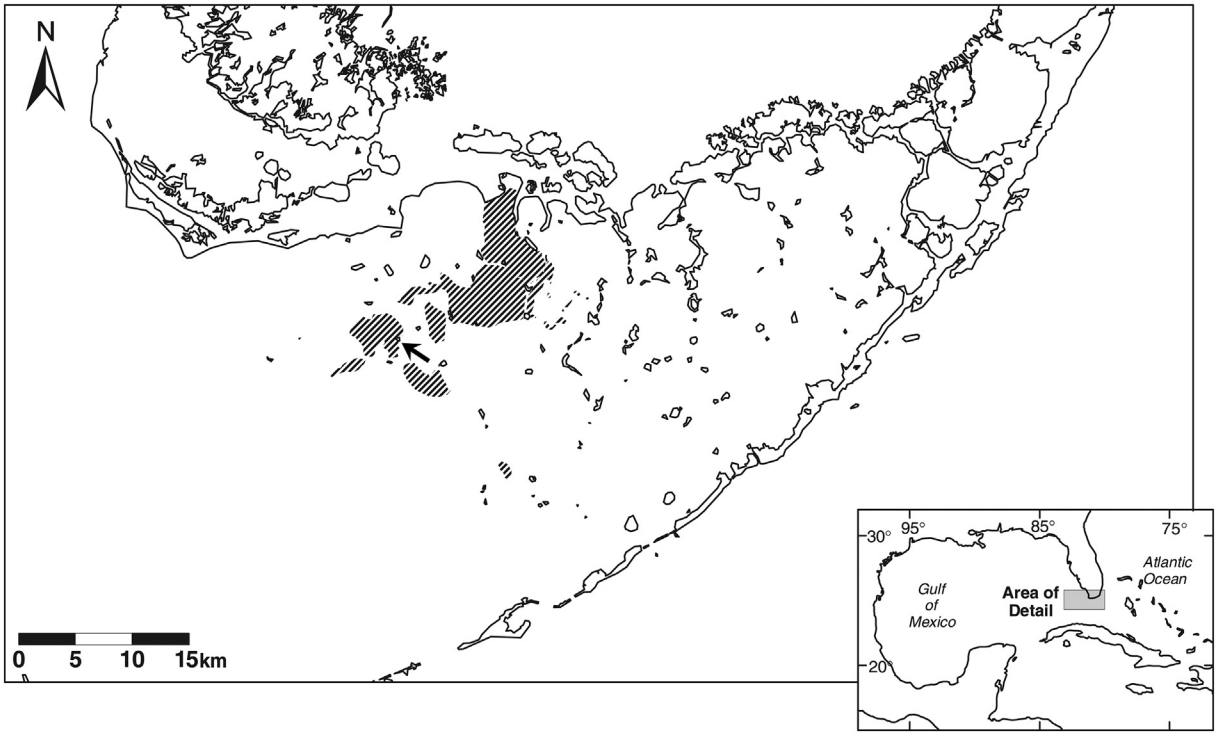

Fig. 1. Location of the *Thalassia testudinum* core collection site adjacent to Johnson Key (indicated by arrow) in the northwest region of Florida Bay at the southern most terminus of the Florida Peninsula (USA). Hatching denotes areas affected by the 2015 seagrass die-off event in Florida Bay (Hall et al., 2016).

mortality.

Seagrass die-off in Florida Bay has been correlated to high total sulfide (H_2S, HS^-, S^{2-}) concentrations in the sediment porewater ($\geq 2000\,\mu M$) and low O_2 and hypersalinity (up to 70) in the overlying water column when water temperatures are maximum (Robblee et al., 1991; Carlson et al., 1994; Zieman et al., 1999; Koch and Erskine, 2001; Borum et al., 2005; Koch et al., 2007c,d). The two major die-off events in Florida Bay in 1987 (95 km^2) and 2015 (88 km^2) occurred during the summer/early fall when salinity levels were > 50 at the surface and > 65 on the benthos (Hall et al., 2016). These mortality events occur in very dense *T. testudinum* beds with high sediment organic matter and an accumulation of H_2S in the porewater reaching total sulfide levels > 2000 μM.

Elevated temperature in combination with low O_2 in estuarine sediments stimulates microbial organic matter decomposition via increased sulfate reduction rates (Canfield et al., 1993; Carlson et al., 1994; Holmer and Kristensen, 1996; Holmer et al., 2003; Koch et al., 2007b,c). Tropical carbonate sediments typically lack the capacity to bind sulfides into sulfur precipitates (e.g., pyrite) due to their low concentration of iron. High sulfate reduction rates without hydrogen sulfide (H_2S) sequestration by iron exposes belowground seagrass tissues to elevated concentrations of H_2S, a potent phytotoxin (Ingold and Havill, 1984; Koch and Mendelssohn, 1989; Koch et al., 1990; Goodman et al., 1995; Holmer and Bandgaard, 2001). Hydrogen sulfide enters seagrass roots and moves by gas phase diffusion to shoot meristems and leaves via gas space tissue or aerenchyma (Pedersen et al., 2004). Although H_2S in porewaters has been implicated in seagrass die-off in many regions, seagrasses have several mechanisms to detoxify sulfides within the plant (Hasler-Sheetal and Holmer, 2015). They have the capacity to detoxify H_2S through either radial O_2 loss from their roots (Sand-Jensen et al., 2005; Frederiksen and Glud, 2006) or internal sequestration of H_2S into oxidized (e.g, ^oS, SO_4^{2-}) or organic sulfur compounds (Hasler-Sheetal and Holmer, 2015). Seagrasses also utilize O_2 from photosynthesis during the day and from the overlying water at night to oxidize sedimentary H_2S. Seagrasses, along with other wetland plants, have the ability to diffuse O_2 from their leaves to roots and form an oxidized boundary layer at the root-sediment interface (rhizosphere) which prevents H_2S intrusion into the plant (Holmer and Bandgaard, 2001).

Although seagrasses have been shown to possess mechanisms to detoxify porewater H_2S via oxidation at the root surface, an oxidized rhizosphere can collapse at night and H_2S intrusion occur (Pedersen et al., 2004). Low night-time O_2 partial pressure has been measured in the meristem of *T. testudinum* in Florida Bay at active die-off sites coincident with sediment H_2S intrusion (Borum et al., 2005) implicating sulfide toxicity. However, Koch et al. (2007a,c) showed a relatively high tolerance of *T. testudinum* belowground tissue exposure to total sulfides > 2000 μM in porewater under ambient salinity. Further, Koch and Erskine (2001) identified the importance of multiple stressors in combination, including hypersalinity, elevated temperature and sulfide accumulation, to simulate die-off conditions observed in the field (reviewed in Koch et al., 2007d). Moreover, *T. testudinum* nighttime O_2 consumption was shown to increase with increasing salinity (35, 45, 55, and 65) across a range of water column O_2 hypoxia levels (Koch et al., 2007a), implying a high respiratory O_2 cost to hypersalinity exposure (e.g., osmoregulation). At salinity of 65, total sulfides increased in the sediment of intact cores of *T. testudinum* in contrast to cores at salinity ≤55, regardless of hypoxia conditions in the overlying water or sulfide injections up to 6000 μM (Koch et al., 2007a). These results lead us to suggest that the capacity for rhizosphere oxidation is compromised at 65 salinity. Thus, while H_2S is a well-established phytotoxin that underlies large-scale seagrass mortality in Florida Bay and globally, and seagrasses have shown a relatively high H_2S tolerance, the mechanism linking hypersalinity and H_2S-induced seagrass die-off events remains elusive.

To establish the potential linkage between hypersalinity, hypoxia in the water column and sulfide toxicity in *T. testudinum*, O_2 and H_2S dynamics were investigated within the shoot meristem, the tissue showing necrosis in the field during active die-off events. Microsensor probes inserted into the shoot meristem were used to measured O_2 and H_2S dynamics in the dark under a series of sequential reductions in water column pO_2. The experiments were run using intact cores with