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Thalassia testudinum response to the interactive stressors hypersalinity, sulfide and hypoxia

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Abstract

A large-scale mesocosm (sixteen 500 L tanks) experiment was conducted to investigate the effects of hypersalinity (45–65 psu), porewater sulfide (2–6 mM) and nighttime water column hypoxia (5–3 mg L^{-1}) on the tropical seagrass *Thalassia testudinum* Banks ex König. We examined stressor effects on growth, shoot survival, tissue sulfur (S^0, TS, $\delta^{34}S$) and leaf quantum efficiencies, as well as, porewater sulfides ($\sum TS_{pw}$) and mesocosm water column O_2 dynamics. Sulfide was injected into intact seagrass cores of *T. testudinum* exposing below-ground tissues to 2, 4, and 6 mM S^{2-}, but rapid oxidation resulted in $\sum TS_{pw} < 1.5$ mM. Hypersalinity at 65 psu lowered sulfide oxidation and significantly affected plant growth rates and quantum efficiencies ($F_v/F_m < 0.70$). The most depleted rhizome $\delta^{34}S$ signatures were also observed at 65 psu, suggesting increased sulfide exposure. Hypoxia did not influence $\sum TS_{pw}$ and plant growth, but strengthened the hypersalinity response and decreased rhizome S^0, indicating less efficient oxidation of $\sum TS_{pw}$. Following nighttime hypoxia treatments, ecosystem level metabolism responded to salinity treatments. When O_2 levels were reduced to 5 and 4 mg L^{-1}, daytime O_2 levels recovered to approximately 6 mg L^{-1}; however, this recovery was more limited when O_2 levels were lowered to 3 mg L^{-1}. Subsequent to O_2 reductions to 3 mg O_2 L^{-1}, nighttime O_2 levels rose in the 35 and 45 psu tanks, stayed the same in the 55 psu tanks, and declined in the 65 psu tanks. Thus, hypersalinity at 65 psu affects *T. testudinum's* oxidizing capacity and places subtle demands on the positive O_2 balance at an ecosystem level. This O_2 demand may influence *T. testudinum* die-off events, particularly after periods of high temperature and salinity. We hypothesize that the interaction between hypersalinity and sulfide toxicity in *T. testudinum* is their synergistic effect on the critical O_2 balance of the plant.

Keywords: Salinity; Hypersalinity; Sulfide; Hypoxia; Anoxia; Seagrass; *Thalassia testudinum*; Florida Bay

1. Introduction

In subtropical/tropical estuaries and coastal lagoons, large contiguous seagrass meadows support a diversity of higher consumers, promote sedimentation, assist in sediment stabilization and enhance nutrient retention. Therefore, it is important to understand large-scale mortality events of meadow forming seagrass (Robblee et al., 1991; Seddon et al., 2000; Plus et al., 2003). In 1987 approximately 40 km^2 of *Thalassia testudinum* Banks ex König meadows experienced a major "die-off" in Florida Bay, a shallow semi-enclosed estuary in South Florida (Robblee et al., 1991) and since this time has been followed by smaller (<1 km^2) patchy episodes of mortality on an annual basis (Zieman et al., 1999). It is hypothesized that exposure to one or a combination of environmental stressors such as high temperature, salinity, porewater sulfide and/or a biological agent (*Labyrinthula* sp.) contribute to sudden mortality events of *T. testudinum* (Robblee et al., 1991; Carlson et al., 1994; Zieman et al., 1999; Koch and Erskine, 2001; Borum et al., 2005; Koch et al., 2007a,b).

In Florida Bay, hypersalinity (>50 psu), driven by high heat loads and evaporation, is frequently found at the end of the dry season, particularly during periods of drought (Boyer et al., 1999). High temperatures that promote hypersaline conditions in the bay also stimulate microbial sulfate reduction rates,

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important for organic matter decomposition in coastal marine sediments (Canfield, 1993; Holmer et al., 2003; Koch et al., 2007b). Accelerated sulfate reduction rates increase exposure of seagrass roots and rhizomes to porewater sulfide, a potent phytotoxin to aquatic macrophytes (Ingold and Havill, 1984; Koch and Mendelssohn, 1989; Koch et al., 1990; Goodman et al., 1995; Holmer and Bondagaard, 2001). Sulfide accumulates in sediments of Florida Bay (Carlson et al., 1994) and other tropical regions because of high temperatures (Holmer and Kristensen, 1996; Koch et al., 2007b) and the low capacity of carbonate sediments to bind sulfide into solid-phase forms, particularly pyrite.

While porewater sulfide can cause stress in seagrass and other emergent marine macrophytes, our mesocosm and field experimental work has shown that *T. testudinum* can grow and maintain high shoot densities in porewater with sulfide concentrations in the millimolar range (2–10 mM; Erskine and Koch, 2000; Koch et al., 2007b). We have also found in a short-term hydroponic experiment that *T. testudinum* can exhibit a ''die-back'' response when exposed to high sulfide (6 mM), high temperature (35 °C) and hypersalinity (55–60 psu) in combination (Koch and Erskine, 2001). The research presented herein is a continuation of this work using large-scale mesocosms (detailed in Koch et al., 2007a) to examine the synergistic effects of various levels of hypersalinity (45–65 psu for 60 days) and porewater sulfide (2–6 mM for 40 days). These longer-term experiments accommodate the use of intact seagrass cores and a slow rate of salinity increase allowing plants to osmotically adjust, simulating field conditions (Koch et al., 2007a). In the present study, we also examined the interaction of sulfide and salinity with nighttime water column hypoxia (5–3 mg L^{-1}) found to influence sulfide intrusion into seagrass below-ground tissues (Pedersen et al., 2004; Borum et al., 2005).

2. Materials and methods

2.1. Plant collection and mesocosm design

Intact *T. testudinum* cores (~15 cm diameter × 20 cm depth, 2840 cm^{-3} sediment, ~2 L porewater) were collected 25 July, 2003 from Florida Bay (25°02′47.0″N, 80°45′11.4″W). Cores were transported to the FAU Marine lab (Boca Raton, FL) and placed into mesocosm tanks (1 m ht × 1 m diameter with 1000 W metal halide lights) with coastal Atlantic seawater for 2 weeks (36 psu, 27 ± 1 °C, 12:12 h light–dark cycle; PAR of 582 ± 56 μmol m^{-2} s^{-1}). Mesocosm tanks were equipped with one powersweep for circulation at canopy height and one for surface to bottom circulation with aeration. The mesocosms were run as a closed system with deionized water amended to compensate for evaporation and coastal seawater added weekly to maintain nutrient levels.

2.2. Experimental design

We determined the response of *T. testudinum* Banks ex König to four salinity (36 [ambient], 45, 55, and 65 psu) and four sulfide treatments (0, 2, 4, and 6 mM) and their interactions. After 60 days of salinity and 40 days of sulfide treatments, a hypoxia treatment was initiated. Hypersalinity, sulfide and hypoxia and their interactions were tested for their effects on plant growth, shoot density, leaf quantum efficiency, tissue sulfur (TS, S^0) and isotopic ratios ($\delta^{34}S$), porewater sulfide levels, and water column O_2 dynamics.

Salinity (Instant Ocean Inc.) was raised 1 psu day^{-1} (11 August 2003) to approximate in situ evaporative rates on shallow carbonate banks in the bay (0.5 psu day^{-1}; Koch et al., 2007a). After 29 days all tanks were at salinity treatment level and sulfide treatments were initiated. Sulfide was injected via syringe through two horizontal sippers (~0.5 cm diameter tubes) with small holes alternately drilled along the tube which was previously inserted across the center of the core in opposite directions to distribute sulfide throughout the core. One end of the tube was closed and the other fitted with a three-way valve extending into the water. Sippers were left in the cores throughout the experiment. Tests of the sulfide injection system were conducted using dye tracers in extra cores to ensure that sulfides were not readily advected to the overlying water. Deoxygenated (N_2) artificial seawater was adjusted to porewater pH (7.0) with NaOH and sulfide added as $NaS{\cdot}7H_2O$. At the initiation of the sulfide injection treatments, 60 mL of 2 mM sulfide was added to each of the sulfide treatment cores and ambient artificial seawater injected into the controls. Sulfide concentrations in the injections were raised 1 mM until injection treatment levels were reached. Due to a lack of sulfide accumulation in the cores after 4 days using 60 mL injections adding 0, 120, 240, and 360 μmol S^{2-} day^{-1}, the injection volume was raised to 120 mL day^{-1} adding 0, 240, 480, and 720 μmol S^{2-} day^{-1}. Based on minimum sulfate reduction estimates from *T. testudinum* cores in our mesocosms (33 μmol L^{-1} day^{-1}, Koch et al., 2007b) and those measured in Florida Bay (154 μmol L^{-1} day^{-1}, Jensen and Koch, unpublished data) and 2 L of porewater, our sulfide amendments would have increased the total sulfide load ~2 to 5 times.

During the last 9 days of the sulfide × salinity experiment, nighttime hypoxia was simulated in 8 of the 16 tanks (two from each salinity treatment) and tank aeration tubes on the upper powersweeps removed. Nitrogen gas was bubbled in the tank water for 15–20 min at the initiation of the 12 h dark cycle (~17:00 h) until O_2 was lowered to 5 mg L^{-1} (YSI 85). On days 2–4, O_2 level was lowered to 4 mg L^{-1} and on days 5–9 O_2 was lowered to 3 mg L^{-1}. The hypoxia treatments were run for 9 days along with sulfide additions. The experiment was terminated after 60 days of exposure to hypersalinity, 40 days of daily sulfide injections and 9 days of hypoxia.

2.3. Plant response measurements

Plant growth as leaf elongation rates (Zieman, 1974) and net shoot numbers as percent survival were determined weekly. Leaf quantum efficiency (F_v/F_m) was also measured weekly on dark adapted (5 min) leaves using a diving PAM (Pulse