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RELATIONSHIP OF SEDIMENT SULFIDE TO MORTALITY OF *THALASSIA TESTUDINUM* IN FLORIDA BAY

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ABSTRACT

Sediment porewater sulfide concentrations in Florida Bay seagrass beds affected by the catastrophic mortality of *Thalassia testudinum* (Turtle-grass) were considerably higher than those of seagrass beds in the Indian River, Charlotte Harbor, or Tampa Bay. Sulfide concentrations in apparently healthy seagrass beds were highest in fall and might have contributed to chronic hypoxic stress of *Thalassia* roots and rhizomes. High porewater sulfide concentrations measured in dying areas of seagrass beds suggest that sulfide produced by microbial degradation of dying *Thalassia* might exacerbate stress on adjacent, surviving seagrass. Sulfide concentrations in recent die-off areas initially were higher than in adjacent, surviving grass beds. By the end of the study, however, the pattern was reversed apparently due to depletion of *Thalassia*-derived organic matter in the sediments of die-off areas. In June 1990, high sulfide concentrations preceded a die-off episode at one site, suggesting (1) elevated sulfide concentrations might be involved in a suite of factors that trigger die-off episodes or (2) elevated porewater sulfide results from death and decomposition of belowground *Thalassia* tissue before necrosis of shoots becomes visible. In either case, elevated porewater sulfide concentrations might be of value in predicting die-off. We conclude that porewater sulfide probably is not the primary cause, but a synergistic stressor, which has acted in concert with factors (such as hyperthermia, hypersalinity, and microbial pathogens) suggested by other researchers, to cause *Thalassia* die-off in Florida Bay.

Catastrophic mortality of the seagrass *Thalassia testudinum* Banks ex König (Turtle-grass) has occurred in Florida Bay since 1987. Robblee et al. (1991) estimated that 4,000 ha of highly productive *Thalassia*-dominated seagrass beds had been almost completely denuded, and an additional 23,000 ha had been affected to a lesser degree; recurring "die-off" episodes since 1991 have further increased the amount of *Thalassia* lost. *Thalassia testudinum* is the dominant macrophyte species of Florida Bay (Zieman et al., 1989), and loss of *Thalassia* could affect the function of Florida Bay in providing juvenile habitat for pink shrimp and other species and winter habitat for wading and diving birds (Schomer and Drew, 1982).

Among the possible causes for *Thalassia* die-off are increases in area, density, and biomass of seagrass communities due to high salinities in Florida Bay resulting from water management activities and a decade-long drought in south Florida (Zieman et al., 1989). The same authors have also suggested that the lack of a major hurricane in the past 27 years has caused high levels of inorganic and organic sedimentation that, in turn, have restricted circulation and increased summertime salinity and temperature stress. A pathogen might also play a role in die-off: Porter and Muehlstein (1990) reported the presence of a potentially pathogenic strain of the slime mold *Labyrinthula* in lesions on *Thalassia* leaves from die-off sites. Because many areas affected by die-off are located far from potential sources of anthropogenic nutrients and toxic compounds, pollution is not considered a contributing factor (Robblee et al., 1991).

As part of a collaborative research group studying *Thalassia* die-off in Florida Bay, we have focused on the role of sediment sulfide in the die-off process. Sulfide is produced in anaerobic marine sediments by bacteria that use sulfate as a ter-

minal electron acceptor in the degradation of organic matter (Goldhaber and Kaplan, 1975; Sorensen et al., 1979). High temperatures, abundant organic matter, and low sulfide-binding capacity can result in extremely high porewater sulfide concentrations in sediments of Florida Bay seagrass beds (Barber and Carlson, 1993). Sulfide is highly toxic to many plants and animals (Joshi et al., 1975; Smith et al., 1976; Bradley and Dunn, 1989; Koch and Mendelssohn, 1989) because of direct poisoning of cellular metabolism and indirect hypoxia due to reaction of sulfide with molecular oxygen.

We hypothesized that sulfide might play two roles in *Thalassia* mortality: (1) a chronic, but widespread, role of direct toxicity effects and indirect effects of hypoxia of *Thalassia* roots and rhizomes throughout Florida Bay; and (2) an acute role, during active die-off episodes, of amplified toxicity and hypoxia affecting surviving *Thalassia* as nearby, dead *Thalassia* roots and rhizomes are degraded by bacteria. Although *Syringodium filiforme* Kutz (Manatee-grass) and *Halodule wrightii* Aschers (Cuban shoal-grass) might also be affected by die-off, Robblee et al. (1991) noted that dense *Thalassia* beds appear to be most vulnerable to die-off.

We anticipated that, if sulfide-induced hypoxia contributed to the die-off phenomenon, *Thalassia* might be more vulnerable than other seagrass species because *Thalassia* has a high root : shoot ratio (Zieman, 1982; Fourqurean and Zieman, 1991). To determine spatial and temporal variations in porewater sulfide concentrations and the relationship of porewater sulfide to *Thalassia* die-off, we measured porewater sulfide concentrations in visibly healthy and in die-off-affected *Thalassia* beds at four sites in Florida Bay over an 18-month period from April 1989 to October 1990. We also resampled some sites in October 1992 to determine the effects of lower surface water salinity in Florida Bay on porewater sulfide concentrations.

Study Area

Florida Bay is roughly triangular in shape; it is bounded by the Florida peninsula on its north side, the Florida Keys on the southeast side, and is open to the Gulf of Mexico on the west side (Fig. 1). Intertidal or shallow subtidal carbonate mud banks divide Florida Bay into several basins (Schomer and Drew, 1982). Stunted, sparse stands of *Thalassia* grow on banks, but *Thalassia* standing crop within basins ranges from 30 g dry weight·m^{-2} in the northeast to 130 g dry weight·m^{-2} at the western edge of the bay. *Halodule* is also common but has considerably lower standing crop than does *Thalassia*. *Syringodium* grows mainly in areas with strong oceanic influence, especially along the south and west margins of the bay (Zieman et al., 1989).

Our sampling was concentrated in four areas (Johnson Key Basin, Rabbit Key Basin, Rankin Lake, and Sunset Cove) that differed in morphology, hydrography, and the severity and pattern of *Thalassia* die-off (Fig. 1). In all study areas, however, dense *Thalassia* beds appear to have been more vulnerable than sparse beds (Robblee et al., 1991). The three basins in the western bay (Johnson Key Basin, Rabbit Key Basin, and Rankin Lake) experienced major die-off episodes between September 1987 and June 1988. The initial die-off episode killed over 60% of the seagrass in Rankin Lake, leaving widely scattered patches of surviving *Thalassia*. Rabbit Key Basin had one large (63 ha) die-off patch in its southeast corner, and smaller patches were scattered throughout the rest of the basin. Numerous small die-off patches occurred in Johnson Key Basin, primarily along the margins of banks on the east and west sides of the basin.

Freshly-killed die-off patches were present in Johnson Key Basin and Rabbit Key Basin in spring 1989, indicating that some die-off had occurred the previous fall and winter. Die-off also occurred in winter and early spring 1989 near the National Park service dock in Sunset Cove, located along the west side of Key Largo, far removed from sites in the western bay.

A small die-off episode occurred in the northeast corner of Johnson Key Basin in late summer 1990, and a major episode began in Johnson Key Basin in late summer 1991. By spring 1992, the latter die-off killed large areas of previously undamaged seagrass in Johnson Key Basin and banks along the west and south sides of the basin.

Surface water salinity ranges during the study period in 1989 and 1990 were 37‰ to 48‰ in

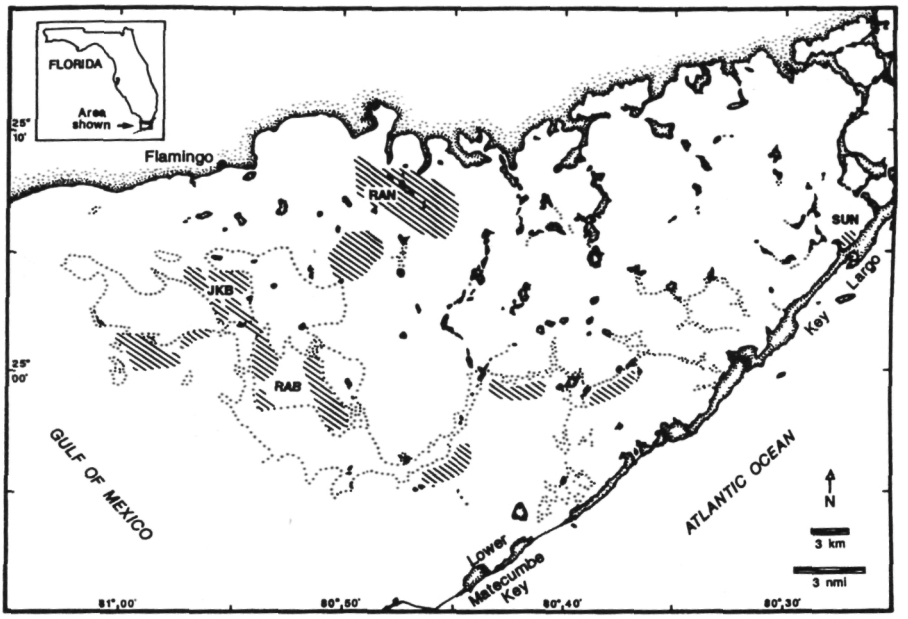

Figure 1. Basins of Florida Bay affected by *Thalassia* die-off (after Robblee et al., 1991). JKB is Johnson Key Basin, RAN is Rankin Lake, RAB is Rabbit Key Basin, and SUN is Sunset Cove. Hatched marks indicate areas where die-off has occurred; stippled areas are shallow carbonate mud banks.

Johnson Key Basin and Rabbit Key Basin; 42‰ to 65‰ in Rankin Lake; and 25‰ to 42‰ in Sunset Cove. Highest salinities at all sites occurred in late spring, and lowest values occurred in fall. Sunset Cove was exposed to low (<25‰) salinities for a long period in fall 1988 when the South Florida Water Management District opened the floodgates of the C-111 canal. Water temperatures for all basins ranged from 19°C to 32°C.

Surface water salinity throughout Florida Bay was lower in fall 1992 than in fall 1989 and 1990 (Everglades National Park–South Florida Research Center, unpubl.). When sites in the western bay were resampled in October 1992, salinity was 36‰ in Johnson Key Basin, 35‰ in Rabbit Key Basin, and 27‰ in Rankin Lake.

Methods

Sediments along the margins of an active die-off area (or "patch") were sampled during and after a die-off episode in Sunset Cove (Fig. 1). Die-off at this site ended as we began sampling in April 1989. From April to December 1989, we sampled a fixed transect from a healthy *Thalassia* bed into a die-off patch every 2 months. Triplicate sediment cores were collected at 1-m intervals beginning at a point 3 m inside the healthy bed and extending 3 m into the die-off patch.

Seasonal and spatial variations in porewater sulfide concentrations were sampled in three other basins, Johnson Key Basin, Rabbit Key Basin, and Rankin Lake (Fig. 1). In April 1989, recent die-off patches in each basin were identified by the presence of *Thalassia* shoot "stubble." Three patches in each basin were selected randomly. In the vicinity of each patch, we sampled three vegetation zones: the dead zone within the die-off patch which was denuded of *Thalassia,* a fringe zone of surviving *Thalassia* around the edge of the die-off patch, and a healthy zone located at least 1 m inside a visibly healthy *Thalassia* bed. Triplicate cores were collected within each zone of each patch for a total of 27 cores per basin.

Between April 1989 and October 1990, we sampled the same patches repeatedly: Johnson Key Basin patches were sampled every two months, and other basins were sampled every 4 months. Sites were sampled again in October 1992 to determine the effects of declining surface water salinity in Florida Bay.

Porewater sulfide concentrations were determined on surficial (0–2 cm) and rhizome-depth (8–10

Table 1. Porewater sulfide concentrations in seagrass beds of four Florida estuaries, summer 1988 (data are means and standard deviation of four replicate samples)

Estuary/Site	Porewater sulfide (μM)
Tampa Bay	
Bunces Pass	122 (23)
Bonne Fortune Key	333 (70)
Charlotte Harbor	
Catfish Creek	228 (139)
Sandfly Key	279 (139)
Cape Haze	32 (14)
Indian River	
Jim Island	64 (60)
Little Jim Bridge	123 (68)
Round Island	158 (89)
Florida Bay	
Die-Off Site	2,143 (664)

cm) sediments of triplicate cores collected from each zone of each die-off patch. Cores were collected in 60-ml plastic syringe barrels, stoppered, and transported to the laboratory while submersed in cold (4–10°C) seawater. Potentiometric determinations of porewater sulfide concentrations were made with an ion-specific electrode by the procedure of Carlson et al. (1983). Sediment water content (a measure of sediment porosity) was determined by measuring the volume of the central segment of each core and drying it to constant weight at 60°C.

Statistical analyses were performed using SAS release 5.18 (SAS Institute, 1990). Kolmogorov-Smirnov tests of normality indicated that porewater sulfide concentrations were normally distributed. Analysis of Variance and Duncan's Multiple Range tests were used to determine the effects of sampling date, basin, patch, zone, and sediment depth on porewater sulfide concentrations. Preliminary statistical analyses indicated that rhizome-depth (8–10 cm) porewater sulfide concentrations were significantly higher than those of surface (0–2 cm) samples, so results reported below are limited to rhizome-depth samples.

Results

Preliminary sampling in summer 1988 indicated that porewater sulfide concentrations were considerably higher in Florida Bay die-off areas than in healthy *Thalassia* beds in other estuaries (Table 1). Mean porewater sulfide concentrations in Tampa Bay, Charlotte Harbor, and Indian River were generally lower than 350 μM, while porewater sulfide concentrations in Florida Bay die-off areas were over 2,000 μM.

Porewater sulfide concentrations along the Sunset Cove transect increased abruptly at the edge of the die-off patch (Fig. 2). Concentrations inside the healthy grass bed were generally less than 1,000 μM, but concentrations in the die-off patch ranged from 1,000 μM to nearly 3,000 μM. Although die-off ended and the patch margin at this site stabilized in April 1989, high sulfide concentrations persisted in the denuded sediments of the die-off patch throughout the remainder of the year.

Sediment water content for western bay sites varied between 0.72 and 0.81; values were significantly higher in Rankin Lake than in Rabbit Key Basin and Johnson Key Basin (Table 2). Dead-zone water content was significantly lower than live-zone values; fringe zone values were intermediate.

Porewater sulfide concentrations exhibited significant seasonal variations in all three basins (Table 2). Highest porewater sulfide concentrations for all three basins occurred in October 1990; slightly lower values occurred in October 1989. Lowest

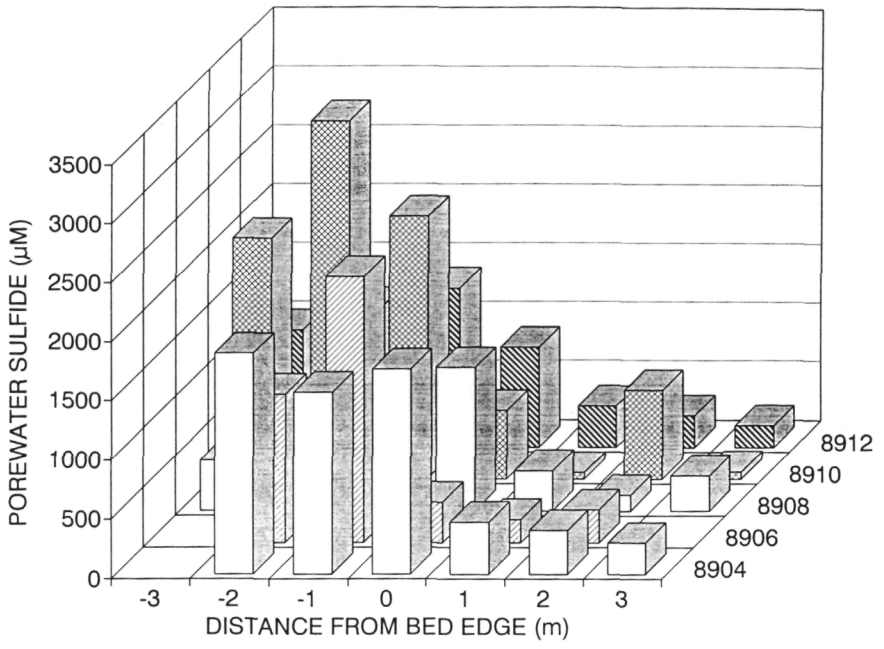

Figure 2. Porewater sulfide concentrations along a transect from a healthy *Thalassia* bed into a die-off patch. Positive distances indicate the end of a transect within healthy *Thalassia*. Sampling date in yymm format is shown along right side of figure. Empty cells indicate missing data.

values for 1989 and 1990 occurred in June and February, respectively. Porewater sulfide concentrations ranged from 1,450 μM in Rankin Lake, to 1,250 μM in Johnson Key Basin, and 1130 μM in Rabbit Key Basin, but differences among basins were not statistically significant. A significant difference among patches was determined to be an artifact of high porewater sulfide concentrations in Rankin Lake patch 3 and Rabbit Key Basin patch 3. Likewise, the significance of the combined effects of sample date and basin on porewater sulfide concentrations depends primarily on one extremely high value (3,020 μM) for Rankin Lake in October 1990.

Within Johnson Key Basin, porewater sulfide concentrations varied significantly among sampling dates, among die-off patches within the basin, and among zones within die-off patches (Table 3). In a pattern similar to those of other basins, highest sulfide concentrations in Johnson Key Basin occurred in October of both sampling years. Lowest concentrations occurred in August 1989 and February 1990. Patches 1 and 2, both located near margins of shallow banks, had higher sulfide concentrations than did patch 3, which was located in slightly deeper water.

Porewater sulfide concentrations at Johnson Key Basin patch 1, located on the shallow bank at the northeast corner of Johnson Key Basin, reflected the influence of both recent die-off and seasonal changes in water temperature (Fig. 3). At the beginning of the study, when die-off had just ended at this site, dead-zone sulfide concentrations were considerably higher than those of the healthy zone. During the course of the study, however, the pattern reversed: healthy-zone sulfide concentrations were higher than those of the dead zone.

In June 1990, significantly elevated porewater sulfide concentrations of the healthy and fringe zones of Johnson Key Basin patch 1 (Fig. 3) preceded, by 4–

Table 2. Comparisons of rhizome-depth sediment water content and porewater sulfide concentrations among basins in Florida Bay. ANOVA values are *F*-ratios, and results of multiple range test are mean water content and porewater sulfide concentrations, expressed in units of ml water·cm^{-3} sediment and μM, respectively. Values with the same letter are not significantly different. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

Independent variable	Water content	Porewater sulfide
Analysis of Variance Results		
Sample date	6.65*	26.23***
Basin	87.40***	1.58
Patch	4.01*	4.86**
Zone	20.03***	0.90
Sample date × Basin		2.81**
Sample date × Zone		1.55
Multiple Range Test Results		
Sample date		
June 1989		575 b
October 1989		1,930 a
February 1990		715 b
June 1990	0.76 a	625 b
October 1990	0.74 b	2,280 a
Basin		
Rankin Lake	0.81 a	1,450 a
Johnson Key Basin	0.73 b	1,250 a
Rabbit Key Basin	0.72 b	1,130 a
Zone		
Healthy	0.77 a	1,290 a
Fringe	0.75 ab	1,160 a
Dead	0.72 b	1,370 a

6 weeks, the recurrence of die-off at this site. Necrosis of *Thalassia* shoots began in late July 1990, and die-off persisted through December 1990. During this die-off episode, porewater sulfide concentrations of the previously healthy zone reached 5,700 μM (Fig. 3), and values as high as 13,000 μM were measured nearby at other freshly killed patches. Significantly elevated porewater sulfide concentrations also occurred during June 1990 in fringe and healthy zones of Johnson Key Basin patch 3, but die-off did not occur at this patch.

In fall 1992, porewater sulfide concentrations of visibly healthy *Thalassia* beds in Johnson Key Basin, Rabbit Key Basin, and Rankin Lake were lower than in fall 1990 (Fig. 4), coinciding with lower surface water salinity throughout Florida Bay (Everglades National Park–South Florida Research Center, unpubl.). However, the decline of porewater sulfide concentrations in Rabbit Key Basin and Rankin Lake sediments was not statistically significant.

Discussion

The high porewater sulfide concentrations measured in Florida Bay seagrass sediments and the demonstrated toxicity of sulfide to other plant species (Joshi et al., 1975; Bradley and Dunn, 1989; Koch and Mendelssohn, 1989) suggest that sulfide may play a role in *Thalassia* die-off. Porewater sulfide concentrations in apparently healthy seagrass beds of Florida Bay were considerably higher than values we have measured in other Florida estuaries, and concentrations in active die-off patches were extremely high. Mean porewater sulfide concentrations for

Table 3. Factors affecting porewater sediment sulfide concentrations in Johnson Key Basin. Samples were collected at depths of 8–10 cm in sediments. ANOVA values are *F*-ratios, and results of multiple range tests are mean porewater sulfide concentrations expressed as μM. Values with the same letter are not significantly different. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

Independent variable	Porewater sulfide
Analysis of Variance	
Sample date	40.96***
Patch	11.83***
Zone	6.40***
Multiple Range Tests	
Sample date	
April 1989	728 cd
June 1989	427 d
August 1989	388 d
October 1989	2,690 b
December 1989	582 cd
February 1990	469 d
April 1990	538 cd
June 1990	1,033 c
October 1990	3,235 a
Patch	
1	1,415 a
2	1,440 a
3	747 b
Zone	
Dead	899 b
Fringe	1,176 a
Healthy	1,411 a

Johnson Key Basin, Rabbit Key Basin, and Rankin Lake during this study ranged from 1.0 mM to 1.4 mM, whereas values measured in Charlotte Harbor, Tampa Bay, and Indian River *Thalassia* beds during summer 1988 were generally less than 0.5 mM (Table 1). During active die-off periods at Johnson Key Basin patch 1, porewater sulfide concentrations rose to 5.7 mM, and values over 13 mM were measured in nearby die-off patches. The high sulfide concentrations that we measured in Florida Bay sediments probably resulted from the combined effects of (1) rapid microbial sulfate reduction rates in subtropical seagrass sediments (Oremland and Taylor, 1976; Hines and Lyons, 1982) and (2) the low iron content and limited capacity of carbonate sediments to precipitate sulfide (Berner, 1984).

Sulfide inhibits the growth of several wetland plant species: rice, a crop grown in flooded soils (Joshi et al., 1975); *Spartina alterniflora,* a salt marsh grass species (Bradley and Dunn, 1989; Koch and Mendelssohn, 1989); and the red mangrove, *Rhizophora mangle* (Lin and Sternberg, 1992). Less is known of the toxicity of sulfide to seagrass species. Pulich (1989) reported that growth of *Ruppia maritima* L. (Widgeon-grass) was inhibited but growth of *Halodule wrightii* was stimulated in sediments with higher nitrogen and sulfide concentrations. Stable sulfur-isotope ratios of plant tissue confirmed that *Ruppia* growth was faster when root δS^{34} values indicated low amounts of sulfide-S incorporation, whereas faster *Halodule* growth rates were correlated with more negative δS^{34} values. Stable sulfur-isotope analyses of *Spartina alterniflora* (Carlson and Forrest, 1982) and other seagrass and mangrove species (Fry et al., 1982) indicate that porewater sulfide may be incorporated by plants growing in anaerobic sediments, presum-

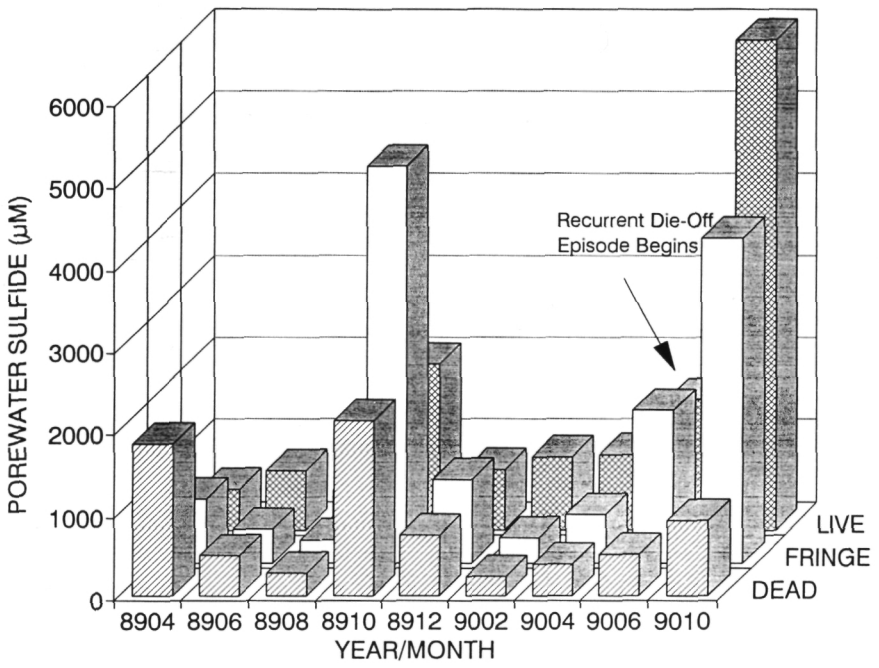

Figure 3. Variation of porewater sulfide among vegetative zones of northeast Johnson Key Basin die-off patch 1. DED denotes dead-zone, FRI is fringe zone, and HLT is visibly healthy *Thalassia* zone.

ably when the capacity of the plant to maintain an aerobic environment in its belowground tissue is exceeded.

The prevalence of gas-conducting tissue (aerenchyma) in roots and rhizomes of flood-tolerant and submerged aquatic plant species suggests this tissue is a primary defense against sulfide toxicity. Aerenchyma provides a path for photosynthetically produced oxygen to move from leaves to roots and rhizomes (Armstrong, 1979), and *Thalassia* shoots, roots, and rhizomes have large lacunar spaces (Tomlinson, 1969). Thursby (1984) has shown that photosynthetically produced oxygen diffuses from the roots of *Ruppia maritima* and oxidizes the surrounding sediments, and Penhale and Wetzel (1983) found that aerenchyma volume was greater and root respiration rates were lower for *Zostera marina* L. (Eel-grass) in intensely reducing sediments than in more oxidized sediments. Smith et al. (1988) found that photosynthetic oxygen production and shoot-to-root transport was generally sufficient to maintain aerobic conditions in *Zostera* roots and rhizomes during the day, but anaerobic conditions occurred in roots and rhizomes at night.

Because the capacity of *Thalassia* to maintain aerobic conditions in its roots and rhizomes depends on the oxygen productivity and oxygen-conducting ability of the plant and on the sulfide-driven chemical oxygen demand of the sediments, the high ratio of belowground biomass to aboveground biomass and the deep-rooted growth habit of *Thalassia testudinum* may make it more vulnerable to hypoxia than other seagrass species. For three sites in the lower Florida Keys, Zieman (1982) reported that roots and rhizomes composed 85–90% of total *Thalassia* biomass, whereas *Halodule* and *Syringodium* roots and rhizomes composed 67–90% and 53–84% of their total biomass, respectively. For stunted bank-top

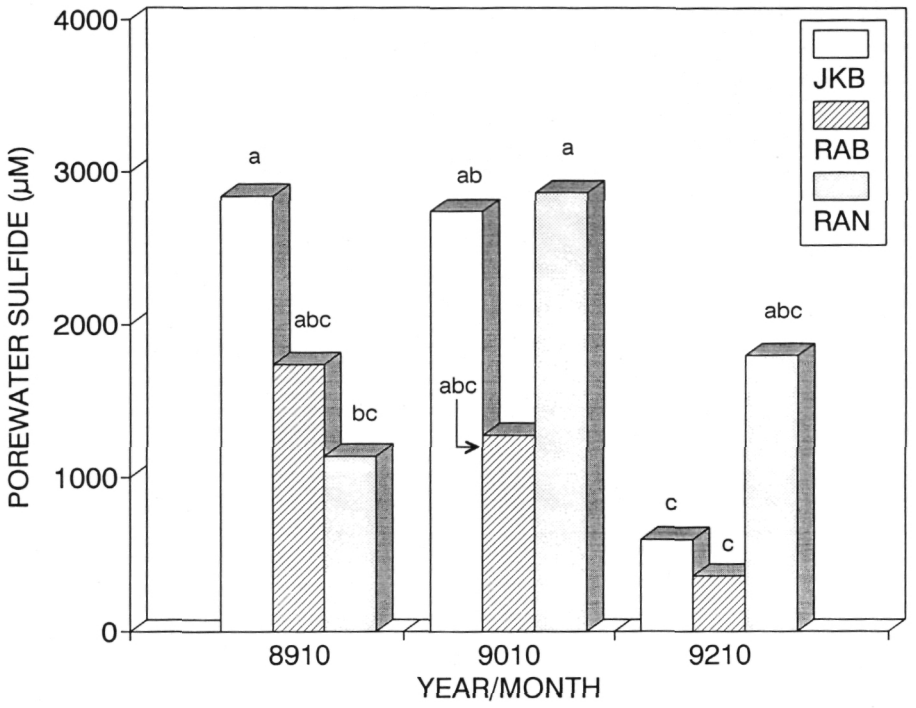

Figure 4. Fall porewater sulfide concentrations in healthy-zone sediments of Florida Bay *Thalassia* beds. Bars with the same letter superscript are not significantly different.

communities in Florida Bay, Powell et al. (1989) found *Thalassia* roots and rhizomes composed 79–87% of total *Thalassia* biomass, whereas *Halodule* roots and rhizomes accounted for only 59% of total *Halodule* biomass.

High porewater sulfide concentrations measured along the margin of the active die-off patch in Sunset Cove (Fig. 2) indicate that dying *Thalassia* roots and rhizomes stimulate the microbial production of sulfide in sediments and suggest that elevated sulfide concentrations exacerbate hypoxia on the belowground tissue of surviving *Thalassia* along the fringe of active die-off patches. Because belowground tissue represents a large fraction of total biomass in *Thalassia,* high sulfide concentrations in die-off-patch sediments are not surprising. Assuming (1) belowground biomass of *Thalassia* in dense beds is 500 $g \cdot m^{-2}$, (2) carbon content of roots and rhizomes is 0.4 g $C \cdot g^{-1}$ dry weight, and (3) the mean stoichiometric ratio of porewater ΣCO_2 : porewater sulfide is 4:1 (Barber and Carlson, 1993), we estimate that approximately four moles of sulfide are produced in each square meter of sediment of die-off patches as roots and rhizomes decompose. Depending on actual biomass, rate of decomposition, depth distribution of root and rhizomes, ΣCO_2:$S^=$ stoichiometry, and sediment porosity, porewater sulfide concentrations greater than 10 mM may be expected in active die-off patches. While this estimate agrees with concentrations measured in northeast Johnson Key Basin sediments during the fall 1990 die-off episode, accumulation of sulfide in porewater probably depends on site-specific rates of bioturbation, sulfide diffusion and oxidation, and potential for sulfate depletion.

Although Kenworthy and Thayer (1984) found that *Thalassia* rhizomes decayed slowly in Florida Bay bank sediments, they found that roots decayed more rapidly,

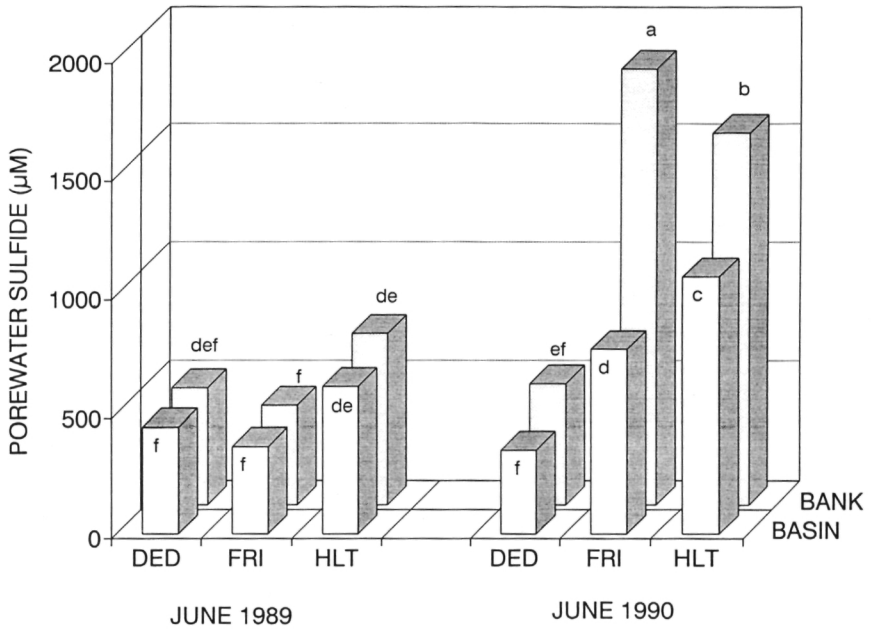

Figure 5. Comparison of June 1989 and June 1990 porewater sulfide concentrations in two Johnson Key Basin die-off patches (1 and 3). Bars with the same letter superscript are not significantly different. DED denotes dead-zone, FRI is fringe zone, and HLT is visibly healthy *Thalassia* zone.

losing 55% of their weight in the first 14 days of incubation. Therefore, much of the sulfide produced early in active die-off episodes may derive from the microbial decomposition of *Thalassia* roots and labile components of the rhizomes, whereas the persistence of high porewater sulfide concentrations for up to a year after die-off is probably supported by slower degradation of rhizomes. The persistence of high sulfide concentrations in denuded sediments for months after die-off ceases may also predispose surviving *Thalassia* to recurrent episodes of die-off.

Variation of porewater sulfide concentrations over time in die-off patches (Fig. 3) indicates that sulfide concentrations decline as supplies of dead organic matter are exhausted. Sediment water content showed a similar pattern as die-off patches aged; values were significantly lower in October 1990 than in June 1990 and lower in dead-zone sediments than in healthy-zone sediments (Table 2). The drop in dead-zone porewater sulfide concentrations over time (Fig. 3) also explains the lack of a statistically significant zone effect in basin comparisons of porewater sulfide concentrations (Table 2). As a result, the seasonal pattern of sulfide concentrations in 1990 (for all patches except Johnson Key Basin patch 1) is probably more representative of natural conditions than is the pattern of concentrations in 1989.

The occurrence of elevated porewater sulfide concentrations before the 1990 die-off episode at Johnson Key patch 1 is significant because it may provide a technique for identifying incipient die-off areas before symptoms are visible. It is not clear, however, whether the elevated porewater sulfide concentrations that preceded visible symptoms of die-off at patch 1 were associated with factors

which subsequently caused *Thalassia* to die or resulted from death of *Thalassia* belowground tissue which preceded visible symptoms on shoots.

The lack of die-off at Johnson Key Basin patch 3, which also had elevated porewater sulfide concentrations in June 1990 (Fig. 5), requires explanation. If porewater sulfide is involved in the suite of factors which cause die-off, a threshold sulfide concentration higher than the values circa 1 mM measured at patch 3 might be required to initiate die-off. Alternatively, elevated sulfide concentrations alone might not be sufficient to cause die-off. If porewater sulfide concentrations reflect death of belowground tissue, moderately elevated porewater sulfide concentrations might reflect the death of some but not all belowground tissue.

Although our evidence supports the role of sulfide as a synergistic, secondary stressor in *Thalassia* die-off, the primary causes or "triggers" for die-off episodes are still unknown. Zieman et al. (1988) suggested that diversion of water away from historical drainage patterns, a decade-long drought, warmer than normal summer and fall temperatures (Zieman, this conference), and an unusually long interval between major hurricanes in Florida Bay have created a high background level of salinity and temperature stress and allowed *Thalassia testudinum* to become very dense and accumulate large amounts of leaf litter. Carlson et al. (1989) and Yarbro and Carlson (1989) suggested that sulfide-induced hypoxia of *Thalassia* belowground tissue might be involved in die-off, particularly during fall when (1) high tissue respiration persists because of warm water temperatures and (2) shortening days causes the photosynthetic supply of oxygen to decline. Our data show that sediment sulfide concentrations peak in fall, so it is reasonable to expect that *Thalassia,* weakened by cumulative hypersalinity, hyperthermia, and hypoxia, might succumb to opportunistic pathogens in fall.

This scenario is similar to Rasmussen's (1977) explanation of climatic effects on eelgrass wasting disease, but the timing of die-off episodes is not completely consistent with this explanation. Even though some die-off episodes have occurred in fall, the 1990 episode in Johnson Key Basin began in late July when sulfide concentrations elsewhere were quite low. The 1988–1989 episode in Sunset Cove occurred between November 1988 and February 1989, a period when water temperatures and salinities, sediment sulfide concentrations, and respiratory oxygen demands of *Thalassia* were presumably low. Although salinities and temperatures in Johnson Key Basin were lower in 1991 than in 1987 or 1988 (Everglades National Park, unpubl.), the most damaging die-off episode to date in Johnson Key Basin occurred in 1991.

The results of fall 1992 sampling (Fig. 4) are also ambiguous regarding the role of environmental stressors such as hypersalinity in *Thalassia* die-off. While declines in porewater sulfide concentrations coincided with declines in surface water salinity between 1990 and 1992 in Johnson Key Basin, Rabbit Key Basin, and Rankin Lake, only the decline in Johnson Key Basin was statistically significant.

Like eelgrass wasting disease, several characteristics of *Thalassia* die-off suggest that a pathogenic microorganism is involved. Outbreaks are rapid, affect dense *Thalassia* beds more than sparse beds, and have occurred in a previously healthy area (Sunset Cove) that might have been "inoculated" by boats traveling from die-off areas in western Florida Bay to the National Park Service dock in Sunset Cove. Although the pathogenic slime mold *Labyrinthula* sp. has now been identified as the primary causative agent of eelgrass wasting disease (Short et al., 1987; Muehlstein et al., 1988) and *Labyrinthula* has been suggested as a possible pathogen in *Thalassia* die-off (Porter and Muehlstein, 1990), the sequence of

symptoms and events associated with the 1990 die-off event in northeast Johnson Key Basin suggests that the role of *Labyrinthula* in *Thalassia* die-off is secondary.

Even though a plausible pathogen has not yet been identified for *Thalassia* die-off, a pathogen might act synergistically with the high sediment sulfide concentrations of Florida Bay to kill *Thalassia.* As noted above, the capacity of *Thalassia* to maintain aerobic conditions in its roots and rhizomes depends on the oxygen productivity and oxygen-conducting ability of the plant and on the sulfide-driven chemical oxygen demand of the sediments. A pathogen that reduces the production or transport of oxygen within *Thalassia* might make it vulnerable to sulfide toxicity.

As time passes since the initial die-off episodes in 1987 and 1988, the primary causes and processes of *Thalassia* die-off become more difficult to determine. Hurricane Andrew, which devastated portions of Everglades National Park (Davis, in prep.[1]) had minimal impacts on Florida Bay. However, secondary effects of die-off, such as the increased epiphytism, sediment resuspension, and decreased water clarity predicted by Zieman et al. (1988), are now occurring. Increased rainfall in 1991 and 1992 has also lowered salinities throughout Florida Bay, but most dramatically in Rankin Lake and other interior basins of the bay (Everglades National Park, unpubl.). Widespread and persistent algal blooms have occurred in 1991, 1992, and 1993, perhaps as the result of increased rainfall, secondary effects of die-off, or both. The hot, hypersaline, and transparent water of 1987 and 1988 has now been replaced by cooler, turbid, and less saline water. The future course of die-off in Florida Bay is, therefore, uncertain.

Conclusions

Porewater sulfide concentrations of Florida Bay seagrass beds were higher than those measured in other Florida estuaries. Sulfide concentrations in apparently healthy seagrass beds were highest in fall and might have caused chronic hypoxic stress of *Thalassia* roots and rhizomes, contributing to seagrass die-off. High porewater sulfide concentrations measured in dying areas of seagrass beds suggest that sulfide produced by microbial degradation of dying *Thalassia* might exacerbate stress on adjacent, surviving seagrass. The occurrence of elevated porewater sulfide concentrations before necrosis of *Thalassia* leaves and shoots became visible during one die-off episode suggests that porewater sulfide concentrations might be used in surveys of Florida Bay to locate areas of incipient die-off. The weak relationship of lower porewater sulfide concentrations and lower surface water salinity in October 1992 could reflect a link between environmental conditions and levels of porewater sulfide which are potentially stressful to *Thalassia.* Additional work is needed to determine the causes of die-off and the specific role of porewater sulfide in the process, but changes in the Florida Bay ecosystem since 1991 might obscure the etiology of the original die-off episodes.

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[1] Davis, G. E. An assessment of Hurricane Andrew impacts on natural and archaeological resources of Big Cypress National Preserve, Biscayne National Park, and Everglades National Park.

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