

# The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*

J. BORUM, O. PEDERSEN\*, T. M. GREVE†, T. A. FRANKOVICH‡, J. C. ZIEMAN‡, J. W. FOURQUREAN§ and C. J. MADDEN¶

Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, DK-3400 Hillerød, Denmark,

†Department of Marine Ecology, National Environmental Research Institute, Vejlsovej 25, DK-8600 Silkeborg,

Denmark, ‡Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903, USA,

§Department of Biological Sciences and South-east Environmental Research Program, Florida International

University, Miami, Florida 33199, USA, and ¶Coastal Ecosystems Division, South Florida Water Management

District, West Palm Beach, Florida 33406, USA

## Summary

**1** Oxygen and sulphide dynamics were examined, using microelectrode techniques, in meristems and rhizomes of the seagrass *Thalassia testudinum* at three different sites in Florida Bay, and in the laboratory, to evaluate the potential role of internal oxygen variability and sulphide invasion in episodes of sudden die-off. The sites differed with respect to shoot density and sediment composition, with an active die-off occurring at only one of the sites.

**2** Meristematic oxygen content followed similar diel patterns at all sites with high oxygen content during the day and hyposaturation relative to the water column during the night. Minimum meristematic oxygen content was recorded around sunrise and varied among sites, with values close to zero at the die-off site.

**3** Gaseous sulphide was detected within the sediment at all sites but at different concentrations among sites and within the die-off site. Spontaneous invasion of sulphide into *Thalassia* rhizomes was recorded at low internal oxygen partial pressure during darkness at the die-off site.

**4** A laboratory experiment showed that the internal oxygen dynamics depended on light availability, and hence plant photosynthesis, and on the oxygen content of the water column controlling passive oxygen diffusion from water column to leaves and below-ground tissues in the dark.

**5** Sulphide invasion only occurred at low internal oxygen content, and the rate of invasion was highly dependent on the oxygen supply to roots and rhizomes. Sulphide was slowly depleted from the tissues when high oxygen partial pressures were re-established through leaf photosynthesis. Coexistence of sulphide and oxygen in the tissues and the slow rate of sulphide depletion suggest that sulphide reoxidation is not biologically mediated within the tissues of *Thalassia*.

**6** Our results support the hypothesis that internal oxygen stress, caused by low water column oxygen content or poor plant performance governed by other environmental factors, allows invasion of sulphide and that the internal plant oxygen and sulphide dynamics potentially are key factors in the episodes of sudden die-off in beds of *Thalassia testudinum*. Root anoxia followed by sulphide invasion may be a more general mechanism determining the growth and survival of other rooted plants in sulphate-rich aquatic environments.

*Key-words:* die-back of wetland plants, microelectrodes, oxygen, seagrass die-off, sediment, sulphide, *Thalassia*

*Journal of Ecology* (2005) **93**, 148–158  
doi: 10.1111/j.1365-2745.2004.00943.x

## Introduction

Sudden seagrass die-off has been reported in several parts of the world (Rask *et al.* 2000; Seddon *et al.* 2000; Plus *et al.* 2003). In late summer 1987, a major episode of mass mortality affecting the tropical seagrass *Thalassia testudinum* Banks ex König (turtle grass) occurred in the western and central part of Florida Bay (Robblee *et al.* 1991), and since then episodes of die-off have been regularly reported, although at more limited scales (Carlson *et al.* 1994; Hall *et al.* 1999; Zieman *et al.* 1999). The first noticeable symptom of this die-off was the detaching of leaf bundles around the apical meristem of the short shoots, which left defoliated spots of bare sediment with characteristic stubbles of leaf sheaths as the only remains of the short shoots. In small-scale events, seagrass mortality gradually spreads radially outwards from scattered centres of initial die-off, creating progressively expanding patches of bare sediment bounded by dense, apparently healthy stands of *Thalassia*. The causes of the *Thalassia* die-offs are still under debate, but combinations of stress factors such as high salinity, high temperature, infection by the slime mold *Labyrinthula* sp. and self-poisoning due to overcrowding have been suggested as some of the more likely causes (Porter & Muehlstein 1989; Robblee *et al.* 1991; Carlson *et al.* 1994; Zieman *et al.* 1999). The physiological mechanisms responsible for mortality are not well understood, but it has been hypothesized that poor oxygen supply to below-ground tissues and subsequent uptake of phytotoxic sulphide from the sediment into the plants may be the direct cause of short shoot mortality (Carlson *et al.* 1994; Durako & Kuss 1994).

Angiosperms rooted in flooded soils, or growing submerged in aquatic environments, constantly face problems in supplying oxygen to below-ground tissues for the support of aerobic respiration and for protection against invasion and accumulation of reduced toxic metabolites and ions from the sediment (Armstrong 1979; Crawford & Braendle 1996; Vartapetian & Jackson 1997). Seagrasses have well-developed aerenchyma to enable efficient oxygen supply to roots, rhizomes and to the highly metabolically active meristematic tissue situated at the interface of sediment and water column (Tomlinson 1969; Penhale & Wetzel 1983; Greve *et al.* 2003). Oxygen is supplied to below-ground tissues both from photosynthetic oxygen release in leaves during daylight and from passive diffusion of oxygen from the water column through leaves during darkness (Pregnall *et al.* 1984; Pedersen *et al.* 1998; Greve *et al.* 2003). However, poor oxygen supply may result if photosynthesis is persistently low, and/or plant respiration is high, as a result of environmental stress factors (Carlson *et al.* 1989; Durako & Kuss 1994). Under these conditions, metabolism of below-ground tissues must rely on anaerobic respiration, with the potential risks of poor energy utilization and of accumulation of internally produced anaerobic metabolites and/or toxic metabolites present in the sediment (e.g. Pregnall *et al.* 1984;

Smith *et al.* 1988; Raven & Scrimgeour 1997). Not much is known about the oxygen dynamics of *Thalassia* (see Oremland & Taylor 1977; Carlson *et al.* 1989) and it is therefore not known whether differences in the internal oxygen dynamics among different sites in Florida Bay are correlated with the occurrence of die-off episodes.

Sulphide, produced by reduction of sulphate within the anoxic sediment, is a potent phytotoxin, and sulphide accumulation has been identified as causing mortality in plants growing in flooded soils (e.g. Vámos & Köves 1971; Koch *et al.* 1990; Goodman *et al.* 1995). Sulphide has a negative impact on photosynthesis, aerobic metabolism, growth and nutrient uptake of rooted plants (Bradley & Dunn 1989; Goodman *et al.* 1995; Raven & Scrimgeour 1997; Holmer & Bondgaard 2001), and sulphide concentrations may be extremely high in the carbonate sediments of tropical seagrass beds, where the low availability of iron reduces the sulphide binding capacity (Carlson *et al.* 1994; Lee & Dunton 2000; Chambers *et al.* 2001). Detoxification of sulphide may occur through formation of S-containing organic compounds (Raven & Scrimgeour 1997), by non-enzymatic catalysis by metals and by mitochondrial oxidation (Lee *et al.* 1999). The most efficient defence against sulphide toxicity would, however, be to prevent sulphide intrusion by promoting the re-oxidation of reduced sulphur by molecular oxygen, a process mediated by bacteria in the oxic microzone around roots and rhizomes. Signals describing the stable isotope composition of sulphur occasionally have negative  $\delta^{34}\text{S}$  values, suggesting that, in these cases, sulphide does invade the tissues and become incorporated in wetland plants and seagrasses (Carlson & Forrest 1982; Fry *et al.* 1982; Raven & Scrimgeour 1997). Furthermore, addition of iron to Florida Bay *Thalassia* beds has been shown to increase the  $\delta^{34}\text{S}$  values of the seagrass leaves, presumably by complexing with the isotopically depleted, microbially produced sulphide, and thereby decreasing the invasion of sulphide into the plants (Chambers *et al.* 2001). Sulphide intrusion has been experimentally documented in eelgrass (Pedersen *et al.* 2004), but the exact conditions allowing sulphide invasion in *Thalassia testudinum* and the occurrence of *in situ* sulphide intrusion have not been examined.

Until recently, the means by which oxygen dynamics has been described inside and around seagrass below-ground tissues has been either indirect, crude or destructive (e.g. Oremland & Taylor 1977; Carlson *et al.* 1989) and the same is true for sulphide (e.g. Carlson *et al.* 1994; Lee & Dunton 2000). The development of micro-electrode techniques has, however, provided means by which oxygen (Revsbech 1989) and gaseous sulphide ( $\text{H}_2\text{S}$ , Jeroschewski *et al.* 1996) can be measured with high precision and at high spatial and temporal resolution. We therefore conducted a series of measurements in the laboratory and in the field, using oxygen and sulphide microelectrodes to investigate the internal dynamics in *Thalassia testudinum* in Florida Bay. The objectives were to evaluate whether differences among

sites in the internal oxygen dynamics of *Thalassia* were consistent with its hypothesized role in die-off and to examine whether gaseous sulphide actually does invade *Thalassia* tissues and, if so, under what conditions.

## Methods and materials

### STUDY AREA

Florida Bay is a large, shallow lagoon bounded by the Florida peninsula to the north and by the Florida Keys to the south-east, while open to exchange with the Gulf of Mexico along the western margin (Fig. 1). The bay harbours over 2200 km<sup>2</sup> of seagrass beds, with *Thalassia testudinum* as the dominant species (Zieman *et al.* 1989; Fourqurean *et al.* 2001). Florida Bay is divided into numerous basins by intertidal or shallow submerged mud banks, and the biomass and species composition of the seagrass beds vary substantially among basins. Field measurements were conducted at three sites. At Porjoe Key (25°07.5' N, 80°28.2' W) the density of *Thalassia* was sparse and the sediment layer was only 10 cm thick above the solid limestone. At both Rabbit Key (24°58.5' N, 80°50.5' W) and Barnes Key (24°56.5' N, 80°47.5' W) plant density was high and the sediment layer thick (> 40 cm). Plant density was highest at Barnes Key, where active die-off was observed during the study, albeit on a small scale (c. 1000 m<sup>2</sup>).

### PLANT AND SEDIMENT DATA

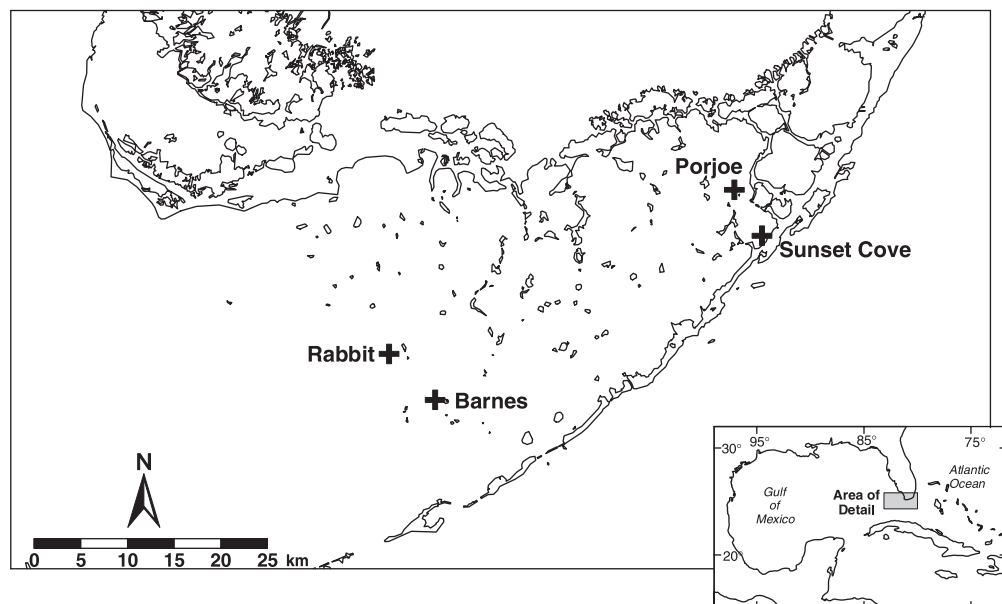
At each sampling site the shoot density of *Thalassia* was recorded by counting all short shoots within 6–10 randomly distributed frames of 200 cm<sup>2</sup> (10 cm by 20 cm, *n* = 6–10). Sediment samples were collected in triplicate to a depth of 15 cm (10 cm at Porjoe Key) by

use of perspex corers with a diameter of 4.4 cm. The upper 1 cm of the cores was discarded and live rhizome and root material was removed. The sediment was dried at 60 °C for 48 hours, homogenized and analysed for organic matter, including dead leaves, rhizomes and roots.

Sediment profiles of oxygen and gaseous sulphide (H<sub>2</sub>S) down to a depth of around 7 cm were measured at all sites by use of an automatic profiler (MiniProfiler MP4, Unisense, Aarhus, Denmark) mounted with oxygen and sulphide minielectrodes with tip diameters of 500 µm (OX-500 and H<sub>2</sub>S-500, Unisense). Oxygen and sulphide partial pressures were measured at 1-mm intervals. In water, total sulphide is found as dissolved gaseous sulphide (H<sub>2</sub>S) and as HS<sup>-</sup> and S<sup>2-</sup> ions, forming a chemical equilibrium depending on pH of the medium. The sulphide microsensor is not sensitive to HS<sup>-</sup> and S<sup>2-</sup> but only to gaseous sulphide (Jeroschewski *et al.* 1996), which is, however, also the species of sulphide invading the plant tissues by lipid-solution permeation of the plasmalemma (Raven & Scrimgeour 1997). During laboratory as well as *in situ* measurements, the temperature of the water and sediment was recorded by means of type-K thermocouples connected to a laptop computer via a resistance converter (TH-08, PicoTech, St. Neats, England). The individual temperature dependence of all electrodes was determined, and electrode signals were corrected for changes in temperature during field or laboratory measurements. In addition, all electrodes were individually calibrated to standard partial pressures of oxygen or sulphide at known temperatures before and after the measurements (Pedersen *et al.* 2004).

### LABORATORY EXPERIMENT

For the laboratory experiment terminal rhizomes of *Thalassia* with three to five short shoots were planted in



**Fig. 1** Location map of Florida Bay showing the three study sites at Porjoe, Rabbit and Barnes keys and the sampling site in Sunset Cove. During this study, a small-scale die-off of *Thalassia testudinum* occurred at Barnes Key.

perspex aquaria containing 10 cm of homogenized sediment from the collection site at Sunset Cove (25°03.0' N, 80°30.0' W). Cut ends of rhizomes and roots were sealed with silicone grease. The aquaria with plants were stored for up to 3 days at 0.5-m depth in Sunset Cove until the experiments were conducted. In the laboratory, the perspex aquaria were placed in a large aquarium with freshly collected seawater. Water circulation was provided by a submersible pump and oxygen partial pressure (pO<sub>2</sub>) was controlled by bubbling with mixtures of atmospheric air and nitrogen using compressed gasses and a gas mixer (Brooks 5850TR, Brooks, Venedal, the Netherlands). Light was provided by a Tungsten halogen lamp (Schott, Mainz, Germany). During the experiments, the pO<sub>2</sub> in the water was continuously monitored with a Clark-type oxygen minielectrode with a tip diameter of 500 µm (OX-500, Unisense). Water column concentrations of sulphide (H<sub>2</sub>S) were also measured by minielectrodes (H2S-500) during the experiments, but no sulphide was detected in the water column.

After gently removing the sediment to expose a portion of the rhizome, oxygen and sulphide microelectrodes (OX-25 and H2S-25) were inserted until a relatively constant signal was recorded. The electrodes record an integrated response from a sphere around the electrode tip of about the same radius as the tip diameter. The intra-plant measurements were conducted by use of electrodes with tip diameters of 25 µm representing oxygen and sulphide concentrations of small cell clusters or lacunal air spaces. The exact position of the electrode tip, whether within lacunae or nearby cell clusters, could not be determined. After insertion, the exposed tissue was again carefully covered with 2–3 cm of sediment. The microelectrodes were connected to an eight-channel pA meter (PA8000, Unisense) and the outputs were logged on a computer using an analogue-digital-converter (ADC-16, PicoTech). After mounting the microelectrodes, the set-up was left to equilibrate for at least 2 hours in order to allow re-establishment of the biogeochemical profiles around roots and rhizome (Pedersen *et al.* 2004). During the experiment, changes in the internal partial pressures of oxygen and gaseous sulphide were measured after manipulating plant oxygen supplies by dark–light shifts and by changing water column oxygen concentrations around the plants.

#### IN SITU MEASUREMENTS

Similar electrode and data logging equipment as described above was used for the field measurements of

internal oxygen and sulphide dynamics *in situ* in meristems and rhizomes of *Thalassia testudinum* at water depths of 0.8–1.0 m for part or all of a diel cycle. The microelectrodes with micromanipulators were mounted on aluminium supports, and the electrodes were inserted into the basal, meristematic part of the short shoots after carefully removing the old leaf sheaths of formerly shed leaves. The positioning of the oxygen electrodes was conducted by following the electrode signal during insertion until a constant oxygen signal was obtained at a depth of approximately one-third of the tissue diameter. The electrode tip was most likely to be positioned within compact tissue because the meristematic region has poorly developed lacunae. The diel changes in surface irradiance, water column and sediment temperature, water column oxygen content and internal pO<sub>2</sub> in the meristems of *Thalassia* were recorded from the afternoon throughout the dark period until late in the morning at Porjoe, Rabbit and Barnes Keys.

Contemporaneous diel measurements of oxygen and sulphide partial pressures in *Thalassia* rhizomes were conducted in the die-off patch at Barnes Key. As changes in sulphide signals could not be recorded during insertion of the sulphide electrode in daylight, the electrode tip was positioned at approximately the same depth into the tissue as the oxygen electrodes.

## Results

#### SEAGRASS AND SEDIMENT CHARACTERISTICS

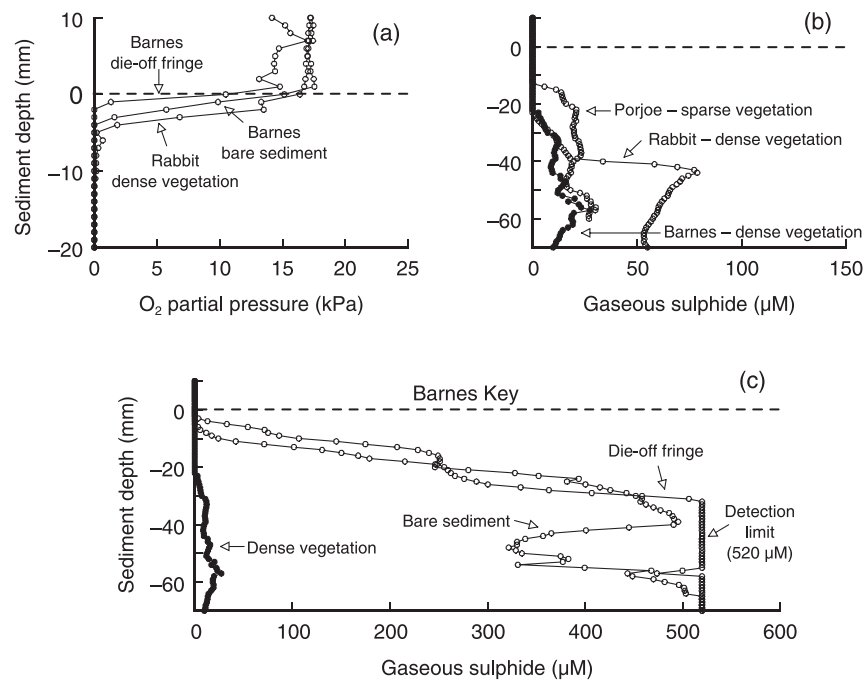
The shoot density of *Thalassia* was relatively low at Porjoe Key, higher at Rabbit Key, and the highest at Barnes Key, where it exceeded 1500 short shoots m<sup>-2</sup> (Table 1). The organic content of the sediment also varied among stations. The sediment content of organic matter including plant litter was substantially higher in the densely vegetated beds at Rabbit and Barnes Keys than at Porjoe Key. The sediment content of gaseous sulphide was relatively low within the healthy stands at all three sites but very high in bare sediments and in the die-off fringe at Barnes Key (Table 1, Fig. 2b and c).

#### OXYGEN AND SULPHIDE PROFILES IN THE SEDIMENT

Oxygen profiles across the water–sediment interface followed rather similar patterns at the different plant densities and sites (Fig. 2a). Oxygen was depleted within the upper 5 mm of the sediment, and the sediment

**Table 1** Shoot density of *Thalassia testudinum* ( $n = 6–10$ ), sediment organic matter including dead plant material ( $n = 3$ ) and concentrations of gaseous sulphide (range) in the sediment at the three different sites in Florida Bay. Means  $\pm$  95% CL or ranges

	Porjoe Key	Rabbit Key	Barnes Key
Shoot density (shoots m <sup>-2</sup> )	665 $\pm$ 139	1233 $\pm$ 190	1533 $\pm$ 137
Organic matter (% of dry weight)	7.2 $\pm$ 1.1	18.8 $\pm$ 3.3	13.6 $\pm$ 1.5
Gaseous sulphide, 30–70 mm (µM)	15–30	10–80	15–520+



**Fig. 2** Sediment profiles of oxygen and gaseous sulphide measured at different sites and positions in Florida Bay. (a) Oxygen profiles from a dense and healthy stand of *Thalassia testudinum* at Rabbit Key and from bare sediment and the fringe of a die-off patch at Barnes Key. (b) Profiles of gaseous sulphide in healthy stands of *T. testudinum* with sparse vegetation at Porjoe Key and dense vegetation at Rabbit and Barnes keys. (c) Profiles of gaseous sulphide in bare sediment, the die-off fringe and in a dense stand of *T. testudinum* at Barnes Key. The detection limit for gaseous sulphide was 520  $\mu\text{M}$ .

remained anoxic with increasing depth. Microzones of oxygen may have existed around roots and rhizomes but none were detected by the relatively large (tip diameter 500  $\mu\text{m}$ ) microelectrodes during profiling. Gaseous sulphide could not be detected within the upper 13 mm of the sediment at the sparsely vegetated site at Porjoe Key (Fig. 2b). Below this zone the concentration of gaseous sulphide was low (15–30  $\mu\text{M}$ ) and relatively constant with depth. In the dense *Thalassia* bed at Rabbit Key gaseous sulphide was detected below 25 mm depth and levels were relatively high (up to 80  $\mu\text{M}$ ) in deeper sediment layers (Fig. 2b). Within the healthy looking part of the *Thalassia* bed adjacent to the die-off patch at Barnes Key, the profile of gaseous sulphide was similar to that at Porjoe in deeper sediments and to Rabbit nearer the surface (15–25  $\mu\text{M}$ , Fig. 2b).

The profiles of gaseous sulphide were very different in bare sediments within the die-off patch and within the zone of active die-off at Barnes Key (Fig. 2c). In bare sediment, less than 2 m from the healthy seagrass stand, gaseous sulphide appeared at 3 mm depth in the sediment and increased to levels above detection limit (> 520  $\mu\text{M}$ ) at 60–70 mm depth. In the zone of active die-off (i.e. between the healthy stand and the bare patch) gaseous sulphide appeared at 6 mm depth and increased to above detection limit at 30 mm depth (Fig. 2c).

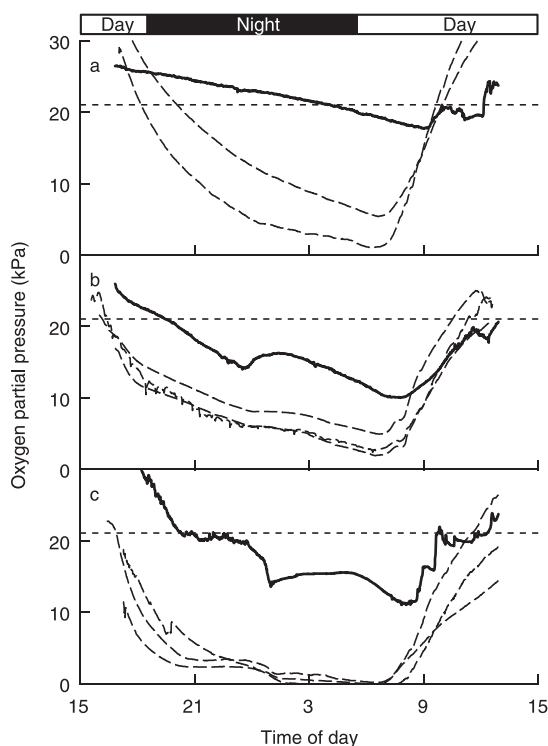
#### IN SITU MEASUREMENTS OF PLANT OXYGEN CONTENT

Oxygen partial pressures ( $\text{pO}_2$ ) in the water column and meristematic tissues of *Thalassia* at three of the sites in

Florida Bay exhibited similar, systematic patterns on a diel basis, with supersaturation relative to atmospheric  $\text{pO}_2$  during daylight and gradually declining  $\text{pO}_2$  to minimum levels at the end of the dark period (Fig. 3). At the sparsely vegetated site at Porjoe Key the water column was slightly supersaturated until after midnight and declined to minimum values of around 80% of air saturation 2 hours after sunrise (Fig. 3a). The amplitude of variations in meristematic internal  $\text{pO}_2$  was much larger than in the water column, corresponding to almost 150% of air saturation during daylight and below 25% at the end of the dark period. The internal  $\text{pO}_2$  decreased rapidly during the afternoon from supersaturation relative to water column oxygen to hyposaturation during the night. The rate of decline in internal oxygen gradually decreased with increasing difference between water column and internal plant oxygen. Minimum meristematic oxygen content was recorded around sunrise, after which the internal  $\text{pO}_2$  increased rapidly, reaching supersaturation relative to water column oxygen at around 09.00 (Fig. 3).

The diel changes in water column oxygen in the very dense and apparently healthy *Thalassia* bed at Rabbit Key (Fig. 3b) were substantially stronger than in the sparsely vegetated bed at Porjoe Key (Fig. 3a). The meristematic internal  $\text{pO}_2$  within three different short shoots was very similar and followed about the same diel pattern as at Porjoe. Supersaturation relative to water column oxygen during daylight was not as pronounced but at Rabbit Key oxygen was present within the meristematic tissues throughout the diel cycle.

In the die-off area at Barnes Key, water column oxygen also changed markedly on a diel basis but in addition



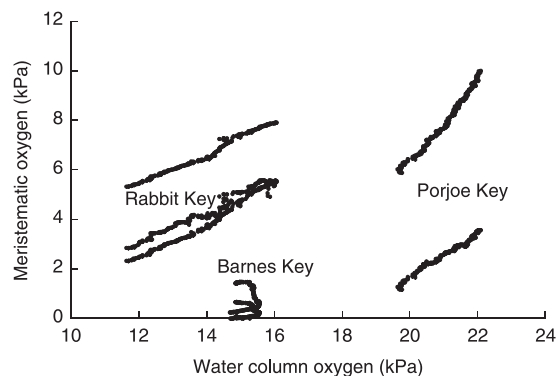
**Fig. 3** Diel changes in the oxygen partial pressure of the water column (solid lines) and in individual meristems (dashed lines) of *Thalassia testudinum* at Porjoe Key (a), Rabbit Key (b) and at a die-off site near Barnes Key (c). The horizontal, dashed lines indicate the oxygen partial pressure of the atmosphere.

fluctuated substantially (Fig. 3c), probably because of heterogeneity in the different water bodies passing through the *Thalassia* bed. Changes in meristematic  $pO_2$  were recorded for plants in the middle and periphery of the die-off patch at Barnes Key; all of them followed a similar pattern, with much steeper decreases in internal oxygen late in the afternoon and much lower oxygen contents during night compared with those at Porjoe and Rabbit Keys. The meristematic tissues turned anoxic, or close to anoxic, late at night, and the meristematic tissue of one plant in the middle of the die-off patch was without detectable oxygen from around 02.00 to after 07.00 (Fig. 3c). All three plants had lower oxygen contents than the water column during most of the recording period.

During the dark period from 02.00 to 06.00 the meristematic oxygen partial pressure exhibited substantial differences among plants and sites and changed with water column oxygen content (Fig. 4). At Porjoe Key and Rabbit Key,  $pO_2$  varied considerably among different plants within the same site. In the die-off area at Barnes Key plant oxygen content was uniformly very low, even though the water column oxygen content was as high as at Rabbit Key.

#### CONTEMPORANEOUS *IN SITU* MEASUREMENTS OF INTERNAL OXYGEN AND SULPHIDE

Spontaneous invasion of sulphide was recorded in the meristem of *Thalassia* growing at the margin of the

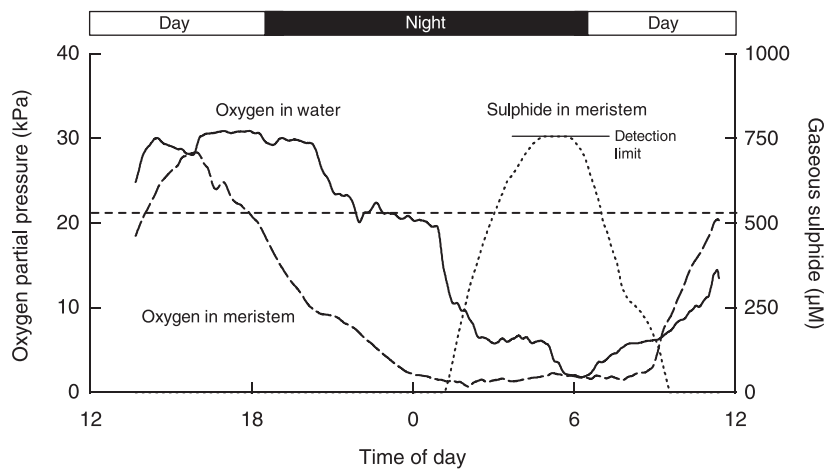


**Fig. 4** Relationships between the oxygen partial pressure in meristems of *Thalassia testudinum* and in the water column measured during darkness (02.00–06.00) at Porjoe, Rabbit and Barnes Keys, Florida Bay.

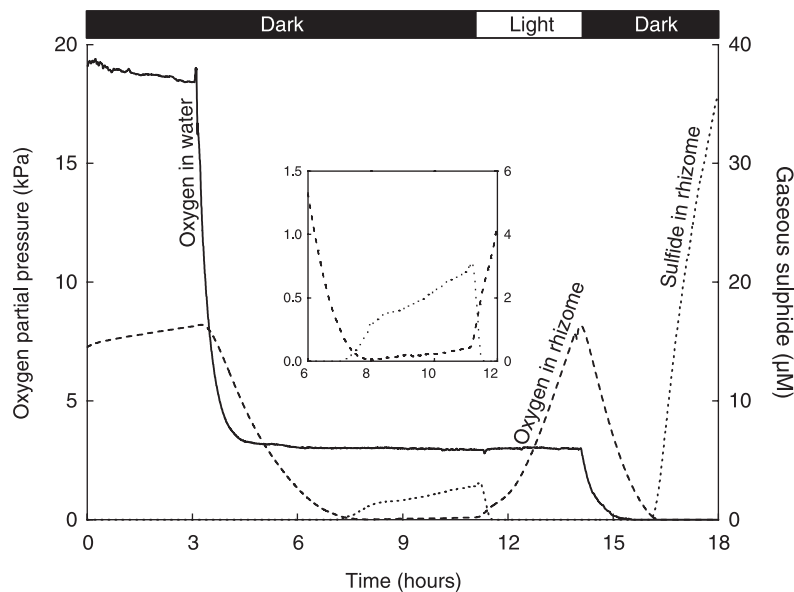
die-off patch at Barnes Key (Fig. 5). Water column  $pO_2$  varied from supersaturation during the day to night-time oxygen content of down to 10% of air saturation. Meristematic  $pO_2$  declined to 0–2 kPa at around midnight and remained low until 2 hours after sunrise. Gaseous sulphide invading from the sediment was detected at around 02.00 and increased to 750  $\mu M$  (= maximum recordable level of the sulphide electrode) at the end of the dark period. Internal gaseous sulphide began to decrease before sunrise and had disappeared by 10.00, when internal plant oxygen had increased to above 8 kPa. Throughout the period with detectable sulphide within the meristem, sulphide coexisted with oxygen at  $pO_2$  ranging from 1 to 8 kPa (Fig. 5).

#### LABORATORY EXPERIMENT

Oxygen and sulphide variability within a rhizome of *Thalassia testudinum*, under controlled conditions in the laboratory, confirmed the tight coupling between external light and oxygen conditions and internal gas dynamics (Fig. 6). Rhizome oxygen content remained above 30% of air saturation during darkness as long as there was high water column oxygen partial pressure. When water column oxygen was reduced to between 10 and 15% of air saturation, the rhizome oxygen declined to almost zero and invasion of sulphide was detected. Molecular oxygen and gaseous sulphide coexisted at internal oxygen contents in the range 0.01–0.6 kPa and the concentration of sulphide increased steadily until the plant was illuminated (Fig. 6). When the plant received light, photosynthetic activity generated a rapid increase in internal  $pO_2$ , with subsequent depletion of the pool of gaseous sulphide. By turning off the light and depleting the water column of oxygen, the internal plant oxygen declined rapidly and a fast invasion of gaseous sulphide was recorded. The complete disappearance of internal oxygen resulted in a steeper increase in internal sulphide, and substantially higher sulphide concentrations (> 30  $\mu M$ ) were found compared with the situation with coexistence of oxygen and sulphide in the preceding period.



**Fig. 5** *In situ* measurements of diel changes in the oxygen content of the water column and of the oxygen partial pressure and content of gaseous sulphide in the meristematic tissue of *Thalassia testudinum* from a die-off patch at Barnes Key. Upper detection limit (= 750 µM) of the sulphide microelectrode is indicated. The horizontal, dashed line corresponds to the oxygen partial pressure of the atmosphere.



**Fig. 6** Changes in the oxygen partial pressure and content of gaseous sulphide in the rhizome of *Thalassia testudinum* after manipulation of light availability (panel above) and water column oxygen partial pressure (solid line) in a laboratory experiment. The inset figure shows details of oxygen and sulphide coexistence during a dark–light shift at low water column oxygen content at an enlarged scale.

### Discussion

The microelectrode techniques allowed us to conduct measurements of oxygen and gaseous sulphide in water, sediment and plants at high spatial and temporal resolution, both in the field and in the laboratory. The oxygen partial pressure of *Thalassia testudinum* meristems was highly variable over diel cycles but agreed with the early results reported by Oremland & Taylor (1977) and Carlson *et al.* (1994), who used more traditional techniques to extract and subsequently analyse gas samples from rhizomes of *Thalassia*. The diel changes in meristematic oxygen partial pressure ( $pO_2$ ) also followed a pattern very similar to that described for the temperate seagrass, *Zostera marina* (Greve *et al.*

2003), with supersaturation relative to the  $pO_2$  of the water column during the day, when light supports leaf photosynthesis, and hyposaturation at night. The present study also directly documented invasion of sulphide into seagrass tissues. Hitherto, sulphide invasion and incorporation has been indicated by low isotopic  $^{34}S : ^{32}S$  ratios in below-ground tissues (Carlson & Forrest 1982; Raven & Scrimgeour 1997; Stribling *et al.* 1998) or leaves (Chambers *et al.* 2001) of aquatic plants, and sulphide intrusion has been demonstrated for *Zostera marina* in the laboratory (Pedersen *et al.* 2004), but the present measurements showed that sulphide invasion does also occur under *in situ* conditions. The highly variable  $pO_2$  within the seagrass tissue over the diel cycle and the occurrence of sulphide invasion

under low internal  $pO_2$  demonstrate that marine angiosperms, such as seagrasses, are potentially challenged by hypoxia and sulphide invasion (Raven & Scrimgeour 1997).

The oxygen balance of seagrasses is determined by a complex combination of processes: leaf photosynthesis driven by irradiance; oxygen loss or gain in leaves through exchange with the water column depending on concentration gradients and water movement; plant respiration affected by physiological activity and temperature; and loss of oxygen to the sediment through roots and rhizomes probably determined by the reducing power of the sediment (Greve *et al.* 2003; Borum *et al.* 2005). The large diel changes in the meristematic oxygen content of *Thalassia testudinum* recorded at the three sites in Florida Bay seemed mainly to be driven by the changes in irradiance, supporting high photosynthetic rates in the leaves during the day, while respiratory oxygen consumption within tissues and sediment reduced the internal pools of oxygen in the dark. During daylight, leaf photosynthesis resulted in oxygen hyper-saturation within the meristems relative to the  $pO_2$  of the water column, and it is reasonable to assume that in daylight the rhizomes and roots of *Thalassia* were sufficiently supplied with oxygen to support aerobic metabolism and maintain oxic microzones on root surfaces, as has been seen for other seagrass species (Pedersen *et al.* 1998; Borum *et al.* 2005).

In this study, critically low oxygen contents or anoxia in meristems of *Thalassia* were only observed during periods of darkness. In the dark, the fast depletion of internal plant oxygen showed that the oxygen pools built up by leaf photosynthesis during daylight were insufficient to fully sustain night-time plant respiration and maintain oxic conditions in below-ground seagrass tissues at Barnes Key. It has been shown that maintenance of internal oxygen conditions in the dark requires a continuous influx of oxygen from water column to leaves and further on to rhizomes and roots (Pedersen *et al.* 1998; Greve *et al.* 2003; Pedersen *et al.* 2004). The present study underlines the role of the water column as an important source of internal plant oxygen during darkness. In the laboratory experiment, the rhizome  $pO_2$  could be controlled solely by changing water column oxygen concentrations without photosynthetic oxygen release from the leaves. In addition, night-time  $pO_2$  of *Thalassia* meristems recorded *in situ* showed close relationships between plant and water column  $pO_2$  within the different sites. Accordingly, passive diffusion of oxygen from water column to meristems and below-ground tissues of *Thalassia* in the dark may be sufficient to support aerobic metabolism under conditions with moderate plant respiration and low sediment oxygen consumption.

The internal oxygen partial pressure of *Thalassia* meristems varied, however, during darkness among and within the different sites independently of differences in water column oxygen conditions. Part of this variability might be attributed to between-shoot differences

as observed within the individual sites. Such variability could be caused by differences in plant composition (e.g. variable above- to below-ground biomass ratio) but also by differences in the position of the microelectrodes within the plants. The further away the electrodes are placed from the leaf bundles (the sources of oxygen), the lower the internal  $pO_2$  is expected to be due to the respiratory drain within rhizomes and roots. Hence, marked differences in the recorded oxygen partial pressure in plants within and among sites may be found under similar environmental conditions, and the variable within-site  $pO_2$  observed at Porjoe and Rabbit Keys may reflect stochastic differences among plants or variable positioning of the microelectrodes.

The consistently low oxygen partial pressure during darkness within plants at the die-off site near Barnes Key could, however, hardly be assigned to coincidence. Neither could low water column oxygen concentrations explain the systematically lower meristematic  $pO_2$ , as water  $pO_2$  during darkness at Barnes Key was similar to that at Rabbit Key with higher meristematic  $pO_2$ . Therefore, the low  $pO_2$  at the die-off site must have been caused by either higher plant respiration or a larger oxygen drain within the sediment. Higher plant respiration could occur as a result of higher water temperature (Masini & Manning 1997), salinity stress (Biebl & McRoy 1971) or infection by pathogens, such as the slime mould, *Labyrinthula* sp. (Durako & Kuss 1994). During the major die-off in the late 1980s, a regional drought resulted in salinities well over 50‰ in central Florida Bay (Fourqurean *et al.* 1993; Nuttle *et al.* 2000), and abnormally high water temperatures occurred in late summer and early autumn (Zieman *et al.* 1994). These conditions may have induced poor plant oxygen conditions and might have been responsible for the initiation of the large die-off at that time. However, during our study, the water temperature was not systematically different among the sampling sites, and the salinity was not exceptionally high at Barnes Key. In addition, pathogen lesions on *Thalassia* leaves at Barnes did not seem more frequent than at the other study sites. Therefore, the lower meristematic  $pO_2$  at Barnes Key compared with at the other sites was more likely to be caused by differences in sediment conditions. The sediment was anoxic below 5–10 mm depth at all sites but the sediments could differ substantially with respect to redox potential and potential oxygen consumption. The organic content of the sediment including leaf litter was indeed very high (14–18% of dry weight) both at Barnes and Rabbit Keys. In addition, the pool of gaseous sulphide in the sediment ( $> 520 \mu M$ ) of the die-off patch at Barnes Key was above detection limit, indicating a large oxygen drain and debt related to reoxidation of total sulphide within the sediment.

Invasion of sulphide was provoked in the laboratory and occurred spontaneously in plants at the die-off patch near Barnes Key. In both situations sulphide must have invaded from the sediment through below-ground tissues as no sulphide was present in the water column



during the measurements. As also shown by Pedersen *et al.* (2004), sulphide invasion was tightly coupled to the oxygen balance of the plants and only occurred at relatively low  $pO_2$  in meristems or rhizomes. The meristematic oxygen content is only a proxy for the oxygen conditions of rhizomes and roots, but we assume that very low  $pO_2$  within the meristem or rhizome is indicative of root anoxia at least in distal root sections. Furthermore, we assume that anoxia immediately on the root surfaces is a prerequisite for sulphide invasion into the plants because sulphide reoxidation mediated by sediment bacteria is usually very fast, allowing zones of oxygen and sulphide coexistence of less than  $100 \mu\text{m}$  (Jørgensen & Revsbech 1983). Hence, the rate of sulphide invasion and the maximum level of sulphide accumulating within the plant tissues should be functions of the surface area of anoxic below-ground tissues, the sediment sulphide concentration and of the rates of sulphide reoxidation inside plant tissues fuelled by oxygen transported from the leaves. The rate of sulphide invasion could be controlled by changing the rhizome  $pO_2$  in the laboratory experiment, but the present observations of sulphide invasion were too few to allow a more detailed analysis of the different controlling factors.

When gaseous sulphide has entered the root tissues, the transport of sulphide to the meristematic region and further on to leaves is relatively fast because it occurs as simple gas-phase diffusion, as does oxygen diffusion in the air-filled lacunae (Pedersen *et al.* 2004). The expected high rates of sulphide transport were supported by the observations of coexistence of oxygen and sulphide inside the lacunae but this is also indicative of low rates of sulphide reoxidation. Spontaneous molecular reoxidation of gaseous sulphide in air or water is in the order of 10 000 to 100 000 times slower than bacteria-mediated and 10 to 100 times slower than enzyme-mediated reoxidation (Jørgensen & Revsbech 1983). The half-life of sulphide pools undergoing molecular reoxidation is in the order of 1 hour (Almgren & Hagström 1974) but less than 1 second when mediated by bacteria (Jørgensen & Revsbech 1983). Accordingly, the observed coexistence of significant levels of sulphide and oxygen within the tissues of *Thalassia* strongly suggests that the plant does not possess effective biological mechanisms to accelerate sulphide reoxidation. This conclusion is further supported by the relatively slow depletion of the lacunal sulphide pools after oxygen supplies were re-established by increasing water column  $pO_2$  or by photosynthetic oxygen release within the leaves. The time patterns of sulphide displacement revealed half-lives of 0.5–1 hour, similar to those seen with simple molecular sulphide reoxidation. The lack of effective mechanisms to immobilize or detoxify sulphide in *Thalassia* means that vulnerable tissues, such as the meristems, may be exposed to toxic internal levels of sulphide for several hours following invasion.

The different oxygen and sulphide dynamics described from the three sites in Florida Bay are consistent with the observations of die-off episodes being restricted to

sites of high plant density and organically rich and highly reduced sediments (Zieman *et al.* 1989; Carlson *et al.* 1994; Durako & Kuss 1994). Low night-time oxygen partial pressure or even anoxia in meristematic tissues of *Thalassia* was only recorded at the die-off site in Barnes Key basin. The results are also consistent with the proposed effect of plant oxygen release on the concentrations of sediment sulphide described by Carlson *et al.* (1994). The concentrations of sediment sulphide we measured were moderate ( $< 100 \mu\text{M}$ ) at all sites except within and in the margin of the die-off patch, where concentrations of gaseous sediment sulphide exceeded  $520 \mu\text{M}$ . The iron-poor carbonate sediment characteristic of Florida Bay means that there is little iron to immobilize sulphide (Chambers *et al.* 2001), and therefore the concentration of sulphide primarily depends on the balance between sulphate reduction and sulphide reoxidation by oxygen supplied from the plants. The observed pattern suggests that healthy *Thalassia* plants fuel sediment reoxidation of sulphide via transport of oxygen from water column and leaf photosynthesis to the sediment, thereby preventing sulphide from accumulating to very high levels under normal growth conditions (Carlson *et al.* 1994).

The direct links between growth conditions, plant performance, invasion of sediment sulphide and subsequent shoot mortality have still not been clearly demonstrated, and probably will not be, because of the complex nature of the interactions among the controlling factors. However, there is no doubt that high sediment sulphide concentrations can have negative impacts on growth and survival of wetland plants and seagrasses (e.g. Havill *et al.* 1985; Bradley & Dunn 1989; Koch & Mendelsohn 1989), and that the removal of sulphides by iron additions increases the growth rate of *Thalassia* in Florida Bay (Chambers *et al.* 2001). Sulphide is highly toxic, and the concentrations of gaseous sulphide measured in *Thalassia* greatly exceeded the  $10 \mu\text{M}$  known to be toxic to plant tissues (Raven & Scrimgeour 1997). Sulphide invasion may not be the proximate factor initiating the episodes of seagrass die-off, but the accumulation of high sulphide concentrations in the fringe of die-off patches may be responsible for the radial progression of the die-off by affecting plant physiology and internal oxygen balance or by exerting an additional oxygen drain required for sulphide reoxidation at the fringe of the patch (Carlson *et al.* 1994). The initiation of die-off episodes is more likely to be caused by disturbance of plant oxygen balances induced by environmental factors, such as hypersalinity or high temperatures (Zieman *et al.* 1999; Koch & Erskine 2001), pathogen infection increasing plant respiration (Porter & Muehlstein 1989; Durako & Kuss 1994), overcrowding and accumulation of high amounts of organic matter in the sediment (Zieman *et al.* 1989) or combinations of these environmental stresses (Zieman *et al.* 1999). However, our results strongly support the hypothesis advanced by Carlson *et al.* (1994) that root-rhizome hypoxia followed by

sulphide invasion from the sediment is the most likely mechanism responsible for short shoot mortality of *Thalassia testudinum* during die-off episodes in Florida Bay. The mechanism could probably also be responsible for the events of more gradual dieback reported from wetland plant species growing in certain salt marshes (e.g. Mendelssohn & McKee 1988; Chambers *et al.* 1998; Fogli *et al.* 2002). Reduced plant growth and increased mortality have been observed among stands of *Spartina alterniflora* and *Phragmites australis* growing under conditions with poor root oxygen supplies and high soil concentrations of sulphide (Mendelssohn & McKee 1988; Fogli *et al.* 2002). Hence, root anoxia followed by sulphide invasion could be a mechanism that more generally may determine the growth and survival of rooted plants in sulphate-rich aquatic environments.

### Acknowledgements

This work was supported by the Carlsberg Foundation (grants # 990530/10 and # 0052) and used equipment partly provided by an EC grant (EVK3-CT-2000-00044). Logistic support with laboratory and boat facilities were kindly provided by Everglades National Park and the FCE-LTER programme at FIU, funded by the US National Science Foundation (Agreement # DEB-9910514). This is contribution # 2004-08 in the SFWMD Coastal Ecosystems Technical Science Series and contribution # 323 from the SFWMD Coastal Ecosystems Division.

### References

- Almgren, T. & Hagström, I. (1974) The oxidation rate of sulphide in sea water. *Water Research*, **8**, 395–400.
- Armstrong, W. (1979) Aeration in higher plants. *Advances in Botanical Research*, Volume 7 (ed. H.W. Woolhouse), pp. 225–332. Academic Press, London.
- Biebl, R. & McRoy, C.P. (1971) Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Marine Biology*, **8**, 48–56.
- Borum, J., Sand-Jensen, K., Binzer, T., Pedersen, O. & Greve, T.M. (2005) Oxygen movement in seagrasses. *Seagrass Biology: a Treatise* (eds A.W.D. Larkum, R.J. Orth & C.M. Duarte). Kluwer Academic, Dordrecht.
- Bradley, P.M. & Dunn, E.L. (1989) Effects of sulfide on the growth of three salt marsh halophytes of the southeastern United States. *American Journal of Botany*, **76**, 1707–1713.
- Carlson, P.R. & Forrest, J. (1982) Uptake of dissolved sulfide by *Spartina alterniflora*: evidence from natural sulfur isotope abundance ratios. *Science*, **216**, 633–635.
- Carlson, P.R., Yarbro, L.A. & Barber, T.R. (1994) Relationship of sediment sulfide to mortality of *Thalassia testudinum*, Florida Bay. *Bulletin of Marine Science*, **54**, 733–746.
- Carlson, P.R., Yarbro, L.A., Sargent, W.B. & Barber, T.R. (1989) Factors influencing the oxygen supply of *Thalassia testudinum* rhizomes. 10th Biennial Estuarine Research Federation Conference, abstract. Estuarine Research Federation, Lawrence.
- Chambers, R.M., Fourqurean, J.W., Macko, S.A. & Hoppenot, R. (2001) Biogeochemical effects of iron availability on primary producers in a shallow marine carbonate environment. *Limnology and Oceanography*, **46**, 1278–1286.

- Chambers, R.M., Mozdzer, T.J. & Ambrose, J.C. (1998) Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. *Aquatic Botany*, **62**, 161–169.
- Crawford, R.M.M. & Braendle, R. (1996) Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany*, **47**, 145–159.
- Durako, M.J. & Kuss, K.M. (1994) Effects of *Labyrinthula* infection on the photosynthetic capacity of *Thalassia testudinum*. *Bulletin of Marine Science*, **54**, 727–732.
- Fogli, S., Marchesini, R. & Gerdol, R. (2002) Reed (*Phragmites australis*) decline in a brackish wetland in Italy. *Marine Environmental Research*, **53**, 465–479.
- Fourqurean, J.W., Jones, R.D. & Ziemann, J.C. (1993) Processes influencing water column nutrient characteristics and phosphorus limitation of phytoplankton biomass in Florida Bay, FL, USA: inferences from spatial distributions. *Estuarine, Coastal and Shelf Science*, **36**, 295–314.
- Fourqurean, J.W., Willsie, A., Rose, C.D. & Rutten, L.M. (2001) Spatial and temporal pattern in seagrass community composition and productivity in south Florida. *Marine Biology*, **138**, 341–354.
- Fry, B., Scalan, R.S., Winters, J.K. & Parker, P.L. (1982) Sulfur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. *Geochemica et Cosmochimica Acta*, **46**, 1121–1124.
- Goodman, J.L., Moore, K.A. & Dennison, W.C. (1995) Photosynthetic responses of eelgrass (*Zostera marina* L.) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany*, **50**, 37–47.
- Greve, T.M., Borum, J. & Pedersen, O. (2003) Meristematic oxygen dynamics in eelgrass (*Zostera marina*). *Limnology and Oceanography*, **48**, 210–216.
- Hall, M.O., Durako, M.J., Fourqurean, J.W. & Ziemann, J.C. (1999) Decadal changes in seagrass distribution and abundance in Florida Bay. *Estuaries*, **22**, 445–459.
- Havill, D.C., Ingold, A. & Pearson, J. (1985) Sulphide tolerance in coastal halophytes. *Vegetatio*, **62**, 279–285.
- Holmer, M. & Bondgaard, E.J. (2001) Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. *Aquatic Botany*, **70**, 29–38.
- Jeroschewski, P., Steuckart, C. & Köhl, M. (1996) An amperometric microsensor for the determination of H<sub>2</sub>S in aquatic environments. *Analytical Chemistry*, **68**, 4351–4357.
- Jørgensen, B.B. & Revsbech, N.P. (1983) Colorless sulfur bacteria, *Beggiatoa* spp. & *Thiovulum* spp., in O<sub>2</sub> and H<sub>2</sub>S microgradients. *Applied Environmental Microbiology*, **45**, 1261–1270.
- Koch, M.S. & Erskine, J.M. (2001) Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: interactions with light, salinity and temperature. *Journal of Experimental Marine Biology and Ecology*, **266**, 81–95.
- Koch, M.S. & Mendelssohn, I.A. (1989) Sulphide as a soil phytotoxin – differential responses in 2 marsh species. *Journal of Ecology*, **77**, 565–578.
- Koch, M.S., Mendelssohn, I.A. & McKee, K.L. (1990) Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. *Limnology and Oceanography*, **35**, 399–408.
- Lee, K.S. & Dunton, K.H. (2000) Diurnal changes in pore water sulfide concentrations in the seagrass *Thalassia testudinum* beds: the effects of seagrasses on sulfide dynamics. *Journal of Experimental Marine Biology and Ecology*, **255**, 201–214.
- Lee, R.W., Kraus, D.W. & Doeller, J.E. (1999) Oxidation of sulfide by *Spartina alterniflora* roots. *Limnology and Oceanography*, **44**, 1155–1159.
- Masini, R.J. & Manning, C.R. (1997) The photosynthetic responses to irradiance and temperature of four meadow-forming seagrasses. *Aquatic Botany*, **58**, 21–36.

- Mendelssohn, I.A. & McKee, K.L. (1988) *Spartina alterniflora* die-back in Louisiana: time-course investigation of soil waterlogging effects. *Journal of Ecology*, **76**, 509–521.
- Nuttle, W.K., Fourqurean, J.W., Cosby, B.J., Zieman, J.C. & Robblee, M.B. (2000) Influence of net freshwater supply on salinity in Florida Bay. *Water Resources Research*, **36**, 1805–1822.
- Oremland, R.S. & Taylor, B.F. (1977) Diurnal fluctuations of O<sub>2</sub>, N<sub>2</sub> and CH<sub>4</sub> in the rhizosphere of *Thalassia testudinum*. *Limnology and Oceanography*, **22**, 566–570.
- Pedersen, O., Binzer, T. & Borum, J. (2004) Sulfide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell and Environment*, **27**, 595–602.
- Pedersen, O., Borum, J., Duarte, C.M. & Fortes, M.D. (1998) Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. *Marine Ecology Progress Series*, **169**, 283–288.
- Penhale, P.A. & Wetzel, R.G. (1983) Structural and functional adaptations of eelgrass (*Zostera marina* L.) to the anaerobic sediment environment. *Canadian Journal of Botany*, **61**, 1421–1428.
- Plus, M., Deslous-Paoli, J.-M. & Dagault, F. (2003) Seagrass (*Zostera marina* L.) bed re-colonisation after anoxia-induced full mortality. *Aquatic Botany*, **77**, 121–134.
- Porter, D. & Muehlstein, L.K. (1989) A species of *Labyrinthula* is the prime suspect as the cause of massive die off of the sea grass, *Thalassia testudinum* in Florida Bay. *Mycological Society of America Newsletter*, **40**, 43.
- Pregnall, A.M., Smith, R.D., Kursar, T.A. & Alberte, R.S. (1984) Metabolic adaptation of *Zostera marina* (eelgrass) to diurnal periods of root anoxia. *Marine Biology*, **83**, 141–147.
- Rask, N., Bondgaard, E.J., Rasmussen, M.B. & Laursen, J.S. (2000) Ålegræs-udbredelse før og nu. *Vand og Jord*, **7**, 51–54 (in Danish).
- Raven, J.A. & Scrimgeour, C.M. (1997) The influence of anoxia on plants of saline habitats with special reference to the sulphur cycle. *Annals of Botany* (Suppl. A) **79**, 79–86.
- Revsbech, N.P. (1989) An oxygen microsensor with a guard cathode. *Limnology and Oceanography*, **34**, 474–478.
- Robblee, M.B., Barber, T.R., Carlson, P.R., Durako, M.J., Fourqurean, J.W., Muehlstein, L.K. et al. (1991) Mass mortality of the tropical seagrass *Thalassia testudinum*, Florida Bay (USA). *Marine Ecology Progress Series*, **71**, 297–299.
- Seddon, S., Conolly, R.M. & Edyvane, K.S. (2000) Large-scale seagrass dieback in northern Spencer Gulf, South Australia. *Aquatic Botany*, **66**, 291–310.
- Smith, R.D., Pregnall, A.M. & Alberte, R.S. (1988) Effects of anaerobiosis on root metabolism of *Zostera marina* (eelgrass) – implications for survival in reducing sediments. *Marine Biology*, **98**, 131–141.
- Stribling, J.M., Cornwell, J.C. & Currin, C. (1998) Variability of stable sulfur isotopic ratios in *Spartina alterniflora*. *Marine Ecology Progress Series*, **166**, 73–81.
- Tomlinson, P.B. (1969) On the morphology and anatomy of turtlegrass, *Thalassia testudinum* (Hydrocharitaceae). II. Anatomy and development of the root in relation to function. *Bulletin of Marine Science*, **19**, 57–71.
- Vámos, R. & Köves, E. (1971) Role of the light in the prevention of the poisoning action of hydrogen sulphide in the rice plant. *Journal of Applied Ecology*, **9**, 519–525.
- Vartapetian, B.B. & Jackson, J.B. (1997) Plant adaptations to anaerobic stress. *Annals of Botany*, **79**, 3–20.
- Zieman, J.C., Davis, R., Fourqurean, J.W. & Robblee, M.B. (1994) The role of climate in Florida Bay seagrass dieoff. *Bulletin of Marine Science*, **54**, 1088.
- Zieman, J.C., Fourqurean, J.W. & Frankovich, T.A. (1999) Seagrass die-off in Florida Bay: long-term trends in abundance and growth of Turtle Grass, *Thalassia testudinum*. *Estuaries*, **22**, 460–470.
- Zieman, J.C., Fourqurean, J.W. & Iverson, R.L. (1989) Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. *Bulletin of Marine Science*, **44**, 292–311.

Received 21 February 2004

revision accepted 21 July 2004

Handling Editor: Anthony Davy