Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events

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Abstract

Survival, metabolism and growth of *Zostera marina* exposed to hypoxia and sulfides in the water column were examined during a 3-week experiment. *Z. marina* was collected in the Archipelago of South Fyn, Denmark and kept under controlled laboratory conditions where they were exposed to low oxygen concentrations and two concentrations of sulfides in the water column. Survival of *Z. marina* was negatively influenced by the presence of sulfides, and photosynthetic activity stopped after 6 days of exposure to high sulfide concentrations (100–1000 μM). Rates of photosynthesis also decreased in plants exposed to hypoxia, but the plants remained alive during the 3-week incubation. Rates of leaf elongation (exposed: 0–13.5 mm mm$^{-1}$ d$^{-2}$; control: 24 mm mm$^{-1}$ d$^{-2}$) and number of leaves per shoot (exposed: 3.2–4.2; control: 5.1) decreased in all treatments compared to control plants indicating that hypoxia and sulfides have negative impacts on *Z. marina* metabolism. The non-structural carbohydrate reserves in roots were reduced by up to 81% compared to the controls, whereas the reserves of starch in the rhizomes remained similar to the controls. The exposure to hypoxia and sulfides resulted in loss of above-ground biomass, most severe in the sulfide treatment (55% decrease in shoot:root ratio), suggesting that both parameters may play important roles during die-back events of seagrasses. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Z. marina*; Hypoxia; Sulfides; Growth; Photosynthesis

1. Introduction

Seagrasses are key inhabitants of the coastal zone and extend to depths receiving about 10–11% of the irradiance at the surface (Duarte, 1991). Seagrasses are characterized by their...
ability to survival in habitats with low light availability and anoxic and reduced sediments. Anoxia tolerance in the temperate seagrass *Zostera marina* involves structural features that provide O$_2$ (Pedersen et al., 1998) and photosynthetically derived carbohydrates to below-ground tissues (root and rhizome) (Zimmerman et al., 1995). Non-structural carbohydrates are stored in the root-system when the carbon balance is positive at high light intensities (Burke et al., 1996), and can be used during energy starvation, e.g. at limiting light conditions (Kraemer and Alberte, 1995; Zimmerman et al., 1995; Burke et al., 1996).

Increased eutrophication of coastal zones often has resulted in reduced light availability for seagrasses by stimulating growth of phytoplanktonic and periphytic algae and fast-growing macroalgae (Vermaat et al., 1997; Hemminga, 1998). As a result of lower light levels in the water column, the daily period of light-saturated photosynthesis decreases, and the light levels below the saturation level become even less favorable for photosynthesis. This shifts the balance between the fixation of inorganic carbon by photosynthesis and the consumption of organic carbon by respiration towards the latter process. Reduced light levels in the coastal zones have caused decline of seagrasses and lowered depth limits, probably due to a negative carbon balance in seagrasses at depth limits (Duarte, 1991; Olesen, 1996; Borum and Sand-Jensen, 1996). The distribution of seagrasses shows large year-to-year fluctuations close to the depth limits indicating that the seagrasses are vulnerable at these depths (Rask et al., 1999). Reduced light availability caused by blooms of phytoplankton may contribute to additional stress on the seagrasses, as the blooms often are followed by a rapid sedimentation of phytoplanktonic detritus. This detritus is a source of labile organic matter in the sediments, compared to seagrass detritus, and may increase the sediment oxygen demand significantly (Borum and Sand-Jensen, 1996). Enhanced growth of macroalgae within the seagrass beds due to increased nutrient availability during eutrophication may also increase the organic matter mineralization and sediment oxygen consumption in the seagrass beds (Krause-Jensen et al., 1996). In the marine environment sulfate reduction is an important mineralization pathway (Jørgensen, 1982), and enhancement of sulfate reduction by input of labile organic matter may result in high sulfide concentrations in the sediments if the iron-buffering capacity is exhausted (Thamdrup et al., 1994). This increases the chances of exposure to stress in the below-ground tissues due to increased concentrations of phytotoxins. The evidence for the toxicity of sulfide to seagrasses is not fully conclusive (Hemminga, 1998; Terrados et al., 2000), but sulfides are known inactivators of metallo-enzymes (e.g. in photosystem II) and stunted roots and blockage of vascular and gas-pathways have been found (Goodman et al., 1995; Armstrong et al., 1996; Fürtig et al., 1996). There is, however, evidence that some seagrasses require sulfides for optimal growth (Pulich, 1989). High levels of porewater sulfides (1–2 mM) have been implicated as a synergistic stressor acting in concert with factors such as hyperthermia, hypersalinity and microbial pathogens to cause mass mortality of the seagrass *Thalassia testudinum* in Florida Bay (Carlson et al., 1994). Sulfides were considered to play at least two roles in *T. testudinum* mortality, a direct chronic toxicity effect on the roots and an acute more severe toxicity effect during the die-off episodes due to degradation of dead roots and rhizomes.

The aim of this study was to investigate the physiological response of *Z. marina* plants to oxygen depletion events with sulfides present in the water column. The effects of low oxygen and increasing sulfide concentrations on the photosynthetic response of *Z. marina*
were tested, as well as the effects on growth and non-structural carbohydrate reserves in below-ground tissues.

2. Materials and methods

2.1. Collection of plants and experimental design

Eelgrass plants (Z. marina) were collected during summer 1997 from a sandy site with a water depth of ~1 m and a sediment organic content of 0.1–0.2% dry wt in the Archipelago of South Fyn, Denmark (55°58’0”N, 10°36’6”E). The plant density at the site was 2380 ± 291 m⁻² (n = 9, ±SEM). Vegetated blocks of sediment were dug up and transferred into buckets (i.d. 21 cm). The transplants were transported to the laboratory growth facilities and kept in 350 l aquaria (six buckets) at in situ salinity (16‰) at 15°C. The irradiance was 400–500 μmol photons m⁻² s⁻¹ and the transplants were kept in a 16 h photoperiod and acclimated for 1–2 weeks before initiation of treatments. The transplants were then placed in gas-tight polyamide/polythene bags (Kruse, 1993) with a final water volume of 25 l, and the concentrations of oxygen and sulfides in the water column were manipulated as described below. The experiment consisted of a control group and three manipulations with two to three eelgrass transplants in each treatment. Control (C): full-oxygenated water column, low oxygen (LO): oxygen concentration <63 μM, no sulfides, low sulfide (LS): no oxygen, sulfide concentration 50–100 μM, high sulfide (HS): no oxygen, sulfide concentration 100–1000 μM. The photon irradiance was above saturation (>125 μmol m⁻² s⁻¹; Denison and Alberte, 1985) in the bags. The eelgrass transplants were incubated for 3 weeks, and five to 10 shoots were sampled every 3–7 days for measurements of photosynthesis and chemical composition of the Z. marina plants. Growth was followed in 10–20 shoots as described below. The concentrations of oxygen and sulfide were kept at the initial concentrations during the incubation period by flushing the water column with N₂ and adding H₂S solution during sampling. Half of the seawater in the bags was renewed at each sampling in order to maintain the concentration of dissolved inorganic carbon higher than 2.5 mM and a pH between 7.6 and 7.8. The transplants were harvested at the end and total above and below-ground biomass was measured in addition to the parameters described above.

2.2. Rates of photosynthesis and respiration in leaves

Photosynthesis and respiration rates were measured by oxygen production or consumption according to McRoy and McMillan (1977) on shoots (n = 5–10) from the transplants. Segments from leaf number 2 (2–3 cm) were placed in closed glass bottles (25 ml) with water sampled from the transplants and exposed to seven irradiances (0–450 μmol photons m⁻² s⁻¹) for 4–6 h. Respiration was measured by incubation in darkness. The concentration of total dissolved inorganic carbon was elevated to about 12 mM to ensure sufficient supply of inorganic carbon during the incubations. Initial and final oxygen concentrations were determined by the Winkler technique. Light-saturated photosynthetic rates (P_max) and the initial slope of the P–I curve (α) were estimated according to Fourqurean and Zieman (1991).
2.3. Growth measurements

Before initiation of the manipulations, plants \((n = 10–20)\) were marked for growth measurements by pushing a sewing needle through all leaves just above the leaf sheath (Zieman, 1974, 1975). Leaf growth was carefully measured in the transplants every 3–7 days as the length of the displacement of the holes in the leaves relative to leaf sheath. At the end of the incubation all plants were harvested. Harvesting was performed 2 h into the light period and the total number of shoots, the total number of leaves per shoot and the leaf lengths and widths were measured. Seagrass material for chemical composition was immediately frozen \((-20^\circ C)\) and analyzed as described below. Dry weights were determined by freeze-drying.

2.4. Chemical composition of eelgrass plants

Leaf chlorophyll concentration was measured spectrophotometrically at 665 nm after 14 h extraction of freeze-dried material with ethanol. The concentration of sucrose and starch in leaves, roots and rhizomes were determined according to Hendry et al. (1993) on freeze-dried material.

3. Results

3.1. Photosynthesis and growth

The eelgrass showed negative photosynthetic response to the LO concentration and presence of dissolved sulfides in the water column. \(P_{\text{max}}\) was reduced by 40% in the LO and LS and 80% in HS after 6 days of treatment (Fig. 1). \(P_{\text{max}}\) remained at the reduced level during the incubation, and for the HS treatment, \(P_{\text{max}}\) decreased further and there was no photosynthetic activity at the end of the incubation period. The light efficiency coefficient

![Fig. 1](image.png)

Fig. 1. Time course of the maximum photosynthetic rate \((P_{\text{max}})\) in the control plants and the three treatments. Symbols represent mean \((\pm \text{range/SEM}, n = 2–3)\).
Table 1
Initial (day 0) and final (day 18–21) values of light efficiency coefficient ($\alpha$) and Chl a content in leaf number 2 in the different treatments. Initial values represent mean ($\pm$SEM, $n = 5$) and final values represent mean ($\pm$range/SEM, $n = 2–3$)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\alpha$ (\mu mol O$_2$ g$^{-1}$ (dry wt) h$^{-1}$ (\mu mol photons m$^{-2}$ s$^{-1}$))</th>
<th>Chl a (mg g$^{-1}$ (dry wt))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.78 $\pm$ 0.06</td>
<td>2.23 $\pm$ 0.13</td>
</tr>
<tr>
<td>Control</td>
<td>0.84 $\pm$ 0.09</td>
<td>2.16 $\pm$ 0.01</td>
</tr>
<tr>
<td>Low oxygen</td>
<td>0.25 $\pm$ 0.03</td>
<td>1.40 $\pm$ 0.02</td>
</tr>
<tr>
<td>Low sulfides</td>
<td>0.56 $\pm$ 0.01</td>
<td>1.60 $\pm$ 0.10</td>
</tr>
<tr>
<td>High sulfides$^a$</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

$^a$ There was no photosynthetic activity in the leaves in the HS treatment at day 18.

($\alpha$) remained at the initial level in the control plants, whereas it decreased by 28–68% in the LO and LS treatments (Table 1).

The rate of leaf elongation increased with approximately 58% in the control plants, whereas it remained at the initial level in the LO plants (13.5 mm m$^{-1}$ d$^{-1}$). The leaf elongation was low from the start in LS (7 mm m$^{-1}$ d$^{-1}$) and decreased further to approximately 5 mm m$^{-1}$ d$^{-1}$ (Fig. 2). The shoot density was higher in LS than the other transplants, which may have caused self-shading and lowered the initial leaf elongation rates. In HS leaf elongation was high initially, but stopped after 2 weeks of exposure. The plants were clearly affected by the HS concentration, as the meristematic regions were damaged and showed signs of degradation with soft spots in the tissue. There were several shoots, which were released from the rhizomes, and the shoot density was reduced by 31% at harvest in one of the transplants, whereas no plants survived in the second transplant (Table 2). Similar signs were found in the other two treatments, although not as extensive, and shoot density only

Fig. 2. Time-dependent changes in leaf elongation rates in the control plants and the three treatments. Mean values ($\pm$SEM, $n = 10–20$).
Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial density (shoots m(^{-2}))</th>
<th>Final density (shoots m(^{-2}))</th>
<th>Final shoot: root ratio</th>
<th>Marked shoots, initial/final (No.)</th>
<th>Initial leaves (No. per shoot)</th>
<th>Final leaves (No. per shoot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2339 ± 16</td>
<td>16</td>
<td>0.51 ± 0.09</td>
<td>9/9</td>
<td>4.9 ± 0.2</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Low oxygen</td>
<td>2069 ± 223</td>
<td>223</td>
<td>0.38 ± 0.06</td>
<td>20/17</td>
<td>4.8 ± 0.1</td>
<td>4.2 ± 0.2(ab)</td>
</tr>
<tr>
<td>Low sulfides</td>
<td>3247 ± 1146</td>
<td>1146</td>
<td>0.25 ± 0.07</td>
<td>17/17</td>
<td>4.2 ± 0.2</td>
<td>3.7 ± 0.2(c)</td>
</tr>
<tr>
<td>High sulfides(d)</td>
<td>2769</td>
<td>1910</td>
<td>0.25</td>
<td>20/9</td>
<td>4.7 ± 0.1</td>
<td>3.2 ± 0.4(b, e)</td>
</tr>
</tbody>
</table>

\(a\) Significantly different mean values between initial and final sampling \((p = 0.05)\).

\(b\) Significantly different mean values between control and treatment \((p = 0.06)\).

\(c\) Significantly different mean values between control and treatment \((p < 0.002)\).

\(d\) Only one transplant as all plants died in the second transplant.

\(e\) Significantly different mean values between initial and final \((p < 0.001)\).

decreased in LO. The shoots were still attached to the rhizomes at the end of the incubation in these treatments, but several shoots were unhealthy with soft spots in the meristematic region. The number of leaves per shoot decreased by up to two leaves in the treatments compared to the controls (Table 2). The total plant biomass varied among the mesocosms as these were collected from a natural eelgrass bed with some spatial variation in plant parameters, and there were no significant difference in total biomass at the end of the experiment (data not shown). The shoot:root ratios were reduced between 31 and 55% in the treatments compared to the controls, indicating that the above-ground biomass was negatively affected by exposure to LO and sulfides (Table 2).

### 3.2. Chemical composition

Chl \(a\) concentrations were measured in leaf number 2 in photosynthetically active plants. The Chl \(a\) concentration in all leaves decreased during the incubation, including the control plants. The most significant decline was found in the LO and LS treatment (Table 1).

The non-structural carbohydrate reserves of the eelgrass plants showed a negative response to the LO and presence of sulfides, as the reserves were almost depleted in the below-ground tissues compared to control plants (Table 3). The sucrose concentration also decreased in the leaves in the LS and HS treatments, most significantly in the HS, where sucrose levels were reduced by 60% during the experiment (Fig. 3). The sucrose content was highly variable in the control and LO treatment but showed a tendency to higher concentrations after 3 weeks incubation. This was not followed by higher pools in the root system at LO, where levels of sucrose in the rhizome and roots were low compared to the control plants. The starch content was also lower in the roots, where pools were reduced by approximately 50% compared to the control plants, whereas the pools were less affected in the rhizomes. There were no major differences in the carbohydrate reserves in the root system between the LO, LS and HS treatments.
Table 3
The concentration of sucrose and starch in the below-ground tissues after 3 weeks of incubation in the control plants and the three treatments. Concentrations are given for rhizomes and roots collected in the 0–6 cm sediment layer. Mean values (±SEM, n = 2–3 transplants with three replicate determinations of each transplant).

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Treatment</th>
<th>Sucrose (mg g⁻¹ (dry wt))</th>
<th>Starch (mg g⁻¹ (dry wt))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizome</td>
<td>Control</td>
<td>20.24 ± 2.24</td>
<td>40.70 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>Low oxygen</td>
<td>3.55 ± 2.45</td>
<td>35.97 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>Low sulfides</td>
<td>4.06 ± 2.34</td>
<td>34.64 ± 5.31</td>
</tr>
<tr>
<td></td>
<td>High sulfides</td>
<td>7.99 ± 4.95</td>
<td>25.33 ± 9.25</td>
</tr>
<tr>
<td>Roots</td>
<td>Control</td>
<td>0.58 ± 0.11</td>
<td>34.13 ± 7.01</td>
</tr>
<tr>
<td></td>
<td>Low oxygen</td>
<td>0.03 ± 0.01</td>
<td>16.25 ± 1.86</td>
</tr>
<tr>
<td></td>
<td>Low sulfides</td>
<td>0.07 ± 0.02</td>
<td>17.96 ± 2.47</td>
</tr>
<tr>
<td></td>
<td>High sulfides</td>
<td>0.06 ± 0.04</td>
<td>14.82 ± 1.45</td>
</tr>
</tbody>
</table>

Fig. 3. Time-dependent changes in the concentration of sucrose in leaf number 2 in the control plants and the three treatments: (A) control and LO; (B) LS and HS. Mean values (±SEM, n = 5–6).

4. Discussion

Eelgrass growth and survival were negatively affected by the poor oxygen conditions and the presence of sulfides in the water column. The effects were more severe when eelgrass
was exposed to sulfides suggesting that the plants were highly susceptible to sulfides. The plants showed negative physiological responses as the photosynthetic activity decreased and the respiration increased. Goodman et al. (1995) also found that rates of photosynthesis decreased with increasing porewater sulfide concentrations. Morphological changes were found in the plants exposed to sulfides as the cells in the meristematic region were in decay. Studies have shown that especially actively growing tissue as meristem or young root cells, are sensitive to anoxia and sulfides and stunted growth and bud death have been found (Armstrong et al., 1996). Greve and Borum (2000) found rotting meristems in *Z. marina* kept under anoxic conditions. The number of leaves per shoot and thus the biomass of individual shoots decreased, and an increasing number of shoots were breaking off between the meristem and the first segment of the rhizomes. This caused a progressive reduction in shoot density during the 3-week exposure, although not as dramatic as observed at field sites (Carlson et al., 1994; Rask et al., 1999). Although most of the plants exposed to sulfides had soft meristems at the end of the experiment, the shoots were still attached to the rhizomes probably because the plants were kept under calm laboratory conditions.

In addition to the physiological and morphological effects of anoxia on the above-ground parts, the lack of oxygen in the water column may affect the conditions in the below-ground tissues. In an oxidized environment seagrasses keep their root system aerobic by transport of oxygen produced from photosynthesis during the day or by passive transport of oxygen from the water column through the aerenchyma to the roots during the night (Pedersen et al., 1998; Greve and Borum, 2000). The roots and the rhizosphere are thus oxic, although the oxic area around the roots is reduced during the night, and the root metabolism and energy production is aerobic under normal conditions. However, if oxygen is depleted in the water column, the roots are then only supplied with oxygen through photosynthetic mediated transport, and function anaerobically to a greater extent, e.g. during low irradiance and at night. In this experiment the exposed plants were probably only able to maintain the roots aerobic during the day by the diffusion of oxygen from photosynthesis, and turned anoxic during the night (Greve and Borum, 2000). The root anoxia period may have been even longer in the exposures to sulfides, as the photosynthetic production of oxygen was reduced compared to the control plants. Anoxic conditions in the root/rhizome system blocks sucrose transport in eelgrass, thereby limiting the sucrose pool present in below-ground tissues to the size at the onset of the anoxia (Zimmerman and Alberte, 1996). There were several signs of inhibited or limited translocation in the exposed plants. The concentration of sucrose in the leaves increased under hypoxia suggesting that there was no translocation of sucrose from leaves to roots. There was no accumulation of sucrose in the leaves of plants exposed to sulfides probably due to low production of sucrose at the reduced photosynthetic activity. The reserves of sucrose were low in the rhizome and roots of the exposed plants suggesting that sucrose was not renewed but consumed during root metabolic processes. The mobilization of starch appeared to be inhibited as the concentration of starch in the rhizomes remained similar to the control plants despite the depletion of the sucrose pools in the roots. The conversion and translocation of starch stored in the rhizomes back into sucrose is an energy demanding process (Bertani et al., 1981; Burke et al., 1996) and may have been limited due to a reduced ATP production in the plants.

The period of root anoxia is also of importance for the concentration of sulfides in the rhizosphere, as there is no release of oxygen from the roots under anoxic conditions (Greve
A lack of oxygen release will probably decrease the reoxidation of sulfides in the rhizosphere significantly. There are alternative ways to remove sulfides from the rhizosphere, e.g. through oxidized iron, but the reoxidation potential is controlled by the availability of oxygen, as the oxidized iron and manganese pools are relatively low and have to be reoxidized by oxygen (Thamdrup et al., 1994). It was visually observed that the plants exposed to LO and sulfides were not able to oxidize the sediments as the sediments were very sulfidic (black and smelly) at the end of the experiment compared to the control mesocosms.

Whereas light availability normally leads to significant changes in morphological parameters related to chloropigment distribution, such as length, width or thickness of seagrass leaves (Duarte, 1991; Olesen and Sand-Jensen, 1993; Vermaat et al., 1997), the reduced oxygen levels and LS concentration only affected the chlorophyll concentration in the leaves. Chlorophyll concentration decreased by 18–35%, and thereby followed the negative trend in photosynthetic capacity of the leaves. The dead leaves in the HS treatment were green as long as they were kept under anoxic and sulfidic conditions, but turned yellow when returned to oxidized conditions. It has been found that the degradation of Chl a is much slower under anoxic conditions (Sun et al., 1991).

The results of this experiment show strong negative effects of water column hypoxia and presence of sulfides on growth and survival of eelgrass. The effects were most severe, when eelgrass was exposed to sulfides, as the plants started to rot in the meristematic region, but also plants exposed to LO concentrations were severely affected with loss of above-ground biomass and depletion of sucrose reserves in the roots. This is similar to observations of seagrass die-back events suggesting that these two factors play important roles possibly acting in concert with reduced light conditions and high water temperatures (Carlson et al., 1994).

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