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Eelgrass (*Zostera marina*) tolerance to anoxia

Cristina Pulido, Jens Borum *

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

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ABSTRACT

The tolerance of eelgrass (*Zostera marina* L.) to anoxia was assessed experimentally to evaluate the potential role of short-term anoxia on eelgrass performance. Eelgrass ramets (terminal leaf bundles with rhizomes and roots) were submerged in anoxic seawater for variable periods of time (0.5 to 48 h) at three temperatures (20, 25 and 30 °C) in darkness. Photosynthetic efficiency (Fv/Fm), leaf growth and shoot mortality were assessed as response parameters.

Photosynthetic efficiency and leaf growth of ramets exposed to anoxia were closely coupled and declined with both increasing exposure time and temperature. At 20 °C, negative effects of anoxia occurred after 12 h (Fv/Fm) and 24 h (leaf growth). Shoot mortality appeared after 24 h. The negative impacts of anoxia on photosynthesis and growth increased markedly with increasing temperature. At 30 °C, Fv/Fm declined after 1 h, leaf growth after 2 h and no plants survived 8 h of anoxia.

The results show that eelgrass is surprisingly intolerant to anoxia and support the hypotheses that anoxia may induce sudden eelgrass die-off and that high temperature strengthens the negative impact of anoxia. The study documents that anoxia in itself may have strong negative impacts on eelgrass performance within ecologically relevant time scales.

1. Introduction

Tissue anoxia, possibly combined with invasion of sulfide from the sediment, has been suggested as a key parameter in events of sudden seagrass mass mortality (Plus et al., 2003; Greve et al., 2003; Borum et al., 2006). The question of what initiates and controls events of seagrass mortality has been addressed in many studies, of which however most have focused on effects of sediment sulfide invading the plants through roots and rhizomes (e.g. Goodman et al., 1995; Holmer and Bondgaard, 2001; Koch et al., 2007). It seems well documented, that tissue anoxia – at least root anoxia – is a prerequisite for sulfide invasion, because sulfide reoxidation within the sediment occurs extremely fast in the presence of oxic microzones around plant roots (Crawford, 1992; Pedersen et al., 2004; Borum et al., 2005). However, it is poorly known under what conditions seagrass tissue anoxia may occur, how often and for how long, and, in addition, seagrass tolerance to tissue anoxia *per se* has not been described. We, therefore, experimentally examined the tolerance to anoxia of the north-temperate seagrass, eelgrass (*Zostera marina* L.), to find out whether plant anoxia in itself affects photosynthesis, growth and survival within ecologically relevant time scales.

Under normal field conditions (high water column oxygen concentrations, moderate temperature and sediments of low organic content and oxygen consumption), internal oxygen concentrations in seagrasses are sufficiently high to support aerobic metabolism within leaves, rhizomes and roots throughout the diel cycle (Pedersen et al., 1998; Greve et al., 2003; Borum et al., 2006). Plant oxygen concentrations may vary considerably on a diel basis (Greve et al., 2003; Borum et al., 2005). In light, photosynthesis builds up internal oxygen pools, and oxygen is readily supplied to rhizomes and roots through the airfilled lacunae, while in darkness, oxygen supply is efficiently maintained by passive diffusion from the water column through leaves to roots (Pedersen et al., 1998; Borum et al., 2006). Seagrasses may, however, experience tissue anoxia under conditions with low water column oxygen and under plant stress at for example high temperature (Greve et al., 2003; Borum et al., 2005; Borum et al., 2006). Such conditions can be created experimentally but are difficult to catch under field conditions and have to our knowledge only been documented directly during a die-off event in the tropical seagrass, *Thalassia testudinum* (Borum et al., 2005). However, Plus et al. (2003) reported widespread eelgrass mortality after a period of water column anoxia, and data on sulfur isotopic composition in eelgrass strongly suggest that temporary tissue anoxia may actually occur quite frequently in eelgrass (Frederiksen et al., 2006).

Sudden seagrass mortality is most often observed during summer periods of high water temperature (Plus et al., 2003; Rask et al., 1999), and high temperature has been shown to induce anoxia in meristematic tissues of eelgrass (Greve et al., 2003). The importance of temperature for internal plant oxygen concentrations may be explained by the stimulating effect of increasing temperature on

* Corresponding author. Tel.: +45 3532 1900; fax: +45 35321901.
E-mail address: JBorum@bio.ku.dk (J. Borum).

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eelgrass respiration and oxygen consumption (Marsh et al., 1986; Zimmerman et al., 1989). Hence, eelgrass tissues should become anoxic faster when exposed to low external oxygen concentrations at high temperature. In addition, high temperature may accelerate anaerobic metabolism and accumulation of potentially toxic metabolites during anoxia.

Tissue anoxia has substantial impacts on plant physiology and performance (Crawford, 1992; Crawford and Braendle, 1996; Drew, 1997). Anaerobic metabolism is 5–10 times less energetically efficient than aerobic metabolism and, therefore, constitutes a strong sink on seagrass carbon reserves (Zimmerman et al., 1989). Anaerobic metabolites, such as ethanol, may be toxic, and anaerobiosis may result in cell acidosis (Drew, 1997). Furthermore, reactive oxygen species and acetaldehyde can be formed upon return to oxic conditions resulting in post-anoxic injuries (Crawford, 1992; Crawford and Braendle, 1996), and in the field, anoxia may lead to invasion of reduced, toxic compounds in addition to sulfide from the sediment (Carlson and Forrest, 1982; Pedersen et al., 2004). All these factors act in concert and are difficult to separate. Our aim here was not to analyze the different physiological injuries but merely to assess the integrated effects of tissue anoxia alone on eelgrass photosynthesis, growth and mortality at different temperatures.

2. Materials and methods

The experimental approach was to expose whole eelgrass ramets (terminal leaf bundles with rhizomes and roots) to variable periods of anoxia in the laboratory and subsequently measure responses in photosynthetic efficiency, leaf growth and shoot mortality at temperature conditions corresponding to normal summer weather (20 °C), warm periods (25 °C) and extremely warm weather (30 °C).

2.1. Plant material

Eelgrass ramets, water and sediment were collected during winter and spring (temperature range 4–12 °C) in Roskilde Fjord, Denmark (55°57′29 N, 11°57′37 E), and transferred to the laboratory where healthy looking ramets consisting of one terminal leaf bundle with a minimum of four undamaged leaves and six root bearing internodes were selected. The ramets were kept submerged in cooled, aerated water in dim light for no longer than 12 h before being used in experiments. The ramets were not temperature acclimated prior to the experiments because growth and survival of eelgrass cannot be maintained in cultures for longer periods at 25 and 30 °C (Evans, 1983; Zimmerman et al., 1989). The experiment at 20 °C was conducted first followed by experiments at 25 and 30 °C, which means that the plants were exposed to similar, sudden temperature increases of 15 to 20 °C in all three experiments.

2.2. Experimental design

To create tissue anoxia, eelgrass shoots were submerged in anoxic seawater continuously bubbled with nitrogen gas for variable periods of time ranging from 0.5 to 48 h. The time taken for plants to become anoxic following transfer from air saturated to anoxic water was assessed in the dark in eelgrass ramets similar to those used in the experiments. Internal oxygen partial pressure in basal meristematic tissues was measured using oxygen microelectrodes with tip diameters of 25 µm (OX25, Unisense, Denmark). When ramets were exposed to oxic water conditions, the oxygen partial pressure remained at 8–9 kPa (corresponding to about 40% of air saturation) at both 20 and 30 °C, but the oxygen partial pressure declined to below detection limit after 20 and 15 min, respectively, when exposed to anoxic water at 20 and 30 °C.

The experiment was conducted in 5 replicated containers consisting of 2 L glass jars placed in a temperature controlled water bath. The plants were kept submerged to avoid direct leaf contact with air. Incubations were run in the dark to avoid photosynthetic oxygen production. Eelgrass ramets incubated under similar conditions but continuously bubbled with atmospheric air functioned as controls. To assess the combined effect of anoxia and temperature the experiments were conducted at 20 °C, 25 °C and 30 °C representing different summer weather conditions.

Sulfide concentrations in the water were measured during the incubations at 25 and 30 °C to assess whether sulfide had unintentionally accumulated and potentially confounded plant responses. Concentrations of total sulfide were analyzed according to Cline (1969).

After exposure to oxic or anoxic conditions for variable periods of time ranging from 0.5 to 48 h, two ramets from each replica were planted in plastic boxes ($18 \times 13 \times 10$ cm) with sandy sediment low in organic matter content (0.28 ± 0.04% of DW (mean ± 95% CL)). The boxes were placed randomly in aquaria (120 L) under continuous irradiance (150 µmol photons m^{-2} s^{-1}, PAR), and the plants were allowed to grow for 96 h. Temperature was kept constant at 20 °C and the water was continuously bubbled with air and stirred by submersible pumps.

2.3. Response parameters

Immediately after exposure, photosynthetic efficiency was measured in dark adapted leaves of between three and five plants from each treatment and time period. We used a photosynthetic efficiency analyzer (PEA MK2 Hansatech, Germany) to measure maximum quantum efficiency of PS II ($Fv/Fm = (Fm - Fo)/Fm$), with Fm being the maximum fluorescence yield (observed when all reaction centres of PSII are "closed"), Fo the initial or minimum fluorescence yield (when all reaction centres of PSII are "open") and Fv, the variable fluorescence yield (Krause and Weis, 1991; Larkum et al., 2006). The ratio Fv/Fm was determined on the outer side of leaf number two (number one being the youngest) which consistently gave the highest and least variable value when measured across control plants. The sampling position was 2–5 cm above the leaf sheath of the second leaf as it has fully developed pigments but low epiphyte cover (Durako and Kunzelman, 2002). The flash produced at 90% of saturating light during 2 s achieved maximum photosynthesis efficiency values.

Leaf growth was measured using the leaf-marking technique described by Sand-Jensen (1975). Before transplanting ramets to boxes with sediment, all leaves were marked with a waterproof felt pen above the leaf sheath of the oldest leaf. Leaf growth was measured after 96 h as the cumulated displacement of the marks on younger leaves relative to the reference mark of the oldest plus visible parts of new leaves.

Shoot mortality occurred in two different forms. Acute mortality was defined as the absence of growth during the incubation in light after exposure to anoxia. However, mortality also occurred during the incubation in light, hereafter called post-mortality. Shoots exhibiting post-mortality were initially able to grow slightly in the light, but growth was low (<25% of control plants). The leaves showed clear visual damage with loss of cell pigments, and the basal meristematic tissue became brownish and degraded at the end of the incubation in light, when leaf bundles also easily detached from the rhizome.

2.4. Statistical analysis

All data were analyzed using non-parametric Kruskal–Wallis tests due to lack of variance homogeneity ($p<0.05$; Bartlett's test). Dunn's post hoc test was used to identify at what exposure time a significant response was observed. The statistics were conducted using Graphpadprism® and all values are presented as means ± SE ($n = 5$).