***In situ* oxygen dynamics in rhizomes of *Posidonia sinuosa* – impact of light, water column oxygen, current speed and wave velocity**

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ABSTRACT

The presence of oxygen in seagrass tissues, which plays a role in preventing seagrass die-off, is partly regulated by environmental conditions. Here, we examined the relationship between oxygen (O_2) in the rhizomes of *Posidonia sinuosa* and key environmental variables at Garden Island, Western Australia. We made *in situ* measurements of internal oxygen partial pressure (pO_2) of rhizomes using fiber optic optodes and compared these to pO_2 in the water column, photosynthetically active radiation (PAR) and wave and current velocities within the seagrass canopy. During daytime, tissue pO_2 was regulated by PAR, whereas in darkness, both near-bed mean current and wave orbital velocities were important in influencing pO_2 . Tissue pO_2 was positively correlated with current speed at night up to a threshold of $\sim 0.045 \text{ m s}^{-1}$, likely because of a reduction in the thickness of the diffusive boundary layer surrounding the plant, allowing for more rapid exchange of O_2 with the surrounding water. The flow velocities in the meadow were generally low and at times near stagnant, and pO_2 in rhizomes declined to critical levels at night. This may explain the lack of recovery of seagrasses in the area despite management efforts that have improved water quality. Our observations of tissue pO_2 in *P. sinuosa* show remarkable similarities to previous laboratory and field studies across a range of seagrass species, suggesting that the relationships to hydrodynamic conditions and light levels that are described here are general across taxa.

KEYWORDS

seagrass, photosynthesis, diurnal, hypoxia, PAR, rhizosphere, currents, waves

INTRODUCTION

The internal oxygen partial pressure (pO_2) in seagrasses depends on a balance between several processes: photosynthesis, respiration, transport from leaves to belowground tissues, and radial oxygen efflux to the sediment (Pedersen et al. 1998; Greve et al. 2003; Borum et al. 2005). During daytime, leaf pO_2 can be as high as 400 hPa, which corresponds to approximately 200 % of air saturation (Greve et al. 2003; Borum et al. 2005). The oxygen produced through photosynthesis is supplied to roots, rhizomes and meristems via gas-filled aerenchyma in leaves, rhizomes and roots that facilitate internal diffusion of oxygen and enables aerobic metabolism in belowground tissues. The presence of aerenchyma is therefore an adaptation to allow seagrasses to grow in anoxic sediment that is typical of seagrass environments (Borum et al. 2005, 2006; Pedersen et al. 1998). Oxygenation of belowground tissues prevents invasion of reduced compounds (e.g. sulfide) from the sediment that can be harmful to seagrass growth and may lead to large-scale losses of seagrass (e.g. Borum et al. 2005).

During the night, oxygen is rapidly lost from plant tissue through respiration and radial oxygen efflux from roots (Borum et al. 2005) and the oxygen present in the aerenchyma is typically unable to sustain aerobic respiration for more than 15 minutes after photosynthesis has ceased (Sand-Jensen et al. 2005). When leaf pO_2 drops below the pO_2 of the water column, the water column can act as a source of oxygen. Oxygen from the surrounding water enters the plant by passive diffusion and the plant pO_2 becomes closely correlated to pO_2 in the water column (Pedersen et al. 2004). The rate of oxygen transported by this process depends on properties of the cell wall and tissue of the plant, but also the oxygen pressure gradient (i.e. the difference between

the internal and external pO_2) and the thickness of the diffusive boundary layer (DBL) that forms along the leaves in water with convective flow (Pedersen et al. 1998; Larkum et al. 1989). Low water column oxygen content can cause internal oxygen stress in the plant and water column hypoxia will eventually lead to tissue anoxia (Greve et al. 2003; Pedersen et al. 2016).

The thickness of the DBLs around seagrass leaves can range from 50 to 1000 μm depending on local flow velocities (Larkum et al. 1989). When flow in a seagrass meadow is weak, a thicker DBL develops and the transport of solutes through diffusion across the DBL may become a limiting factor in plant metabolism (Koch et al. 2006). Flow speed determines the rate of passive gas exchange between plant tissue and the surrounding water and, thereby playing a role in controlling internal tissue pO_2 . Flow rates are particularly important during nighttime, when the plant O_2 becomes depleted and the water column is a source of oxygen to the plant.

Oxygenation of both above- and belowground tissues is closely linked to flow speed, as the O_2 entering the plant is transported through porous lacunae driven by a gradient in pO_2 from leaves to rhizomes and roots (Borum et al. 2006). Laboratory experiments have shown that, in darkness, pO_2 in the rhizomes of *Cymodocea nodosa* increased with the flow rate of the water and was saturated at velocities $\geq 7 \text{ cm s}^{-1}$ (Binzer et al. 2005). Flow speed was more important for tissue aeration at lower water column pO_2 ; rhizomes would become anoxic when the water column was at $\sim 20\%$ of air saturation in flowing water but in stagnant water at $\sim 30\%$ of air saturation. While the flow velocity of water within a seagrass canopy is a key driver of oxygenation of the plant in darkness under laboratory conditions (Binzer et al. 2005), a relationship between

flow rate and internal oxygen dynamics of seagrasses has never been measured in the field.

In the field, flow is made up of two components: 1) a mean current component that can be driven by e.g. tidal variability, wind stresses imposed on the surface of the water and wave forces; and 2) an oscillatory wave component driven by surface waves that may be generated locally by winds or remotely-generated as longer period swell waves. In the absence of waves, the drag imposed by a seagrass canopy attenuates current speed within the canopy (e.g. Nepf 2012a) and the reduced velocity within the canopy promotes development of a relatively thick DBL along the leaves of the plant (Larkum et al. 1989; Nepf 2012b). If the current is sufficiently fast that this attenuation results in an inflection point near the top of the canopy, large vortices can be generated that enable exchange between the top of the canopy and overlying flowing water (Finninigan 2000; Ghisalberti and Nepf 2005, 2006). For current-dominated conditions, oxygen transport from water column to the plant is likely to be directly related to current velocities in the canopy. However, many field sites are located in regions also exposed to wave activity. For these canopies, the inertial forces associated with waves can be similar to, or larger than, drag forces imposed by the canopy (Luhar et al. 2010). Consequently, wave velocities are less attenuated than current velocities of the same magnitude (Lowe et al. 2005). Thus, when the current is low or even absent, waves can drive sufficient water movement within the canopy to thin the DBLs and maintain high rates of oxygen diffusion.

In this study we hypothesized that waves and currents would not significantly affect the pO_2 in the plant during daytime when photosynthesis is the primary source of O_2

and Photosynthetically Active Radiation (PAR) is the main driver. Conversely, at night-time, when the water column is the source of O₂ to the plant, we hypothesized a linear relationship would develop between water pO_2 and tissue pO_2 , with the slope and intercept related to changes in wave and current velocities. To test these hypotheses, we examined the relationship between internal tissue aeration in rhizomes of the seagrass *Posidonia sinuosa* and key environmental variables using *in situ* measurements of pO_2 of the rhizome, pO_2 of the water column, temperature, light availability and the wave and current velocities.

MATERIALS AND METHODS

Study site

In situ measurements were made in a shallow *Posidonia sinuosa* meadow on the Eastern side of Garden Island, Western Australia (32.192°S; 115.682°E; Fig. 1). Six deployments of 18–46 h duration were undertaken in October 2015 (Table 1). The first two deployments were undertaken in a section of the meadow that was 2.6 m deep (Stn 1; Fig. 1b). The subsequent four deployments were undertaken in a nearby section of meadow at 1.6 m depth (Stn 2; Fig. 1b). The tidal range during the experiment varied between 0.3–0.7 m.

Rhizome oxygen measurements

The pO_2 inside the rhizome tissue of *P. sinuosa* was measured *in situ* using oxygen micro-optodes housed inside 500 µm hypodermic needles (OXF500PT; Pyroscience, Aachen, Germany) (e.g. Pedersen et al. 2016). Optodes were calibrated at known temperature in water at air equilibrium and in anoxic water as described by Pedersen et al. (2016). In the field experiment, four optodes were connected to a FireStingO2

(Pyroscience). The oxygen measurements were corrected *in situ* for temperature with a thermocouple connected to a TEX4 temperature module (Pyroscience) placed adjacent (within 25 mm) to the optode tip. Scuba divers located terminal rhizomes of four individual plants by gently wafting the sediment away. Steel stakes were fixed in the sediment adjacent to the apical shoot of each plant and fitted with micromanipulators (MM33; Unisense A/S, Aarhus, Denmark; Fig. 1c). A thermocouple was mounted to the stake next to one of the plants. An optode was then mounted on each micromanipulator allowing full fine-scale control and enabled the tip of the optode to be inserted approximately 2 mm into the rhizome tissue immediately behind the first node of the shoot. The signals from O₂ optodes and thermocouples were logged every minute using a Pyro Oxygen Logger (Pyroscience) and a laptop housed inside a dry-box sitting on top of a scaffold platform located approximately 1.5 m above the mean water level. An operator on the platform monitored the output to ensure a constant signal from each optode was obtained. The correct positioning of the needle inside the rhizome was detected as a distinct dip in pO_2 compared to the surrounding water followed by a smaller increase and stabilising of the signal. The rhizomes and the temperature probes were then gently re-covered in sediment and left to log overnight (typically after a logging period of 18–20 h, except for deployment 6 where measurements lasted 46 h). Despite one or two sensors becoming dislodged overnight, reliable data were obtained from 2–3 individual plants during each deployment.

After each optode deployment, the measured plants including all shoots on the terminal end of the rhizome from the optode insertion point were harvested and brought back to the laboratory. Each rhizome was examined for the probe insertion

point and once located, a cross section was cut by hand and observed under 10 X magnification to confirm penetration depth of the probe (Fig. 1d,e). The number of shoots and leaves per shoot at the terminal end of the insertion point were recorded for each plant. We obtained leaf areas by scanning the leaves and measuring the area using the software WinRhizo 2009 (Regent Instruments Inc).

Environmental parameters

Key environmental parameters were measured at 1-minute intervals at the site during the period of *in situ* O₂ measurements. Photosynthetically active radiation (PAR) was recorded with a HOBO Micro Station logger (H21-002, Onset) at the seabed within the seagrass meadow. Temperature, dissolved oxygen, pH, salinity and water depth were measured by three multi sonde EXO loggers (YSI, Xylem) deployed at three different depths; immediately below the water surface, just above the seagrass canopy and within the seagrass canopy approximately 10 cm above the seabed. Wind speed and direction, air temperature and rainfall were recorded at 1-minute intervals at the Bureau of Meteorology weather station (Garden Island Station #009256), which is situated at 6 m above mean sea level.

Hydrodynamics

Two upward facing Nortek Acoustic Doppler Profilers (ADPs) operating in high-resolution (pulse-coherent) mode were deployed on the seabed within 40 m of Stn 2. For logistic reasons, it was not possible to conduct the measurements closer, but we are confident that the wave and current velocities measured are representative of the conditions where the *p*O₂ measurements were made: The bathymetry was near uniform between the location of the ADPs and Stn 2 and the seagrass meadow

extended well beyond the measuring locations. The flow was, therefore, expected to be fully developed at both locations and the measurements characteristic of the hydrodynamic climate at a canopy scale. Each ADP obtained a continuous 1 Hz vertical current profile that ranged from ~23–92 cm above the bed with a resolution of 30 mm. Three Nortek Acoustic Doppler Vectors (ADV) were co-located with the ADPs and were positioned 23 cm, 46 cm and 80 cm above the bed. The ADVs obtained 34 min sample bursts at 8 Hz each hour. The raw velocity measurements from the ADPs and ADVs were assembled into 15-minute data bursts. Data associated with low signal correlations (<40%) were first removed and only data records where the number of points removed was <50% were retained for further analysis. The data was then subjected to a kernel-based despiking algorithm (Goring and Nikora 2002) to remove velocity spikes (e.g., caused by bubbles or debris in the sample volume). The velocity in each direction was time-averaged to produce velocity ('current') profiles in Cartesian (East-North) coordinates. The ADP profile data was also subjected to a 5th Order Median Filter that removed velocity spikes that differed significantly from the vertically adjacent measurements. For each burst of profile data, the depth averaged current were calculated, which included an above canopy profile, within canopy profile and total water column profile. To calculate the velocity associated with the propagation of locally generated wind waves, the time-averaged velocity profiles in Cartesian coordinates were removed from the cleaned ADV velocity data to produce an oscillatory (wave) velocity time series. Each burst of data was then rotated into a coordinate system defined by the maximum velocity variance and the Root-Mean-Squared (RMS) orbital velocity, which can be interpreted as an average velocity associated with the waves, for each vertical measurement cell was calculated. Noise in the hydrodynamics measurements within the canopy made these

measurements generally unsuitable for wave analysis. However, segments of the time series that were acceptable showed very little attenuation within the canopy when compared to measurements obtained higher in the water column. Thus, we use the measurements obtained at the top of the canopy in this analysis. No hydrodynamic measurements were obtained for the first two O₂ optode deployments (Table 1).

Meadow Characteristics

Shoot density of the seagrass meadow was estimated by counting all shoots within three randomly placed 30 cm x 30 cm quadrats. Canopy height, defined as the 80th percentile of shoot heights within the quadrats (Duarte and Kirkman 2001), was measured at three locations within the meadow. Biomass was estimated from three cores (10 cm diameter) taken to rooting depth from within the meadow. Cores were processed by separating living and dead material and according to tissue type. Dry weights of above and belowground materials were obtained after drying at 60°C for 72 h.

Data analysis

All environmental and plant oxygen data were averaged into 5-minute intervals prior to analysis. As the drivers of plant pO_2 were expected to differ between daytime and night-time, we examined these time periods separately. Daytime was defined as the time approximately one hour after sunrise to one hour before sunset (6:30–17:30) and night-time as the time approximately one hour after sunset to one hour before sunrise (19:30–4:30). To account for the unique pO_2 signal of each plant, which may for example be influenced by differences in shoot size and distance from the measuring

point to the meristem, we calculated “normalised rhizome O₂” by dividing the rhizome *p*O₂ values by the maximum rhizome *p*O₂ measured for that plant. To allow comparison with the hydrodynamic data, we analysed the environmental and plant oxygen data using 15-minute bursts of data. Data were analysed using R version 3.3.2 (R Core Team 2016), and graphed with the ‘ggplot2’ package (Wickham 2009).

RESULTS

Seagrass characteristics

The *Posidonia sinuosa* meadow had a shoot density of 400 ± 88 shoots m⁻² and a canopy height of 35 ± 3 cm. The above-ground biomass was 272.3 ± 32.8 g m⁻² of leaves and 293.6 ± 14.4 g m⁻² sheaths, whereas below-ground biomass was 564.8 ± 92.1 g m⁻² of roots and 449.8 ± 35.1 g m⁻² of rhizomes.

Successful O₂ microelectrode measurements were obtained from 2–3 plants per deployment totalling 13 individual plants of *P. sinuosa*. The measured plants had on average 2.8 ± 0.7 shoots with 1.6 ± 0.1 leaves shoot⁻¹ and a total leaf area of 5528 ± 1737 mm² to the terminal end of the optode insertion point. Cross sections of the harvested rhizomes confirmed that the tips of the optode needles had been inserted into the cortical tissue (Fig. 1).

Environmental and hydrodynamic conditions

Water temperature, pH and salinity were consistent across the experimental period and were very similar between Stn 1 and Stn 2 (Table 1). The mean current speeds above the canopy typically ranged from 0.02 m s⁻¹ to 0.07 m s⁻¹ (average 0.03 m s⁻¹; Fig. S1) with some near still-water (zero flow) conditions also recorded during the

experiment. Within the seagrass canopy, currents were substantially lower than above canopy (average 0.008 m s^{-1}) and for most of the experiment current speed was $<0.01 \text{ m s}^{-1}$ (Fig. S1). The depth-averaged currents were predominantly directed alongshore of Garden Island with an average speed of 0.02 m s^{-1} . Waves at the site were small with Root-Mean-Squared (RMS) wave velocities averaging 0.04 m s^{-1} both within the canopy and in the overlying water column (Fig. S1). However, when the wind was directed onshore, locally-generated wind waves resulted in RMS velocities up to 0.24 m s^{-1} in the overlying water column. There was no difference between daytime and night-time mean current or RMS wave speeds (Fig. S2) and the diurnal mean water flow speed was fairly constant (Fig. S3).

Plant tissue and water column oxygen profiles

The *in situ* measurements of $p\text{O}_2$ in *P. sinuosa* rhizomes revealed typical diel patterns that closely followed levels of PAR (Fig. 2). Rhizome $p\text{O}_2$ increased rapidly after sunrise reaching a peak of 12.2–28.2 kPa around or shortly after solar noon followed by a steady decline that continued through the night. Tissue minima of 0.2–6.4 kPa were observed around dawn and the rhizomes in a few of the plants were hypoxic for several hours during the night (Fig. S4). Assuming a linear decline in rhizome $p\text{O}_2$ after sunset, oxygen would theoretically be depleted after 16–17 h (10 to >24 h) in darkness (Fig. S4).

The water column $p\text{O}_2$ followed diurnal patterns similar to those in the rhizomes, but values were around 10–15 kPa higher (Fig. 2). Oxygen within the seagrass canopy never dropped below 14 kPa and reached a daytime maximum of ~30 kPa. The $p\text{O}_2$ near the surface was typically within 3 kPa of the $p\text{O}_2$ inside the seagrass canopy, but

top-to bottom gradients in the water column were present (Fig. S5). During the night, pO_2 was highest at the surface and lowest at the seagrass canopy level. After sunrise, pO_2 increased most rapidly inside the seagrass canopy quickly reversing this gradient. The canopy showed the steepest decline in pO_2 as light intensity declined in the afternoon and a top-to bottom gradient of decreasing pO_2 developed.

Normalised rhizome pO_2 increased linearly with PAR intensity between sunrise (5:30 am) and noon (Fig. 3a). After this, normalised rhizome pO_2 remained fairly constant until light decreased below $\sim 150 \mu\text{mol m}^{-2} \text{s}^{-1}$ when it declined steeply (Fig. 3b).

Oxygen in the rhizomes of *P. sinuosa* was strongly coupled to pO_2 of the water column and their relationship was linear during both daytime and night-time (Fig. 4). The slope of the relationship during daytime – when plant O_2 is regulated by net photosynthesis (Fig. 4a) – was steeper than during the night-time when the only supply of O_2 to the plant is through diffusion from the water column (Fig. 4b). By extrapolating these relationships, we estimated that, given the average flow conditions during the measuring period, rhizomes of *P. sinuosa* would theoretically reach zero kPa for a water column pO_2 of ~ 14 kPa.

Flow velocity within the canopy had an effect on the internal oxygen concentrations in rhizomes of *P. sinuosa* during night-time (Fig. 5). Rhizome pO_2 and the oxygen ratio (pO_2 rhizome: pO_2 water column) both increased with the speed of flow inside the seagrass canopy until a plateau was reached, beyond which, oxygen levels remained fairly constant at ~ 7.5 kPa (rhizome:water column ratio ~ 0.35). The patterns

were similar for flow associated with the currents, wave RMS velocities, and the combination of currents + waves, and indicated ‘threshold’ speeds of ~ 0.014 , 0.04 and 0.045 m s^{-1} , respectively (Fig. 5). In near stagnant conditions ($\sim 0 \text{ m s}^{-1}$), the mean rhizome $p\text{O}_2$ was $\sim 2.5 \text{ kPa}$ and the rhizome: water column ratio < 0.2 , suggesting that an increase in the ‘total’ water flow to $> 0.045 \text{ m s}^{-1}$ corresponded to a 3-fold increase in internal $p\text{O}_2$ in the rhizome and a 1.5-fold increase in the rhizome:water column O_2 ratio.

DISCUSSION

We assessed diurnal oxygen concentrations in rhizomes of *Posidonia sinuosa* *in situ* using O_2 fibre optic optodes and simultaneously measured, irradiance, water column $p\text{O}_2$ and water flow velocity. Tissue $p\text{O}_2$ displayed a clear diurnal pattern following the ambient light intensity. The effect of PAR on tissue $p\text{O}_2$ overwhelmed any potential effect of water flow speed during daytime. At night, however, flow speed was positively correlated with tissue $p\text{O}_2$ up to a threshold of $\sim 0.045 \text{ m s}^{-1}$ as increasing flow speed reduced the thickness of the DBL thus allowing for more rapid exchange of O_2 between the plant and the surrounding water. While relationships between light and O_2 production are well established, this is the first time the effect of flow speed on seagrass tissue $p\text{O}_2$ has been quantified *in situ*. A handful of studies have measured the relationship of PAR and internal seagrass tissue oxygen dynamics. Despite the studies involving different species, geographic locations, different tissues, and being carried out in the laboratory or *in situ*, we observe adherence between the observations in the present study to patterns previously described, both qualitatively and quantitatively. Below, we discuss our results in relation to observations made for

other seagrass taxa and the implications of the findings for the health of *P. sinuosa* at Garden Island.

Patterns in seagrass tissue pO_2 largely follow diurnal changes in irradiance. Maximum pO_2 is typically reached in early afternoon, when tissue pO_2 sometimes exceeds water column pO_2 , e.g. in meristems of *Zostera marina* (Borum et al. 2006) and *Thalassia testudinum* (Borum et al. 2005). Overnight, tissue pO_2 declines gradually until sunrise and the minimum pO_2 is often close to zero even in meristematic tissue. The differences in the absolute maximum and minimum values depend on ambient light conditions, specific plant metabolic rates as well as sediment oxygen demand. The amplitudes measured in *P. sinuosa* (min = 0.2 – 6.5 kPa; max = 11.8 – 27.8 kPa) were similar to those reported from other seagrass taxa, e.g. meristem of *Thalassia testudinum* 1.1 – 30 kPa (Borum et al. 2005), meristem of *Zostera marina* 2.5 – 37 kPa (Borum et al. 2006), meristem of *Zostera muelleri* 0 – 40 kPa (Brodersen et al. 2017), rhizome of *Zostera marina* 3.1 – 40.8 kPa (Greve et al. 2003), and shoot base of *Thalassia hemprichii* and *Enhalus acoroides* with min pO_2 of 0 and max pO_2 of 32 – 53 kPa and 35 – 51 kPa respectively (Pedersen et al. 2016). Some plants sustain tissue pO_2 above 2 kPa throughout the night, but it is common for tissues to fall below this value for extended periods of time. Seagrass growing in extreme conditions were particularly prone to low pO_2 , e.g. meristems of *Zostera muelleri* measured on a cloudy day under turbid conditions were < 2 kPa for 11 h (Brodersen et al. 2017) and shoot bases of *Thalassia hemprichii* and *Enhalus acoroides* growing under extreme heat stress in shallow tidal pools were < 2 kPa for 5.5 – 12 and 5 – 9.5 h respectively (Pedersen et al. 2016). Some of the rhizomes of *P. sinuosa* in the present study

remained below 2 kPa for up to 11 h demonstrating that they may be particularly vulnerable.

In darkness, the relationship between pO_2 in seagrass tissue to pO_2 of the water column is almost always linear with a slope typically close to 1, but the slope can show considerable individual variation even for the same species at the same site, e.g. 0.39 – 1.1 for *Zostera muelleri* (Brodersen et al. 2017). The majority of slopes are slightly below 1 (Binzer et al. 2005, Borum et al. 2005, 2006, Brodersen et al. 2017), including that of *P. sinuosa* in the present study at 0.83, but can exceed this; e.g. the slope for meristems of *Thalassia testudinum* measured *in situ* at Porjoe Key, Florida was 1.5 (Borum et al. 2005). The projected x-axis intercepts, which correspond to the water column pO_2 where the seagrass tissue theoretically becomes anoxic depend on the balance between supply and consumption, which are influenced by characteristics of the seagrass as well as external factors including temperature and water flow speed (Greve et al. 2003, Pedersen et al. 2004). Intercepts that indicate anoxia in seagrasses span a broad range of 2.5 – 18.4 kPa (Binzer et al. 2005, Borum et al. 2005, 2006, Brodersen et al. 2017), which encompasses the value found for *P. oceanica* in the present study (14 kPa). We initially hypothesized that, during night-time, the rate of flow speed would be related to the slope and intercept of the linear relationship between pO_2 in the tissue to pO_2 of the water column. We were not able to demonstrate this as the slopes encountered were very similar among the different plants measured, possibly due to the limited range of flow speeds encountered at our study site and due to the fact that the mean flow rates were similar among the nights sampled.

Previous studies of O₂ dynamics in seagrasses have demonstrated the dependence of the plant on the water column for night-time O₂ supply (e.g. Greve et al. 2003, Borum et al. 2005). During this time, water flow speed is thought to be critically important for tissue oxygenation as it determines the thickness of the DBL and thus the rate of gas exchange between the water column and the plant. Only one previous study has quantified the relationship between water flow speed and plant *p*O₂ in a controlled laboratory setup (Binzer et al. 2005) and our study is the first to confirm the influence of flow speed *in situ*. There is considerable scatter in the field data, but it is remarkable that the threshold velocity identified at $\sim 0.045 \text{ m s}^{-1}$ corresponds well to the threshold of $0.05 - 0.07 \text{ m s}^{-1}$ found in the laboratory study. Seagrasses with different morphologies, e.g. plants that differ in their above- to belowground biomass ratio, are likely to respond differently to flow speed. Seagrasses with a larger relative surface area for O₂ diffusion above ground compared to belowground biomass, which is associated with respiratory demand are likely to sustain a higher night-time *p*O₂ in the rhizome or meristem for the same water column *p*O₂ and might be less vulnerable to reduced flow conditions. We still lack information to identify general taxonomic and geographic patterns that would enable us to predict which seagrasses are most vulnerable to low water column oxygen and which are most likely to be able to establish and survive in areas of limited flow.

During the night, *p*O₂ in rhizomes of *Posidonia sinuosa* declined rapidly and we estimated the projected oxygen depletion to >12 h, some individual rhizomes in this study did become anoxic after only ~ 10 h. We took our measurements relatively close to the apical leaf meristem where oxygen concentrations are highest (Pedersen et al. 2004; Binzer et al. 2005; Sand-Jensen et al. 2005). As the internal oxygen

concentrations typically follow a gradient of high to low pO_2 from leaves to rhizomes to roots (Borum et al. 2006), more distally located tissues like roots and portions of rhizome further away from the shoot may become hypoxic even sooner. In addition, although rhizome pO_2 remained above 0 at night, tissues may not have had enough oxygen to achieve capacity respiration (Zimmerman et al. 1989). In fact, the night-time patterns observed suggest that the respiratory capacity of the rhizomes might be O_2 -limited throughout the night (Zimmerman et al. 1989). Low oxygen therefore can have a direct impact even in the absence of sulfide intrusion, and anoxia tolerance by belowground tissues is a key trait to the ecological success and survival of seagrasses in anoxic sediments (Pregnall et al. 1984, Smith et al. 1984, Smith et al. 1988, Zimmerman and Alberte 1996).

Seagrasses at many sites in Cockburn Sound, including Garden Island, have experienced limited recovery since large-scale losses took place between 1967 and 1999 (Cambridge & McComb 1984; Kendrick et al. 2002), despite marked improvements in water quality (Mohring & Rule 2013; Fraser et al. 2016). A combination of low tissue pO_2 and intrusion of toxic sulfides is thought to be contributing to this lack of recovery (Fraser & Kendrick 2017). The low rhizome pO_2 observed at night in the present study suggests the seagrass may have limited ability to prevent sulfide intrusion. For another species of seagrass, *Zostera marina*, sulfide intrusion occurred when rhizome pO_2 fell below 7.4 kPa and critical concentrations of H_2S in the plant were observed for $pO_2 < 2.1$ kPa (Pedersen et al. 2004). The pO_2 in rhizomes of *P. sinuosa* at Garden Island regularly declined below these values in our study, in fact, all of the rhizomes had $pO_2 < 7.4$ kPa at the end of the night suggesting they may be vulnerable to sulfide intrusion. Given that just a short period of low pO_2

can be enough for sulfides to enter seagrass tissue and cause dieback (Pedersen et al. 2004), understanding the temporal variability in oxygen dynamics is important to predict the future trajectory of the seagrass meadows at Garden Island. It is likely that the hydrodynamic conditions we observed contribute to the vulnerability of the seagrass as the combined wave and current speed was low inside the *Posidonia sinuosa* meadow.

Conclusions

Our observations of tissue oxygenation in *Posidonia sinuosa* showed remarkable similarities to previous laboratory and field studies across a range of species, suggesting that the relationships to ambient flow conditions and light levels transcend taxonomic boundaries. While relationships between light and oxygenation are well established, the importance of flow rates during night-time has been less explored. This study has, for the first time, demonstrated the effect of flow speed on seagrass tissue pO_2 during night-time in a natural field setting. To better understand the role of water flow rates on seagrass tissue oxygenation in darkness, there is a need to carry out *in situ* measurements across a wider current regime and to broaden the taxonomic scope as, to date, only two species have been tested. This would allow us to assess whether there are general patterns based on morphological or anatomical traits that can enable us to predict which seagrasses are most vulnerable to low water column oxygen and which are most likely to be able to establish and survive in areas of limited flow.

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TABLES

Table 1. Experimental setup and parameters measured during the experiment.

Deployment	1	2	3	4	5	6
Dates (Oct 2015)	12-13	13-14	20-21	21-22	26-27	27-29
Location	Stn 1	Stn 1	Stn 2	Stn 2	Stn 2	Stn 2
Duration (h)	18	13	18	20	19	46
Rhizomes measured (#)	2	2	2	2	3	2
Depth (m)	2.6	2.6	1.6	1.6	1.6	1.6
Temperature (°C)						
air	16.5–29.9	17.4–23.1	13.1–23.3	13.9–24.1	14.4–25.2	16.1–23.5
water	19.5–20.1	19.3–19.9	19.3–20.8	19.7–20.6	19.6–21.1	19.8–20.7
Salinity (ppt)	36.2	36.2	36.3	36.3	36.4	36.4
pH	8.14–8.28	8.15–8.29	8.17–8.38	8.13–8.35	8.20–8.31	8.11–8.35
Wind Speed (km h ⁻¹)						
9 am	13 NE	13 NW	9 S	13 NE	19 ESE	15 NE
3 pm	17 WSW	17 W	26 SW	24 SW	26 SW	24 SW
Hydrodynamic measurements	No	No	Yes	Yes	Yes	Yes

FIGURES

Figure 1. (a) Map of Cockburn Sound indicating the extent of seagrass meadows in green. The study area is shown by the black rectangle. (b) Study area showing the depth contours in meters and the location of the two sampling sites (yellow circles). The other panels show: (c) diver setting up a rhizome measurement, (d) rhizome of *Posidonia sinuosa* showing insertion point of the optode needle, (e) cross-section of *P. sinuosa* rhizome showing the scar from the optode needle, cortex (C), fiber bundles (F) and centre stele (S).

Figure 2. An example from October 27–28th of diurnal cycles from (a) *in situ* pO_2 dynamics in two rhizomes of *Posidonia sinuosa* and the overlying water column and (b) incident light and temperature at canopy height. The dashed line in (a) represents water column-air equilibrium of O_2 (20.6 kPa).

Figure 3. Normalised tissue oxygen in *Posidonia sinuosa* rhizomes as a function of PAR: (a) sunrise (5:30) to noon, and (b) noon to sunset (18:30) for all deployments. The line in (a) is a linear fit with the equation $y = 0.0005x + 0.17$ ($F = 1121$, $p < 0.001$, r^2 adjusted = 0.63) with 95% confidence intervals shown in grey. The fitted line in (b) has the equation $y = 0.94x / (14.3 + x)$ (Pearson correlation coefficient = 0.78).

Figure 4. Measurements of *Posidonia sinuosa* rhizome and water column pO_2 during (a) daytime (6:30–17:30) and (b) night time (19:30–4:30) for all deployments. The transition periods two hours around sunrise and sunset are not included. The linear relationship during daytime (a) is described by the equation $y = 1.3x - 18.7$ ($F =$

2206, $p < 0.001$, r^2 adjusted = 0.64) and during night-time (b) by the equation $y = 0.83x - 11.54$ ($F = 2206$, $p < 0.001$, r^2 adjusted = 0.64). By extrapolating the linear relationships, we estimate that rhizomes would become anoxic at water column pO_2 of 14.4 kPa and 13.9 kPa for daytime and night-time respectively. The dashed lines are 1:1 lines.

Figure 5. The relationship between water flow (measured at the top of the seagrass canopy) and *Posidonia sinuosa* rhizome pO_2 (top) and rhizome:water column O_2 ratio (bottom) during night-time (19:30–4:30) for all deployments at Stn 2. Water flow is shown as current, Root-Mean-Squared (RMS) wave and total (current + RMS wave) flow. The fitted lines are based on locally weighted polynomial regression (LOESS) and the gray area indicates the 95% confidence interval.

FIGURES

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Supplementary Materials

Figure S1. Speed of water flow measured inside the canopy of *Posidonia sinuosa* (left) compared to in the overlying water column (right). Flow speeds are shown as current (a, b), root-mean-squared wave (c, d) and total speed (e, f) during the optode deployments. The dashed lines represent the mean speed, which is also annotated on each panel.

Figure S2. Water flow measured at the top of the canopy of *Posidonia sinuosa* during daytime (6:30–17:30, left) and night time (19:30–4:30, right) of the optode deployments as current (a, b), root-mean-squared wave (c, d) and total speed (e, f). The dashed lines represent the mean speed, which is also annotated on each panel.

Figure S3. Diurnal water flow measured at the top of the canopy of *Posidonia sinuosa* during the optode deployments as current (a), root-mean-squared wave (b) and total speed (c). The shaded area shows the time from sunset (18:30) to sunrise (5:30).

Figure S4. *Posidonia sinuosa* rhizome pO_2 measured from sunset to sunrise. The fitted linear model in panel a) with the equation $y = -0.0065x + 7.2$ ($F = 435$, $p < 0.001$; 95% confidence interval indicated in gray) indicates that rhizome O₂ would be depleted after, on average, 18 h in darkness. Fitted linear models for the individual rhizomes in panel b) suggest that some rhizomes would become depleted much sooner – as early as after 10 h – whereas others would theoretically remain oxygenated for well over 24 h.

Figure S5. Diurnal oxygen profiles measured at three positions in the water column over a *Posidonia sinuosa* meadow. Measurements were made at the top of the water column, immediately above the canopy and within the canopy (approximately 1, 0.3 and 0.1 m above the seafloor respectively). Lines are generalised additive models of measurements from four deployments at Stn 2 (see Table 1 for details). The dashed line represents water column-air equilibrium of O_2 (20.6 kPa). The shaded area is darkness (18:30–5:30) and the unshaded area daylight (5:30–18:30).