

In situ oxygen dynamics in rhizomes of the seagrass *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 fibre 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.

KEY WORDS: Seagrass · Photosynthesis · Diurnal · Hypoxia · PAR · Rhizosphere · Currents · Waves

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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 (Pedersen et al. 1998, Borum et al. 2005, 2006). Oxygenation of belowground tissues prevents invasion of reduced compounds (e.g. sulphide) 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 min 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, 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 2 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 (Finnigan 2000, Ghisalberti & 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 hypothesised 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 nighttime, when the water column is the source of O_2 to the plant, we hypothesised that 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 2 deployments were undertaken in a section of the meadow that was 2.6 m deep (Stn 1; Fig. 1b). The subsequent 4 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 and 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 μ m hypodermic needles (OXF500 PT; Pyrosience; 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, 4 optodes were connected to a FireStingO2 (Pyrosience). The oxygen measurements were corrected *in situ* for temperature with a thermocouple connected to a TEX4 temperature module (Pyrosience) placed adjacent (within 25 mm) to the optode tip. Scuba divers located terminal rhizomes of 4 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; 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 O_2 optodes and thermocouples were logged every minute using a Pyro Oxygen Logger (Pyrosience) 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 that a constant signal from each optode was obtained. The correct positioning of the needle inside the rhizome was

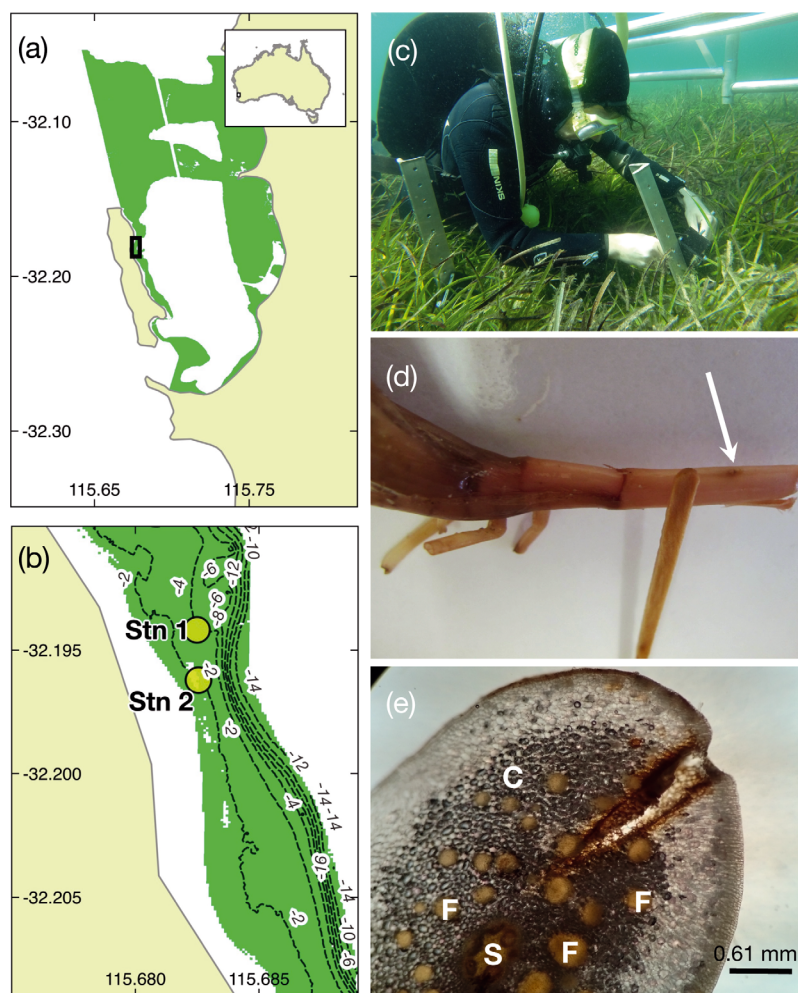


Fig. 1. (a) Cockburn Sound, Western Australia, indicating the extent of seagrass meadows in green. Black rectangle is the study area. (b) Study area showing the depth contours (m) and the location of the 2 sampling sites. (c) Diver setting up a rhizome measurement. (d) Rhizome of *Posidonia sinuosa* showing insertion point of the optode needle. (e) Cross-section of *P. sinuosa* rhizome showing the scar from the optode needle; C: cortex, F: fibre bundles, S: centre stele

Table 1. Experimental setup and parameters measured during the experiment

Deployment	1	2	3	4	5	6
Dates (October 2015)	12–13	13–14	20–21	21–22	26–27	27–29
Location (Stn)	1	1	2	2	2	2
Duration (h)	18	13	18	20	19	46
Rhizomes measured (n)	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), direction						
09:00 h	13 NE	13 NW	9 S	13 NE	19 ESE	15 NE
15:00 h	17 WSW	17 W	26 SW	24 SW	26 SW	24 SW
Hydrodynamic measurements	No	No	Yes	Yes	Yes	Yes

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 for a logging period of 18–20 h, except for deployment 6 where measurements lasted 46 h). Despite 1 or 2 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× 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).

Environmental parameters

Key environmental parameters were measured at 1 min intervals at the site during the period of *in situ* O_2 measurements. 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 3 multisonde EXO

loggers (YSI, Xylem) deployed at 3 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 min intervals at the Bureau of Meteorology weather station (Garden Island Station no. 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 pO_2 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, 46 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 min 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 were then subjected to a kernel-based despiking algorithm (Goring & 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 was 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 hydrodynamic 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 used the measurements obtained at the top of the canopy in this analysis. No hydrodynamic measurements were obtained for the first 2 O₂ optode deployments (Table 1).

Meadow characteristics

Shoot density of the seagrass meadow was estimated by counting all shoots within 3 randomly placed 30 cm × 30 cm quadrats. Canopy height, defined as the 80th percentile of shoot heights within the quadrats (Duarte & Kirkman 2001), was measured at 3 locations within the meadow. Biomass was estimated from 3 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 min intervals prior to analysis. As the drivers of plant *p*O₂ were expected to differ between daytime and night-time, we examined these time periods separately. Daytime was defined as the time approximately 1 h after sunrise to 1 h before sunset (06:30–17:30 h) and night-time as the time approximately 1 h after sunset to 1 h before sunrise (19:30–04:30 h). To account for the unique *p*O₂ 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 each rhizome *p*O₂ value by the maximum *p*O₂ measured for that plant. To allow comparison with the hydrodynamic data, we analysed the environmental and plant oxygen data using 15 min 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 (mean ± SE). 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 (mean ± SE).

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 (mean ± SE) 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 to 0.07 m s⁻¹ (average 0.03 m s⁻¹; Fig. S1 in the Supplement at www.int-res.com/articles/supp/m590p067_supp.pdf) 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 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 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 *pO*₂ in *P. sinuosa* rhizomes revealed typical diel patterns that closely followed levels of PAR (Fig. 2). Rhizome *pO*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 *pO*₂ after sunset, oxygen would theoretically be depleted after 16–17 h (10 to >24 h) in darkness (Fig. S4).

The water column *pO*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 *pO*₂ near the surface was typically within 3 kPa of the *pO*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 (05:30 h) and noon (Fig. 3a). After this, normalised rhizome *pO*₂ remained fairly constant until light decreased below ~150 μmol m⁻² 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 O₂ is regulated by net photosynthesis (Fig. 4a)—was steeper than during the night-time when the only supply of O₂ to the plant is

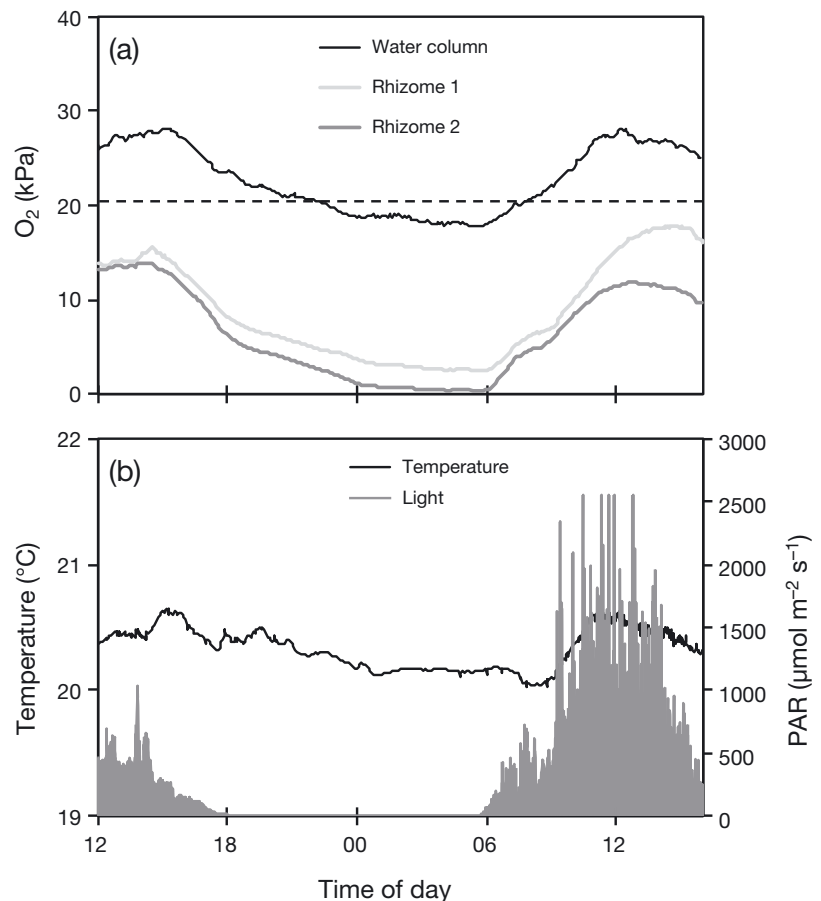


Fig. 2. Example from 27–28 October of diurnal cycles from (a) *in situ* *pO*₂ dynamics in 2 rhizomes of *Posidonia sinuosa* and the overlying water column and (b) incident light and temperature at canopy height. The dashed line in (a) represents water column–air equilibrium of O₂ (20.6 kPa); PAR: photosynthetically active radiation

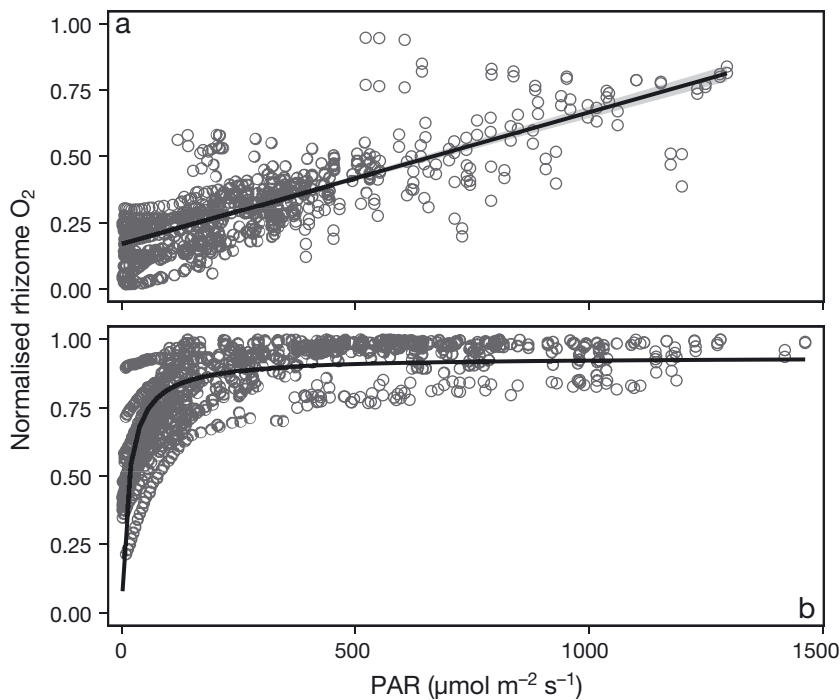
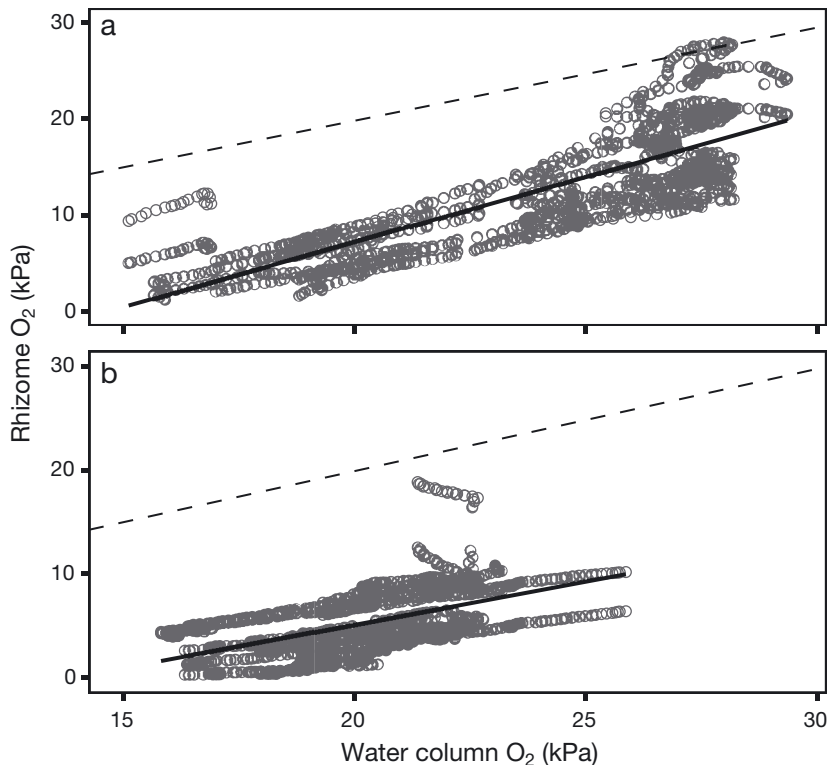


Fig. 3. Normalised tissue oxygen in *Posidonia sinuosa* rhizomes as a function of PAR: (a) sunrise (05:30 h) to noon, and (b) noon to sunset (18:30 h) for all deployments. The line in (a) is a linear fit with the equation $y = 0.0005x + 0.17$ ($F = 1121$, $p < 0.001$, r^2 adj = 0.63) with 95% CI in grey. The fitted line in (b) has the equation $y = 0.94x / (14.3 + x)$ (Pearson corr. coeff. = 0.78)



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 0 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 (~ 0 $m\ s^{-1}$), the mean rhizome pO_2 was ~ 2.5 kPa and the rhizome:water column ratio was < 0.2 , suggesting that an increase in the 'total' water flow to > 0.045 $m\ s^{-1}$ corresponded to a 3-fold increase in internal pO_2 in the rhizome and a 1.5-fold increase in the rhizome:water column O_2 ratio.

DISCUSSION

We assessed *in situ* diurnal oxygen concentrations in rhizomes of *Posidonia sinuosa* using O_2 fibre optic

Fig. 4. Measurements of *Posidonia sinuosa* rhizome pO_2 during (a) daytime (06:30–17:30 h) and (b) night-time (19:30–04:30 h) for all deployments. The transition periods 2 h 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 adj = 0.64) and during night-time (b) by the equation $y = 0.83x - 11.54$ ($F = 2206$, $p < 0.001$, r^2 adj = 0.64). The dashed lines are 1:1 lines

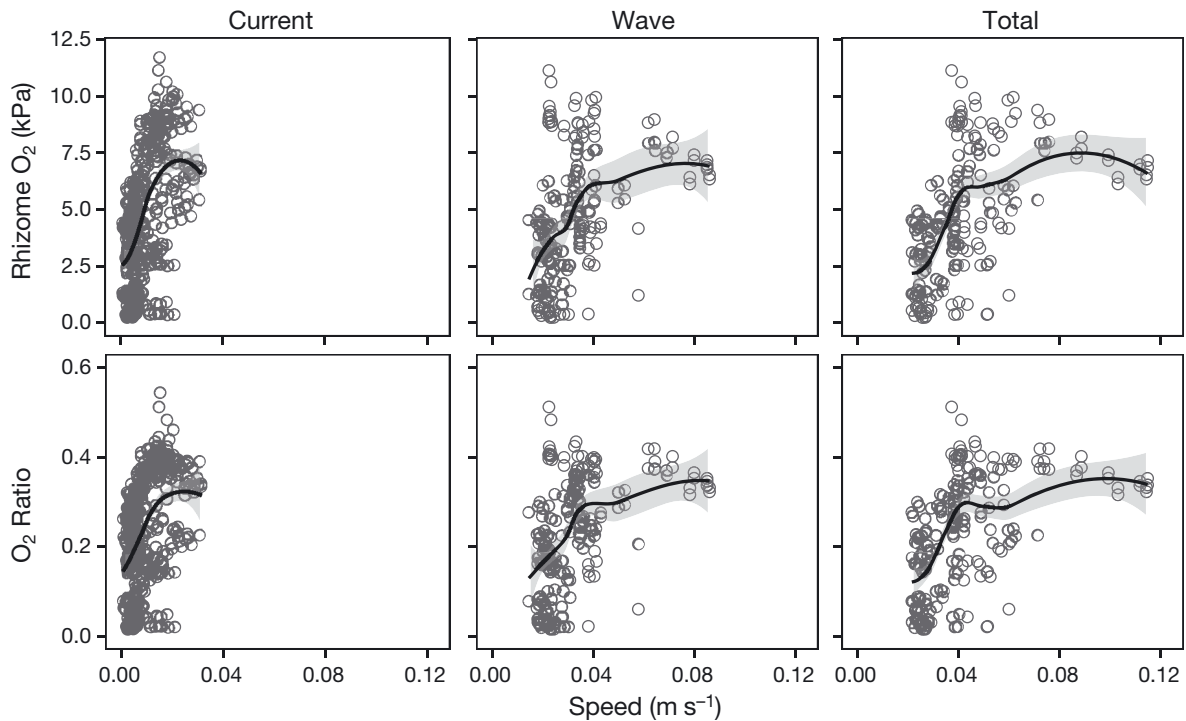


Fig. 5. Relationship between water flow (measured at the top of the seagrass canopy) and *Posidonia sinuosa* rhizome pO_2 (top row) and rhizome:water column O_2 ratio (bottom row) during night-time (19:30–04:30 h) for all deployments at Stn 2. Water flow is shown as current, root mean squared (RMS) wave and total (current + RMS wave) flow. The fitted lines are based on locally weighted polynomial regression (LOESS), and the grey area indicates the 95% CI

optodes and simultaneously measured irradiance, water column pO_2 and water flow velocity. Tissue pO_2 displayed a clear diurnal pattern following the ambient light intensity. The effect of PAR on tissue pO_2 overwhelmed any potential effect of water flow speed during daytime. At night, however, flow speed was positively correlated with tissue pO_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 pO_2 has been quantified *in situ*. A handful of studies have measured the relationship of PAR and internal seagrass tissue oxygen dynamics (e.g. Greve et al. 2003, Borum et al. 2005, Pedersen et al. 2016). Despite the studies involving different species, geographic locations, different tissues, and being carried out in the laboratory or *in situ*, we found 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 0 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 *T. testudinum* 1.1–30 kPa (Borum et al. 2005), meristem of *Z. marina* 2.5–37 kPa (Borum et al. 2006), meristem of *Z. muelleri* 0–40 kPa (Brodersen et al. 2017), rhizome of *Z. marina* 3.1–40.8 kPa (Greve et al. 2003) and shoot base of *T. 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. Seagrasses growing in extreme conditions are particularly prone to low pO_2 , e.g. meri-

stems of *Z. muelleri* measured on a cloudy day under turbid conditions were <2 kPa for 11 h (Brodersen et al. 2017) and shoot bases of *T. hemprichii* and *E. 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 *Z. 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 *T. testudinum* measured *in situ* at Porjoe Key, Florida (USA), 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. sinuosa* in the present study (14 kPa). We initially hypothesised 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 O_2 dynamics in seagrasses have demonstrated the dependence of the plant on the water column for night-time O_2 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 pO_2 in a controlled laboratory setup (Binzer et al. 2005), and our study is the first to con-

firm 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 $0.05\text{--}0.07 \text{ m s}^{-1}$ 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 O_2 diffusion aboveground compared to belowground biomass, which is associated with respiratory demand, are likely to sustain a higher night-time pO_2 in the rhizome or meristem for the same water column pO_2 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, pO_2 in rhizomes of *P. sinuosa* declined rapidly and we estimated the projected oxygen depletion to >12 h, although 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 sulphide 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, 1988, Zimmerman & 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 sulphides 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 sulphide intrusion. For another species of seagrass, *Z. marina*, sulphide 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 sulphide intrusion. Given that just a short period of low pO_2 can be enough for sulphides 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 *P. 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 2 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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