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Published in:
New Phytologist

DOI:
10.1111/nph.13900

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Heat stress of two tropical seagrass species during low tides – impact on underwater net photosynthesis, dark respiration and diel in situ internal aeration

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Summary

- Seagrasses grow submerged in aerated seawater but often in low O2 sediments. Elevated temperatures and low O2 are stress factors.
- Internal aeration was measured in two tropical seagrasses, Thalassia hemprichii and Enhalus acoroides, growing with extreme tides and diel temperature amplitudes. Temperature effects on net photosynthesis (PN) and dark respiration (RD) of leaves were evaluated.
- Daytime low tide was characterized by high pO2 (54 kPa), pH (8.8) and temperature (38°C) in shallow pools. As PN was maximum at 33°C (9.1 and 7.2 μmol O2 m−2 s−1 in T. hemprichii and E. acoroides, respectively), the high temperatures and reduced CO2 would have diminished PN, whereas RD increased (Q10 of 2.0–2.7) above that at 33°C (0.45 and 0.33 μmol O2 m−2 s−1, respectively). During night-time low tides, O2 declined resulting in shoot base anoxia in both species, but incoming water containing c. 20 kPa O2 relieved the anoxia. Shoots exposed to 40°C for 4 h showed recovery of PN and RD, whereas 45°C resulted in leaf damage.
- These seagrasses are ‘living near the edge’, tolerant of current diel O2 and temperature extremes, but if temperatures rise both species may be threatened in this habitat.

Introduction

Completely submerged plants rely on gas exchange with the surrounding water and flooded sediments are often anoxic only a few millimetres below the surface but can contain elevated CO2 (e.g. Ponnamperuma, 1984; Gundersen & Jorgensen, 1990). During the day, submerged plants can sustain aerobic respiration of belowground tissues by photosynthetically derived O2 produced in the shoots (e.g. Pedersen et al., 1995, 1998) moving to belowground tissues via gas-filled aerenchyma. This gas phase diffusion is 10 000-fold faster than diffusion in water (Armstrong, 1979). However, during the night, the only source of O2 available to respiration is the O2 dissolved in the water that enters the shoot and diffuses along the concentration gradient into, and along, aerenchyma of the leaf bases, rhizomes and roots buried in anoxic sediments (Pedersen et al., 2006; Holmer et al., 2009); O2 present in the aerenchyma at sunset is typically unable to sustain respiration for > 15 min (Sand-Jensen et al., 2005). Therefore, night-time respiration can only be sustained as long as the pool of dissolved O2 in the water remains, and declining levels of dissolved O2 resulting in water column hypoxia (levels of O2 limiting aerobic respiration) will eventually lead to tissue anoxia (zero O2) (Greve et al., 2003; Borum et al., 2005).

Seagrasses typically grow permanently submerged in seawater and a large volume of water with dissolved O2 normally prevents severe hypoxia in the water column (Borum et al., 2006). However, elevated temperature (increasing O2 demand of seagrass tissues, macroalgae and microorganisms) or low water volume (high shoot biomass per unit of water volume) or a combination of both, can lead to critically low O2 concentration in the water column and thus also in seagrass tissues (Greve et al., 2003). Low tides can sometimes result in low volume of water, and if low tide occurs during the night in systems with seagrasses forming dense canopies, the large respiring biomass in addition to sediment O2 consumption may deplete the water of O2 and result in severe hypoxia in rhizomes and roots. By contrast, when the low tide occurs during the day, dissolved O2 can increase owing to the large photosynthetic biomass but the solar radiation may soon heat up the shallow water resulting in reduced net photosynthesis (PN) if heat stress occurs, and possibly also increased photorespiration (Beer, 1989) because the O2:CO2 ratio increases when CO2 is depleted and O2 is produced.

Species distribution of seagrasses among temperate and tropical seas is controlled primarily by temperature (McMillan, 1984). This is reflected in tropical seagrass species having higher temperature optima for photosynthesis of 30°C (25–35) than for...
temperate species at 23°C (16–35) (median, with range in brackets) (data extracted from Lee et al., 2007). The response of $P_N$ to temperature can often be described using a Gaussian model (Staehr & Borum, 2011) with a clear temperature optimum. By contrast, dark respiration ($R_D$) normally increases exponentially (Gifford, 2003) with increasing temperature up until $R_D$ rapidly declines to zero when, at a critical temperature, the enzymes denaturate (e.g. for terrestrial leaves; Atkin & Tjoelker, 2003; O’Sullivan et al., 2013). Plants may survive periods where $R_D$ exceeds $P_N$ on a diel basis, whereas growth and long-term persistence in the environment requires a net production of carbohydrates. Moreover, when heat rises to levels resulting in permanent damage to cells of leaves, the critical threshold temperature for photosynthesis is reached before that of respiration (e.g. Phaseolus vulgaris, Hûve et al., 2011; Eucalyptus pauciflora, O’Sullivan et al., 2013). Improved understanding of the temperature responses of $P_N$ and $R_D$ in plants is of importance for refinement of global climate models (cf. O’Sullivan et al., 2013) and for predicting possible responses of species and communities to changes in climate, including extreme temperature events (e.g. marine heat waves; Wernberg et al., 2013; Fraser et al., 2014).

Reefs in the Kimberley region of Australia have the largest tides of any tropical location in the world (up to 11 m) with significant time for some components of the benthic communities to be partially or fully exposed to the air (c. 3 h; Rossé & Veron, 2011). Seagrasses living in the intertidal zone behind reef crests may be partially air exposed in ponded water twice daily, conditions that heavily influence their survival and growth (Short & Neckles, 1999; Collier & Waycott, 2014). Diurnally, they are exposed to extreme light levels, temperatures, $O_2$ concentrations and changes in pH that can inhibit photosynthesis and ultimately cause leaf tissue photodamage (Ralph, 1998; Beer et al., 2006), mortality and seagrass die-off (Erftemeijer & Herman, 1994; Stapel et al., 1997). Climate change projections for the Kimberley region are for increases in air temperatures by 2–3°C and 2–5% loss in rainfall by 2050, as well as 2.2–4.0°C increases in mean sea surface temperatures (Moise et al., 2015) and these changes will exacerbate heat stress and threaten the future persistence of intertidal communities.

In the field, temperatures above 40°C may occur in the shallow water during spring tides (McMillan, 1984) and some tropical seagrass species allegedly are able to tolerate short-term water temperatures of up to 42°C (Collier & Waycott, 2014). For example, species of Halodule and Halophila have been observed to survive short-term exposure to 42°C in the shallow waters of Fiji and Micronesia (McMillan, 1984). More prolonged exposure to elevated temperatures, however, eventually results in mortality but Halodule wrightii and Enhalus acoroides both survived 120 h of exposure to 39°C, whereas Thalassia hemprichii died after only 48 h of exposure to 39°C (McMillan, 1984).

In the present study, we measured internal tissue aeration in two tropical seagrass species, T. hemprichii and E. acoroides, that co-dominate at the field location, along with key environmental variables in a tropical shallow tidal seagrass meadow characterized by large tides and substantial diel temperature and $O_2$ amplitudes. We hypothesized that low tide during the night would lead to water column hypoxia and tissue anoxia, whereas with low tide during the day we expected tissues to remain aerobic even if heat stress resulted in declining $P_N$ in leaves (and increasing $R_D$ in the nonphotosynthetic tissues) as the water temperature increased. In laboratory experiments, we tested how temperature influenced $P_N$ and $R_D$ of leaves of the two species, enabling us to compare their physiology. We hypothesized that the temperature responses for leaves of T. hemprichii and E. acoroides would reveal greater sensitivity to heat stress of $P_N$ than of $R_D$ both in terms of rates of these metabolic processes at high temperatures and the capacity for recovery following heat stress. Our results highlight the similar tolerances to high temperatures of the two species and the threat of heat stress to seagrasses in shallow pools, as exemplified by these reef flats with seagrasses ‘living near the edge’ of their physiological tolerances.

**Materials and Methods**

**Plant material and the environment**

The Kimberley region of Australia is macro-tidal, semi-diurnal tidal fluctuations range (over a spring–neap cycle) typically from c. 4 to 10 m and reach up to 11 m; the largest depth range of any tropical region of the world (Holloway, 1983). The study location was an extensive high intertidal platform and lagoon behind a 2-m high coralline algal terrace on Tallon (Jalan) Island (16°24.132'S, 123°08.374'E) in the Sunday Island group (Richards & O’Leary, 2015). The reef platform is wide (c. 1.4 km) and sits above mean sea level, offshore water levels during low tide can fall up to 4 m below the platform whereas on the platform tidal range is attenuated to mean sea level (Lowe et al., 2015). The seagrasses Thalassia hemprichii (Ehrenb.) Asch. and Enhalus acoroides L.C. Rich ex Steud were abundant as mixed species beds near mangroves that fringed the shore.

Shoot density, above- and belowground biomass and productivity were measured in April/May (end of wet season) and October/November (end of dry season) over three calendar years. At each time, three quadrats 20 cm × 20 cm were measured. Within each quadrat, the numbers of shoots were counted to determine shoot density; all shoots were marked for leaf productivity using a standard hole-punching technique (Short & Duarte, 2001). After 4 d, the quadrats were sampled by removal of all plants. The distances of the hole-punch marks above leaf sheaths were measured and converted into productivity per day. Then, seagrasses were sorted into above- (leaf blades and sheaths) and belowground (roots and rhizomes) components, oven-dried at 60°C and weighed to obtain dry mass (DM). Data of each season were pooled together from 2013, 2014 and 2015. Water temperature was logged at 30 s intervals during each of the sampling times using a CTD logger (RBRconcerto; RBR Ltd, Ontario, Canada). Data are shown in Supporting Information Table S1.

**Internal aeration**

The shoot base $P_O_2$ of T. hemprichii and E. acoroides was measured *in situ* in a seagrass meadow on the reef using the approach...
of, for example, Greve et al. (2003), Borum et al. (2005) and Holmer et al. (2009). Mini O₂ optodes built into 500-μm syringes (OXF500PT; Pyroscience, Aachen, Germany) were used to measure tissue pO₂. Key environmental parameters (water column O₂, pH, temperature and depth) were monitored using a multi sonde (EXO1 Water Quality Sonde, YSI, Yellow Springs, OH, USA) or in the case of light, small light loggers (HOBO Pendant; Onset, Bourne, MA, USA). O₂ probes were calibrated at known temperature in water at air equilibrium (20.6 kPa O₂) and in anoxic water (0 kPa O₂) containing 100 mol m⁻³ sodium ascorbate and 100 mol m⁻³ NaOH. Temperature correction of the readings was achieved using thermocouples placed within 10 mm of each optode (described below).

In the field, an eye-dropper pipette with seawater was used to gently excavate the basal part of the vertical shoot. Steel stands were fixed in the sediment adjacent to each plant measured and mounted with micromanipulators (MM33; Unisense A/S, Aarhus, Denmark). The optode was mounted on the micromanipulator enabling full control of the optode in the x, y and z plane with a resolution of 0.050 mm in the x and y plane and 0.010 mm in the z plane (the plane that controls the position relative to the tissue surface). The tip of the O₂ optode (detection limit and thus criterion for apparent anoxia is 0.020 kPa) was inserted c. 1 mm into the white chlorophyllous part of the shoot base. The surface of the tissue was detected as a slight increase in pO₂ compared to the surrounding water (Borum et al., 2005). The excavation was refilled with sediment after positioning of the optode was complete to enable re-establishment of the biogeochemical profiles of the sediment (Pedersen et al., 2004). The vertical level of insertion into tissues was approximately at the sediment surface but varied from replicate to replicate depending on where the white shoot base started. Four optodes with 4-m-long cables (connected to a 4-channel FireStingO₂; Pyroscience) with individual temperature correction (thermocouple placed within 10 mm of the optode) using a 4-channel temperature module (TeX4; Pyroscience) allowed for four simultaneous measurements so at each measurement set we positioned two optodes in two T. hemprichii shoots and two in E. acoroides shoots. On one occasion, one of the four sensors was lost due to misalignment of the optode during the 21 h of continuous measurements possibly caused by disturbance from crustaceans, fish or water birds foraging inside the research area. Typically, it took 2 h to mount optodes and thermocouples in four plants during low tide in the early morning hours and so, the in situ pO₂ measurements cover the periods from 11:00 h in the morning until 09:00 h the following morning. The signals from O₂ optodes and thermocouples were logged every minute using a Pyro Oxygen Logger (Pyroscience) and a standard laptop computer within a ‘dry-box’ placed c. 2.5 m above the sediment on a 4-m-tall scaffold with platform.

Water depth and water column pO₂, temperature and pH were monitored 5 cm above the sediment surface using the Water Quality Sonde (details given above) programmed to log data with 1-min intervals. Light 5 cm above the sediment was also logged with 1-min intervals using the pendant loggers that measure light (luminous flux per unit area, lux) and temperature. The luminous flux per unit area was converted into photon flux density (PFD) using a conversion factor of 0.0185 μmol photons m⁻² s⁻¹ per lux valid for sunlight (Apogee Instruments, Logan, UT, USA); unconverted values of luminous flux are provided in Fig. S1.

Underwater net photosynthesis and dark respiration

Underwater net photosynthesis (Pₐ) and dark respiration (Rₐ) of leaf (lamina) segments were measured in a laboratory located c. 10 km from the field site using freshly collected shoots (transported back to the laboratory in darkness at c. 25°C) and the approach described in Pedersen et al. (2013). In this approach, tissue samples are incubated in a defined medium (see later in this section) for a known time in closed transparent vials with gentle mixing and held at a constant temperature (with a light source or in darkness, as appropriate), after which the O₂ evolution (Pₐ) or consumption (Rₐ) by the tissue is measured. Five replicate leaves (youngest fully expanded from five different plants) were taken from each of the two species. Five- (Pₐ) to 10- (Rₐ) mm-long leaf segments (projected area of c. 30–150 mm²) were excised from the middle third of the lamina. Underwater Pₐ and Rₐ (n = 5) were measured at 25–50°C with 5°C intervals using 25-ml glass vials with two glass beads added to ensure mixing as the vials rotated according to the method of Pedersen et al. (2013) with photosynthetically active radiation (PAR) inside the vials of 760 ± 76 μmol photons m⁻² s⁻¹ (mean ± SE, n = 4) provided from two horizontally-positioned metal halide lamps (BT28 Metalarc Metal Halide M/MS 250W; Sylvania, Danvers, MA, USA). The incubation medium was 0.7-μm-filtered seawater with a salinity of 35%o with initial pO₂ near air-equilibrium. Vials without leaf segments served as blanks.

Following incubations of known duration (90–150 min), the dissolved O₂ concentration in each vial was measured using an O₂ mini-electrode (OX-500; Unisense A/S, Aarhus, Denmark) connected to a 1 channel picoampere meter (Oxymeter; Unisense A/S). The electrode was calibrated as described above for the O₂ optodes used in the in situ measurements of O₂ dynamics. Projected area was measured for each individual leaf segment using digital photos and subsequent image analysis in IMAGE (Schneider et al., 2012). Samples were then initially sun-dried and subsequently oven-dried and DM recorded. A relationship between DM and area enabled calculation of specific leaf area (SLA, m² kg⁻¹ DM) based on one-sided area.

Recovery of Pₐ and Rₐ was tested following exposure of whole shoots to elevated temperatures in laboratory incubations. Intact shoots with roots and horizontal rhizomes were collected and were incubated in seawater in the light (760 μmol photons m⁻² s⁻¹, PAR) for 4 h at 35, 40 or 45°C, n = 5 for each treatment. The shoots were left in darkness overnight in seawater at 25°C for 12–16 h and Pₐ and Rₐ of all treatments were then measured the next morning at 35°C (close to the optimum temperature for Pₐ, see the section Data Analysis) on leaf segments following the procedure described above in this section.
Use of bicarbonate as source of inorganic carbon

Long-term incubations were used to test how far \( R_N \) of *T. hemprichii* and *E. acoroides* could extract dissolved inorganic carbon; that is, to deplete \( \mathrm{CO}_2 \) and \( \mathrm{HCO}_3^- \) and drive up pH and thus referred to as the ‘pH–drift approach’ (Sand-Jensen et al., 1992; Pedersen et al., 2013). The end pH can be used as a crude diagnostic tool for \( \mathrm{HCO}_3^- \) use so that a final pH below 9.0 indicates that only \( \mathrm{CO}_2 \) is used, whereas a pH above 9.0 indicates that concentrations of free \( \mathrm{CO}_2 \) are below 1 mmol m\(^{-3}\) (Borum et al., 2016) and that acquisition of inorganic carbon must be based primarily on \( \mathrm{HCO}_3^- \) use (Allen & Spence, 1981; Maberly, 1990). Incubation vials were prepared to have approximately equal amounts of tissues (1 leaf segment 30 mm in length) and to minimize \( \mathrm{O}_2 \) build-up in the water and the risk of photorespiration during extended incubation, a headspace of air was left in the vials to enable escape of \( \mathrm{O}_2 \) into the gas phase (one volume of gas can hold 52-fold more \( \mathrm{O}_2 \) than 35\% \( \mathrm{CO}_2 \) seawater at 35\°C resulting in lower \( \rho_{\mathrm{O}_2} \) in the liquid phase than without the headspace). After incubation in the light (PAR = 760 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) for 18 h at 35\°C, pH was measured directly in the vials using a calibrated flat membrane pH electrode (Lot403; Mettler Toledo, Columbus, OH, USA).

External conversion of \( \mathrm{HCO}_3^- \) into \( \mathrm{CO}_2 \) can be achieved by proton pumps acidifying the cell wall spaces and diffusive boundary layer (DBL) resulting in conversion of \( \mathrm{HCO}_3^- \) into \( \mathrm{CO}_2 \) (Prins et al., 1980). We used a TRIS buffer (Tris [hydroxymethyl]aminomethane) to reduce acidification of the extracellular spaces and DBL and thereby effectively inhibiting the influence of proton pumps (Beer et al., 2002). Rates of \( R_N \) of leaf segments incubated in filtered seawater with TRIS buffer (final concentration = 50 mmol m\(^{-3}\) and adjusted to pH 8.0, the equilibrium pH of air-bubbled seawater at 35\°C) were compared to rates obtained in filtered seawater without TRIS using the approach described in the section ‘Underwater net photosynthesis and dark respiration’ but with \( n = 4 \).

Tissue chlorophyll and nitrogen concentrations

The youngest fully expanded leaf was sampled from each species \( (n = 5) \), immediately frozen at \(-18\°C \) and brought back to the University of Western Australia (UWA). Tissue samples were freeze-dried until constant weight and ground to a fine powder in a ball mill. Tissue powders were extracted in 100% methanol at 4\°C in darkness, centrifuged and the absorbance of the supernatant was measured at 665 nm on a spectrophotometer (Model 1601; Shimadzu, Japan). Chlorophyll \(_a\) (Chl\(_a\)) concentrations were calculated using the equations determined for 100% methanol by Wellburn (1994). Tissue total N was measured on a CHN analyser-mass spectrometer consisting of a 20/22 mass spectrometer connected to an ANCA-S1 preparation system (Sercon, Crewe, UK) at the Western Australian Biogeochemistry Centre at UWA. For detailed procedures, see Fraser et al. (2012).

Data analysis

\( Q_{10} \) was estimated from a standard exponential model \( (R_0 = a \times e^{B \times Temp}) \) using the approach of Lloyd & Taylor (1994), where \( a \) is a constant and \( B \) is the exponential quotient; \( B \) relates to \( Q_{10} \) as \( \log(Q_{10})/10 \); statistics on each variable were calculated using the nonlinear regression module in GraphPad PRISM 6 (Motulsky, 2014). The optimum temperature \( (T_{opt}) \) and the maximum net photosynthesis \( (P_{max}) \) were estimated from a standard Gaussian model \( (\max) \) available as a built-in model \( (Y = \text{Amplitude} \times e^{(0.5 \times (\text{Temp} – \text{Mean}/\text{SD})^2)}) \) in GraphPad PRISM 6 (Motulsky, 2014). In the case of data in Figs 3 and 4 (see later), the Sidak test was used as post hoc test.

Results

**In situ internal aeration and key environmental parameters**

The *in situ* measurements of internal aeration and key environmental parameters revealed large diel fluctuations as influenced by time of day as well as tide. In general, the low volume of water at low tide resulted in huge amplitudes in water column \( \rho_{\mathrm{O}_2} \), pH and temperature, whereas the high volume of water present at high tide dampened fluctuations in those parameters (Fig. 1; Table 1).

Low tide during the daytime was characterized by high water column \( \rho_{\mathrm{O}_2} \), pH and temperature in the shallow water. At low tide, the water depth was often <10 cm resulting in high shoot biomass in a low volume of water as the canopy collapsed with leaves packed in layers on top of one another. Nevertheless, the high insolation-fuelled underwater \( R_N \) and \( \mathrm{O}_2 \) built up to 54.3 kPa in the water surrounding the shoots (Table 1). The slow exchange of gases with the atmosphere not only resulted in build-up of \( \mathrm{O}_2 \) but also draw-down of \( \mathrm{CO}_2 \) with minimum \( \mathrm{CO}_2 \) concentrations of c. 0.5 mmol m\(^{-3}\) and with maximum water temperatures briefly exceeding 40\°C; such conditions would promote photorespiration (Beer, 1989). Incoming tidal water originating from the surrounding ocean caused steep declines in \( \rho_{\mathrm{O}_2} \), pH and temperature when high tide occurred during the daytime. Dissolved \( \mathrm{O}_2 \) and \( \mathrm{CO}_2 \) were close to air equilibrium \( (\rho_{\mathrm{O}_2} \text{ ranged from 19.6 to 22.2 kPa and } \mathrm{CO}_2 \text{ concentration from 7 to 8 mmol m}^{-3}) \) in the incoming tidal water and the water temperature declined by 9\°C in only 20 min to c. 28\°C, as compared with the shallow pools at low tide.

During the night, the chemical and physical parameters basically followed the opposite pattern of those observed in the light of day. The dense shoot biomass packed in a small volume of water quickly consumed most of the dissolved \( \mathrm{O}_2 \) resulting in severe water column hypoxia, that is <1 kPa, with \( \mathrm{CO}_2 \) concentrations c. three-fold atmospheric equilibrium (Table 1). The water temperature was little affected by tidal status, whereas high tide during the night brought in fresh supplies of dissolved \( \mathrm{O}_2 \) restoring water \( \rho_{\mathrm{O}_2} \) to c. 20–22 kPa (Table 1). Surprisingly, \( \rho_{\mathrm{O}_2} \), pH and temperature all increased at the transition from high to low tide, even in the dark. We suggest that this is due to the fact that the warm water, which also is \( \mathrm{O}_2 \)-rich and \( \mathrm{CO}_2 \)-poor,
present at the end of the previous daytime low tide has a tendency not to mix with the incoming cooler oceanic water. Instead, a front is likely formed and the warm water is ‘pushed’ into the fringing mangrove. When the water again recedes off the reef with the next low tide, the warm, O₂-rich and CO₂-poor water returns from the mangrove causing a rise in pO₂, pH and temperature at the seagrass meadow; these transient increases occur even after sunset and subsequently pO₂, pH and temperature then decrease in these remaining pools as respiration dominates and the shallow water cools during the darkness of night (Fig. 1).

The huge amplitudes in the key environmental parameters were also reflected by the dynamic internal pO₂ in the shoot base of both T. hemprichii and E. acoroides. At dawn, shoot base pO₂ increased from severely hypoxic or even anoxic conditions to air equilibrium or above within 1.5 h. The steep increase in stem base pO₂ levelled out before noon and stayed more or less constant until mid-afternoon when a combination of lower insolation and incoming tide with lower water column pH led to a drop in pO₂ below detection limit before the advent of lower pH (Fig. 1). When evaluated for the period between 1 h after sunrise and 1 h before sunset, tissue pO₂ could vary from below detection limit of 0.02 kPa in the early morning hours and up to above 50 kPa in mid-afternoon (Table 1). Similarly, shoot base pO₂ ranged from below detection limit late in the night to above 30 kPa for early in the evening; E. acoroides with smaller amplitude than for T. hemprichii (Table 1).

Both species experienced periods of tissue anoxia when measured in the shoot base. The period varied from 3 h and 30 min and up to almost half a day in T. hemprichii (11 h 15 min; Table 1). In the case of E. acoroides, periods of anoxia were as short as half an hour but for other individuals up to 8 h and 15 min (Table 1). Tissue anoxia was caused by the prolonged periods of water column hypoxia at low tide during the night.

Table 1

<table>
<thead>
<tr>
<th>Tissue-related parameters</th>
<th>Thalassia hemprichii (n = 5)</th>
<th>Enhalus acoroides (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue pO₂ in light; 1 h after sunrise till 1 h before sunset (kPa)</td>
<td>c. 0.8–53.1</td>
<td>c. 0.8–51.4</td>
</tr>
<tr>
<td>Tissue pO₂ in darkness; 1 h after sunset till 1 h before sunrise (kPa)</td>
<td>c. 0.8–15.9</td>
<td>c. 0.8–30.2</td>
</tr>
<tr>
<td>Duration of tissue anoxia (h : min)</td>
<td>3:30–11:15</td>
<td>0:30–8:15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water column-related parameters</th>
<th>Low tide (n = 6)</th>
<th>High tide (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water column pO₂ (kPa)</td>
<td>0.7–54.3</td>
<td>9.5–22.6</td>
</tr>
<tr>
<td>Water column pH</td>
<td>7.61–8.84</td>
<td>8.12–8.18</td>
</tr>
<tr>
<td>Water column free CO₂ (mmol m⁻³)</td>
<td>0.4–32.0</td>
<td>7.0–8.0</td>
</tr>
<tr>
<td>Water column temperature (°C)</td>
<td>26.8–38.2</td>
<td>27.4–28.0</td>
</tr>
</tbody>
</table>

Values are ranges extracted from data in Fig. 1 and Supporting Information Figs S2 and S3.

*Detection limit = 0.02 kPa.
because O₂ dissolved in the water column is the only source of O₂ in darkness.

Photosynthesis vs temperature and estimation of optimum temperature

The in situ measurements of key environmental parameters showed that the seagrasses experienced large diel temperature fluctuations (e.g. 27–38°C; Table 1) influenced by the tide as well as solar radiation (Fig. 1). Thus, we established the response of leaves for underwater photosynthesis (PN) and also respiration (R₀) in the temperature range from 25 to 50°C.

Underwater PN increased from the rates at 25°C and up to the temperature optimum in a similar fashion for both T. hemprichii and E. acoroides (Fig. 2). The temperature optimum for PN was c. 33°C and did not differ significantly between the two species (Table 2). At higher temperatures, PN declined steeply reaching 0 μmol O₂ m⁻² s⁻¹ at 45°C and both species had negative O₂ balance (i.e. net consumption) in the light at 50°C (Fig. 2).

PNmax differed between the two species with T. hemprichii having a higher area-specific photosynthetic rate than E. acoroides (9.1 vs 7.2 μmol O₂ m⁻² s⁻¹, respectively; Table 2). The higher PNmax of T. hemprichii per unit of projected area at saturating light levels was caused neither by thicker leaves nor by a higher Chl a concentration (Table 2) because the area-specific Chl a was higher in E. acoroides than in T. hemprichii (118 vs 95 mg Chl a m⁻², respectively).

Dark respiration vs temperature and estimation of Q₁₀

The response to temperature of R₀ of leaf segments was very different to that of PN. R₀ increased exponentially with increasing temperature and the response was similar for both T. hemprichii and E. acoroides (Fig. 2). Under the present experimental conditions with an incubation time of 1.5–2.0 h, the leaf tissue of both species respired at high rates even at the extreme temperature of 50°C, although there was a tendency for the respiration to level off in the case of T. hemprichii. The Q₁₀ for R₀ of E. acoroides (2.7) was slightly higher than that of T. hemprichii (2.0), although this was not a statistically significant difference when estimated based on projected area. However, when estimated based on DM, the Q₁₀ of T. hemprichii (2.8) was significantly larger than that of E. acoroides (2.1) (Table 2); see caption for Table 2 for details on statistics.

Diel O₂ balance, and inferred C balance, of leaf segments

The contrasting response of PN and R₀ to increasing temperature prompted us to estimate the net diel O₂ balance of leaf segments. Assuming 12 h of saturating light and a photosynthetic quotient of 1 (Pokorny et al., 1989) – that is, 1 mol O₂ produced per mol inorganic carbon fixed – resulted in positive O₂ balance of 375 and 290 mmol O₂ m⁻² d⁻¹ at 35°C (Fig. 2) for T. hemprichii and E. acoroides, respectively, corresponding to 63 and 48 mmol hexose equivalents m⁻² d⁻¹. By contrast, the extreme
Fig. 3 Recovery of underwater net photosynthesis ($P_N$, a) and dark respiration ($R_D$, b) of two tropical seagrass species, Thalassia hemprichii and Enhalus acoroides. Whole plants were exposed to 35, 40 or 45°C for 4 h in light and then left to recover overnight at 25°C in the dark before measurements at 35°C were taken 12–18 h later. Different letters indicate significant differences ($p < 0.05$, Sidak-test) within each species (mean ± SE, $n = 5$).

Bicarbonate use

The high pH observed in the light during low tides in the field situation, which at times exceeded 8.8 (Table 1), prompted us to test if, in addition to CO$_2$, Thalassia hemprichii and Enhalus acoroides were utilising bicarbonate as source of inorganic carbon. The ‘pH–drift approach’ revealed that both species were capable of driving pH up to 9.6 (caption of Fig. 4) after 18 h in the light at 35°C, demonstrating that bicarbonate is used as source of inorganic carbon (Allen & Spence, 1981; Maberly, 1990). The bicarbonate use was further supported by the strong effect of applying TRIS buffered seawater during measurements of underwater $P_N$ (Fig. 4), showing that acidification of the cell walls is among the mechanisms used by both Thalassia hemprichii and Enhalus acoroides to access CO$_2$ from bicarbonate. In the case of Thalassia hemprichii, $P_N$ declined to 27% and similarly for Enhalus acoroides (down to 37%) when measured in TRIS buffered seawater compared to controls in seawater without TRIS, all at 35°C. Hence, both species were able to grow at high pH and vice versa.

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Table 2: Specific leaf area (SLA), leaf Chl, leaf N, optimum temperature ($T_{opt}$) for net photosynthesis ($P_{max}$), maximum photosynthesis ($P_{max}$) and $Q_{10}$ for dark respiration ($R_D$) of two tropical seagrass species, Thalassia hemprichii and Enhalus acoroides

<table>
<thead>
<tr>
<th>Species</th>
<th>SLA (m$^2$ kg$^{-1}$ DM)</th>
<th>Leaf Chl (mg g$^{-1}$ DM)</th>
<th>Leaf N (mg g$^{-1}$ DM)</th>
<th>$T_{opt}$ (°C) (area-based $P_N$)</th>
<th>$T_{opt}$ (°C) (DM-based $P_N$)</th>
<th>$P_{max}$ at $T_{opt}$ ($\mu$mol O$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>$P_{max}$ at $T_{opt}$ (nmol O$_2$ g$^{-1}$ DM s$^{-1}$)</th>
<th>$R_D$ at $T_{opt}$ ($\mu$mol O$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>$R_D$ at $T_{opt}$ (nmol O$_2$ g$^{-1}$ DM s$^{-1}$)</th>
<th>$Q_{10}$ (area-based $R_D$)</th>
<th>$Q_{10}$ (DM-based $R_D$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassia hemprichii</td>
<td>27.2 ± 1.5</td>
<td>2.59 ± 0.38</td>
<td>2.73 ± 1.5</td>
<td>32.8 ± 0.6</td>
<td>32.1 ± 0.6</td>
<td>9.1 ± 0.7</td>
<td>359.7 ± 23.1</td>
<td>0.45 (0.12–1.10)</td>
<td>9.0 (3.5–18.1)</td>
<td>2.0 (1.8–2.3)</td>
<td>2.8 (2.5–3.1)</td>
</tr>
<tr>
<td>Enhalus acoroides</td>
<td>18.6 ± 1.6</td>
<td>2.19 ± 0.23</td>
<td>20.9 ± 1.8</td>
<td>33.8 ± 0.3</td>
<td>33.3 ± 0.7</td>
<td>7.2 ± 0.7</td>
<td>239.1 ± 13.1</td>
<td>0.33 (0.04–1.03)</td>
<td>7.2 (2.4–16.0)</td>
<td>2.7 (2.3–3.2)</td>
<td>2.1 (1.9–2.3)</td>
</tr>
</tbody>
</table>

The tissues were collected on a tropical reef, Tallon Island, Western Australia as intact plants and used within 24 h of collection. $T_{opt}$ and $P_{max}$ were estimated from a Gaussian model, and $R_D$ and $Q_{10}$ were derived from an exponential model (see Fig. 2); error estimates were calculated using the nonlinear module in GraphPad Prism 6. Values are means ± 95% C.L. or in the cases of $R_D$ and $Q_{10}$, 95% C.L. in brackets.

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Recovery of net photosynthesis and dark respiration following heat stress

At 45°C, underwater $P_N$ was severely depressed and $R_D$ greatly increased as compared to measurements taken at 35°C (Fig. 2). Therefore, we tested if $P_N$ and $R_D$ were able to recover following exposure to elevated temperatures. Whole shoots exposed to 35, 40 and 45°C for 4 h in light in seawater in laboratory incubations were left to recover overnight in darkness at 25°C before measurements of $P_N$ and $R_D$ were taken at 35°C the next morning, using the same approach as outlined above.

The recovery experiment demonstrated that both $P_N$ and $R_D$ of Thalassia hemprichii and Enhalus acoroides recovered when exposed to 40°C with no significant differences between tissues exposed to 35 or 40°C (Fig. 3; see figure caption for details on statistics). By contrast, exposure to 45°C resulted in damage to the photosynthetic apparatus that did not recover during the following 12–18 h when in aerated seawater in darkness at 25°C. The $P_N$ rates of tissues exposed to 45°C were negative and similar to those exposed directly to 45°C with no opportunity to recover (Fig. 3). Similarly, $R_D$ also did not recover although there was still a considerable residual $R_D$ present in both species (63% and 42% of the rate at 35°C in Thalassia hemprichii and Enhalus acoroides, respectively).

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temperature of 50°C resulted in a strongly negative diel O$_2$ balance and thus negative C balance equivalent to c. 30 mmol hexose equivalents m$^{-2}$ d$^{-1}$ for both species (derived from Fig. 2; c. 180 mmol O$_2$ m$^{-2}$ d$^{-1}$ and assuming a ratio of 6 O$_2$ to 1 hexose). The estimated diel O$_2$ balance shows the largest difference between the two species in the interval from 30 to 40°C, whereas the difference diminished at both ends of the temperature range tested.

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at least partly sustain photosynthesis by bicarbonate utilization when pH was high and availability of free CO2 low, such as occurs during low tide and high irradiance.

Discussion

In the seagrass species *Thalassia hemprichii* and *Enhalus acoroides*, at times of low tides, measurements in the field of shoot base pO2 revealed long periods of tissue anoxia in the dark followed by high water temperature and hyperoxia exceeding 40 kPa (twofold atmospheric equilibrium) during the day, water column measurements also showed low CO2 in the pools of remaining water during daytime low tides. The challenging environmental conditions at low tide resulted from the seagrass canopy occurring in a relatively small volume of water, resulting in quick consumption of O2 by respiration in the dark – whereas CO2 declined and O2 built up in light – and a marked increase in temperature during the daytime. Below, we discuss these field-based findings and responses to temperature of leaf PN and Rn and the ability for bicarbonate use in PN in the two species, in the context of this extreme environment, with its heightened risks of potential tissue injury due to anoxia, hyperoxia and high water temperatures.

The environment

The persistence of seagrasses *T. hemprichii* and *E. acoroides* in such an elevated intertidal lagoonal environment is because water is trapped behind raised coralline algal terraces, a common feature of these macrotidal Kimberley reefs (Rosser & Veron, 2011; Lowe et al., 2015; Richards & O’Leary, 2015). Nevertheless, the consequences of trapped water are reduced water flow, high water temperatures, variable O2 concentrations from supersaturated during the day to hypoxic during the night and associated biogeochemical transformations. We have demonstrated that the two seagrass species studied persist under extremes of temperature during the day and hypoxia/anoxia during the night. These diel environmental fluctuations led to recurrent tissue stress caused by high temperature and low tissue O2 in the present study, in contrast with previous studies on temperature stress and tissue anoxia in seagrass that occur on a seasonal basis (Borum et al., 2005). The future of these seagrass species in these high intertidal lagoons is threatened by global warming. Climate change modelling for the terrestrial Kimberley environment predicts 2–3°C increases in air temperature and recent coral bleaching events suggest a similar level of warming in the ocean (2.2–4.1°C by 2090) under a high scenario (RCP8.5, i.e. no change in human activity; Moise et al., 2015). Our current data show that such temperature increases above those during the hottest periods of the daytime low tides would exceed the present heat tolerances, threatening both species, although the limits for survival would also depend upon possible further acclimation to thermal stress (discussed below).

Heat stress

The underwater net photosynthesis (PN) and dark respiration (Rn) of both species were sensitive to elevated temperature but, nevertheless, the estimated optimum temperature for PN was higher than previously reported. Temperature effects upon underwater PN of seagrasses have been studied for temperate as well as for tropical species. As anticipated, temperate species of seagrasses generally have a lower temperature optimum for PN (23°C; 16–35; median and range) than tropical species (30°C; 25–35) (Lee et al., 2007). This median optimum temperature of 30°C for PN of tropical seagrass species (Lee et al., 2007) is lower than the assessed optimum temperature in the present study both for *T. hemprichii* (32.8°C, Fig. 2) and for *E. acoroides* (33.3°C, Fig. 2). Moreover, the optimum temperature for PN of 32.8°C in *T. hemprichii* and of 33.3°C in *E. acoroides* (present study) are both well above those reported previously for these two species (27°C for both species; Agawin et al., 2001).

It is possible that the high optimum temperature for PN is a local phenomenon restricted to the reef population of *T. hemprichii* and *E. acoroides* experiencing high water temperature at low tide on a regular basis. Unfortunately, we were unable to locate subtidal populations of the two species in the area and we were thus unable to test if the intertidal populations had acclimated, or possibly adapted, to higher temperatures common on the reef flat. However, for *Zostera marina* it has been reported that the optimum temperature for PN can vary between 21.7 and 23.9°C depending on time of year (Staehr & Borum, 2011) and between 16 and 35°C depending on latitude (Lee et al., 2007). Thus, based upon the observations available for *Z. marina* it is quite possible that *T. hemprichii* and *E. acoroides* also possess some potential plasticity enabling acclimation to different

Fig. 4 Inhibition of bicarbonate use as a source of inorganic carbon in underwater net photosynthesis (PN) of two tropical seagrass species, *Thalassia hemprichii* and *Enhalus acoroides*, using TRIS (50 mol m−3) added to seawater and pH adjustment to 8.00 to prevent acidification of the cell wall spaces and diffusive boundary layer (Hellblom et al., 2001) as compared with controls in seawater. We also used a ‘pH drift experiment’ with seawater (see the Materials & Methods section) as a diagnostic test of bicarbonate use and this indicated that both species were able to utilize bicarbonate as a source of inorganic carbon at 35°C with an end pH of 9.61 ± 0.01 and 9.61 ± 0.02 (median ± SE, n = 8) for *T. hemprichii* and *E. acoroides*, respectively. Different letters indicate significant differences within each species (P < 0.05, Sidak-test).
temperatures, and thus explaining the very different optimum temperatures for $R_N$ obtained in the present study compared to those previously reported by Agawin et al. (2001).

Many plant species possess a potential for acclimation to the temperature of the environment, both for $R_N$ (Berry & Björkman, 1980) and for $R_D$ (Atkin & Tjoelker, 2003). Acclimation to increasing temperature can influence both the optimal temperature for $R_N$ and also the critical temperature above which damage occurs. As an example, Quercus suber seedlings subjected to heat shock (40°C for 36 h) had a 3°C increase in the critical temperature for Photosystem II stability (Ghouil et al., 2003). We found that $P_N$ by the two seagrass species did not recover following exposure to 45°C, demonstrating damage to the photosynthetic apparatus, so that the critical temperature lies in the range between 40 and 45°C. It is unknown whether T. hemprichii and E. acoroides can acclimate to warmer temperatures so that the critical temperature increases when faced with heat stress, but interestingly four tropical seagrass species subjected to daily 2.5-h exposures to 43°C for 6 d suffered moderate (Cymodocea rotundata) or almost complete (Halodule uninervis, Halophila ovalis, Thalassia hemprichii) shoot mortality, whereas the damage was less with exposures to 40°C (Collier & Waycott, 2014). The issue of possible acclimation to high temperatures of both $P_N$ and $R_D$, in a range of seagrasses, should be evaluated.

In the present study, direct exposure of leaf tissue to 40°C depressed $P_N$ and stimulated $R_D$ significantly in both species when compared to rates at 35°C. However, exposure to 40°C for 4 h and following a night of recovery at 25°C there was no adverse effect on $P_N$ or on $R_D$ when again measured the following day at 35°C. In the field situation, T. hemprichii and E. acoroides both experienced prolonged periods with temperatures up to 40°C, suggesting also that leaf tissues in the field would face times of declining rates of $P_N$ and increasing rates of $R_D$. There is some indication that the heat stress is reflected in declining water $pO_2$ and also lower shoot base $pO_2$, but because light is declining in this period as well (Fig. 1), we were unable to separate for the field plants the effects caused by heat stress from those caused by declining light. In contrast to the recovery after exposure to 40°C, leaf tissue exposed to 45°C for 4 h did not recover by the next morning. $P_N$ was approximately zero (E. acoroides) or even negative (T. hemprichii) for tissues previously exposed to 45°C and measured at 35°C (Fig. 3), corresponding to values obtained during direct exposure to 45°C (Fig. 2) and indicating a more permanent damage to the photosynthetic apparatus from this higher temperature. The damage could be restricted to Rubisco which is known to be sensitive to heat stress (Salvucci & Crafts-Brandner, 2004a,b; Carino-Silva et al., 2012) but the damage could also be at the level of chloroplasts (Allakhverdiev et al., 2008). In a previous study, Thalassia hemprichii also was found to be relatively tolerant to high water temperatures but suffered permanent damage to the photosynthetic apparatus when exposed to 45°C (Campbell et al., 2006). Interestingly, whilst $R_D$ also did not recover, indicating more widespread damage to cells than to the photosynthetic apparatus, there was still substantial respiration in the leaf tissue when measured at 35°C on the following day (64% and 43% in T. hemprichii and E. acoroides, respectively, compared to the rate measured directly at 35°C). Cleary, the $R_D$ rates indicate that the tissues were not dead, although a proportion of cells could have died or been damaged (e.g. as found in heat-stressed Phaeolus vulgaris; Hivre et al., 2011). Based upon the present experimental design, we are unable to rule out the possibility of recovery of both $P_N$ and $R_D$ if the tissues were offered a longer recovery period. Future work to reveal the dynamics of tissue damage resulting from heat stress of various shorter durations, which can occur within 5 min of exposure to temperatures above a threshold level (Hivre et al., 2011), should be of priority for these and other seagrasses.

The $R_D$ of both seagrass species increased exponentially with increasing temperature within the 25–50°C interval tested in the present study. The temperature where $R_D$ reaches its maximum, $T_{max}$ (cf. O’Sullivan et al., 2013) consequently was not necessarily reached. The $R_D$ response to increasing temperature followed an exponential response for both T. hemprichii ($Q_{10}$ of 2.0 on a leaf area basis) and E. acoroides ($Q_{10}$ of 2.7). Experiments using continuous measurements of leaf $R_D$ with step-ups of temperature to achieve ‘high-resolution temperature response curves’ have shown a ‘burst’ in $R_D$ at a leaf temperature of c. 45°C, where damage to Photosystem II was also observed (O’Sullivan et al., 2013); the method used in the present study with longer incubations might not have resolved these dynamics, if present. Importantly, the present data on two tropical seagrass species showed, in agreement with the earlier work by O’Sullivan et al. (2013) and others (see Introduction), that photosynthesis was more sensitive than respiration to heat stress.

Bicarbonate use

The low CO$_2$ concentrations observed during daytime low tides and large increases of O$_2$ in the water and seagrass shoots which would result from photosynthesis, indicated that both species must have relied on alternative sources of inorganic carbon for photosynthesis. In addition to dissolved CO$_2$ in water, some seagrasses also have been shown to use atmospheric CO$_2$ during air exposure (Silva et al., 2005) but the most common alternative source of inorganic carbon is bicarbonate (HCO$_3^-$) dissolved in the water (Touchette & Burkholder, 2000) and present at concentrations of c. 2 mol m$^{-3}$ in typical seawater (Raven & Johnston, 1991). Our experiment using the ‘pH–drift approach’ showed that both species drove the pH of the seawater up to 9.6, indicating the capacity to utilize HCO$_3^-$ (Allen & Spence, 1981; Maberly, 1990). There are at least three different ways that HCO$_3^-$ can be utilized in underwater photosynthesis (Larkum et al., 2006): (1) direct H$^+$–driven symport uptake of HCO$_3^-$ followed by internal transformation into CO$_2$; (2) external transformation into CO$_2$ catalysed by carbonic anhydrase in the cell wall; and (3) external transformation into CO$_2$ via acidification of the cell wall space and diffusive boundary layer (DBL). In the present study, we tested if mechanisms (1) and/or (3) were in operation and found that both species indeed responded with greatly reduced photosynthetic rates when the DBL pH was buffered by TRIS to be near pH 8.0 (Fig. 4). At pH 8.0 in the DBL and presumably within the cell wall space the H$^+$ gradient for HCO$_3^-$...
uptake via a symport (if present) would be greatly diminished and also at pH 8.0 < 0.5% of the total pool of dissolved inorganic carbon is present as CO₂ (i.e. substantially reduced low pH-driven conversion of HCO₃⁻ to CO₂ because H⁺ extrusion can no longer effectively acidify the DBL). Thus, the significantly reduced Pₐ of both species in the presence of TRIS-buffered seawater indicates that HCO₃⁻ use by both species occurs either by its uptake via H⁺-driven symport (i.e. the diminished H⁺ gradient would reduce symporter activity), or acidification of the cell wall (i.e. prevention of DBL acidification would reduce conversion to CO₂), or a combination of both of these mechanisms.

The ability of these two species to utilize HCO₃⁻ agrees well with the observed environmental conditions where dissolved CO₂ can be scarce and well below the level of atmospheric equilibrium during the day in the warm, shallow water (Table 1). Furthermore, HCO₃⁻ use also helps reduce photosrespiration as CO₂ can be kept high around Rubisco (Beer, 1989; Reiskind et al., 1989), even under conditions of high temperature and external pO₂ of up to 2.6-fold atmospheric equilibrium (Table 1). Moreover, with the present diagnostic test of DBL acidification, we cannot rule out that one or both of the species tested also uses carbonic anhydrase to speed up the conversion of HCO₃⁻ into CO₂ (cf. Beer et al., 2002).

**Tissue anoxia and hyperoxia**

The challenging environment resulted in extreme variation in tissue O₂ status. The tissue of the shoot base experienced conditions of hyperoxia with pO₂ of up to 51 kPa followed by anoxia during the night (Fig. 1). Hyperoxia is common in submerged plants (e.g. Sand-Jensen et al., 2005; Pedersen et al., 2006) and is a result of the slow exchange of O₂ with the environment caused by the low gaseous diffusion in water compared to that in air. For seagrasses, field observations of daytime shoot base or rhizome pO₂ are in the range of 30–42 kPa; >30 kPa in *T. testudinum* (Borum et al., 2005), 37 kPa in *Syringodium filiforme* (Holmer et al., 2009) and 42 kPa in *Z. marina* (Greve et al., 2003), and so the 53.1 kPa for *T. hemprichii* and 51.4 kPa for *E. acoroides* observed in the present study (Figs 1, S2, S3; Table 1) are substantially higher than previously reported. Yet 6–8 h later, the same tissues experienced anoxia at low tides during the nights.

The duration of tissue anoxia at nighttime was indeed remarkable for seagrass. *In situ* shoot base anoxia of up to 6 h has been reported for *T. testudinum* with only one example from an individual plant in an area already affected by widespread die-off (Borum et al., 2005), whereas shoot base anoxia in *Z. marina* in the field situation has yet to be documented. In the present study, recurrent shoot base anoxia varied from 30 min and up to 11 h and 15 min (almost twice as long as for *T. testudinum*, see above in this section) with few interspecies differences, although there was a tendency for *T. hemprichii* to experience longer periods of anoxia than *E. acoroides* (Table 1). These observations indicate that, although the period of tissue anoxia was sometimes interrupted for up to 3 h when the high tide brought in O₂-containing oceanic water (Figs 1, S2), *T. hemprichii* and *E. acoroides* must be considerably more tolerant to tissue anoxia than the temperate seagrass, *Z. marina* (Pulido & Borum, 2010). Tolerance to tissue anoxia of *Z. marina* is highly dependent on temperature and leaf growth ceases with no recovery after only 8 h of tissue anoxia at 30°C, whereas the survival rate of shoots exposed to anoxia for 8 h at 20°C (normal summer temperature for *Z. marina* was 100% (Raun & Borum, 2013). In the present study, tissue O₂ status was followed in the shoot base, and thus the period of tissue hypoxia in the roots would likely be even longer because hypoxia occurs sooner in the more distally located tissues relative to the water column being the source of O₂ during the night-time (Pedersen et al., 2004; Binzer et al., 2005; Sand-Jensen et al., 2005). Root anoxia has been shown to potentially result in H₂S intrusion when the oxidation of H₂S to SO₄²⁻ fuelled by radial O₂ loss ceases (Pedersen et al., 2004; Borum et al., 2005). However, the risk of sulfide poisoning in the present environment is likely to be insignificant because the sediment is rich in Fe (Anand & Gilkes, 1987) which has a large capacity to bind sulfides in pyrite (Wilkin & Barnes, 1996; Rickard, 1997).

**Conclusions**

The extreme macrotidal environment presents a challenge to the two seagrass species coexisting on the reef flat. During the day, the shallow water was characterized by high pO₂, pH and temperature affecting Pₐ and Rₐ; Pₐ declined due to heat stress and CO₂ availability approached zero whilst Rₐ increased in the warm water. During the night, by contrast, dissolved O₂ declined to hypoxic levels resulting in hours of shoot base anoxia in both *T. hemprichii* and *E. acoroides*. We showed that Pₐ and Rₐ of both species fully recovered during the night from exposure to 40°C for 4 h of the day before, whereas heat stress at 45°C resulted in more permanent tissue damage. In conclusion, these seagrass species are ‘living near the edge’; there is a prominent risk that both species will be damaged or possibly even cease to exist on these reefs due to heat stress or combined heat stress and night-time anoxia if average air and seawater temperatures increase (Moise et al., 2015), and/or if marine heat wave events become more frequent (e.g. for other locations and species in Western Australia; see Wernberg et al., 2013; Fraser et al., 2014) owing to climate change.

**Acknowledgements**

We acknowledge the Bardi Jawi traditional owners for allowing us access to their lands and the WA Department of Parks and Wildlife for permitting the research. This work could not have been accomplished without the sustained support and assistance of the Bardi Jawi rangers. James Brown and staff of the Kimberley Marine Research Station are acknowledged for their logistical support of our work at Cygnet Bay. Funding for this work was provided by the Western Australian Marine Science Institution (WAMSI) Kimberley Marine Research Program (project 2.2.4 to G.A.K.). We also thank the UWA Institute of Advanced Studies for hosting O.P. on his visits to UWA.
Author contributions

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Luminous flux per unit area for the three consecutive deployments.

Fig. S2 Example of a diel cycle with key environmental parameters (deployment no. 2).

Fig. S3 Example of a diel cycle with key environmental parameters (deployment no. 3).

Table S1 Meadow characterization for the two species.

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