Niche partitioning of intertidal seagrasses: evidence of the influence of substrate temperature

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Summary

- The influence of soil temperature on rhizome depths of four intertidal seagrass species was investigated in central Queensland, Australia. We postulated that certain intertidal seagrass species are soil temperature-sensitive and vertically stratify rhizome depths.
- Below-ground vertical stratification of intertidal seagrass rhizome depths was analysed based upon microclimate (soil temperature) and microhabitat (soil type).
- Soil temperature profiles exhibited heat transfer from surface layers to depth that varied by microhabitat, with vertical stratification of rhizome depths between species. *Halodule uninervis* rhizomes maintain a narrow median soil temperature envelope; compensating for high surface temperatures by occupying deeper, cooler soil substrates. *Halophila decipiens*, *Halophila ovalis* and *Zostera muelleri* rhizomes are shallow-rooted and exposed to fluctuating temperatures, with broader median temperature envelopes. *Halodule uninervis* appears to be a niche specialist, with the two *Halophila* species considered as generalist niche usage species.
- The implications of niche use based upon soil temperature profiles and rhizome rooting depths are discussed in the context of species' thermal tolerances and below-ground biomass O_2 demand associated with respiration and maintenance of oxic microshields. This preliminary evidence suggests that soil temperature interaction with rhizome rooting depths may be a factor that influences the distribution of intertidal seagrasses.

Introduction

Understanding what influences the distribution of species in intertidal zones has implications with regard to climate change with subsequent repercussions for how we consider restoring these habitats. Intertidal seagrasses are of particular interest given their decline over recent decades (e.g. Orth *et al.*, 2006; Waycott *et al.*, 2009; Short *et al.*, 2014) and the difficulties and variation in success rates when restoring these habitats (e.g. Valle *et al.*, 2015; Suykerbuyk *et al.*, 2016; York *et al.*, 2017).

A number of existing theories attempt to explain the zonation of intertidal species. Some zonation trends appear relatively straightforward, such as biotic interactions of predation and competition (e.g. Bando, 2006), and abiotic interactions of wave exposure (de Boer, 2007), desiccation (Leuschner *et al.*, 1998; Björk *et al.*, 1999; Lan *et al.*, 2005; Shafer *et al.*, 2007), species' thermal tolerances associated with exposure to air or water (Seddon & Cheshire, 2001; Short *et al.*, 2007; Massa *et al.*, 2009; Kaldy *et al.*, 2015), light availability (de Boer, 2007) and light tolerance (Björk *et al.*, 1999). Shafer *et al.* (2007) identified eight factors that can be attributed to intertidal seagrass zonation from the literature, with no one factor appearing to control all species' intertidal zonation patterns. Of these eight factors, three (desiccation, air exposure and high irradiance) are most commonly investigated regarding potential constraints leading to intertidal zonation (Fig. 1). Although these eight factors have been discussed within the literature, a full understanding of the causes of intertidal zonation of seagrass species still eludes researchers. As Shafer *et al.* (2007) suggest, it may be a combination of factors, including growth strategies, that explain intertidal seagrass zonation.

We postulate an additional factor that has yet to be fully considered which might be limiting the vertical zonation of intertidal seagrass species – the substrate temperature profile and its influence on below-ground biomass, specifically rhizome depth. We note that substrate temperature profiles have not been previously considered as a constraint to intertidal elevation of seagrasses, and rooting depth (of rhizomes, or below-ground biomass) is rarely examined within the context of seagrasses. Similarly, we note that only one seagrass species (*Ruppia maritima*) has root depth records within the Plant Trait (TRY) database (https://www.try-db.org/TryWeb/Home.php).

Soil temperature is a major limiting factor for terrestrial plant growth, seedling establishment, and survival. The importance of

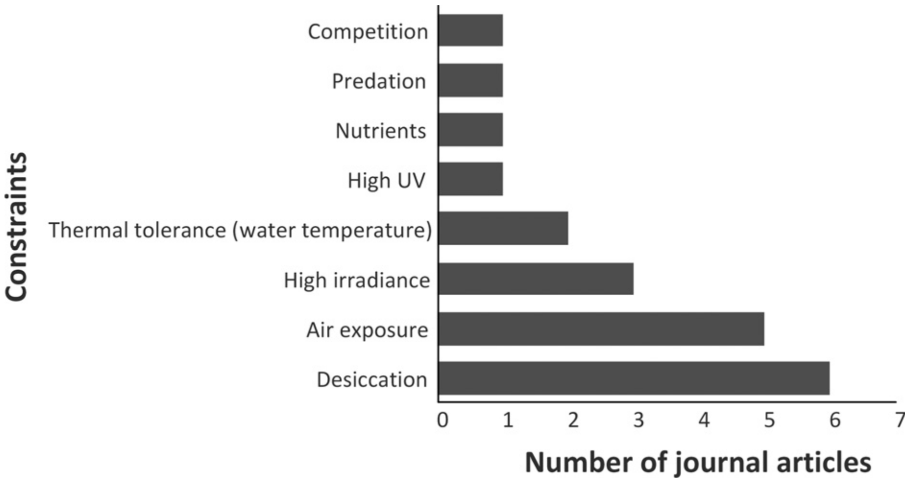

Fig. 1 Constraining factors reported in the literature that influence intertidal seagrass zonation (based on a Scopus search: 1997–2015).

soil temperature is linked, for example, to nutrient acquisition (e.g. Gutiérrez-Girón & Gavilán, 2013), biomass and productivity (e.g. Ityel *et al.*, 2014), soil respiration (specifically carbon dioxide flux) (Fang & Moncrieff, 2001), organic matter decomposition (Davidson *et al.*, 2012), and nitrogen mineralization (e.g. Theodose & Martin, 2003). Yet substrate temperature is often overlooked within a seagrass context in favour of investigating the influence of water temperature (e.g. Koch & Erskine, 2001; Koch *et al.*, 2007; Lee *et al.*, 2007; Collier & Waycott, 2014; Georgiou *et al.*, 2016; McDonald *et al.*, 2016; Wilkinson *et al.*, 2017) and air temperature for intertidal species (Massa *et al.*, 2009).

High water temperature increases seagrass growth (e.g. Lee *et al.*, 2007), reproduction (e.g. Zhou *et al.*, 2014), respiration, and sucrose-P synthase activity (Touchette & Burkholder, 2000). However, when water temperature exceeds a species' thermal tolerance, stress occurs that can interrupt photosystem II, enhance trace metal exposure and uptake from sediment or overlying waters, and increase shoot mortality, all of which are implicated in the biogeographic distributional limits of various tropical and austral seagrass species and can simultaneously increase sediment production of phytotoxins (e.g. sulphide) (Phillips *et al.*, 1983; Marsh *et al.*, 1986; Prange & Dennison, 2000; Seddon & Cheshire, 2001; Campbell *et al.*, 2006; Lee *et al.*, 2007; Short *et al.*, 2007; Devault & Pascaline, 2013; Collier & Waycott, 2014; Mascaró & Pérez, 2014; Hyndes *et al.*, 2016; Pedersen *et al.*, 2016; Stafford-Bell *et al.*, 2016). In general, tropical seagrasses seem more vulnerable to water temperature fluctuations, exhibiting narrower temperature ranges than temperate species (Bulthuis, 1987), and temperature tolerances that are approximately half that of temperate species (Moore, 1963). There is evidence that tropical seagrasses cannot survive exposure to prolonged high air and/or water temperatures (Zieman, 1975; Brouns, 1987; Campbell *et al.*, 2006; Massa *et al.*, 2009; Collier & Waycott, 2014); however, the role of substrate temperature profiles in ameliorating high air and/or water temperatures is unknown. This is particularly pertinent given that future planning and forecasts for climate change scenarios that may affect seagrasses are focused on water temperatures (e.g. Koch *et al.*, 2015) and fail to consider how marine soil temperature (especially intertidal soils) may influence the health, maintenance, spread and survival of seagrasses.

A chance observation of seagrass species' rhizome depths appearing to be correlated with changes in intertidal soil temperature at depth in a number of subtropical, intertidal seagrass meadows in Gladstone Harbour (also known as Port Curtis), Queensland, Australia, led us to postulate that certain intertidal seagrass species are soil temperature-sensitive. We hypothesize that soil temperature provides another dimension of differentiation. As an initial step to investigate this hypothesis, we examined linkages between below-ground vertical stratification based upon microclimate (soil temperature) and microhabitat (soil type). We note that we found no publications that explicitly examine niche breadth of seagrasses within the literature (Scopus and Web of Science searches). This work is a preliminary investigation and, as such, further investigations are required to see if the patterns observed are reflected in other intertidal locales and other species. Specifically, we note that the linkage between soil temperature and O_2 as demonstrated by Borum *et al.* (2005) needs further investigation in intertidal seagrass habitats.

Materials and Methods

The study was conducted in Gladstone Harbour (Fig. 2) during the start of austral summer (October 2013). Sea surface water temperatures vary between a minimum of 19.2°C (August) and a maximum of 29.7°C (January), averaging 24.4°C across the year (Fig. 3). Pelican Banks represents the largest extent of seagrasses in Gladstone Harbour (M. L. Campbell, pers. obs.) and has a mix of different substrates and five species of seagrass: *Zostera muelleri* Irmisch ex Asch (synonym *Z. capricorni*), *Halophila ovalis* (R.Br.) Hook f., *Halophila decipiens* Ostenf., *Halodule uninervis* (Forssk.) Asch. and *Halophila spinulosa* (R.Br.) Asch. (Weatherall *et al.*, 2016). Gladstone Harbour is subtropical and reflects a transition zone between temperate and tropical species. Four species were examined in this study, of which, *H. uninervis* is tropical in extent, with *Z. muelleri, H. ovalis* and *H. decipiens* being both temperate and tropical species (Green & Short, 2003;

Short *et al.*, 2007). *H. uninervis* is potentially near its southern distributional limit and therefore close to its low-temperature extreme.

Sampling occurred at four sites on Pelican Bank, and one site at South Trees Inlet (Fig. 2). All sites were intertidally exposed (*c.* 0.8 m above the Lowest Astronomic Tide) during sampling at low tide between 12:00 h and 15:00 h on 3, 4 and 17 October 2013. The majority of seagrasses on Pelican Banks are intertidal, although some species are subtidal and are rarely exposed even at extreme low tides (e.g. *H. decipiens* and *H. spinulosa*). South Trees Inlet (site 5) is on the coastal mainland in close proximity to industrial wharves and has patches of seagrass in a sandy substrate. Three species (*Z. muelleri*, *H. ovalis* and *H. uninervis*) are present at site 5 and all are intertidal. Owing to the ephemeral presence of species at the sampled sites, not all species were sampled or represented at all sites across the study. Similarly, the distribution of the seagrass species was patchy at all five sites and hence sampling effort was uneven (Table 1).

Substrate temperature depth profiles and seagrass rhizome depth were measured at the five sites, each of which was characterized by a different substrate type: mud; mud/gravel complex; sand; shell/gravel complex; and mud/shell complex (Table 1). These substrate types represent the predominant substrates that seagrasses occupy in Gladstone Harbour. At each site, temperature profiles of the substrate were taken on the surface and to a depth of 10 cm, at 1 cm increments. Temperatures were measured using a portable, waterproof digital thermometer ('Pocket temp' IP65 Digital Probe; HLP Controls, South Windsor, NSW, Australia) with an accuracy of ± 0.1°C that was gently pushed into the substratum to the relevant depth where the temperature was measured. The depth (cm) of the seagrass rhizome within the sediment was measured *in situ* by inserting a probe to detect rhizome depth and verifying rhizome presence by hand, and then measuring and recording the probe depth. All rhizome depth measurements were taken in the vicinity (< 1 m) of temperature profiles.

Measurements were taken at the start of an austral summer period (October 2013). The region is subtropical, typified by two seasons (wet and dry). Sampling occurred during the wet season at low tide when seagrass meadows were exposed. The wet season is the hottest season and is characterized by high rainfall, likely flood events and cyclones, with peaks of high turbidity and summer (October–March) air temperatures (average 29.6°C; average maxima 37.9°C; Fig. 3). The average seawater surface temperature (measured at −0.2 m water depth) during summer is 26.3°C (average maxima 28.3°C; Fig. 3). Turbidity in Gladstone Harbour is highly variable (ranging from 0.1 nephelometric turbidity

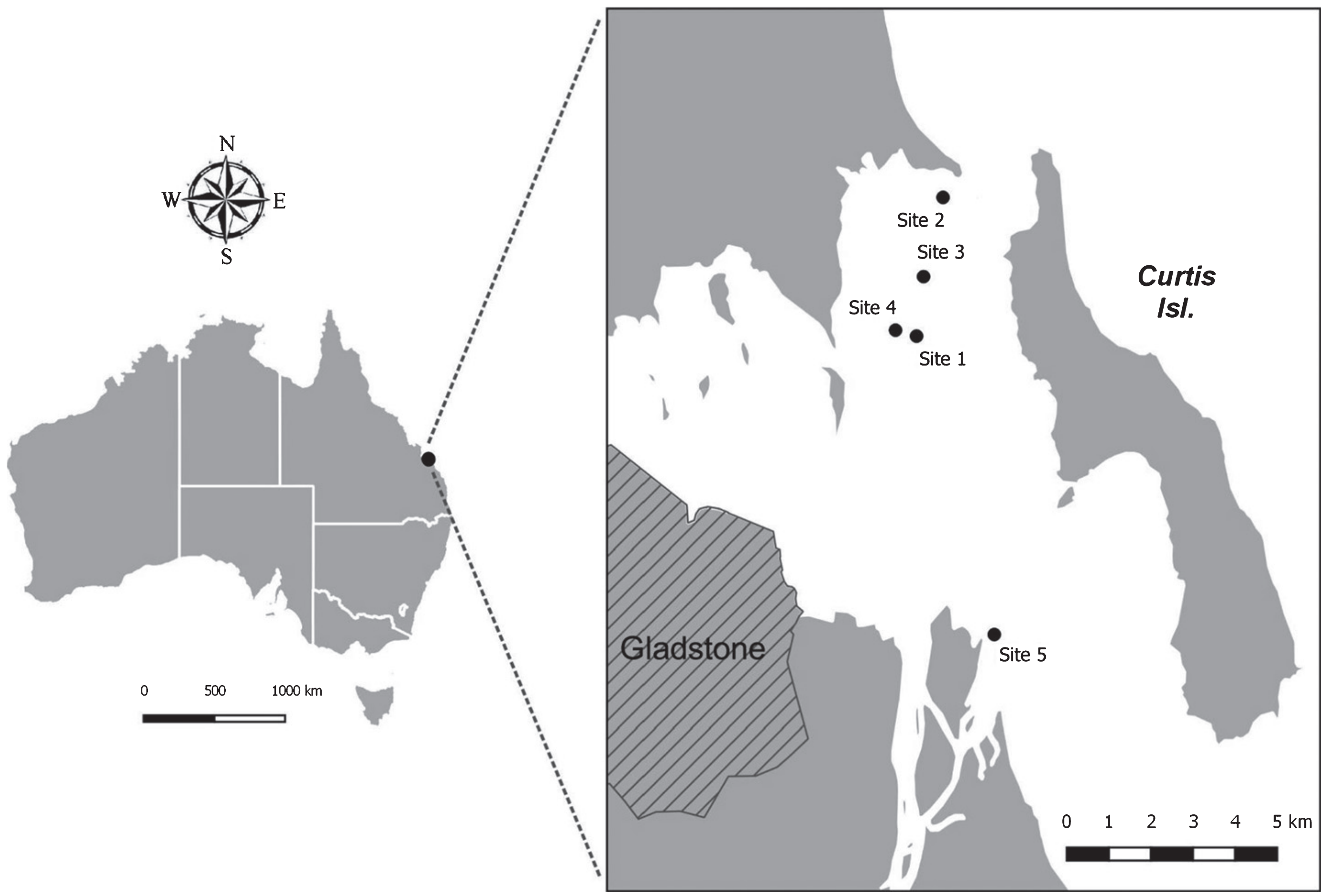

Fig. 2 Sites sampled (sites 1–5) for seagrass (*Halodule uninervis*, *Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) rhizome depth, soil temperature profiles and substrate type in Gladstone Harbour, central Queensland, Australia.

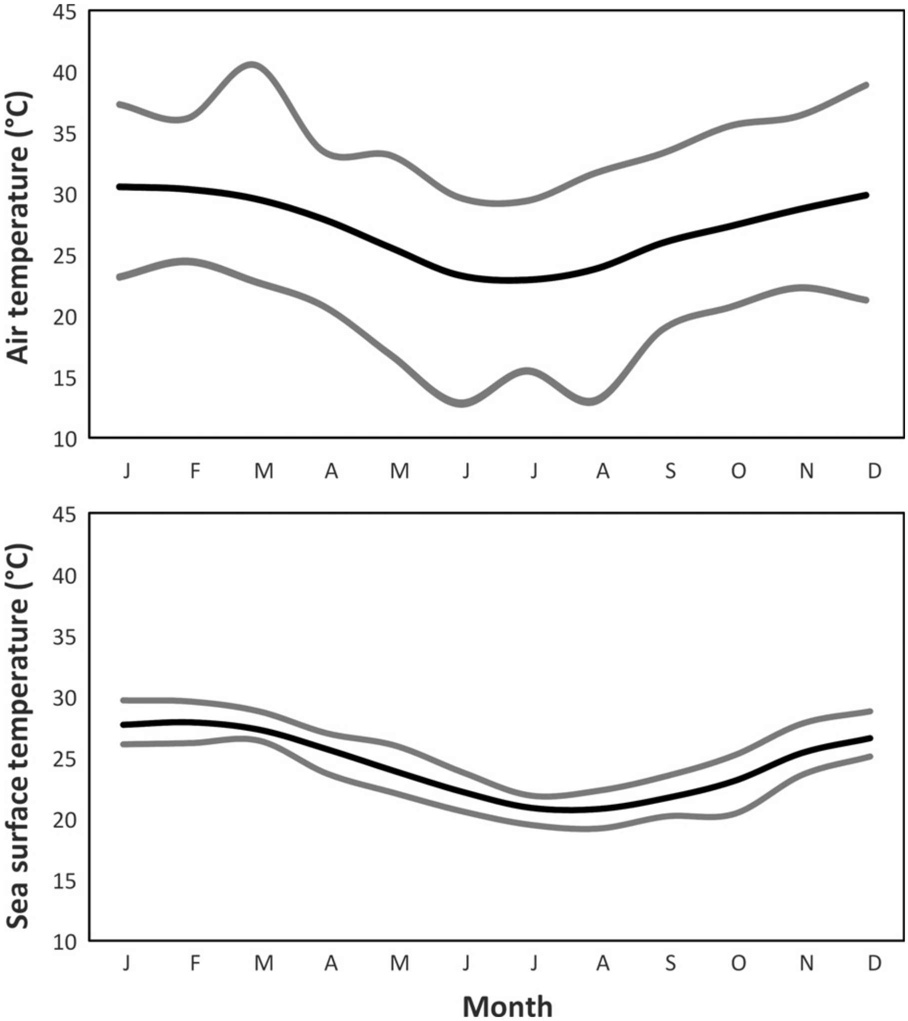

Fig. 3 Monthly mean (black) and minima/maxima (grey) air temperatures (1993–present) and sea surface temperatures (2009–present) in Gladstone Harbour. Air temperatures are from the Australian Bureau of Meteorology, Gladstone Airport Station (http://www.bom.gov.au); sea surface temperatures are from daily satellite readings from the National Oceanic and Atmospheric Administration (NOAA) (https://www.seatemperature.org/australia-pacific/australia/gladstone.htm).

units (NTU) to 1329.5 NTU) with turbidity peaks consistent with flood events (Queensland Department of Environment and Resource Management, 2011).

For comparisons across the landscape (all sites), the species rhizome depth failed normality (Shapiro–Wilk test $P < 0.05$) and thus the nonparametric Kruskal–Wallis ANOVA on ranks was used to examine the between-species differences in rhizome depth. Similarly, the differences between temperature and substrata profiles across all sites was examined using a Kruskal–Wallis ANOVA on ranks. A Dunn's test (all pairwise multiple comparison) was used to isolate species and profile depths that differed.

At individual sites, differences in species substrata depth occupancy were examined using one of three different statistical analyses (dependent upon data meeting assumptions): parametric data where three species were present were analysed using an ANOVA, with a Holm–Sidak (all pairwise multiple comparison) *post hoc* analysis to isolate differences; nonparametric data where three species were present were analysed using a Kruskal–Wallis ANOVA on ranks, with a Tukey *post hoc* test; or when two species were present but the data were nonparametric, a Mann–Whitney rank sum test was used. When examining temperature depth profiles, parametric data were analysed using ANOVA with a Holm–Sidak *post hoc* analysis. Nonparametric temperature depth profiles were examined using a Kruskal–Wallis ANOVA on ranks, with a Dunn's test to isolate groups. Descriptive statistics were used to explain other patterns in the data at individual sites.

A one-way analysis of covariance (ANCOVA) was used to investigate the relationship between substrate (granularity) and rhizome depth, with temperature as the dependant variable. A Holm–Sidak all pairwise multiple comparison was used *post hoc* to examine the pairwise relationships that exist. A significance level of 0.05 was used for all analyses.

Results

Landscape patterns

Site exposures All sites were exposed for > 2.5 h during sampling between 12:00 and 15:00 h. Based on solar irradiance profiles collected by the Australian Bureau of Meteorology site *c.* 3 km away, the sites were exposed to between 3.1 and 6.5 MJ m^{-2} over the period of low tide exposure (*c.* 180 min).

Table 1 Intertidal seagrass species (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) by site, substrate type, sampling effort (number of temperature profiles measured) and the number of rhizome depth replicates measured at a site for a species

Site no.	Species	Substrate type	No. of temperature profiles	No. of rhizome depth replicates
1	*Zostera muelleri*	Mud	6	8
1	*Halodule uninervis*	Mud	6	8
2	*Halophila ovalis*	Mud/shell	4	12
2	*Halodule uninervis*	Mud/shell	7	12
3	*Zostera muelleri*	Shell/gravel	5	10
3	*Halophila ovalis*	Shell/gravel	5	10
3	*Halodule uninervis*	Shell/gravel	8	10
4	*Zostera muelleri*	Mud/gravel	3	10
4	*Halophila ovalis*	Mud/gravel	8	10
4	*Halophila decipiens*	Mud/gravel	4	10
5	*Zostera muelleri*	Sand	6	10
5	*Halophila ovalis*	Sand	1	10
5	*Halodule uninervis*	Sand	1	10

Sampling effort differs based upon seagrass presence at each site.

Cloud cover was intermittent during sampling, and reduced direct solar irradiance at sites 1, 2, 4, and 5.

Species rhizome depth Examination of the rhizomes of different seagrass species demonstrated significant vertical stratification ($H_{(3)} = 73.3$, $P < 0.001$; Fig. 4). With the exception of *Z. muelleri* and *H. ovalis*, all other pairwise comparisons indicated significant differences between the substrata depths that their rhizomes occupy (Table 2). *H. uninervis* rhizomes were consistently found deeper than other species at each site where present (Table 3), at a median depth of 3.8 ± 0.3 cm. The species with the next deepest median rhizome depth was *Z. muelleri* (1.5 ± 0.2 cm), followed by *H. ovalis* (1.0 ± 0.1 cm). At low tide, *H. ovalis* plants were typically found in 'puddles' of seawater, where air exposure was reduced, and the rhizomes were close to the substrate–air interface. *H. decipiens* had the shallowest rhizome depth (median depth of 0.1 cm, $n = 4$) and is typically limited to subtidal environments, with exposure rarely occurring.

Substrate temperature profiles Substrate temperature profiles exhibited a pattern of temperature decreasing with substrate depth (Fig. 5). The temperature depth profile differed in a statistically significant manner ($H_{(10)} = 201.133$, $P < 0.001$). In general, the median temperature was highest at the surface, with substratum depths of 6 cm or deeper being significantly cooler than shallower substratum (Table 4). The substrate (surface–air interface) temperature at depths of 0 to −1 cm had a high range of variability, with a temperature range of > 5°C (Fig. 5). At substratum > 4 cm in depth, the temperature range was reduced between 2.7 and 3°C compared with the surface (Fig. 5), demonstrating that seagrass rhizomes that occupy shallow substratum are exposed to greater temperature fluctuations.

The influence of full sun exposure vs cloud cover when sampling had greatest influence on surface temperatures where samples in full sun were, on average, 1.7°C warmer than samples

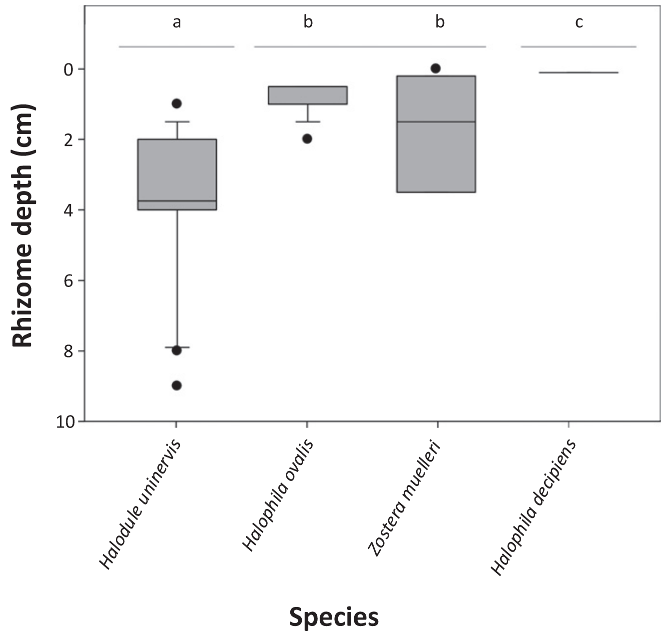

Fig. 4 Median depth of seagrass rhizomes in the substratum for four subtropical species (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) in Gladstone Harbour, Queensland, Australia. Whiskers are 5th and 95th percentiles, black dots denote outliers, and box midlines are the median rhizome depth in the substratum. Dunn's pairwise test result differences at $P < 0.05$ are expressed as groups a, b and c (Table 2).

under cloud. We noted rapid shifts over the course of *c.* 2 min with a maximum change from full sun to cloud of −3.7°C (30.0–26.3°C) between adjacent (*c.* 1 m) surface samples, and rapid warming from cloud to full sun with a maximum change of +4.3°C (26.3–30.6°C). This temperature difference was ameliorated at −1 cm where temperatures for samples in full sun were, on average, 0.6°C warmer than samples under cloud. Rapid surface cooling shifted maximum temperature depths to between −1 and 4 cm.

Substrate type At the landscape scale, the effect of species and soil type together statistically influenced the rhizome depth ($F_{(5,117)} = 70.343$, $P = 0.0000$). Specifically, *H. uninervis* rhizomes are deeper when in mud, shell/gravel and/or sand substrates, compared with *H. ovalis* and *Z. muelleri*.

Both depth and substrate type were significant predictors of temperature. As expected, depth below the substrate surface explained most of the variance in temperature ($F_{(4,1)} = 361.62$, $P < 0.001$); however, substrate type also explained significant variance ($F_{(4,1)} = 39.15$, $P < 0.001$; Table 5; Fig. 6). Shell/gravel substrates were consistently cooler than all other substrate types (Fig. 6). Sand substrates were consistently warmer than other substrate types (Fig. 6).

Species' rhizome temperature differences Maximum and minimum differences between substrate temperatures at rhizome depths and measured surface temperatures demonstrated a consistent median decrease of *c.* 1°C for *H. uninervis* (−0.95°C), *H. ovalis* (−0.9°C) and *Z. muelleri* (−1.2°C) (Fig. 7). The median difference for *H. decipiens* was 2.35°C; however, this was based on its presence at a single site (site 4) with 10 individuals measured. All species experience maximum temperature differences of > 3.0°C; > 45% of individuals in each species experience maximum temperature differences > 2.0°C.

Site patterns

Site 1: species and substrate temperature Site 1 substrate was mud with two species present, *Z. muelleri* and *H. uninervis* (Table 1). The seagrasses at these sites occupied significantly different depth substrata ($t_{(8)} = -9.97$, $P < 0.0001$). *Z. muelleri* rhizomes occupied significantly shallower (−1 ± 0.2 cm) substratum compared with *H. uninervis* rhizomes (−7.5 ± 0.6 cm). The median depth of *Z. muelleri* rhizomes sat within a temperature envelope that ranged between 26.6 and 29°C. *H. uninervis* rhizomes occupied a narrow temperature envelope of 26–26.5°C.

Four of 16 temperature profiles were in cloudy conditions with a mean difference of 1.8°C between full sun and cloud. The substrata profile temperatures showed a decline from surface (0 cm) to depth (−10 cm) ($H_{(10)} = 76.861$, $P < 0.001$). At the surface, the median temperature was 28.5 ± 0.23°C, dropping to 25.9 ± 0.1°C at 10 cm depth. The range of temperatures recorded at shallow depths were more variable (range of 3.9°C) compared with temperatures measured at deeper depths (8, 9 and 10 cm substratum; range of 0.1–0.4°C)(Fig. 8a). Specifically, the temperature declines at the surface (0 cm) to 3 cm depth were significantly higher than temperatures at deeper depths (Table 6; Fig. 8a).

Site 2: species and substrate temperature Site 2 had a mud and shell mixed substrate, with two species present, *H. ovalis* and *H. uninervis* (Table 1). The seagrasses at these sites occupied significantly different depth substrata ($t_{(8)} = 4.69$, $P = 0.0003$), with *H. ovalis* rhizome median depth being significantly shallower (−1 ± 0.12 cm) than that of *H. uninervis* rhizomes (−2.25 ± 0.29 cm). The median depth of *H. ovalis* rhizomes sat within a temperature envelope that ranged between 27.4 and 28°C. *H. uninervis* rhizomes occupied a similarly narrow temperature envelope that was slightly cooler (26.9–27.5°C) than the *H. ovalis* temperature envelope.

Six of 11 temperature profiles were in cloudy conditions with a mean difference of −0.3°C between full sun and cloud. As with site 1, the site 2 substrata profile temperatures showed a decline from surface (0 cm) to depth (−10 cm)($H_{(10)} = 36.489$,

Table 2 Dunn's test results for seagrass (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) rhizome depths, with bold font indicating statistically significant differences

Comparison	Difference of ranks	Q-statistic	*P*-value
Halodule uninervis vs *Halophila decipiens*	95.45	7.17	**< 0.001**
Halodule uninervis vs *Halophila ovalis*	54.41	6.54	**< 0.001**
Halodule uninervis vs *Zostera muelleri*	39.44	4.62	**< 0.001**
Zostera muelleri vs *Halophila decipiens*	56.01	4.18	**< 0.001**
Zostera muelleri vs *Halophila ovalis*	14.98	1.78	0.455
Halophila ovalis vs *Halophila decipiens*	41.04	3.10	**0.012**

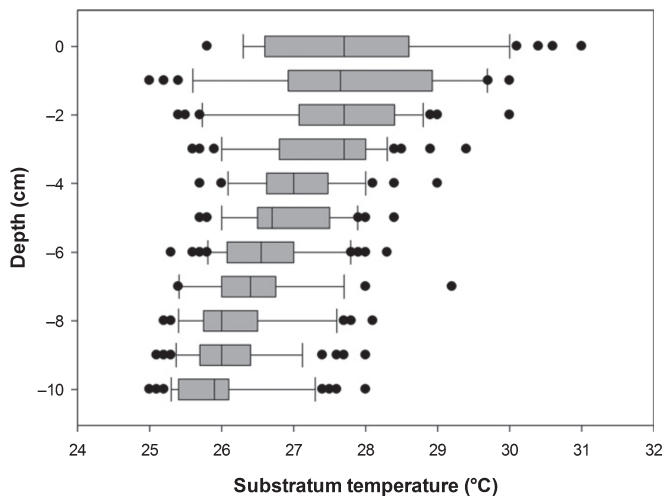

Fig. 5 Range of substratum temperatures (°C) across depth profiles (surface (0 cm) to −10 cm), pooled across the five sampling sites in Gladstone Harbour, Queensland, Australia. Whiskers represent the 5th and 95th percentiles, dots represent outliers, and the box line represents median substratum temperatures.

Table 3 Mean seagrass (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) rhizome depth (cm) at each site, with substrate type

Species	Site 1 Mud	Site 2 Shell/mud	Site 3 Shell/gravel	Site 4 Mud/gravel	Site 5 Sand
Halophila decipiens	–	–	–	−0.1 (± 0.0)	–
Halophila ovalis	–	−1.0 (± 0.1)	−1.2 (± 0.1)	−0.5 (± 0.0)	−1.0 (± 0.0)
Halodule uninervis	−7.2 (± 0.6)	−2.5 (± 0.3)	−2.0 (± 0.2)	–	−4.0 (± 0.0)
Zostera muelleri	−1.3 (± 0.2)	–	−1.4 (± 0.2)	−3.5 (± 0.0)	−0.2 (± 0.0)

'–', a species not present at a site. Values are means ± 1 SE.

Table 4 Dunn's test results (*P*-values) for substratum temperatures across depth (cm) profiles, with bold font indicating statistically significant differences

	0	1	2	3	4	5	6	7	8	9
1	1.000									
2	1.000	1.000								
3	1.000	1.000	1.000							
4	0.761	1.000	1.000	1.000						
5	0.058	0.592	0.254	0.951	1.000					
6	**< 0.001**	**0.008**	**0.002**	**0.016**	0.961	1.000				
7	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.023**	1.000	1.000			
8	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.014**	0.853	1.000		
9	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.073	1.000	1.000	
10	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.008**	0.355	1.000	1.000

Table 5 Holm–Sidak all pairwise multiple comparison results (analysis of covariance *post hoc*) where substrate type is the factor and temperature is the dependent variable, with bold font indicating statistically significant differences

Comparison	Absolute difference of means	*t*-statistic	*P*-value
Sand vs shell/gravel	1.2252	12.108	**< 0.001**
Mud/gravel vs shell/gravel	0.624	7.253	**< 0.001**
Sand vs mud	0.759	6.427	**< 0.001**
Sand vs mud/gravel	0.628	5.925	**< 0.001**
Mud vs shell/gravel	0.494	4.909	**< 0.001**
Shell/mud vs shell/gravel	0.728	4.089	**< 0.001**
Sand vs shell/mud	0.524	2.778	**0.022**
Mud/gravel vs mud	0.131	1.265	0.500
Shell/mud vs mud	0.234	1.250	0.379
Shell/mud vs mud/gravel	0.104	0.577	0.564

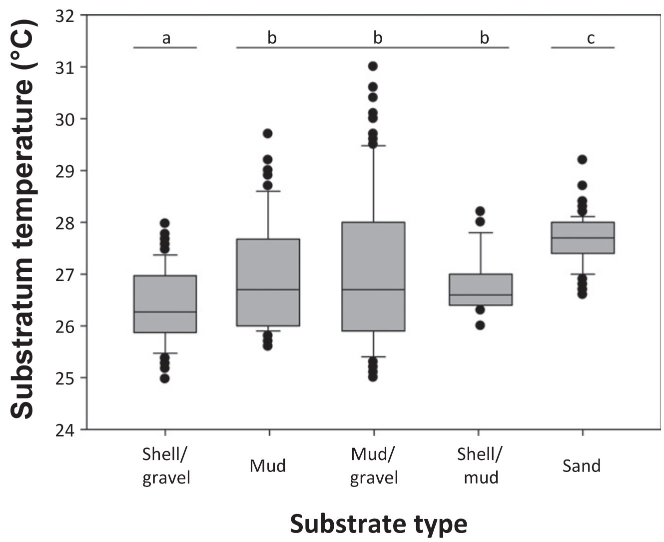

Fig. 6 Median temperature (°C) by substrate type, pooled across depth at each site, in Gladstone Harbour, Queensland, Australia. Whiskers represent the 5th and 95th percentiles, dots represent outliers, and the box line represents median substratum temperatures. Holm–Sidak all pairwise multiple comparison result (analysis of covariance *post hoc*) differences at $P < 0.05$ are expressed as groups a, b and c (Table 5).

$P < 0.001$) (Fig. 8b). However, the temperature differences between depth substrata were less striking than that observed at other sites (temperature range across the site was 0.3°C). The temperature variability at the surface remained a distinctive factor, with a wider temperature range (1.8°C) at the surface than at 10 cm depth (0.4°C). Statistically significant differences in substrata temperatures occurred between 1 and 10 cm depth ($Q = 3.530$, $P = 0.023$) and between 1 and 9 cm depth ($Q = 3.773$, $P = 0.009$).

Site 3: species and substrate temperature Site 3 had a shell and gravel mixed substrate, with three species – *Z. muelleri*, *H. ovalis* and *H. uninervis* – present (Table 1). There was a statistically significant difference in substrata depths that the different species occupied ($F_{(2,27)} = 5.20$, $P = 0.012$), with *H. uninervis* rhizomes occupying a significantly deeper substratum compared with *H. ovalis* rhizomes at this site ($t = 3.09$, $P = 0.013$). The substratum temperature envelope for *H. uninervis* rhizomes was 25.4–28°C (2.6°C range), with the temperature envelope for *H. ovalis* rhizomes being slightly broader (25–28°C; 3°C range).

All temperature profiles were in full sun conditions. The temperature depth profile at site 3 was similar to that at site 1, with a 2.1°C range (Fig. 8c). The temperature depth profiles differed significantly with depth ($H_{(10)} = 41.334$, $P < 0.001$), yet, the statistical patterns at site 3 were less clear-cut than those seen at sites 1, 2 and 5. There were statistically significant differences between substrata temperatures at the surface and at 9 cm ($Q = 3.724$, $P = 0.011$); at the surface and at 10 cm ($Q = 4.437$, $P < 0.001$); at 2 and 10 cm ($Q = 3.449$, $P = 0.031$); at 3 and 10 cm ($Q = 3.979$, $P = 0.004$); at 4 and 10 cm ($Q = 3.976$, $P = 0.004$); and at 5 and 10 cm ($Q = 3.546$, $P = 0.022$).

Site 4: species and substrate temperature Site 4 had a mud and gravel mixed substrate, with three species present, *Z. muelleri*, *H. ovalis* and *H. decipiens* (Table 1). The three seagrass species present occupied different substrata depths ($H_{(2)} = 29.00$, $P < 0.001$), with all three species rhizome depths being statistically different to each other (*Z. muelleri* vs *H. decipiens*, $q = 7.184$, $P < 0.001$; *Z. muelleri* vs *H. ovalis*, $q = 3.592$, $P = 0.03$; *H. ovalis* vs *H. decipiens*, $q = 3.592$, $P = 0.01$). Both *H. ovalis* and *H. decipiens* rhizomes occurred at shallow depths (-0.5 ± 0 and $-0.1 \pm < 0.01$ cm, respectively), with *Z. muelleri* occupying a

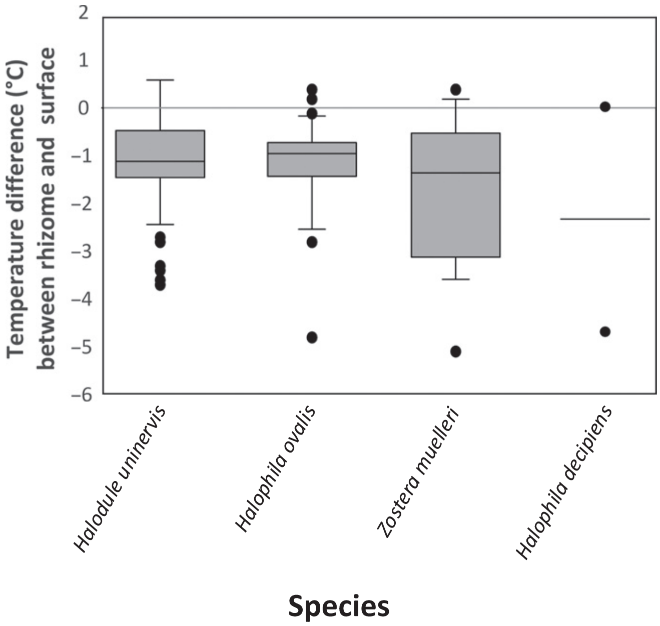

Fig. 7 Median temperature difference (°C) between rhizome and surface, pooled by species (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) across sites, in Gladstone Harbour, Queensland, Australia. Whiskers represent the 5th and 95th percentiles, dots represent outliers, and the box line represents median temperature differences.

−3.5 ± 0 cm substratum. Both *Halophila* species sat within the same temperature envelope of 26.3–31°C, with *Z. muelleri* occupying a narrower and slightly cooler temperature envelope (26–29°C).

Five of 15 temperature profiles were in cloudy conditions with a mean difference of 3.6°C between full sun and cloud. The substrata temperature decline from the surface to 10 cm depth at site 4 had the largest relative temperature range (3.6°C) across the depth profiles examined compared with the other sites (Fig. 8d). The temperature variability at this site was 4.7°C at the surface, reducing with depth (Fig. 8d). Substrata depth temperatures were statistically different ($H_{(10)} = 113.605$, $P < 0.001$); the statistical differences between substrata are summarized in Table 6. The first few centimetres (0 to −2 cm) of substrate were significantly warmer than substrata at 6 cm or deeper (Table 7).

Site 5: species and substrate temperature Site 5 had a sand substrate, with three species present, *Z. muelleri*, *H. ovalis* and *H. uninervis* (Table 1). *Z. muelleri* was the dominant seagrass at this location with both *H. ovalis* and *H. uninervis* being rare. The temperature envelopes that the seagrass species occupied were significantly different from each other ($H_{(2)} = 23.00$, $P < 0.001$), with *Z. muelleri* and *H. uninervis* found at statistically different depths ($q = 6.400$, $P < 0.001$). The temperature envelope for *H. ovalis* was not statistically different from the envelope for *Z. muelleri* ($P = 0.061$) or *H. uninervis* ($P = 0.061$). As with other sites, *Z. muelleri* occupied shallower depths (−0.2 cm substratum) where the temperature range was 26.7–27.7°C. In comparison, *H. uninervis* occupied a deeper substratum (−4 cm) where the temperature was cooler and the envelope range was narrower (0.8°C; ranging from 27.6 to 28.4°C).

All temperature profiles were obtained in cloudy conditions. The temperature depth profile at site 5 was markedly different from that at other sites, with surface temperatures not representing the highest temperature, but rather a temperature peak (28.7°C) occurring at the −2 cm substratum (Fig. 8e). Site 5 was the only site that showed a temperature profile pattern that had peak temperatures below the surface. There was a statistically significant difference in substrata temperatures across the depth profile ($F_{(10,77)} = 3.634$, $P < 0.001$; power 0.930), with the difference occurring between the 3 and 10 cm substrata ($t = 3.517$, $P = 0.040$). The 3 cm depth substratum had the narrowest range of temperatures recorded (0.7°C) of all substrata. Temperature variability at the surface (1°C range) was less than the variability at depth (1.4°C range).

Species temperature patterns

Specific patterns between the species at each site were evident and are summarized in Table 8. In general, *H. uninervis* occupied substrata at individual sites where the temperature envelope was relatively narrow (0.1–0.8°C) (Table 8). However, site 3 was an exception for *H. uninervis*, where it occurred in a relatively shallow substratum, in a temperature range of 2.6°C. Both *Z. muelleri* and *H. ovalis* had broader median temperature envelopes, with *H. decipiens* having the broadest temperature range of the species measured (Table 8). These temperature profiles were a reflection of the depth of substratum that each species rhizome occupied (Fig. 4), with *H. uninervis* tending to occupy deeper and cooler substratum. We note that our temperature envelopes are based upon data from the austral summer/wet season only (i.e., growing season) and that further investigation is needed to develop an understanding of the annual temperature envelope for the species investigated at these locations.

Discussion

This study demonstrates that the four studied seagrass species present in Gladstone Harbour exhibit vertical stratification of below-ground rhizome placement (as a proxy for minimum rooting depth). Rhizome depth varied with substrate; however, rhizomes of *H. uninervis* are consistently and significantly found deeper than the other species' rhizomes, penetrating up to 9.0 cm (average 3.7 ± 0.3 cm), and *Z. muelleri* has average depths > 1 cm (with the exception of sand). Further, we found that this vertical stratification is related to soil temperature. Based on temperature profiles in differing soil types, these species achieve median temperature reductions from measured surface temperatures of at least 1°C, with > 45% of individuals from each species having maximum temperature reductions > 2°C, noting that measured surface temperatures are > 5°C lower than observed maxima (Fig. 3).

Rooting depth is a common plant functional trait examined within terrestrial systems, with a body of literature examining root foraging for nutrients and water, and avoidance for specific soil types (see Kembel & Cahill, 2005; García-Palacios *et al.*,

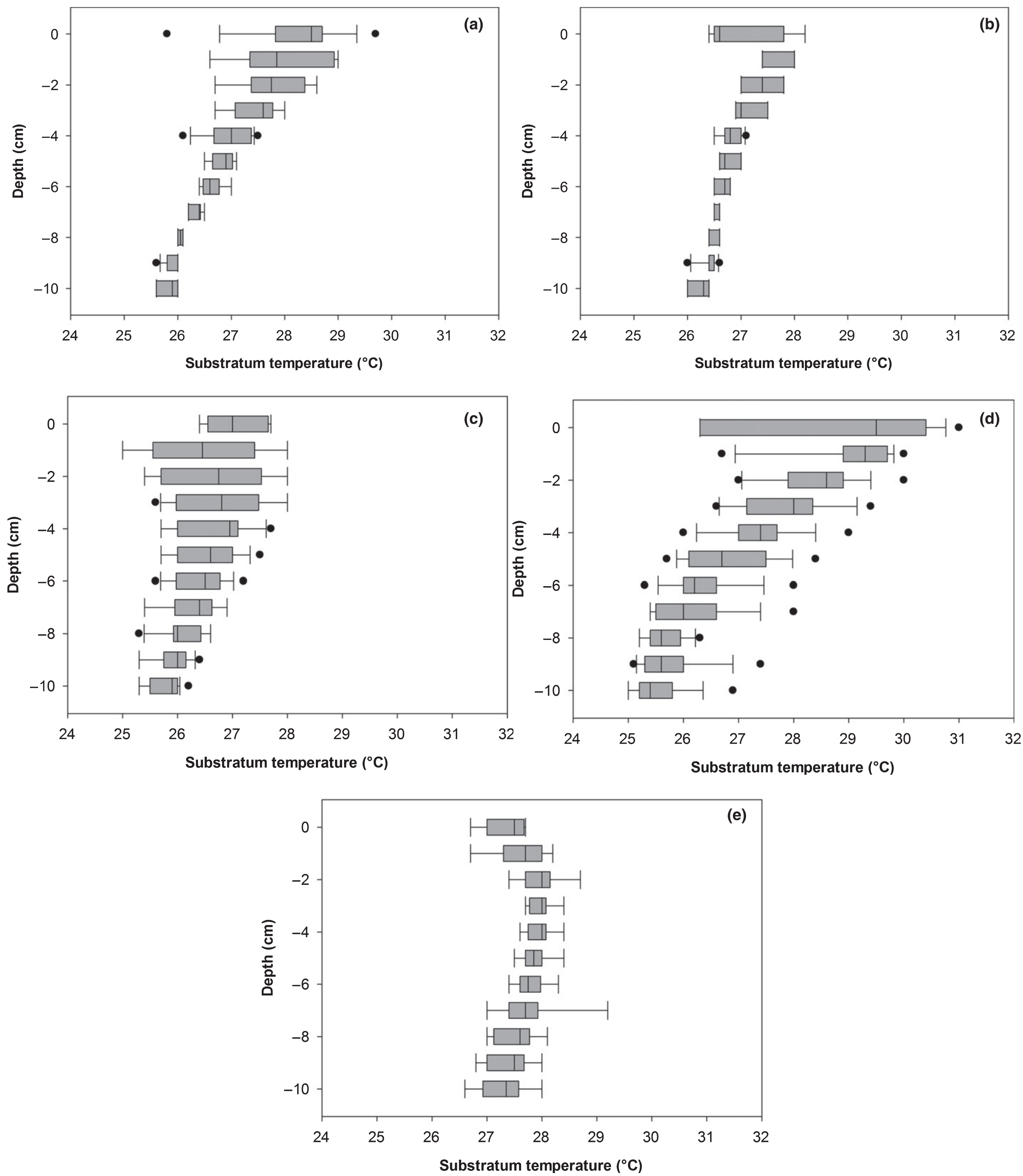

Fig. 8 Range of substratum temperatures (°C) across the depth profiles (surface (0 cm) to −10 cm) in Gladstone Harbour, Queensland, Australia, for: (a) site 1; (b) site 2; (c) site 3; (d) site 4; and (e) site 5. Whiskers represent the 5th and 95th percentiles, dots represent outliers, and box line represents median substratum temperatures.

2012), but rooting depth is variously defined and only occasionally documented for seagrasses (e.g. https://www.try-db.org/TryWeb/Home.php; see Supporting Information Table S1).

Determinants of rhizome and rooting depths have included anchoring (e.g. Balestri *et al.*, 2015), lateral growth/spread (e.g. Han *et al.*, 2012), and nutrient capture (e.g. Duarte *et al.*, 1998;

Table 6 Dunn's test results (*P*-values) for substratum temperatures across depth (cm) profiles at site 1, with bold font indicating statistically significant differences

	0	1	2	3	4	5	6	7	8	9
1	1.000									
2	1.000	1.000								
3	1.000	1.000	1.000							
4	0.544	1.000	1.000	1.000						
5	1.000	1.000	1.000	1.000	1.000					
6	0.404	1.000	1.000	1.000	1.000	1.000				
7	**0.041**	0.568	0.904	1.000	1.000	1.000	1.000			
8	**0.001**	0.064	0.113	0.332	1.000	1.000	1.000	1.000		
9	**< 0.001**	**< 0.001**	**< 0.001**	**0.003**	0.002	0.320	1.000	1.000	1.000	
10	**< 0.001**	**0.002**	**0.005**	**0.019**	0.053	0.649	1.000	1.000	1.000	1.000

Table 7 Dunn's test results (*P*-values) for substratum temperatures across depth (cm) profiles at site 4, with bold font indicating statistically significant differences

	0	1	2	3	4	5	6	7	8	9
1	1.000									
2	1.000	1.000								
3	1.000	1.000	1.000							
4	1.000	1.000	1.000	1.000						
5	0.960	0.069	0.524	1.000	1.000					
6	**0.035**	**0.001**	**0.015**	0.218	1.000	1.000				
7	**0.002**	**< 0.001**	**< 0.001**	**0.018**	0.343	1.000	1.000			
8	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.014**	0.346	1.000	1.000		
9	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.011**	0.304	1.000	1.000	1.000	
10	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.049**	1.000	1.000	1.000	1.000

Table 8 Temperature (°C) envelope range for each seagrass species (*Halodule uninervis, Halophila ovalis*, *Zostera muelleri* and *Halophila decipiens*) at each site when the site is exposed at low tide

	Temperature (°C) range					
Species	Site 1	Site 2	Site 3	Site 4	Site 5	Median
Halophila decipiens	–	–	–	4.7	–	4.7
Halophila ovalis	–	0.6	3	4.7	1.5	2.25
Halodule uninervis	0.1	0.6	2.6	–	0.8	0.7
Zostera muelleri	2.4	–	2.6	3	1	2.5

'–', a species not present at a site.

Balestri *et al.*, 2015), with rhizome and root diameter representing size-related tradeoffs between nutrient uptake efficiency (smaller diameters) and O_2 loss (larger diameters) (Duarte *et al.*, 1998). Han *et al.* (2012) observed a consistent rhizome depth for *Zostera noltii* Hornemann, 1832 and empirically demonstrated that, following addition (burial) and removal (erosion) experiments, *Z. noltii* consistently grew to achieve the 'preferred' rhizome depth and concluded that the ability to rapidly respond to altered sediment depths allowed *Z. noltii* to acclimate at meadow margins. Duarte *et al.* (1998) evaluated below-ground biomass in four subtidal locations and demonstrated substantial vertical stratification, with a finding that larger seagrass species tended to occupy deeper sediment layers, noting an overlap of root structures in shallow sediment layers of mixed species meadows. Vertical stratification of below-ground biomass is typically interpreted as a means of reducing competition for nutrient resources (Wilson, 1988; Kembel & Cahill, 2005). We propose that for some seagrass species, root 'foraging' may be additionally influenced by thermal envelopes.

Unlike most terrestrial plants, seagrasses experience significant tradeoffs between above-ground photosynthetic biomass that also provides access to frequently limiting nutrients such as nitrogen and phosphorus (Stapel *et al.*, 1996; Rubio *et al.*, 2007) and the below-ground biomass that provides access to nutrient resources, but also exposure to anoxic sediments (Hemminga, 1998). Below-ground biomass can represent a significant fraction of living tissue (e.g. Duarte & Chiscano, 1999), creating a high O_2 demand for the organism as a combination of tissue respiration and radial O_2 loss to surrounding sediments (Armstrong, 1980; Isaksen & Finster, 1996; Borum *et al.*, 2006; Brodersen *et al.*, 2014). Radial O_2 loss leads to the establishment and maintenance of 'oxic microshields' surrounding root tips (Pedersen *et al.*, 1998), rhizomes and basal meristems (Brodersen *et al.*, 2014, 2016), which are critical in providing protection from reduced phytotoxins such as sulphide (e.g. Armstrong, 1980; Isaksen & Finster, 1996; Borum *et al.*, 2006; Brodersen *et al.*, 2014, 2015).

Oxygen in above-ground biomass is derived from photosynthesis and air-tissue diffusion (e.g. Colmer, 2003; Borum *et al.*, 2006). Transport of O_2 from above-ground biomass to below-ground biomass is primarily driven by diffusion from high (leaves) to low (roots) partial pressure gradients (Armstrong, 1980; Larkum *et al.*, 1989). Diffusion in seagrasses is further enhanced by a number of anatomical adaptations, morphological features and physiological traits (Colmer, 2003) which increase porosity of plant tissue (e.g. specialized lacunae; Penhale & Wetzel, 1983; Connell *et al.*, 1999), moderate O_2 loss to sediments (e.g. thickened rhizomes and roots; Duarte *et al.*, 1998; Johnson *et al.*, 2016) and reduce exposure of below-ground biomass to anoxic sediments (e.g. short, rapidly replaced roots within the oxic microshield; Johnson *et al.*, 2016).

Additionally, Colmer (2003) proposed that environmental conditions, such as decreased temperature, might reduce O_2 demand in below-ground biomass, noting that maintenance-related respiration may differ significantly from growth-related respiration. In unvegetated subtidal systems, substrate (soil) temperatures are closely correlated with overlying waters (Wheatcroft *et al.*, 2007); however, intertidal substrate temperatures fluctuate between submerged and exposed periods when surface exposure to solar warming results in vertical thermal transfer in the substrate to depths of 50 cm (Kim *et al.*, 2007; Thomson, 2010). Our findings demonstrate that fine scale measurements (1 cm) exhibit rapid temperature reductions of several degrees within the top 10 cm (note that biogenic shell material influences temperature profiles, potentially by altering the surface albedo and substrate porosity). These temperature differentials present an environmental gradient for below-ground rhizome selection during growth.

Rhizomes and roots found at increasing intertidal soil depths experience net decreases in temperature relative to above-ground biomass of at least 1°C, with high proportions of populations having a maximum temperature reduction > 2°C. Given that metabolism scales exponentially with change in temperature, cooler temperatures will therefore decrease metabolic processes, lowering the O_2 tissue demand in the below-ground biomass by slowing respiration (Armstrong, 1980). We estimated the reduction in below-ground respiratory demand using information from Collier *et al.* (2017) for *H. uninervis* ($P_0 = 0.2$; $Q_{10} = 1.89$) and *Z. muelleri* ($P_0 = 0.8$; $Q_{10} = 2.6$)(both measured during austral summer at Moreton Bay, Queensland; *c.* 2° latitude further south of Gladstone Harbour). A 1°C temperature reduction would result in between 6% and 9% reduction in respiration; however, estimations based on field measurements for *H. uninervis* were between 2.8% and 10.5%, and those for *Z. muelleri* were between 3.6% and 24.0%, for maximum and minimum rhizome temperatures, respectively. These potentially represent significant reductions in tissue-specific O_2 demand, resulting in greater pools of O_2 in below-ground tissues, which should enhance radial O_2 loss and enhance the oxic shield. In similar fashion, cooler temperatures will also decrease the metabolism of sulfate reducing microorganisms in the rhizosphere, resulting in increased effectiveness of the oxic microshield.

Our hypotheses that certain species are soil temperature-sensitive and compensate for temperature sensitivity via rhizome rooting depth are supported by our results. We demonstrate that *H. uninervis* rhizomes have consistently deeper rooting depths (Fig. 4) which are statistically cooler (Fig. 5) than the rooting depths that rhizomes of *Z. muelleri*, *H. ovalis* and *H. decipiens* occupy. *H. uninervis* also occupies a narrow, median soil temperature envelope (Table 8). We surmise with caution that, in this location, *H. uninervis* is a soil temperature (or thermal)-sensitive intertidal species that employs the thermal gradient in soil temperatures for placement of below-ground biomass to maintain a restricted thermal envelope. By contrast, *Z. muelleri* and *H. ovalis* appear to be plastic in their thermal response, with much wider thermal envelopes and less active use of the soil temperature gradient (Fig. 4; Table 8).

The implications of our findings have potential relevance to both restoration ecology and climate change-focused management of seagrass species, but require empirical evaluation and validation at biogeographic scales. Surface temperature of Queensland coastal waters has peaked at 41.5°C (2010/2011 summer; http://www.seagrasswatch.org/cairns.html), 11°C more than the reported average seawater temperature during the wet/summer for this region (Queensland Department of Environment and Resource Management, 2011). Increased warming of surface waters and soil temperatures will reduce available O_2 in seagrass meadows, and is likely to influence phytotoxic H_2S concentrations in the substrate (Terrados *et al.*, 1999; Hemminga & Duarte, 2000; Borum *et al.*, 2005), resulting in increased stress upon the plants and shifting previously suitable habitats into sub-optimal areas for seagrass survival in the future.

Climate change and restoration of seagrasses are intrinsically linked, with restoration considered an effective strategy to ameliorate climate change impacts (e.g. Marbà *et al.*, 2015). There are clear concerns within the literature that increases in water temperature and extreme heat events (air temperature) will influence seagrass survival (e.g. Jordà *et al.*, 2012; Collier & Waycott, 2014; Koch *et al.*, 2015; Thomson *et al.*, 2015; Pedersen *et al.*, 2016; Galli *et al.*, 2017). Furthermore, our results demonstrate that intertidal seagrasses occupy species' specific soil temperature envelopes, with *H. uninervis* actively using the soil temperature gradients to reduce temperature fluctuations based on depth of below-ground biomass. Similar patterns have been observed in metacommunities of seagrasses in Portugal, where seagrass-associated species sorting occurs and some species live deeper in the sediment when stress conditions are present (e.g. Dolbeth *et al.*, 2013). We predict that species such as *H. uninervis* should exhibit a latitudinal gradient in depth of below-ground biomass correlated with shifting absolute temperatures and soil temperature profiles. Similarly, climate change-induced shifts in soil temperature profiles should influence seagrass species seeking deeper placement of below-ground biomass in the substrate as an adaptation strategy. An understanding of species soil thermal preferences could aid in modelling potential range shifts that are modulated by climate change.

The capacity for shallow-rooted species such as *Z. muelleri, H. decipiens* and *H. ovalis* (Fig. 4) to adapt to higher soil temperatures and potentially broader soil temperature envelopes remains an unknown at present. It is possible that these species may be vulnerable, as they occupy a niche that is currently exposed to large fluctuations in soil temperature. Phillips *et al.* (1983) has noted that *H. ovalis* and *H. uninervis* are known to tolerate extreme temperature conditions. One mechanism that might aid *H. uninervis* in surviving extreme temperatures may be in its adaption to seek cooler temperature envelopes via more deeply rooted rhizomes (Fig. 4). Thus, *H. uninervis* may not be as vulnerable to climate change influences as shallower-rooted species. Species that are unable to compensate for variable soil temperature fluctuations are likely to be the ones at risk and become climate refugees, via shifting biogeography.

Conclusions

In conclusion, we have demonstrated a correlation between intertidal seagrass species, soil temperature profiles (during low tide) and rhizome rooting depths. In particular, *H. uninervis* is a deep-rooted seagrass that occupies a soil temperature envelope that is relatively restricted, compared with the other species investigated. Soil temperatures followed an anticipated profile of temperatures decreasing with depth, and exhibit high temperature fluctuation within shallow depths, as a function of site sediment type. Implications of how soil temperature profiles influence intertidal seagrasses are relevant to restoration ecology and climate change impacts in these important habitats. We recommend that quantitative exploration of the seagrass species' soil temperature relationships is required to better understand the observations in this study.

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Author contributions

M.L.C. conceived the ideas and designed the methodology with input from C.L.H.; M.L.C., C.L.H. and R.H. collected the data; M.L.C., L.D.H. and C.L.H. analysed the data; M.L.C. and L.D.H. led the writing of the manuscript. M.L.C., L.D.H., R.H., R.M., S.S., A.N.R. and C.L.H. all contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 Seagrass species rhizome depth information reported within the published literature, with supporting references

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