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Accounting for the influence of temperature and location when predicting seagrass (halophila ovalis) photosynthetic performance

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ABSTRACT

We show that the effect of temperature on photosynthesis of a widely distributed and ecological important seagrass species was not consistent among locations, with some evidence of acclimation to the local temperature range. This has important implications for modelling seagrass productivity and the impacts of light reduction on seagrass ecosystems. Reduced light availability is one of the main pressures negatively impacting on seagrass meadows worldwide. Our knowledge of seagrass photosynthetic characteristics is critical to predicting and managing impacts of light reducing activities but suffers from two critical information gaps: first, data is overwhelmingly derived from studies of leaf tissue and not for whole plants, and is unlikely to reflect whole plant performance under light reduction stress; and second, few studies have looked at spatial and temporal variability in photosynthetic performance of whole seagrasses. We investigated temporal and spatial variation at a range of temperatures for whole plants of *Halophila ovalis*, a widely distributed species, by measuring oxygen exchange of intact plants collected from four locations across a latitudinal gradient (10°) at three temperatures (17 °C, 23 °C, 28 °C). For all locations, temperature affected all photosynthetic parameters, with some parameters (NP_{max}, R, I_k) showing a distinct difference between tropical and temperate locations. For example, NP_{max} ranged from 1.35 ± 0.12 to 5 ± 0.16 mg O_2. g DW^{-1}. hr^{-1} in temperate locations and from 4 ± 0.3 to 12 ± 0.68 mg O_2. g DW^{-1}. hr^{-1} in the tropical location. However, the effect of temperature on photosynthesis was not consistent among locations, and often the rate of photosynthesis was greatest at temperatures approaching the mean month maximum temperature for the location, suggestive of acclimation. Time of year also affected photosynthetic rates and how temperature influenced those rates. We conclude that the application of P–I parameters to model, predict or manage the effect of light reduction of *H. ovalis*, and likely other seagrass species, may require site- and time-specific knowledge of P–I relationships.

1. Introduction

Seagrasses are marine angiosperms, highly valued for their productivity and the range of ecological functions and ecosystem services they provide (Orth et al., 2006). Despite their recognised importance, seagrass meadows are among the most threatened ecosystems worldwide. By 1980 anthropogenic activity had resulted in the loss of 35% of the world's seagrass area, and between 1980 and the early 2000s it was declining at a median rate of 5–7% yr^{-1}, comparable to losses reported for coral reefs (27% of world's loss) and tropical rainforests (since 1990 6.4% yr^{-1}) (Achard et al., 2002; Cesar et al., 2003; Waycott et al., 2009). Factors contributing to ongoing seagrass loss are, therefore, of critical concern to the sustainability of seagrass meadows and the communities and industries supported by them (Collier et al., 2011).

The major cause of seagrass decline is reduced availability of light driven by eutrophication, dredging and other anthropogenic activities (Orth et al., 2006; Waycott et al., 2009; Collier et al., 2016). Even small reductions in light can cause decline in seagrass growth and distribution (Ralph et al., 2007). Temperature changes can also have a profound impact on seagrasses (Collier and Waycott, 2014). Globally, sea surface temperatures are projected to increase by 2 °C or more by 2100 in many parts of the world (IPCC, 2014), while sporadic ocean warming events, such as the 2010/2011 marine heatwave (which in some areas increased the sea surface temperature by 5 °C) will also become more frequent (Oliver et al., 2018; Pearce and Feng, 2013). These human-induced impacts on temperature and light do not necessarily occur in isolation. Reduced light availability from activities such as dredging coupled with ocean warming is predicted to result in major reductions in the quality

and quantity of ecosystem services seagrass meadows provide (Hyndes et al., 2016).

Seagrasses have relatively high light requirements due to the respiratory demand of large amounts of non-photosynthetic tissues, including roots, rhizomes and above-ground structural tissue (Beer et al., 2014; Collier et al., 2008). The ratio of carbon fixed in photosynthesis to that consumed through respiration determines the plant's carbon balance (Touchette and Burkholder, 2000), with growth and reproduction requiring a net positive carbon balance. Under reduced light conditions, seagrasses make adjustments to maintain a positive carbon balance, with physiological changes usually the first to occur (Ralph et al., 2007). For example, plants can increase their photosynthetic efficiency (α), reducing the amount of light needed to reach the saturating intensity (I_k), or reallocate carbohydrate reserves to maintain metabolic processes, which can also modify photosynthetic characteristics (Collier et al., 2011). Under persistent stress, morphological changes to leaves and meadow structure allow seagrasses to optimise photosynthesis (Lavery et al., 2009). Most efforts to predict the response of seagrasses to reduced light rely, in some way, on estimates of one or more key photosynthetic parameters, including α and I_k, defined above, as well as P_{max} (the maximum rate of photosynthesis), I_c (the compensation irradiance; that required to balance respiratory carbon losses with carbon fixation) and R (the rate of respiration).

Understanding a seagrass species' carbon balance requires knowledge of its photosynthesis-irradiance (P–I) relationship, which relates the rate of photosynthesis to the intensity of irradiance (Pollard and Greenway, 1993). However, a major limitation of most P–I relationships is that they are determined only for the photosynthetic tissues of the plant (leaves and sheaths) and ignore the non-photosynthetic organs, such as rhizomes and roots. This makes them useful for defining the photosynthetic response to light or other environmental parameters, but they do not reflect the whole plant metabolism and the true light requirement to maintain a positive C balance of the whole plant. Olesen and Sand-Jensen (1993) found a considerably higher I_c for whole plants of *Zostera marina* in comparison to leaf tissue alone. This is because below-ground tissue can make up more than 50% of seagrass biomass, thus whole plants have a greater respiratory demand than leaves alone (Lee et al., 2007). Therefore, measurements of only leaf segments may have limited application in predicting the carbon balance of whole plants. To confidently apply seagrass physiological data to management situations, it is important to know how the P–I relationship is affected by the ratio of photosynthetic to non-photosynthetic material. Collier et al. (2017) found a 34% lower net productivity of whole plants compared to leaves alone, highlighting the importance of accounting for below-ground respiration in seagrass productivity estimates. Using leaf-generated P–I parameters to estimate whole plant or seagrass meadow responses to altered light availability requires assumptions to be made about the respiratory rate of the non-photosynthetic tissue under those conditions. Whole plant P–I curves are more likely to reflect the performance of whole seagrass meadows when scaled up to total biomass, though of course these may be less useful for detailed physiological studies of photosynthesis itself.

A second limitation of most existing seagrass P–I relationships is that they are generalised models determined under a limited set of conditions, and in reality, these models may not be generalisable across a species. Many seagrass species have broad distributions and survive under wide ranges of temperature and light conditions (Hemminga and Duarte, 2000). P–I relationships are particularly sensitive to changes in temperature, and we can assume that adaptation to the temperature regime of their local environment may have led to spatial variation in seagrass P–I relationship (Masini and Manning, 1997; Beer et al., 2014). Further, literature shows that even in the same location the P–I relationship can vary throughout the year (Masini and Manning, 1997), which is likely due to seasonal temperature fluctuations (Staehr and Borum, 2011). Thus, any effort to model the photosynthetic performance of seagrasses under altered light conditions would need to factor

in the temperature regime the plant is likely to be growing under in that place and time, and whether this will affect the P–I relationship.

In this study we aimed to determine whether the whole plant P–I relationship for a widely distributed and ecologically important seagrass species, *Halophila ovalis*, is affected by temperature and whether any effect is consistent among different locations along a latitudinal gradient ($\sim$1200 km) and at different times of year. We hypothesised that: (1) temperature will alter the P–I relationship of *H. ovalis*; (2) the P–I relationships of plants will demonstrate acclimation to the ambient temperatures of the location in which they occur and different seasons; but (3) plants at different sites within the same locations would have the same thermal acclimation pattern.

2. Materials and methods

2.1. Plant collection and acclimation

To test for effects of temperature on the P–I relationship within and across locations, ramets of *Halophila ovalis* were collected between February and May 2016 from four 'Locations' in Western Australia (Table 1; Fig. 1): 'Jurien Bay' 'Perth' and 'South-West' are temperate locations of increasing latitude while 'Coral Bay' is tropical. In each location, plants were collected from two 'Sites' separated by a minimum of 2 km. South-west sites (Cowaramup and Cosy Corner) are small protected embayment's, where seagrass grows in sandy patches interspersed by reef. At the Perth location, Woodman Point is located in a larger embayment, and the Marmion site is protected by reef structure. Seagrass sites in both Jurien and Coral Bay are in larger embayment's, with seagrass growing in sandy patches.

P–I relationships of the plants were determined at three temperatures (17 °C, 23 °C, and 28 °C). The lowest temperature, 17 °C was within the range of the three most southerly locations (South-West to Jurien Bay), 23 °C was within the range of all locations, whereas the upper temperature treatment, 28 °C was the upper range of the Coral Bay location only. Therefore, at the three temperate locations the highest temperature treatment represents conditions plants are not normally exposed to and may be more representative of future conditions under climate change. For the two most southern temperate locations the plants were collected at a temperature within 2 °C of the monthly maximum (20 and 22 °C), but for the most northern, tropical location, it was within 3 °C of the maximum (25 °C). At the temperate location of Jurien Bay plants were collected at 19 °C, which was closer to the monthly minimum. To test for any effect of time of year on either the P–I relationship of *Halophila ovalis* plants or the effect of temperature on the P–I relationship, plants were collected from the same two sites in Perth (Woodman Point and Marmion) on a second collection date, July 2016, and the P–I relationships determined under the same set of temperature treatments as above.

Whole plants were collected by gently fanning sediment away to expose the rhizome, extracting the ramet with at least 4 leaf pairs and placed in a cooler box filled with seawater and aeration for transportation to the laboratory. At each collection time, temperature and salinity were recorded, to determine initial laboratory acclimation conditions. Dark adapted yields were measured on randomly chosen leaves from five different plants at each site using a Waltz™ diving pulse amplitude modulation (PAM) fluorometer. These yield measurements were later used as a reference against dark adapted yields of plants returned to the laboratory to confirm that they had acclimated to the *in vitro* conditions.

In the laboratory, the experimental plants were gently cleaned to remove epiphytes, and each ramet standardised to four leaf pairs preceding an apical meristem. Plants were then planted into plastic containers containing a 7 cm layer of unsorted, washed, quartz river sand overlaid by aerated seawater. Water temperature and salinity (36) emulated field collection conditions. Water temperature was controlled using aquarium heaters. Plants were provided with 180 μmol photons

Table 1

Summary of site data for all locations, including; GPS coordinates, plant collection month, water temperature range for each location, field conditions at time of plant collection, water depth and tidal range. Plants from the Perth location were collected in both summer and winter, with collection month and water temp at time of collection denoted as summer/winter respectively. Biomass data for each site in Fig. S1.

Location	Site	Latitude	Longitude	Collection month	Water temp range (°C)	Water temp at time of collection (°C)	Water Depth (m)	Tidal range (m)
Coral Bay	Five Finger Reef	−23.168	113.762	Apr	21–28	25	1.9–2.5	2.4
	The Ridge	−23.121	113.750				1.9–2.9	
Jurien Bay	Jurien Jetty	−30.299	115.039	May	17–24	19	2–3.1	0.79
	Jurien Harbour	−30.288	115.042				2–3.5	
Perth	Woodman Point	−32.137	115.746	Feb/Jun	16–23	22/16	2	0.86
	Marmion	−31.839	115.749				1.8–2.4	
South-West	Cosy Corner	−34.257	115.028	Mar	16–22	20	2.4	0.9
	Cowaramup	−33.859	114.987				2	

*Water depth based on bathymetry maps.

*Tidal range data extracted from Bureau of Meteorology (2020) to nearest location; Coral Bay (Exmouth), Jurien Bay (Geraldton), Perth (Fremantle), South-West (Bunbury).

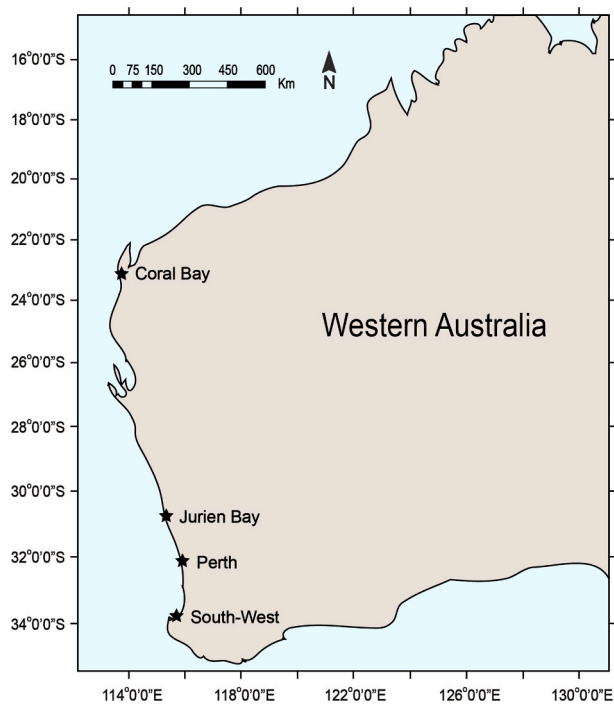*Water temperature data from the Department of Biodiversity and Conservation (DBCA; pers. comm).

Fig. 1. Study Locations within Western Australia where *Halophila ovalis* was collected for P–I determinations. At each Location, plants were collected from two Sites, more than 2 km apart.

$m^{-2}\ s^{-1}$ (approximating saturating irradiance; Strydom et al., 2017) using marine aquarium Light Emitting Diode (LED) modules with a full spectrum light (MarinTech™ Pty Ltd) on a 12/12 light: dark cycle. Light intensity at the base of the canopy was measured using a micro-PAR sensor (In-Situ Marine Optics™). Plants were left for two days before increasing or decreasing temperatures at 1 °C per day until the experimental temperature (17 °C, 23 °C, or 28 °C) was reached, after which they were left for 24 h before taking dark adapted maximum quantum yields on five separate plants to assess their acclimation. Plants were considered healthy and acclimated if they had a dark adapted yield of 0.73–0.75 (Ralph and Burchett, 1995) or yields higher than those taken in the field at the time of collection.

2.2. Photosynthetic-irradiance determinations

Seagrass respiration and photosynthesis were measured via the consumption or production of O_2. Plants were incubated in sealed, transparent, acrylic chambers, (diameter 52 mm, length 150 mm; volume 318 ml). Water within the chambers was circulated using small submersible pumps with a flow rate of 7000 ml h^{-1}. Dissolved oxygen concentrations within the chambers were measured using FireSting™ 3 mm robust REDFLASH technology sensors (Pyroscience) inserted through the chamber wall and connected through a 4-channel meter to a computer recording mg of O_2. To maintain a stable temperature (±0.25 °C), chambers were submerged in a 300 L tank containing 150 L of seawater, which was circulated through a chiller-heater unit set to the appropriate experimental temperature. The internal temperature of the chamber was also measured using a submersible temperature sensor connected to the FireSting O_2 meter. Light was provided by full spectrum LED light units (GrowPro 320; MarinTech™ Pty Ltd) suspended above the chambers, providing intensities from 30 to 300 μmol photons $m^{-2}\ s^{-1}$. Prior to each incubation, the oxygen electrodes were calibrated using the manufacturer's 2-point method (0% and 100% air saturated water). Five replicate plant chambers, each containing four *Halophila ovalis* ramets, and a sixth 'blank' chamber (containing no plant material) were established and placed into the temperature-controlled tank. The chambers were then covered in aluminium foil to exclude light and the inlet of each chamber was connected to its individual pump, to allow the chamber to be flushed whilst the plant material was left to dark adapt for 30 min. Once dark adapted, the chamber outlet was connected to the pump to create a sealed system and the dissolved oxygen concentration monitored every second. Monitoring continued in the dark for at least 20 min after the slope of dissolved oxygen vs time stabilised. The foil was then removed, and photosynthetic rates were measured for 10 min (once the slope had stabilised) at each of 7 light intensities (30, 60, 90, 120, 180, 240, and 300 μmol photons $m^{-2}\ s^{-1}$). At the end of the experiment the plants were removed from the chambers and separated into aboveground (leaves) and below-ground (roots and rhizomes) tissue. Fresh weight was recorded before drying the plants (48 h at 60 °C) and reweighing for dry weight determination.

2.3. Photosynthetic-irradiance curve fitting

For each replicate incubation (and control) at all unique temperature-light intensity combinations, oxygen concentration was plotted against time after discarding the first 2 min of data, which was considered a stabilisation period. The portion of the remaining data used to determine the rate was confined to that where the R^2 value was greater than 0.9. At the two lowest light intensities, due to noise, a lower R^2 value of 0.5 was used. Rates of oxygen exchange were normalised to g^{-1} DW hr^{-1} of whole plant material. Oxygen concentrations within the control chamber (containing no seagrass) were measured throughout

the experiment and experimental units were only used if there was no significant change in control chamber oxygen concentrations.

Photosynthetic parameters were extracted from the P–I curves using the least-squares method. For each incubation, P–I curves were fitted to the data using the hyperbolic tangent model equation of Chalker (1981):

$$P = GP_{max} \times \tanh\left(\frac{\alpha \times I}{GPmax}\right) + R,$$

where P is the rate of photosynthesis (mg O_2. g DW^{-1}. hr^{-1}), GP_{max} is the maximum Gross photosynthesis (mg O_2. g DW^{-1}. hr^{-1}), α (mg O_2. g DW^{-1}. hr^{-1}/(μmol m^{-2} s^{-1})) is the photosynthetic efficiency estimated as the slope for the linear portion (light-limited portion) of the PI curve, I is irradiance (μmol photons m^{-2} s^{-1}), and R (mg O_2. g DW^{-1}. hr^{-1}) is the rate of oxygen consumption in the dark.

NP_{max}, the maximum net rate of photosynthesis (mg O_2. g DW^{-1}. hr^{-1}) was calculated as:

$$NP_{max} = GP_{max} + R$$

The saturating irradiance (I_k; μmol photons m^{-2} s^{-1}) was calculated as:

$$I_k = GP_{max}/\alpha$$

The compensation irradiance (I_c; μmol photons m^{-2} s^{-1}) was calculated via a mathematical approximation that assumes I_c is much smaller than I_k:

$$I_c = (0 - R)/\alpha$$

2.4. Statistical analysis

Because we did not have data for all locations and sites at both times of year, two separate statistical tests were performed to test for: 1) the effects of temperature on the P–I relationship of *H. ovalis* within and across locations; and 2) the effect of time of year on the P–I relationship of *H. ovalis* plants and how temperature affected the relationships.

To test for significant effects of Temperature, Location or Site nested in Location on each P–I parameter, 3-way PERMANOVAs were performed, based on Euclidian distances with 9999 permutations using the PRIMER™ software (Anderson et al., 2008). If the number of unique permutations was less than 100, then a Monte Carlo (MC) simulation was run, and data interpreted in accordance to the MC P-value (Anderson et al., 2008). Temperature and Location were treated as fixed effects, whereas Site(Location) was random.

Because above-ground (leaf; AG) plant tissue has different rates of photosynthesis and respiration, and below-ground (roots and rhizome; BG) tissue has different rates of respiration, the P–I relationships of each replicate might have been affected by the ratio AG:BG biomass. To test for this, a covariate correlation was performed on AG:BG against each P–I variable (α, GP_{max}, NP_{max}, I_k, I_c, and R) in SPSS. The parameters α, GP_{max}, NP_{max}, and R were significantly correlated with AG:BG and in those cases, this was accounted for in subsequent statistical testing by including the ratio in the PERMANOVA as a co-variate. PERMDISP was used to test for homogeneity of variance and a square root or fourth root transformation used where this assumption was not met. Square root transformations of P–I variables were also used when there was a significant correlation with AG:BG ratio, as per Anderson et al. (2008). The Sums of Squares was set to Type III (Partial) SS, however if the co-variate was included in the analyses the Sums of Squares was set to Type I (sequential) SS. Where the PERMANOVA main test indicated a significant main effect or interaction (p < 0.05), pair-wise comparisons were performed to understand the nature of the effect or interaction.

To test for any significant effect of Time (of year), Site, or Temperature on P–I parameters, a 3-way PERMANOVA was performed on each

parameter, based on Euclidian distances, and 9999 permutations using the PRIMER™ software. Time and Temperature were treated as fixed effects, whereas site was a random effect. Treatment of the AG:BG ratio was as above; only α, I_c, and I_k were significantly correlated with AB:BG. PERMDISP was used to check for homogeneity of variance, with a square root transformation used when PERMANOVA assumptions were not met. Where the PERMANOVA main test indicated a significant main effect or interaction (p < 0.05), pair-wise comparisons were performed to explore the differences among the treatments.

3. Results

3.1. Effect of Temperature and location

At all temperatures and for all sites nested in locations *Halophila ovalis* exhibited the typical relationship between photosynthesis and light, without any photoinhibition (Fig. 2, Table S3). The P–I relationships varied among temperatures, and these differences were not consistent across locations, and in some cases not consistent among sites (Table S1).

Across all sites and temperatures, the maximum net photosynthesis rate (NP_{max}) ranged from 1.35 ± 0.12 to 12 ± 0.68 mg O_2. g DW^{-1}. hr^{-1} (Fig. 3a), with rates for tropical Coral Bay plants approximately two-to-three-fold higher than any of the temperate locations. NP_{max} was significantly affected by temperature, but the effect varied spatially with a significant Temperature X Site(Location) interaction (p $\leq$ 0.05), as well as a significant interaction with the leaf to rhizome covariate (AG:BG). At Coral Bay, both sites showed an increase in NP_{max} as temperature increased, and at Jurien Bay both sites had a significantly higher NP_{max} at 23 °C than either of the other temperatures. At the other locations (South-West and Perth) the effect of temperature on NP_{max} differed between sites within the location. Overall, at the most southern temperate location and the tropical location, NP_{max} was highest or equal highest at the highest temperature (28 °C), which for the tropical location aligns with summer *in-situ* ambient water temperatures, while at Jurien Bay it was highest at ambient summer water temperature (23 °C), and the Perth location showed no clear trend.

The rate of respiration (R) ranged from -0.74 ± 0.068 to -4.4 ± 0.28 mg O_2. g DW^{-1}. hr^{-1} and generally increased with temperature (Fig. 3b). Respiration was significantly affected by temperature, but the effect varied within and across locations (Temperature X Site(Location) p $\leq$ 0.05), as well as a significant main effect of the covariate AG:BG on R. The most temperate sites (in South-west location) and the tropical sites in the Coral Bay location had similar rates of R, which were higher than the other temperate locations (Perth & Jurien Bay). Despite the effect of temperature on R varying among sites within each location, R was generally highest or equal highest at 28 °C.

The saturating irradiance (I_k) ranged from 85 ± 5.9 to 156 ± 3.6 μmol photons m^{-2} s^{-1} (Fig. 3c). I_k was significantly affected by an interaction of Temperature and Location (p $\leq$ 0.05); in tropical Coral Bay, I_k increased with temperature while in the three temperate locations I_k decreased with increasing temperature. There was no significant correlation between I_k and AG:BG covariate.

The light compensation point (I_c) ranged from 23 ± 1.4 to 60 ± 6.8 μmol photons m^{-2} s^{-1} (Fig. 3d). There was a significant Temperature X Site(Location) effect on I_c (p $\leq$ 0.05), with no significant effect of the AG:BG covariate. At some sites (Cosy Corner, Marmion, Jurien Jetty and The Ridge) as temperature increased I_c generally increased, while at Cowaramup, Woodman Point, Jurien Harbour, and Five Finger Reef there was no significant effect of temperature on I_c.

Photosynthetic efficiency (α) ranged from 0.022 ± 0.001 to 0.10 ± 0.0039 mg O_2. g DW^{-1}. hr^{-1}/(μmol m^{-2} s^{-1}) (Fig. 3e). There was a significant interactive effect of temperature and Site(Location) on α, which varied among locations and sometimes sites, but with α always greatest or equal greatest at 28 °C (Temperature X Site(Location); p $\leq$ 0.05). At Coral Bay and Jurien Bay, the two sites within each location
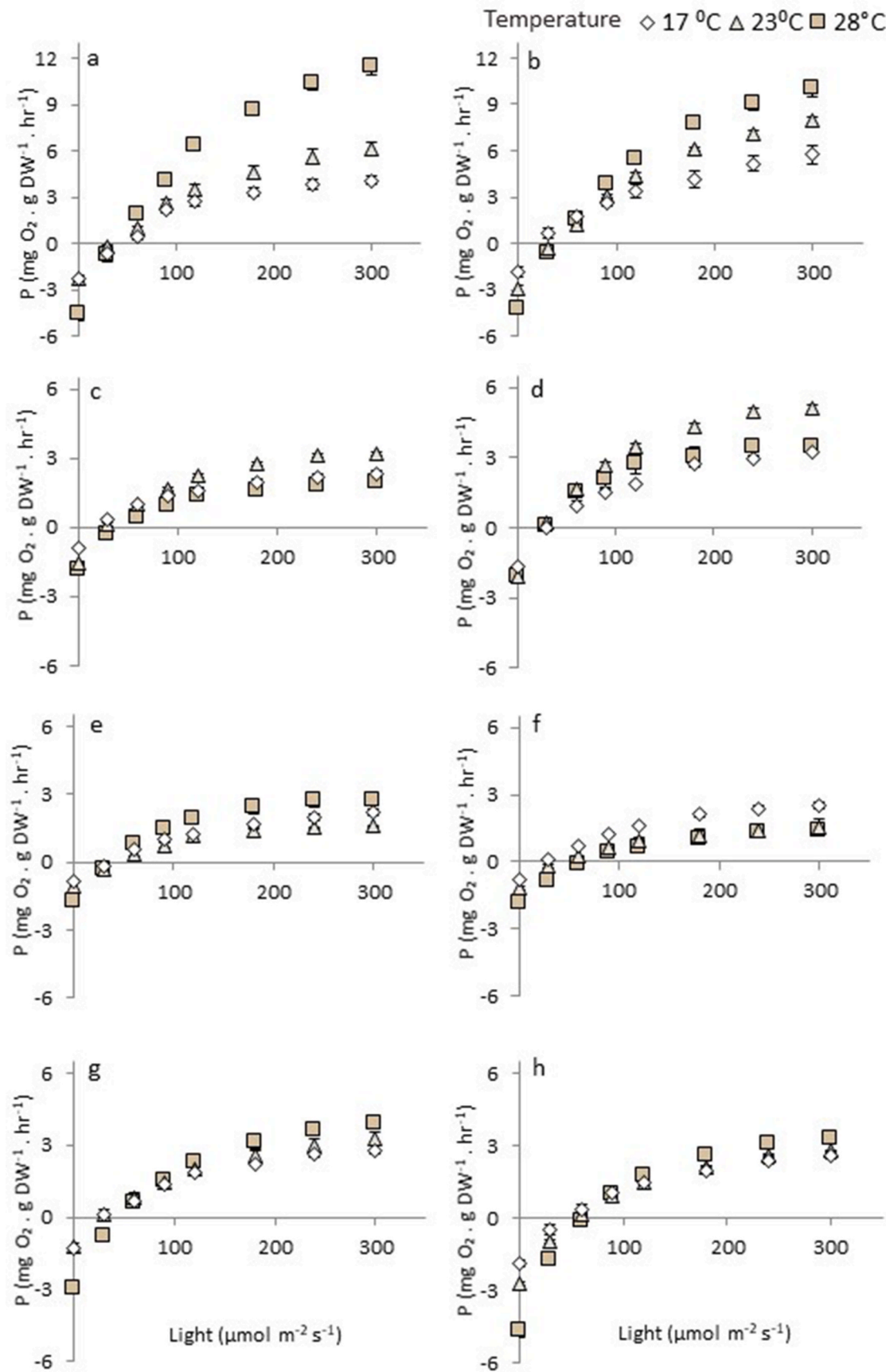
Fig. 2. Photosynthetic-irradiance curves at three temperatures (17 °C, 23 °C, 28 °C) for 8 sites, within four locations for *H. ovalis*. Coral Bay: a) Five Finger Reef b) The Ridge, Jurien Bay: c) Jurien Jetty d) Jurien Harbour, Perth: e) Woodman Point, f) Marmion, South-west: g) Cosy Corner, h) Cowaramup. Means and SE. n = 5.
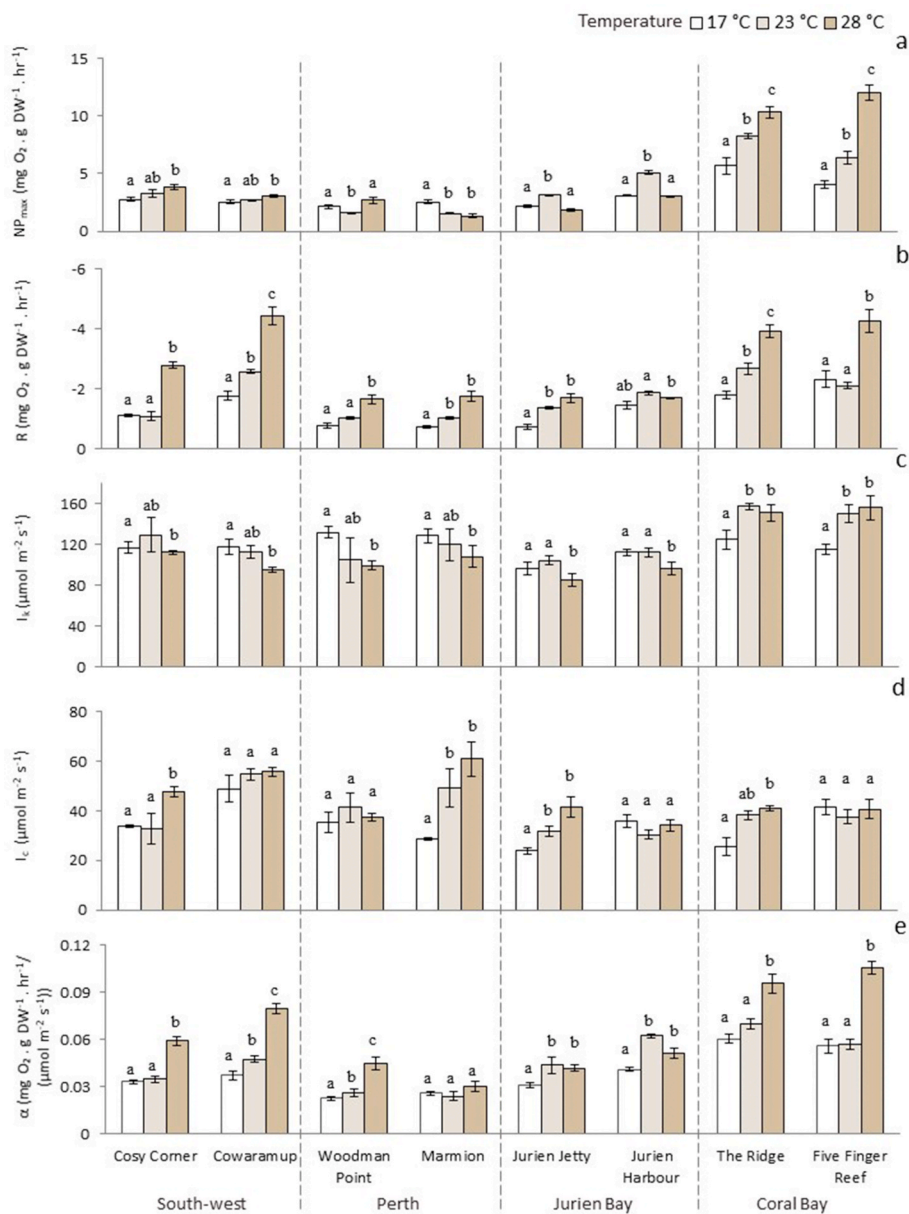
Fig. 3. Effect of temperature on photosynthetic parameters of *H. ovalis* collected from two sites in each of four locations across a latitudinal gradient off the coast of Western Australia. NP_{max} (a), R (b), I_k (c), I_c (d) and α (e). Letters on graphs represent the outcome of pairwise PERMANOVA tests for differences between Temperature within each Site nested in Location (shared letters indicate no significant difference; refer to S1 for main statistical results). Means and SE. n = 5.

showed consistent effects of temperature on α. Plants collected from Coral Bay had a greater photosynthetic efficiency at 28 °C than at 23 °C and 17 °C, and plants collected from Jurien Bay had a higher photosynthetic efficiency at 28 °C and 23 °C than plants exposed to 17 °C. However, sites within the South-west and Perth locations showed different responses, although α generally increased with temperature.

3.2. Time of year

For both sites, in both seasons and at all temperature's plants exhibited the typical relationship between photosynthesis and light, without any photoinhibition (Fig. 4, Table S3). The effect of temperature on photosynthesis significantly differed between time of year and sites (Table S2).

Time of Year significantly affected NP_{max} (Site X TimeofYear X Temperature; $p \leq 0.05$), though the effect of temperature at different times of year varied among the two sites tested. NP_{max} ranged from 1.17 ± 0.044 mg O_2. g DW^{-1}. hr^{-1} (Marmion, in winter at 17 °C) to 4.09 ± 0.19 mg O_2. g DW^{-1}. hr^{-1} (Woodman Point, in summer at 17 °C; Fig. 5a). At Marmion, NP_{max} was highest and not significantly different at 17 °C and 28 °C in winter, but in summer was significantly higher at 17 °C than at 23 °C and 28 °C. At Woodman Point, the same effects of temperature were observed but in the opposite seasons to Marmion.

Photosynthetic efficiencies (α) ranged from 0.022 ± 0.001 mg O_2. g DW^{-1}. $hr^{-1}/(\mu mol\ m^{-2}\ s^{-1})$ at Woodman Point in summer for 17 °C, to 0.075 ± 0.004 mg O_2. g DW^{-1}. $hr^{-1}/\mu mol\ m^{-2}\ s^{-1}$ at Marmion in winter for 28 °C (Fig. 5e). The effect of temperature on α varied across sites and time of year (Site X TimeofYear X Temperature; $p \leq 0.05$), though at both sites and times α was greatest or equal greatest at 28 °C. At Woodman Point, α increased with temperature in summer, however in winter there was no statistically significant effect of temperature,

though the general trend was similar to summer. In contrast, at Marmion there was no effect of temperature in summer but in winter α was greatest at 28 °C, with no significant difference between the other two temperatures.

For all other variables, there was no significant TimeofYear X Temperature interaction, though for I_k there was a significant Site X Temperature effect, with I_k generally decreasing with an increase in temperature (Fig. 5c). I_c had a significant TimeofYear effect, with plants in summer having a greater I_c than plants in winter (Fig. 5d), and R had a Temperature effect, displaying an increase in R as temperature increased (Fig. 5b).

4. Discussion

This study has quantified the effects of both temperature and location on the photosynthesis irradiance (P–I) relationship of *Halophila ovalis*. The P–I relationship was strongly affected by water temperature, however this effect varied spatially over a latitudinal gradient of 1200 km, between sites separated by 2–50 km, and with time of year. Below, we discuss these findings in the context of plant physiology, dredging management and a warming ocean.

4.1. Spatial and temporal variation in photo-physiology

The P–I relationships of *H. ovalis* across locations varies, most notably with higher NP_{max} and respiration rates in plants from the tropical sites. *Halophila ovalis* has one of the widest distributions of all seagrasses species, occurring in temperate and tropical regions and inhabits a diverse range of environmental conditions (Waycott et al. 2004, 2014). Both irradiance and temperature change across the latitudinal gradient studied here, especially between the Temperate (16–24 °C) and
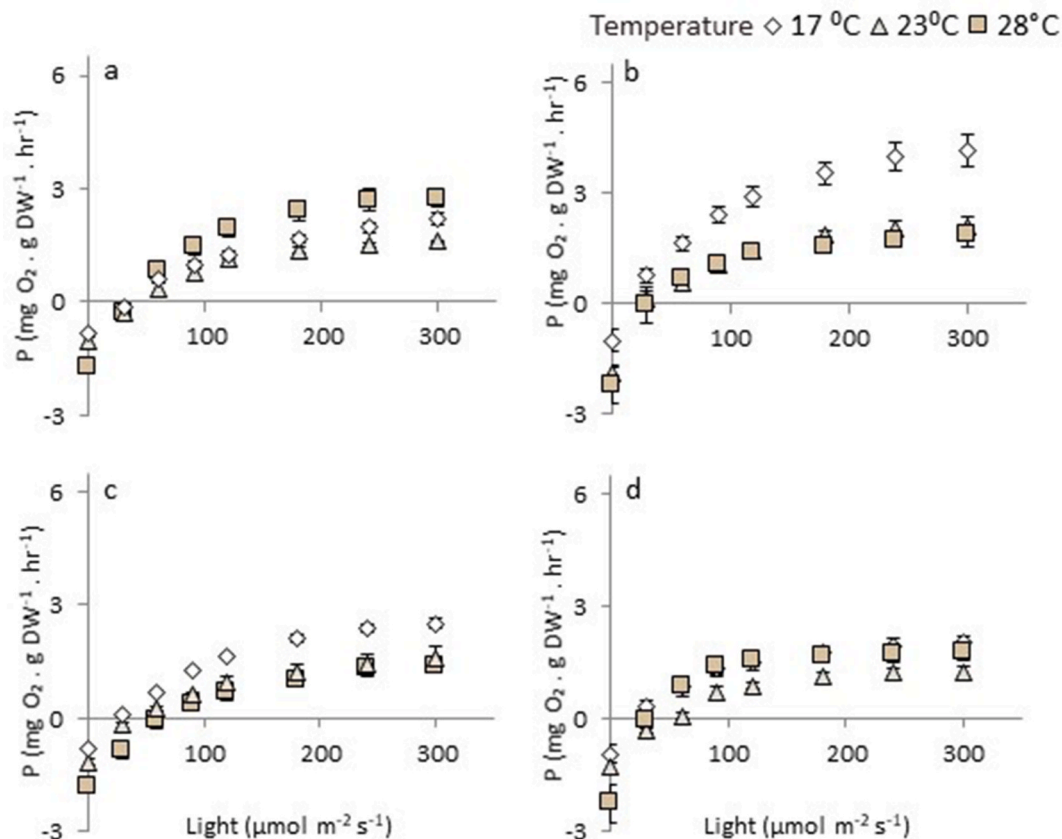

Fig. 4. Photosynthetic-irradiance curves at three temperatures (17 °C, 23 °C, 28 °C) over 2 time periods (austral summer & winter) at 2 sites. a) Woodman Point summer, b) Woodman Point winter, c) Marmion summer, d) Marmion winter. Means and SE. n = 5.
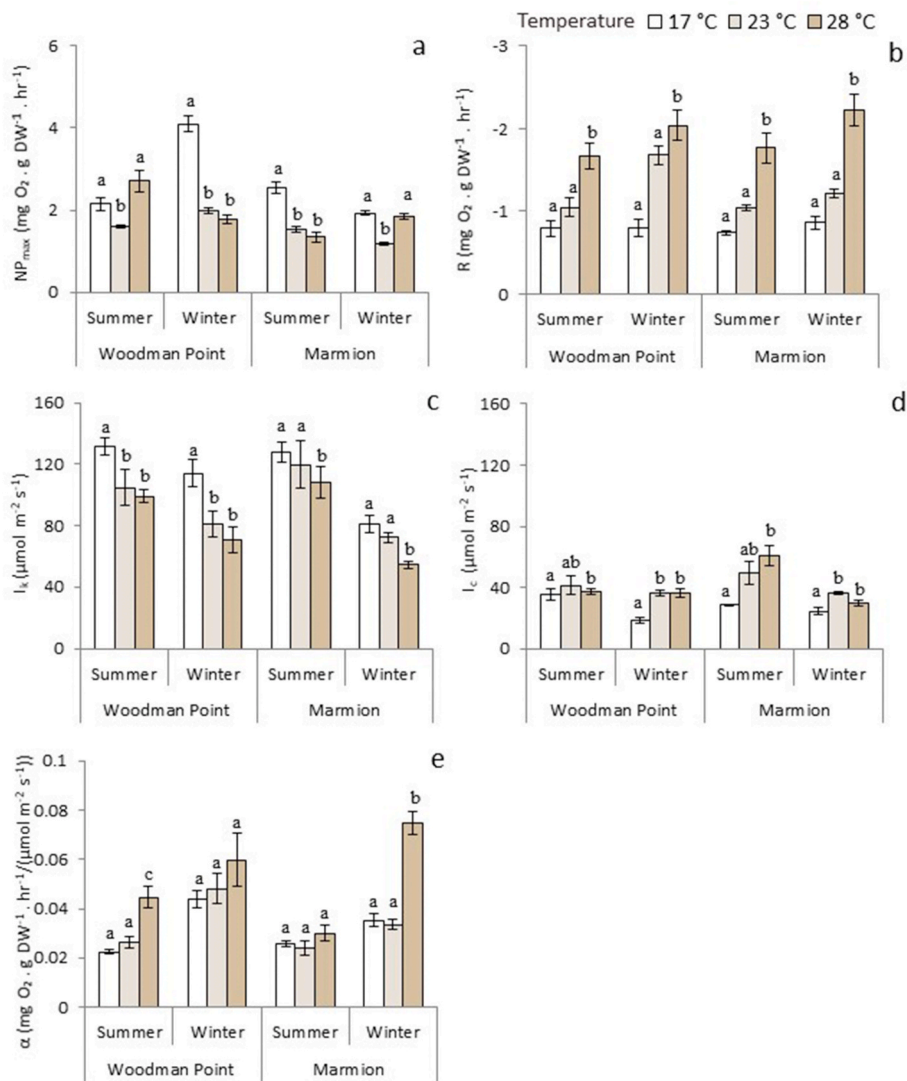
Fig. 5. Effect of time of year and temperature on the P–I characteristics of *Halophila ovalis*. NP_{max} (a), R (b), I_k (c), I_c (d) and α (e) for *Halophila ovalis* collected over two time periods (austral summer & winter) from two sites in Perth. Shared letters on graphs represent no significant difference between temperature treatments at each site and time period (pairwise tests, PERMANOVA; refer to S2 for main statistical results). Means and SE. n = 5.

Tropical locations (23–28 °C). While there are no published studies reporting photosynthetic rates for any other seagrass species across both temperate and tropical regions, tropical species (e.g. *Halodule wrightii*, *Syringodium filiforme*) generally have a higher NP_{max} and thermal optima for photosynthesis than temperate species (*Amphibolis antartica*, *Posidonia australis*, *Posidonia sinuosa*) (Beer and Waisel, 1982; Masini et al., 1995; Masini and Manning, 1997; Major and Dunton, 2000). These climatic zone differences, either among species or, in the case of our study, among populations within the same species, may reflect

adaptation or acclimation of the plants to the different temperature regimes. If populations are adapted/acclimated to their local temperature range, then we might expect NP_{max} to occur at or near the highest temperature they usually grow under (Collier et al., 2011). This local adaptation/acclimation appears to hold for *H. ovalis* in this study. Plants growing at Coral Bay, the most northern site, experience temperatures from 23 to 28 °C (DBCA; pers. comm) and displayed maximum NP_{max} rates at 28 °C. Similarly, at Jurien Bay, *H. ovalis* populations had the highest NP_{max} at 23 °C, which is consistent with the highest water

temperature in Jurien Bay, despite the fact that the plants were collected at a time when the water temperature was closer to the minimum monthly temperature (DBCA; pers. comm). Similar findings have been observed for other angiosperms occupying thermally different habitats (Berry and Bjorkman, 1980). Based on our findings, it cannot be assumed that all *H. ovalis* populations will display the same P–I relationships and, therefore, efforts to model the productivity of this species under changing light and temperature conditions will be improved by taking into account the location-specific difference in physiology.

While plant population adaption/acclimation to local temperature regimes may be the case for the Coral Bay and Jurien Bay locations, it may not be in effect in the Perth and South-west locations. Both Perth sites had highest NP_{max} at 17 °C while both South-west sites showed no decrease in NP_{max} at 28 °C, which far surpasses the normal temperature range plants experience in these regions, 16–23 °C and 16–22 °C respectively (DBCA: pers. comm). This could be due to plants being acclimated to other local conditions such as light and nutrient availability, depth distribution or fine spatial-scale differences in temperature, all of which play a role in controlling photosynthesis (Lee et al., 2007). Although the average sea surface temperature is lower at the South-West sites, *H. ovalis* was collected from small protected embayment's, therefore the plants may be inhabiting an area with less flushing (than a well-mixed ocean) and higher localised warming. Further both seagrass sites in the Perth and South-west locations are shallower (1.8–2.4 m) than the sites in the north-most locations (Jurien Bay and Coral Bay; 1.9–3.5m), which may also have an impact on flushing, impacting localised temperature (Lee et al., 2007). However, there are other plausible reasons for these locations not having maximum net productivity at the local maximum temperature regimes, including an insufficient acclimation period during our experiments. Collier et al. (2011) examined two different species occurring in different climatic regions, *Halodule uninervis* (predominantly a tropical species) and *Zostera muelleri* (a temperate species, but range expanding into tropics for this study). They found that *Z. muelleri* initially (over 5 days) showed no response in leaf photosynthesis to increasing temperature but after 30 days showed a decline with increasing temperature. In our study, the experimental acclimation period was 5–13 days, and based on the findings of Collier et al. (2011) it is possible that if the experiments had been run for longer, a more consistent, and possibly negative, effect could have been observed on plants exposed to temperatures outside of their *in situ* thermal range. Nonetheless, on the basis of our finding, it cannot be assumed that all locations seagrass will have an optimum temperature for NP_{max} at temperatures approaching the maxima experienced at the sites and, therefore, to err on the side of caution local physiological data is most appropriate when modelling responses of *H. ovalis* to different temperature-light regimes.

Plants from temperate and tropical regions showed vast differences in their response to temperature for NP_{max}, and therefore it was expected that other P–I parameters would also respond in the same way. This was true for I_k and I_c. In temperate locations, I_k decreased as temperature increased, contrasting the tropical location where I_k increased with increasing water temperature. This response in the temperate region is consistent with Masini and Manning's (1997) findings for *Posidonia* and *Amphibolis* species, working within the plants' *in situ* water temperature range. However, our study on *H. ovalis* pushed the plants beyond their normal temperature range, resulting in a significant decrease in I_k from the lowest (17 °C) to the highest temperature treatment (28 °C). I_c also had an inconsistent response to temperature across locations, though there was no latitudinal trend. Despite this, the general trend, and in some cases significant effect, was for I_c to increase with temperature. This is, again, consistent with the findings of Masini and Manning (1997) that I_c was less affected by water temperature than I_k, but generally increased with increased water temperature; likely due to greater respiration at higher temperatures.

Our findings indicate that the effect of temperature on the P–I

relationship of *H. ovalis* varies between times of year, but the nature of this variation is not consistent among sites. Collier et al. (2017) looked at time of year and found differences in photosynthetic rates among seasons for *C. serrulata*, but no evidence of this in *H. uninervis*, albeit that this was only done at one site (Morton Bay). Other studies measuring seagrass oxygen evolution have also looked at the effect of time of year on photosynthesis at a single temperature and in those cases time of year affected the P–I relationship (Pollard and Greenway, 1993; Masini and Manning, 1997). The finding that NP_{max} was highest at 17 °C in winter likely reflects acclimation to winter temperatures. Bulthuis (1987) found that the optimum temperature for photosynthesis decreased as irradiance decreased, implying that seagrasses growing in lower light, as is expected in winter, have lower optimum temperatures for photosynthesis than plants growing in the higher light conditions typical of summer. This is also reflected in the photosynthetic efficiencies (α), where generally the plants were more photosynthetically efficient at all temperatures in winter than in summer, exhibiting the typical dark adaptation response to lower light conditions in winter months. It remains unclear why plants at Marmion did not show differences in productivity between winter and summer. In any case, our findings clearly indicate that it is not valid to assume the effects of temperature on P–I relationships will be consistent across different times of the year, nor will any temporal variation necessarily be consistent among different sites. This all points to strong local influences on the P–I characteristics of the plants, and to the effects of temperature on those characteristics. The effect of temperature was not the same for all of the P–I parameters. I_k for example did not show an effect of time of year, and therefore data generated at one time of year for a given temperature may be transferable to other times of year.

4.2. Interpreting photo-physiology data for management criteria

Whilst there are a number of complex approaches to help predict the effects of reduced light on seagrasses, including numerical modelling, most require knowledge of the P–I relationship (Erftemeijer and Robin Lewis, 2006). Our finding that the P–I relationship of *Halophila ovalis* varied spatially, and most dramatically between temperate and tropical locations, argues for the need to apply different P–I parameters or light requirement criteria depending on the location of the plant populations being considered. For example, I_k was significantly higher at the tropical location than those at the temperate locations. In the absence of location-specific data, we recommend that the next most appropriate option would be to use P–I data generated from the same climatic region (temperate, tropical) to model productivity.

Temporal variability in plant pressure-response relationships is also important to consider when developing light requirement criteria for dredging management. Management of dredging regularly incorporates "Environmental Windows", specific time periods when dredging activities are allowed (Suedel et al., 2008; Fraser et al., 2017). Environmental windows should be location specific, minimising the impacts on biological events such as coral spawning (Fraser et al., 2017). Increasingly, environmental windows are being considered to align with the resilience of habitats and the impacts of dredging (Wu et al. 2015, 2017). If seagrass light requirements, as reflected, for example by I_c and I_k, vary with time of year within a location, then this variability could be incorporated into modelling to assess the time of year that is likely to have the least impact on seagrass habitat. There were no time-of-year effects on I_k, however there was a temperature effect and, as temperature varies with time of year, environmental windows may be appropriate. In contrast, if dredging management criteria are based on maintaining a certain level of productivity, based on estimated NP_{max}, then temporal considerations would be required, because time of year affected NP_{max}. These findings indicate two important points to consider when developing light criteria for management. First, location specific P–I data are recommended, although in absence of location specific data, climatic region P–I data may be appropriate. Secondly, it is important to use P–I

data generated at an appropriate temperature (which can have an effect on I_k, I_c and NP_{max}), which coincides with time of year that light reduction impacts are expected to occur. In the case of I_k it may be applicable to use the P–I data with the temperature most relevant to that time of year, however in the case of NP_{max} seasonal temperature specific data should be utilised.

4.3. Ocean warming

This study was not specifically designed to look at climate change impacts, however the findings provide insight into possible impacts of future sea surface temperatures (SST) for *Halophila ovalis* along the coast of Western Australia, particularly in the temperate locations where P–I curves were calculated at temperatures the plants are not currently exposed to. Near Perth (31.5°S), SST rose by approximately 0.13 °C each decade from 1951 to 2002 (Pearce and Feng, 2007) and by about 0.3 °C each decade from 1985 to 2004, leading Lough (2008) to predict an increase of up to 2 °C in southwestern Australian waters by 2100. Across the latitudinal gradient of Western Australia *H. ovalis* maintained productivity at all temperature treatments we exposed it to in all locations, indicating that it is unlikely there will be complete loss of current *H. ovalis* populations in any location due to SST increase alone. Furthermore, there is no evidence in our findings to suggest potential negative effects of projected SST increases on productivity. In fact, in all regions, productivity at the highest temperature (28 °C) was comparable to, or greater than, at lower temperatures which, in the temperate zone locations are more typical of current conditions. However, we need to acknowledge two important qualifiers to this finding. First, our findings relate to plants under laboratory conditions and might not reflect how plants respond to multiple stressors, such as increasing SST and the added pressure of increased herbivory due to the poleward movement of macro-grazers (Hyndes et al., 2016). Second, *H. ovalis* is widely distributed across both tropical and temperate regions. It might, therefore, be expected to display strong physiological plasticity, as would other seagrasses with distributions extending into the temperate zone, such as *Syringodium isoetifolium* and *Halodule uninervis*. However, it remains unclear whether we can expect similar plasticity among the truly temperate species, such as *Amphibolis antarctica* and *Posidonia sinuosa*, which occur on the west coast and are foundational species. Improving our understanding of the ability of these foundation species to acclimate to future temperature changes is critical to understanding the outlook for seagrasses over the remainder of this century.

We conclude that temperature affects the P–I relationship of *Halophila ovalis*, but that the effect is not consistent among climatic regions or, in some cases, among sites within locations. Based on our findings, it is appropriate to use site-specific P–I parameters when modelling seagrass productivity and attempting to predict impacts of reduced light. Our findings also provide insights into possible seagrass response to future climate change scenarios, and we suggest that temperate meadows of *H. ovalis* are likely to be more negatively affected by predicted increases in sea temperature than tropical meadows, though the projected temperature increases are unlikely by themselves to cause the loss of meadows.

Acknowledgments and author contributions

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2021.107414.

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