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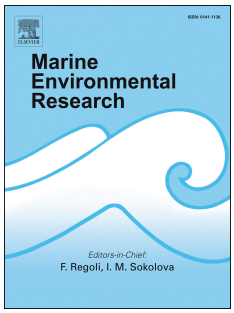

Living at the margins – The response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts

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Running Head: Impact of temp and light on deep-water seagrass

ABSTRACT

Seagrasses inhabit environments where light varies at different timescales, nonetheless are acutely sensitive to reductions in light beyond some conditional bounds. Two tropical deep-water seagrasses, *Halophila decipiens* and *Halophila spinulosa*, from the Great Barrier Reef were tested for their response to defined light and temperature regimes to identify their growth requirements and potential thresholds of mortality. Species were exposed to two light intensities, saturating (75 µmol photons m^{-2} s^{-1}) and limiting (25 µmol photons m^{-2} s^{-1}) light and two temperature treatments (26°C and 30°C) over a four-week period. Wavelength-specific parameters of PSII photochemistry were evaluated for seagrass leaves, as well as shoot density, gas exchange, and pigment content. Both species were sustained under saturating light levels (3.2 mol photons m^{-2} d^{-1}) while limiting light led to decreased shoot density for *H. decipiens* and *H. spinulosa* after two and four weeks, respectively. Wavelength-specific photochemistry was also affected under light-limiting treatments for both species while the functional absorption cross section was highly conserved. Photoacclimation and physiological adjustments by either species was not adequate to compensate for reduced irradiance suggesting these plants reside at the margins of their functional limits. As such, relatively short periods of light attenuating events, like dredging or flood plumes, may be detrimental to deep-water seagrass populations.

Key words: deep-water, seagrass, *Halophila decipiens*, *Halophila spinulosa*, light, temperature; PAM fluorometry; wavelength-specific photochemistry; Great Barrier Reef

1. INTRODUCTION

It is widely accepted that seagrasses are critical to the health and ecosystem function of the coastal marine environment. They provide key inter-habitat connectivity for migrating fauna, feeding grounds for globally threatened turtles and dugong, habitat for commercially important fisheries, sediment trapping and stabilisation, effective nutrient filtration from coastal inputs and carbon sequestration (Duarte et al., 2010;

Heck et al., 2008; Hemminga and Duarte, 2000; Jackson et al., 2001; Orth et al., 2006). Despite being highly valued globally for their contribution to these ecosystem services, seagrass habitats are threatened by a range of anthropogenic activities including coastal development and declining water quality from poor catchment management practices (Costanza et al., 2014; Grech et al., 2012; Waycott et al., 2009), and compounded by natural events such as severe storms and flooding that can accentuate seagrass decline (Rasheed et al., 2014).

The vast majority of seagrass species are located in relatively shallow water habitats with quiescent conditions, favourable sediment chemistry, and where light is adequate to meet gross energy requirements (Hemminga and Duarte, 2000; Koch, 2001). In the Great Barrier Reef World Heritage Area (GBRWHA), research and monitoring programs have detailed the distribution, seasonality, environmental drivers, risks and threats to these specialised plant communities (Bryant et al., 2013; Collier et al., 2012a; Grech et al., 2011; McKenna et al., 2015). However, information on deep-water tropical seagrass communities — generally classified as growing at depths >10-15 m — is limited (Carruthers et al., 2002; Fonseca et al., 2008; Hammerstrom et al., 2006; Josselyn et al., 1986). These deeper meadows are primarily composed of species from the genus *Halophila* (Hydrocharitaceae) and within the GBRWHA have been mapped down to 60 m and modelled to cover over 40,000 km^2 of the seafloor (Coles et al., 2009).

Halophila spp. have size-associated characteristics that are likely to play an important role in their dominance of deep-water seagrass meadows. Small delicate leaves, oval or oblong in shape, occur in pairs that attach directly to either a vertical stem or rhizome via a petiole. Their short canopy height may increase risk of burial from sediment deposition; however, rapid leaf turnover and opportunistic growth can negate this issue (Duarte et al. 1997, Terrados et al. 1998, Cabaço et al. 2008). With leaves only two-cells thick, they have minimal lacunar space and contain densely packed chloroplasts in the epidermal layer (Roberts et al. 1984, Josselyn et al. 1986, Kenworthy et al. 1989, Cambridge & Lambers 1998). Thin leaves allow for quick and efficient gas exchange of evolved oxygen from saturated epidermal cells and reciprocal carbon uptake for fixation (Larkum et al. 2006). Comparatively, seagrasses with high standing biomass (such as *Posidonia* and *Zostera*) have higher diffusive boundary layers which could make living at depth with less wave action and water movement a challenge for gas exchange and acquiring limited resources (Enríquez and Rodríguez-Román, 2006). Minimal below-ground biomass also makes *Halophila* spp. well suited to

grow at greater depths and in shallow turbid areas with chronic low light (Kuo & Kirkman 1995, Durako et al. 2003). Non-photosynthetic below-ground tissues can act as a respiratory burden which may ultimately limit the compensation depth of structurally larger species (Fourqurean & Zieman 1991, Larkum et al. 2006).

The capacity to cope with both a quantitatively low-light environment and a narrowed spectral window of light is critical to living at depth (Duarte, 1991; Ralph et al., 2007); yet little is known about the spectral tuning of deep-water seagrasses. Deep-water seagrasses likely have a reduced threshold of tolerance to low-light because they are growing under lower ambient light intensities, a smaller range of intensities, and a significantly narrowed spectral window from which to harvest light energy (Larkum et al., 2006). Strategies for deep-water seagrasses to maintain a positive carbon balance likely involve the same mechanisms observed in their shallow water counterparts to cope under low-light conditions (Collier et al., 2012b): modifying light harvesting capacity and the efficient use of light (Abal et al., 1994; Enriquez, 2005); adjustments to rates of growth and plant turnover (Collier et al., 2012b); and drawing upon carbohydrate reserves to maintain a positive carbon balance (Burke et al., 1996; Touchette and Burkholder, 2000a). Temperature, which directly affects metabolic rates of carbon fixation and respiration in plants, influences whether photophysiological adjustments to a low-light environment are sufficient to maintain a net positive carbon balance, as opposed to a net negative; the latter of which would lead to plant- or meadow-scale losses (Bulthuis, 1987; Lee et al., 2007).

Some deep-water *Halophila* populations are annual or ephemeral in their above-ground presence, likely in response to seasonally unfavourable conditions to support positive growth and carbon balance (York et al., 2015). In these circumstances the plants may rely on high investment in the production of seeds and a seed bank on which recovery and population maintenance depend (Hovey et al., 2015; Rasheed et al., 2014).

Photoacclimation to low-light environments by seagrasses is similar to that seen in other higher plants (Ralph et al., 2007; Smith, 1982). Changes in accessory pigment content can increase light capture efficiency and its' relative use in the photochemical pathways (Falkowski and Raven, 2007). However, a point can be reached beyond which the self-shading of pigments reduces the effectiveness of this strategy (i.e. the package effect; Cummings and Zimmerman, 2003). While the capacity for photoacclimation to total light

reduction is well documented in higher plants including seagrasses, qualitative shifts in the spectral distribution of available light at depth is largely undescribed for seagrass with an exception by Sharon et al. (2011) on *H. stipulacea* growing at 48 m in the Red Sea. While the spectral distribution of light with depth varies according to the absorption properties of the water, total spectral attenuation of light >600 nm occurs in the GBRWHA at ≥10 m (Appendix Figure 1). Descriptions of seagrass pigment signatures is somewhat typical of a green higher plant with chlorophyll and a suite of accessory pigments that absorb light largely in the blue (400-500 nm) and red bands (650-680 nm) (Costa et al., 2014). How a spectrally-attenuated light climate affects the photosynthetic properties, pigment composition, or light capture efficiency of *Halophila* spp. growing in >10 m is largely undescribed.

Measuring photosynthetic capacity to understand plant condition under varying light intensities with pulse-amplitude modulated (PAM) chlorophyll fluorometry is commonplace in recent years, whether in the laboratory or field (Cosgrove and Borowitzka, 2010; Schreiber, 2004). A PAM fluorometer typically measures variable chlorophyll fluorescence parameters, from which photochemical efficiency of PSII can be calculated, energy transfer efficiency can be measured, and energy dissipation quantified. However, these measurements do not account for variable absorption by PSII across the PAR spectrum which can vary widely by wavelength depending on the pigment composition and its' ambient growing conditions (Schreiber et al., 2012; Szabó et al., 2014).

In this study, we investigate the effects of light intensity and temperature on the two most prevalent deep-water seagrasses in the GBRWHA (Coles et al., 2009), *Halophila decipiens* and *Halophila spinulosa*. *H. decipiens* has a pan-tropical distribution and has a small stature in both above and below-ground tissues (Waycott et al., 2004). *H. spinulosa* is limited to the Indo-Pacific region, has similar oblong leaf pairs, but grows upright on a vertical stem, creating a much larger canopy-forming habitat.

Both species were expressly sourced from deep-water meadows, whereby depth creates unique inherent challenges to the biology and physiology of the plant from that of a turbid shallow water habitat: 1) a unique spectral signature in which light >600nm is absent; 2) lower variation in water quality related to tidal effects, coastal runoff, and sediment re-suspension due to wind and wave activity; and 3) a unique pressure

environment which may impact leaf diffusion and plant physiology (Beer and Waisel, 1982). We assessed morphological, optical and physiological adjustments to both plants when they were exposed to peak growing season light (spectrally-adjusted) and temperature conditions (Chartrand in prep) versus reduced light levels and elevated temperatures. We measured wavelength-dependent photochemical efficiencies, oxygen production, pigment composition, carbohydrate reserves and shoot densities over a four-week period to assess changes in the plants. The aim of this experiment was to i) describe the changes in optical, photochemical, physiological, and physical characteristics used to cope with light and temperature stress events, ii) evaluate wavelength-specific characteristics of light capture in response to the light/temperature treatments, iii) identify the time to detect any such significant changes, and iv) establish an indicative minimum light threshold for *H. decipiens* and *H. spinulosa* to help guide environmental management of tropical deep-water seagrass communities. In addition, we aimed to describe potential differences in physiological responses between two species from the *Halophila* genus.

2. METHODS

2.1 Sample Collection

Halophila decipiens Ostenfeld was collected adjacent to a long-term monitoring site off Green Island (16°45.12354'S, 145°59.5494'E) at approximately 17 m depth below MSL. *H. spinulosa* (R. Brown) Ascherson, was collected approximately 400 km to the south, at a location near Bowen at 10 m depth below MSL (19° 54.4061 'S, 148° 11.0841'E). Plants were harvested in October and November 2013 on SCUBA using a large metal scoop to place transplants and ~7 cm of sediment depth into 26 x 21 x 10 cm plastic tubs in order to minimise disruption to their growing environment. Tubs were transferred overnight to the University of Technology Sydney with enough water to keep shoots wet but not fully submerged, fastened with lids, and kept in the dark during the approximately 18 hour transfer period. On arrival, tubs with samples were maintained in 10-L aquaria with aerated natural seawater (26 °C, pH 8.1, 35 PSU) for one month prior to the start of the experiment. Plants were illuminated using 150W four-channel LED lights (Cidly, China) programmed to simulate an incident deep-water spectral profile with an intensity of 75 µmol photons m^{-2} s^{-1} over a diel light-dark cycle (ramping from 0 to 75 µmol photons m^{-2} s^{-1} from 0500 h to 0700 h and from 75 to 0 µmol photons m^{-2} s^{-1} from 1800 h to 2000 h). The light, temperature, and salinity conditions in the tanks were based on a two year record of water quality monitoring at the collection sites with in situ

loggers (Chartrand pers. obs.). The tank conditions mimic the mean maximum daily light intensity, mean daily temperature, and salinity during the October/November period when plants were harvested.

2.2 Experimental design

Each tub of *H. decipiens* and *H. spinulosa* were randomly allocated to one of four treatments (4 tubs per treatment) manipulating light intensity (*LI*) and temperature (*T*): (1) control (75 µmol photons m^{-2} s^{-1}, 26 °C; equivalent to mean daily irradiance at depth and mean ambient temperature at field sites), (2) elevated temperature only (75 µmol photons m^{-2} s^{-1}, 30 °C), (3) reduced light only (25 µmol photons m^{-2} s^{-1}, 26 °C) and (4) a combination of both reduced light and high temperature (25 µmol photons m^{-2} s^{-1}, 30 °C). The reduced *LI* and elevated *T* levels were chosen to reflect conditions beyond those recorded when plants were actively growing but still found to occur at the collection site at times of the year when seagrasses were absent (Chartrand pers. obs), and therefore realistic as a level of plant stress within their environment. Temperature was controlled using submersible heaters (Aqua One, Australia). Water quality was the same as the holding aquaria, with weekly 30% water changes to maintain salinity within 1 PSU and availability of trace nutrients. Tubs were rotated every other day within tanks in order to remove an effect of location within the tank in relation to the light source or water flow. The experiment was performed over 4 weeks.

The number of seagrass shoots (i.e. *H. decipiens* number of leaf pairs or *H. spinulosa* vertical shoots) in each tub (0.05 m^2) was recorded weekly during the study.

2.3 Oxygen determinations

Oxygen production and respiration rates of both species were measured at the start (T_0) and end (T_f) of the experiment using oxygen optodes (OXSP5-OI, Pyroscience Germany) connected to a O_2 sensor unit (FireStingO2 Fiber-Optic Oxygen Meter, Pyroscience, Germany). Samples were placed in 10 mL glass bottles filled with 0.45 µm filtered seawater from treatment tanks with a magnetic stirrer and connected to an oxygen sensor. Oxygen production and respiration were determined under treatment irradiance and darkness, respectively and rates were calculated as oxygen differentials over the time of incubation and normalised to leaf area.

2.4 Variable fluorescence measurements – wavelength-dependent parameters

The wavelength-dependent functional absorption cross-section of PSII, $\sigma_{II}(\lambda)$, was recorded according to Schreiber and Klughammer (2013; and see Szabó et al., 2014) using a multi-colour pulse amplitude modulated fluorometer (further referred as MC-PAM; Walz GmbH, Germany). Briefly, $\sigma_{II}(\lambda)$ calculations are based on the so-called O-I_1 (or O-J, Strasser and Govindjee, 1992) fluorescence rise kinetics, which corresponds to the photochemical phase of the polyphasic fluorescence rise upon the onset of strong actinic illumination. These measurements were recorded using an automated measuring routine in PamWin v3.2 (Walz GmbH, Germany). At 500 µs after the start of actinic illumination, i.e., before the secondary thermal rise phases contribute significantly to the fluorescence rise, a saturating single-turnover flash (ST) was given to estimate the I_1-level, which represents the fluorescence yield of the fully reduced primary electron acceptor Q_A, with the PQ-pool being oxidized.

Consecutive measurements with the same leaf sample using 440, 480, 540, 590, and 625 nm measuring light (ML) and actinic light (AL) were pre-programmed in a script-file with 10 s dark-time between measurements. For each wavelength, ML intensity and gain settings were programed to give approximately equal F_0 values (designated as 'O' in the O-I_1 terminology). The AL and multiple turnover (MT) flash intensity settings were programmed to obtain similar slopes of the O-I_1 curves for all wavelengths. When kinetics of the O-I_1 fluorescence rise are identical among wavelengths, PAR is directly proportional to changes in $\sigma_{II}(\lambda)$. Values of $\sigma_{II}(\lambda)$ were analysed using a dedicated fitting routine in the PamWin-3 software to determine τ, the time-constant of light-driven Q_A^- reduction (ms) and used in the following equation:

$$\sigma_{II}(\lambda) = \frac{1}{\tau \cdot N_A \cdot E_d}$$

where τ is the time-constant of light-driven Q_A^- reduction (ms), N_A is Avogadro's constant ($3.03 \cdot 10^{23}$ mol photons^{-1}) and E_d is the incident downwelling irradiance (see Schreiber et al. 2012 and Schreiber and Klughammer 2013).

Wavelength-dependent chlorophyll fluorescence parameters, i.e. the effective quantum yield of PSII (Y(II)), relative electron transport rate (rETR(II)) and non-photochemical quenching (NPQ) were determined by using custom-made scripts to record steady-state light curves (SSLC) across the wavelengths provided by the

LEDs of the MC-PAM at a fixed intensity. Essentially, scripts were designed to start with the wavelength with the lowest measured $\sigma_{II}(\lambda)$ then progressing towards greater theoretical wavelength-specific PSII excitation pressure; this equated to a sequence order of 540, 590, 625, 480 and 440 nm. Two scripts were used to determine the chlorophyll fluorescence parameters described above: a sub-saturating (25 µmol photons m^{-2} s^{-1}) and a supra-saturating (150 µmol photons m^{-2} s^{-1}) AL at each wavelength. The F_V/F_M values were calculated upon dark-adaptation for ~5 min, followed by a white saturating pulse (SP). At each of the five wavelengths, AL was set for 3 min at the end of which a SP pulse was given with the same wavelength as the AL to determine F_M'.

2.5 Pigment Characterisation

Seagrass leaves used in MC-PAM measurements were photographed and analysed by imaging software (ImageJ) prior to the extraction and quantification of chlorophyll content. Each leaf sample was ground in 5 mL ice-cold 90% acetone using a mortar and pestle. Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b)* concentrations were determined spectrophotometrically using the equations and extinction coefficients of Ritchie (2006) and normalised to surface area of the leaf.

2.6 Below ground carbohydrates

Additional samples of *H. spinulosa* only were collected at the beginning and end of the experiment for below-ground carbohydrate analysis and were placed in liquid nitrogen to await further processing. Samples were later defrosted, scraped clean and separated into below and above-ground structures. Root and rhizome material was dried at 40˚C for 48 hours when a constant dry weight was obtained. Dried material was ground to a fine powder in a bead mill (Mini-Beadbeater, Biospec) for wet laboratory analysis. Prepared samples were further processed at the University of Queensland where soluble and non-structural carbohydrates (i.e. starch) were extracted and quantified relative to total sample dry weight according to Weir et al. (1977) and Karkalas (1985). Briefly, carbohydrates were extracted by placing each sample in 80% ethanol in an 80°C water bath for 5 min prior to centrifuging (3000 rpm for 5 min). The supernatant was diluted with 80% ethanol to 25 mL and kept for soluble carbohydrate determination. The pellet remaining was dissolved in 10 mL of deionized water and placed in a 95°C water bath for a further 30 min while agitating samples at 10 min intervals to solubilize the starch. After coming to room temperature, samples were digested with

amylase and incubated at 55°C for 30 min prior to dissolving the extracted sample in 20% trichloroacetic acid. Colorimetric determination of starch content was determined using a commercially available glucose oxidase/peroxidase testing kit (GOPOD, Megazyme) with absorbance measured at 510 nm, and soluble carbohydrates colorimetrically determined with a potassium ferricyanide reagent measured at 420 nm.

H. decipiens was not tested for below-ground carbohydrate concentrations due to extremely low below-ground biomass insufficient to perform laboratory analyses.

2.7 Data analysis

Generalized linear mixed-effect models (GLMM) were used to examine the fixed effects of light intensity (*LI*), temperature (*T*), and week (*W*) on shoot count (*SC*) and chlorophyll fluorescence parameters (F_V/F_M, $\Delta F/F_M'$, Q_m) for each species. Each factor was modelled as an additive term and as an interaction with other factors. The factor tub was also included as a random effect in all models to eliminate potential bias resulting from the non-independence of measurements taken from the same tub over the four-week study. Three-way interactions between *LI*, *T* and *W* were considered in the analyses. The response variable *SC* was modelled with a Poisson distribution with logit link function. To assess differences in response variables between species, separate models were run on the effect of species (*SPP*) as an additive and interaction term to avoid over-parameterization of the models.

To determine the optimal model, a global model was created for each response variable where all explanatory variables up to 3-way interactions were considered. Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w), which by definition contain the best set of explanatory factors for adequately predicting each response variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AICc values within 2 of each other were considered strong models and are presented with the chosen model being the simplest of this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All models were validated by assessing Pearson residuals against fitted model values. The best model for *H. decipiens* shoot count data did show some heterogeneity in the residuals versus fitted values. Two influential

tubs were identified using standardized measures of influential data for the point estimates of generalized mixed effects models (Nieuwenhuis et al., 2012) and were removed, which greatly improved the residual patterns while not changing the model selection or significance of the fixed effects in the model output. Multiple comparison procedures using a Bonferroni adjustment were run on least square means when significant overall effects were generated from all best fit models (lsmeans package, Lenth, 2016).

A similar GLMM approach was used to examine the fixed effects of light level (*LI*), temperature (*T*), and the random effect of tub, on oxygen production, respiration rates, P:R ratios, pigment content and all wavelength-dependent fluorescence measurements (Y(II), F, F_m, rETR, NPQ, and $\sigma_{II}(\lambda)$), which were also tested against wavelength (*WV*). Since these response variables were only measured at the start (T_0) and end (T_F) of the study, a separate t-test was performed between T_0 and T_F "control" conditions to test whether the tub conditions affected the status of leaves in addition to the treatment design. Model selection for wavelength-dependent fluorescence measurements was also done using the dredge function, but with a Gaussian or gamma distribution (with logit link) applied for continuous and positive data (Zuur et al., 2009). Model selection for all other response variables (oxygen production, respiration rates, P:R ratios, pigment content) was made using the drop1 command from the lmerTest package (Kuznetsova et al., 2015). Validation steps were the same; Pearson residuals were assessed against fitted model values, and multiple comparison procedures using a Bonferroni adjustment were run on least square means when significant overall effects were generated from all best fit models.

All GLMMs were performed in R using lme, glmmADMB and mgcv packages (Bates et al., 2012; Fournier et al., 2012; Wood, 2006).

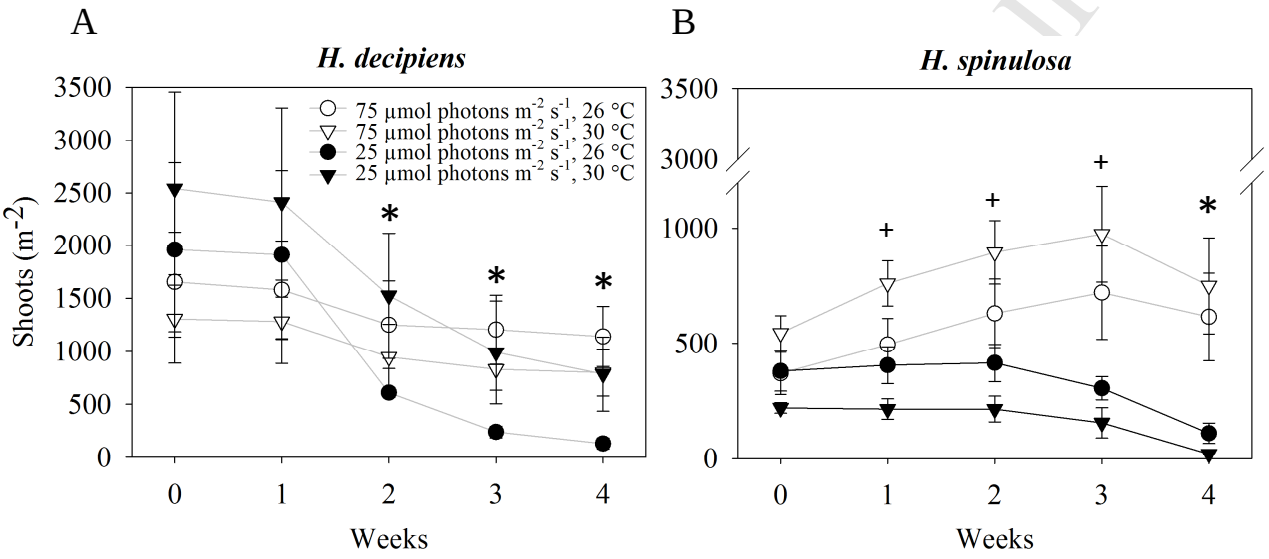
3. RESULTS

3.1 Shoot density

Light deprivation had a negative effect on *Halophila decipiens* and *Halophila spinulosa* shoot density after two and four weeks, respectively (Figure 1). Change in shoot counts of *H. decipiens* were driven by *LI* and *W* with no effect of *T* (Table 1). *H. decipiens* shoots under low *LI* were significantly lower by week 2 with approximately 40% (26˚C) and 60% (30˚C) loss of total shoots and this declined further over the subsequent

two weeks of the study (Figure 1a). Overall, shoots under low *LI* decreased from 1960 ± 826 to 130 ± 52 shoots m^{-2} and 2540 ± 915 to 785 ± 350 shoots m^{-2} from week 0 to week 4, for low and high temperature treatments, respectively.

Figure 1. Shoot density (shoots m^{-2}) for *H. decipiens* (a) and *H. spinulosa* (b) over a four-week study. * indicate significant declines in low *LI* treatments from *T*0 while + indicate significant gains in high *LI* from *T*0. Data symbols and error bars represents mean ± SE. (n = 4).



Shoots of *H. spinulosa* were also driven by *LI* and *W* with no effect of *T* (Table 1). In total, *H. spinulosa* shoots declined under low *LI* from 350 ± 88 to 110 ± 44 shoot m^{-2} (26˚C) and 220 ± 23 to 20 ± 20 shoot m^{-2} (30˚C) from week 0 to week 4. Shoot loss did not begin until after week 2 and was only significant at week 4 (Figure 1b). Conversely, under high *LI* there was a significant gain in shoots from week 1 to week 3 (Figure 2b).

3.2 Below-ground carbohydrates

Below-ground tissues of *H. spinulosa* were significantly affected by *LI* (Table 1) with measurable increases of soluble sugar concentrations under high *LI* treatments compared to the start of the study and low *LI* tubs (Figure 2). In contrast, below-ground starch concentrations were unchanged over time and among treatments.

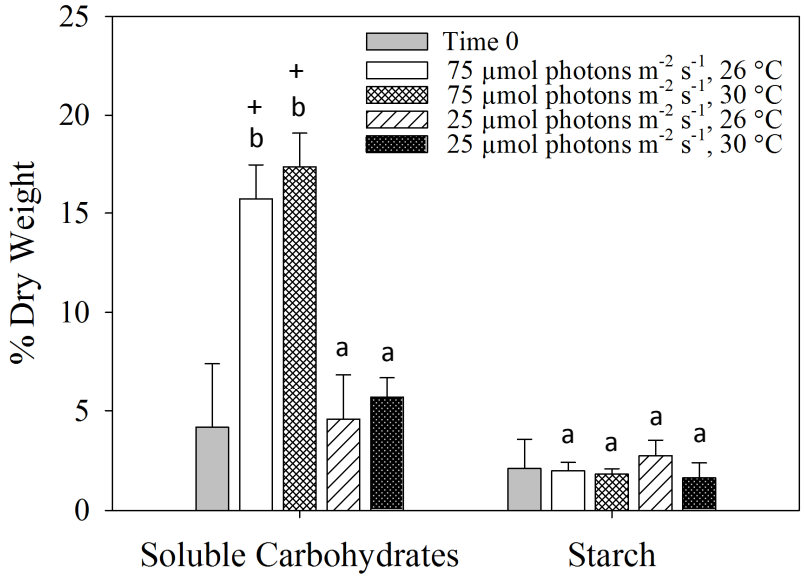
Table 1. *Halophila decipiens* and *H. spinulosa* parameter estimates where significant effects of covariates were found with generalized linear mixed-effects models (GLMM). Effects of light intensity (*LI*), temperature (T*)* and week (*W*) on shoot counts (SC), and wavelength-dependent fluorescence parameters are presented. Wavelength (*WV)* was also included in all models for wavelength-dependent fluorescence parameters. Models with interaction terms also include main effects. Shoot count was modelled with a negative binomial distribution, chlorophyll fluorescence parameters with a beta distribution and oxygen/respiration rates with a gamma distribution (all with logit link function). β_{tub} is the random effect of tub and ε is the error term.

Halophila decipiens					***Halophila spinulosa***				
Model	df	AIC_C	ΔAIC_C	w	Model	df	AIC_C	ΔAIC_C	w
Shoot Count (SC)					Shoot Count (SC)				
LI * W + β_{tub} + ε	**12**	**745.3**	**0.00**	**0.703**	*LI * W + LI * T + β_{tub} + ε*	13	511.8	0.00	0.472
					LI * W + β_{tub} + ε	**11**	**512.2**	**0.34**	**0.398**
$\sigma_{II}(\lambda)$					$\sigma_{II}(\lambda)$				
T + WV + β_{tub} + ε	8	71.6	0.00	0.288	*LI * T + WV + β_{tub} + ε*	8	239.6	0.00	0.386
*LI * T + WV + β_{tub} + ε*	9	71.8	0.22	0.259	*LI + T + WV + β_{tub} + ε*	9	240.1	0.50	0.300
WV + β_{tub} + ε	10	72.6	0.99	0.176	***WV + β_{tub} + ε***	**7**	**240.9**	**1.31**	**0.200**
WV + β_{tub} + ε	**7**	**72.8**	**1.20**	**0.158**					
$YII_{(LL)}$					$YII_{(LL)}$				
LI + WV + β_{tub} + ε	**9**	**-194.8**	**0.00**	**0.956**	***WV + β_{tub} + ε***	**8**	**-290.2**	**0.00**	**0.773**
$YII_{(HL)}$					$YII_{(HL)}$				
WV + β_{tub} + ε	**8**	**-298.9**	**0.00**	**0.930**	***LI + WV + β_{tub} + ε***	**9**	**-434.9**	**0.00**	**0.418**
					*LI * T + WV + β_{tub} + ε*	11	-433.7	1.19	0.231
					LI + T + WV + β_{tub} + ε	10	-433.0	1.92	0.160
$rETRII_{(LL)}$					$rETRII_{(LL)}$				
LI + WV + β_{tub} + ε	**8**	**173.3**	**0.00**	**0.539**	*LI + WV + β_{tub} + ε*	8	146.2	0.00	0.436
					WV + β_{tub} + ε	**7**	**147.6**	**1.36**	**0.221**
					LI + T + WV + β_{tub} + ε	9	148.2	1.99	0.161
$rETRII_{(HL)}$					$rETRII_{(HL)}$				
WV + β_{tub} + ε	**7**	**211.7**	**0.00**	**0.477**	*LI * WV * T + β_{tub} + ε*	22	277.3	0.00	0.250

$WV + T + \beta_{tub} + \varepsilon$	8	213.4	1.66	0.208	$LI * WV + LI * T + \beta_{tub} + \varepsilon$	14	277.7	0.32	0.213
$LI + WV + \beta_{tub} + \varepsilon$	8	213.4	1.72	0.202	$LI * WV + LI * T + LI * WV + \beta_{tub} + \varepsilon$	18	278.3	1.00	0.152
					$LI * WV + T + \beta_{tub} + \varepsilon$	13	278.5	1.18	0.138
					$\boldsymbol{LI * WV + \beta_{tub} + \varepsilon}$	**12**	**278.7**	**1.36**	**0.127**
					$LI * T + WV + \beta_{tub} + \varepsilon$	17	278.8	1.46	0.120
$NPQII_{(LL)}$					$NPQII_{(LL)}$				
$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**-65.1**	**0.00**	**0.616**	$\boldsymbol{WV + \beta_{tub} + \varepsilon}$	**7**	**-87.0**	**0.00**	**0.900**
$T + WV + \beta_{tub} + \varepsilon$	**8**	**-63.8**	**1.37**	**0.310**					
$NPQII_{(HL)}$					$NPQII_{(HL)}$				
$\boldsymbol{LI + WV + \beta_{tub} + \varepsilon}$	**8**	**-95.8**	**0.00**	**0.599**	$\boldsymbol{LI + WV + \beta_{tub} + \varepsilon}$	**8**	**-49.5**	**0.00**	**0.676**
Below-ground Soluble Sugars					Below-ground Soluble Sugars				
–					***LI***	**3**	**65.9**	**0.00**	**0.865**
Below-ground Starch					Below-ground Starch				
–					***Null***	**2**	**39.7**	**0.00**	**0.645**

343

Figure 2. Percent soluble carbohydrates and percent starch in below ground roots and rhizomes at the start (Time 0) and end of the experiment (4 weeks) for *H. spinulosa* under each treatment. Differing letters indicate significant differences among treatments at the end of the study; + indicate difference from time 0 measurements. Data symbols and error bars represents mean ± S.E.M. (n = 3).

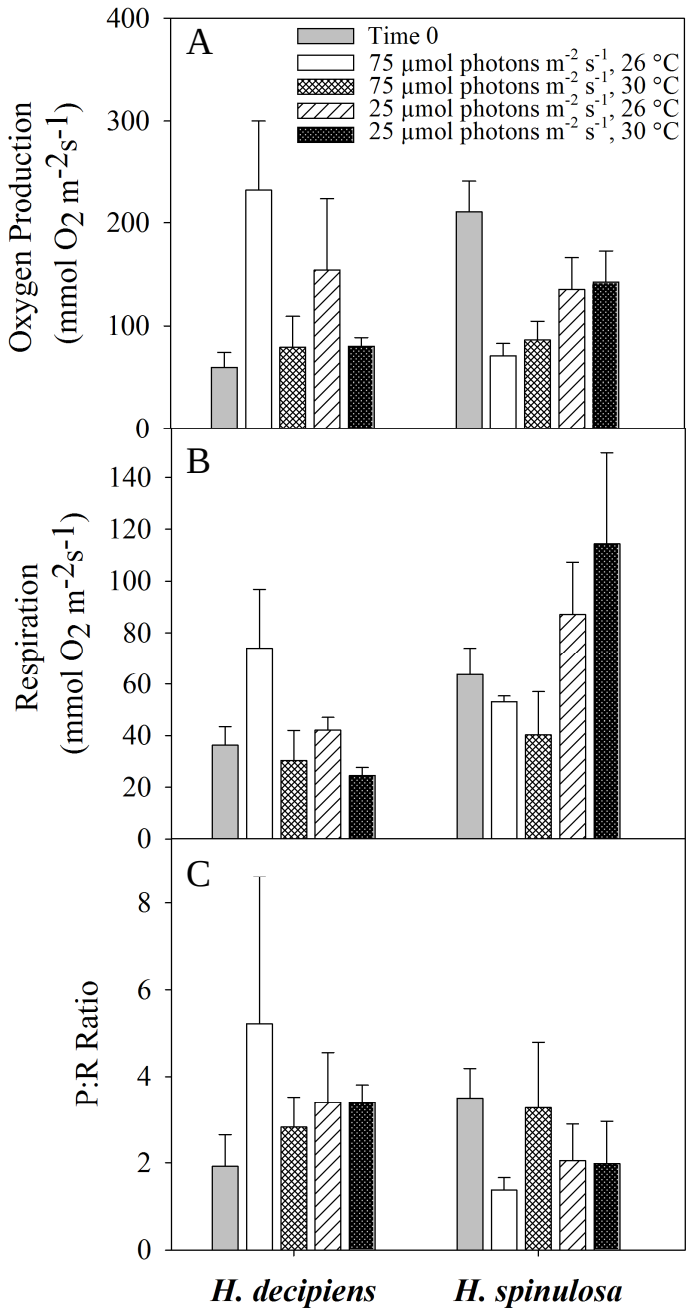


3.3 O_2 gas exchange determinations

Overall, oxygen and respiration measurements of *H. decipiens* leaves were highly variable both within and among treatments (Figure 3). Gas exchange in leaves from the start to the end of the study was not statistically significant despite the appearance of a large increase in both oxygen production and respiration under control conditions at the end of the study due to the measured variance. For *H. decipiens* leaves, significant declines in oxygen production (F = 6.8, p = 0.02) and respiration (F = 5.8, p = 0.03) were related to high T alone, while there were no differences in the P:R ratios among treatments at the end of the study (Figure 3).

In *H. spinulosa* leaves, oxygen production and respiration were correlated with *LI*; relatively higher oxygen (F = 7.1, p = 0.02) and respiration (F = 6.56, p = 0.02) under low *LI* with no measurable patterns in P:R measurements (Figure 3). Oxygen and P:R for *H. spinulosa* was significantly lower at the end of the study compared to the start in control treatments (75 µmol photons m^{-2} s^{-1}, 26 °C; F = 23.0, p = 0.005 and F = 10.1, p = 0.02 respectively).

Figure 3. Oxygen production (µmol O_2 m^{-2} s^{-1}; A), respiration (µmol O_2 m^{-2} s^{-1}; B), and P:R ratios (C) of *H. decipiens* and *H. spinulosa* measured at the start (Time 0; 75 µmol m^{-2} s^{-1}, 26°C) and the end of the experiment. Data symbols and error bars represents mean ± S.E.M. (n = 4).



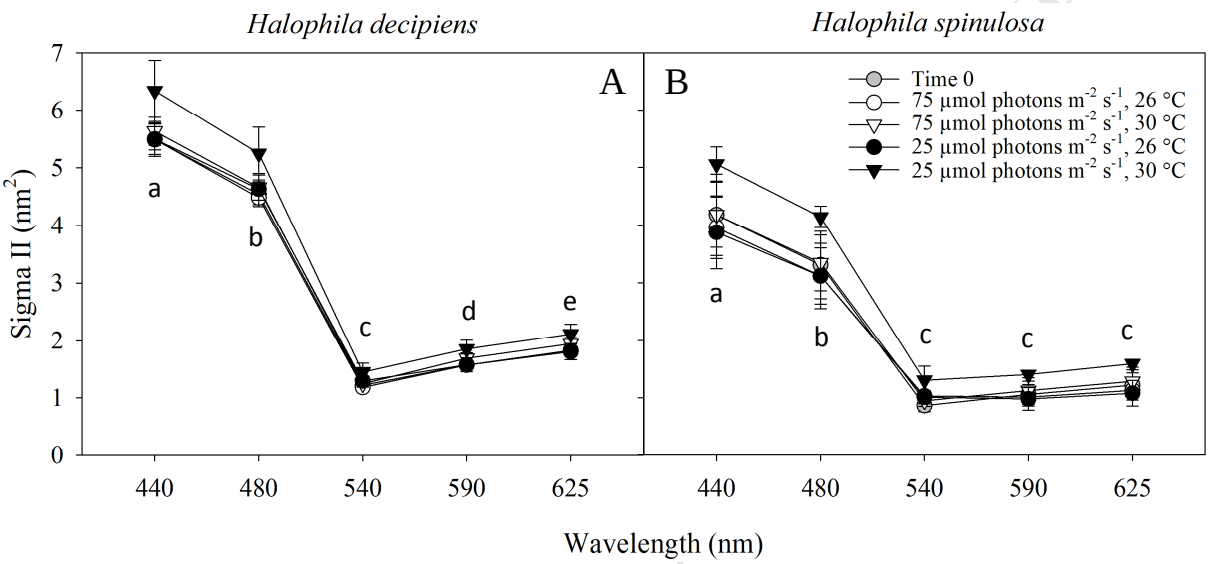
3.4 Wavelength-dependent variable chlorophyll fluorescence

Wavelength-dependent functional absorption cross-section of PSII ($\sigma_{II}(\lambda)$) of both *H. decipiens* and *H. spinulosa* significantly differed by *WV*, but *LI* and *T* had no effect on $\sigma_{II}(\lambda)$ for either species (Figure 4, Table 1). Wavelength (*WV*) also significantly affected effective quantum yield (Y(II)), relative electron transport rate (rETR(II)), and non-photochemical quenching (NPQ) in both *H. decipiens* and *H. spinulosa* in all

375 treatments when exposed to sub- and supra-saturating AL (Figures 5-6; Table 1). These results are in
376 agreement with the $\sigma_{II}(\lambda)$ spectrum (Figure 4); the largest differences among wavelengths were between
377 those most absorbed (440 and 480 nm), whereas smaller differences were recorded between less absorbed
378 wavelengths (540 and 590 nm) for both species.

379

380 Figure 4. Sigma(II)$_\lambda$ for *H. decipiens* (A) and *H. spinulosa* (B) measured across five wavelengths at the start
381 (Time 0) and the end of the study. Data symbols and error bars represents mean ± S.E.M (n = 4).



382

383

384 In addition to a clear separation among wavelengths, a pattern of low *LI* affected both species, but under
385 opposing AL conditions and regardless of *T* (Table 1). For *H. decipiens*, leaves from low *LI* treatments had
386 lower overall Y(II) and rETR(II) across all wavelengths under the sub-saturating AL, while *H. spinulosa*
387 leaves did not have measurable differences under the same sub-saturating AL (Figure 5). Under the supra-
388 saturating AL, leaves of *H. spinulosa* from low *LI* treatments had lower overall Y(II) and rETR(II) across all
389 wavelengths, while *H. decipiens* did not measurably differ among treatments (Figure 6). NPQ for both
390 species was significantly reduced under low *LI*, but only when exposed to supra-saturating AL (Figures 5
391 and 6; Table 1).

392

393 The overall magnitude of wavelength-dependent photochemical parameters differed between the sub- and
394 supra-saturating AL measurements (Figures 5 and 6). Y(II) values, as expected, were depressed under the

supra-saturating AL ranging from a mean of 0.05 – 0.2 (Figures 5) compared to 0.3 – 0.6 (Figures 6) under sub-saturating conditions. rETR(II) and NPQ concomitantly increased under the same conditions.

Y(II) and rETR(II) for *H. decipiens* and *H. spinulosa* under both sub- and supra-saturating AL measurements were significantly lower at 440 and 480 nm compared to all other wavelengths (Figures 5 and 6). The lowest quantum yields of PSII and relative electron transport rates were followed by measurements at 625 nm which were significantly lower than both 540 and 590 nm, the least absorbed wavelengths according to $\sigma_{II}(\lambda)$ measurements. An inverse relationship found for non-photochemical quenching (NPQ), a measure of the plant's capacity to dissipate excess light energy, meant the highest values were recorded at 440 nm in *H. decipiens* and 440 and 480 nm for *H. spinulosa* where light was most absorbed and relative electron transport rates were lowest (Figures 5 and 6).

Overall species differences in wavelength-dependent parameters, irrespective of treatment, were found with both sub- and supra-saturating AL measurements (Table 2); however, stronger patterns were under supra-saturating AL. *H. decipiens* had higher overall $\sigma_{II}(\lambda)$ than *H. spinulosa* (Figure 4; Table 2) but lower Y(II), rETR(II), and NPQ than *H. spinulosa* irrespective of AL (Figures 5 and 6).

Table 2. GLMM model fit for species comparison (*SPP*) of wavelength-dependent variable fluorescence parameters. n =4 ± SE. * p < 0.05, ** p< 0.01, *** p< 0.001

Model	MS	*F*	p
$\sigma_{II}(\lambda)$	55.98	19.30	***
$YII_{(LL)}$	0.04	4.60	*
$YII_{(HL)}$	0.07	23.59	***
$rETRII_{(LL)}$	13.71	5.97	*
$rETRII_{(HL)}$	396.88	23.05	***
$NPQII_{(LL)}$	0.51	10.87	**
$NPQII_{(HL)}$	2.18	42.48	***

Figure 5. Effective quantum yield (YII; A, B), relative electron transport rate (rETRII; C, D), and non-photochemical quenching (NPQII; E, F) for *H. decipiens* (A, C, E) and *H. spinulosa* (B, D, F) measured under sub-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters indicate significant differences among wavelengths at the end of the study based on a Bonferroni correction. Data symbols and error bars represents mean ± S.E.M. (n = 4).

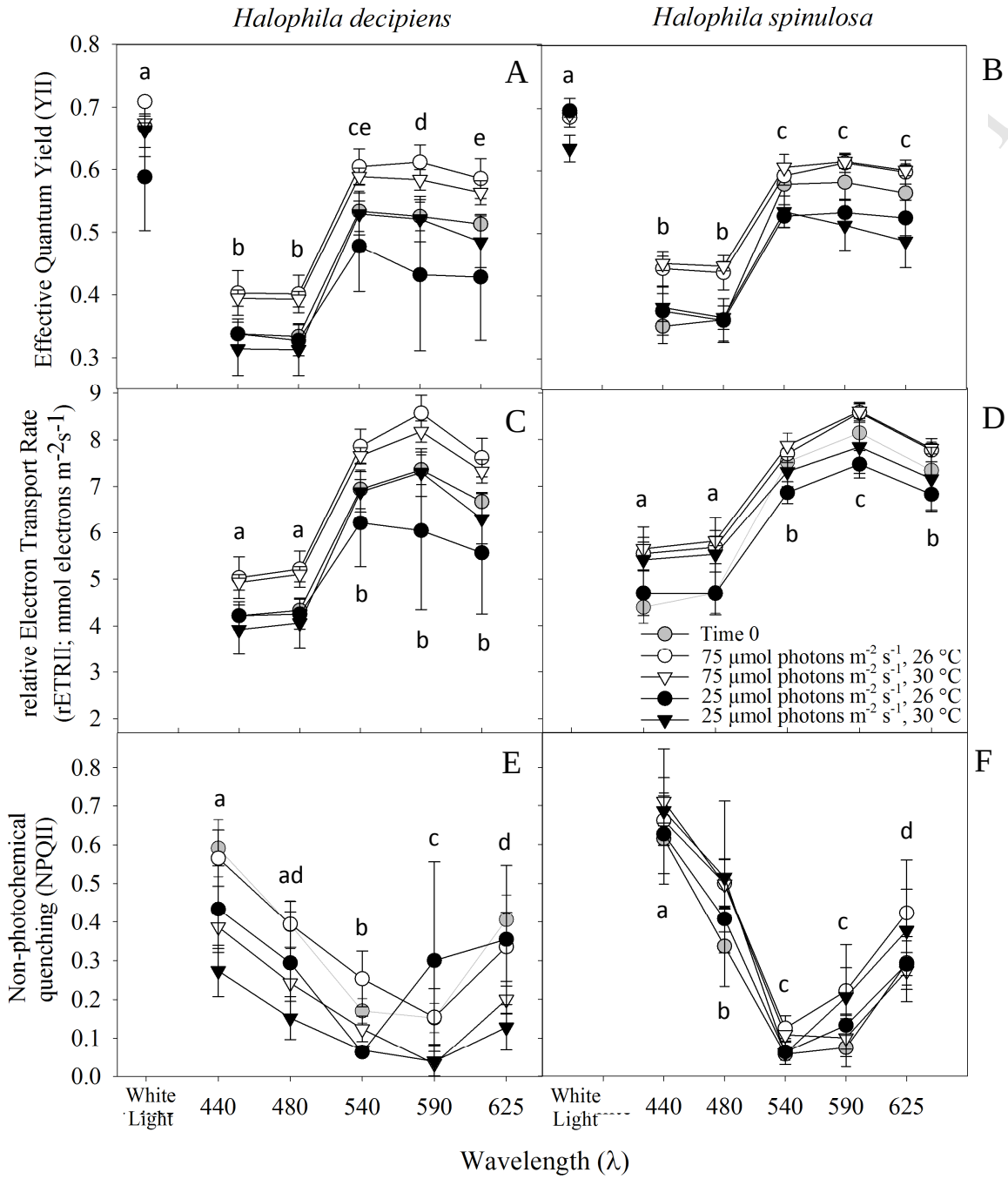
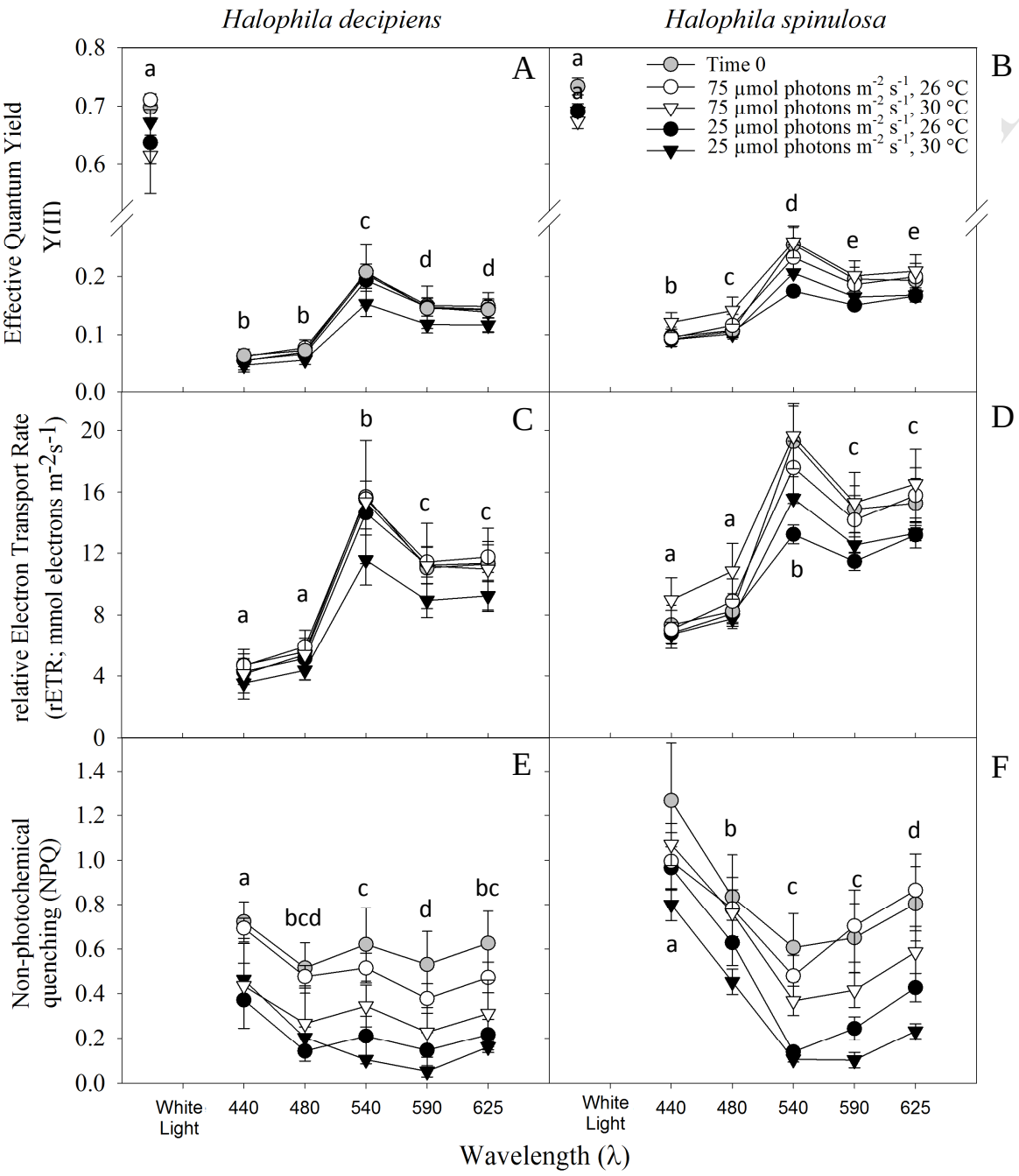


Figure 6. Effective quantum yield (YII; A, B), relative electron transport rate (rETRII; C, D), and non-photochemical quenching (NPQ; E, F for *H. decipiens* (A, C, E) and *H. spinulosa* (B, D, F) measured under supra-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters indicate significant differences among wavelengths at the end of the study. Data symbols and error bars represents mean ± S.E.M. (n = 4).



3.5 Pigment Characterisation

Effect of light and temperature treatments on chlorophyll content differed between the two species (Table 3). *H. decipiens* total chlorophyll, Chl *a*, and Chl *b* were unaffected by treatment, while Chl *b* increased somewhat under low *LI* ($F = 6.1$, $p = 0.02$). Chl *a*:*b* was significantly affected by *LI*, but dependent on *T* with significantly lower Chl *a*:*b* only under low *LI* and low *T* ($F = 7.0$, $p = 0.01$; Table 3).

All measures of chlorophyll content relative to leaf area for *H. spinulosa* leaves were affected in some way by *LI* and *T* treatments (Table 3). Total chlorophyll, Chl *a*, and Chl *b* were highest under low *LI* but with Chl *a* only when under low *T* ($F = 5.65$, $p = 0.03$). Chl *b* was also significantly higher under high *LI* when combined with high *T*. Chl *a*:*b* was significantly lower under high *T* irrespective of *LI* ($F = 16.2$, $p = 0.001$).

450 Table 3. Chlorophyll composition of MC-PAM leaves under two light treatments and two temperature treatments (n =4). Pigment concentrations units are µg cm^{-2}.
451 Differing letters indicate significant differences among treatments for each species when the null model was rejected (Bonferroni correction method).

	Halophila decipiens				***Halophila spinulosa***			
Treatment	Total Chlorophyll	Chl *a*	Chl *b*	Chl *a*:*b*	Total Chlorophyll	Chl *a*	Chl *b*	Chl *a*:*b*
Time 0 (75 µmol m^{-2} s^{-1}; 26°C)	0.54 ± 0.04	0.34 ± 0.02	0.20 ± 0.01[a]	1.68 ± 0.02[a]	0.71± 0.05[a]	0.47 ± 0.03[a]	0.25 ± 0.02[a]	1.89 ± 0.05[a]
75 µmol m^{-2} s^{-1}; 26°C	0.53 ± 0.04	0.33 ± 0.02	0.20 ± 0.01[a]	1.68 ± 0.06[a]	0.64 ± 0.04[a]	0.43 ± 0.03[a]	0.22 ± 0.02[a]	1.98 ± 0.03[a]
75 µmol m^{-2} s^{-1}; 30°C	0.61 ± 0.05	0.36 ± 0.03	0.24 ± 0.02[a]	1.52 ± 0.05[a]	0.86 ± 0.08[a]	0.55 ± 0.05[a]	0.30 ± 0.02[b]	1.81 ± 0.04[b]
25 µmol m^{-2} s^{-1}; 26°C	0.58 ± 0.05	0.34 ± 0.03	0.24 ± 0.02[b]	1.38 ± 0.05[b]	0.98 ± 0.03[b]	0.64 ± 0.02[b]	0.34 ± 0.01[b]	1.90 ± 0.01[a]
25 µmol m^{-2} s^{-1}; 30°C	0.76 ± 0.08	0.46 ± 0.05	0.30 ± 0.03[b]	1.55 ± 0.03[a]	0.87 ± 0.06[b]	0.54 ± 0.04[a]	0.32 ± 0.02[b]	1.67 ± 0.04[b]

4. DISCUSSION

This is the first study to assess two deep-water seagrasses of which neither species' physiology, optical characteristics or morphological response to light and temperature stress has been previously described. Under light spectrally adjusted to mimic natural deep-water conditions, our study showed that a 66% reduction in light availability from 75 to 25 µmol photons m^{-2} s^{-1} caused a decrease in shoots for both *H. decipiens* and *H. spinulosa* after two and four weeks respectively. Surprisingly, temperature did not further affect *H. decipiens* or *H. spinulosa* shoot density under low light. A reduction in light led to characteristic optical changes in the leaves such as increases in Chl *b* concentrations and lower electron transport rates; however, both species lacked the capacity to withstand shoot loss over the 4-week study. The effect of light stress on both *H. decipiens* and *H. spinulosa* followed a characteristic response that has been well documented in studies on other seagrass species (Chartrand et al., 2016; Collier et al., 2012b; Longstaff et al., 1999).

Minimum light requirement and optimal temperature for growth and photosynthesis vary among species due to unique physiological and morphological adaptation (Lee et al., 2007). Previous studies showed light requirements for *H. decipiens* in Cuba and St. Croix, were 4.4 and 8.8% of surface irradiances, respectively (Dennison et al., 1993). Additional work by Erftemeijer and Stapel (1999) off South Sulawesi, Indonesia on similar deep-water *H. ovalis* beds found a light compensation point (i.e. when productivity equals respiration and net carbon balance is zero) of 33 µmol photons m^{-2} s^{-1}, equivalent to approximately 1.4 mol photons m^{-2} d^{-1}. A contiguous deep-water *H. decipiens* meadow off the west coast of Florida was recorded growing year-round at 20 m under light as low as 1.8 mol photons m^{-2} d^{-1} (Hammerstrom et al., 2006). Our study showed that an average irradiance at 75 µmol photons m^{-2} s^{-1} (equal to 3.2 mol photons m^{-2} d^{-1}) which is approximately 4% of surface irradiance (based on a typical midday measurement of 2000 µmol photons m^{-2} s^{-1} at the surface) is an adequate light regime for both *H. decipiens* and *H. spinulosa*. A 66% reduction in light (25 µmol photons m^{-2} s^{-1}, equal to 1.1 mol photons m^{-2} d^{-1}) had a significant effect on shoot density and optical properties of both *H. decipiens* and *H. spinulosa* within 4 weeks.

Elevated temperature had little overall effect on optical and physiological responses and no consequence to shoot loss in either species. Seagrasses respond to light reduction in various ways e.g. including changes in

light absorption properties of the leaves, altering morphology and modifying carbon storage (Abal et al., 1994; Campbell and Miller, 2002; Gordon et al., 1994; Ralph et al., 2007), however temperature is known to have a direct effect on seagrass metabolism, nutrient uptake and enzyme activities (Lee et al., 2007; Short and Neckles, 1999). The optimal temperature for seagrass growth is dependent upon irradiance (Bulthuis, 1987) and an increase in temperature to some optimal level promotes photosynthesis and higher growth rates (Lee et al., 2007). If temperatures increase further without a concomitant increase in light levels to support photosynthesis, metabolic demand will outstrip supply and seagrass condition will deteriorate (Collier et al., 2011; Lee et al., 2007; Masini et al., 1995). In the current study, neither species appeared to be adversely affected – metabolically nor physically — by elevated temperature under either light treatment. Seagrasses are actively growing from July to October in waters of 24-28˚C each year in the meadows where plants were collected. Seagrass die-back occurs rapidly by the following January each austral summer when *in situ* temperatures reach a maximum of 29˚C (Chartrand pers. comm). The 30˚C treatment in this study therefore reflects a biologically-meaningful elevated condition for tropical deep-water seagrasses under a warming climate. Our study showed no changes in P:R ratio in either species suggesting that the high temperature (30˚C) is not beyond some optima for these species and suggests both species are tolerant to minor temperature increases irrespective of the light climate. Testing more extreme temperatures would likely negatively affect plant metabolism and productivity and establish an upper thermal limit for these populations as measured for other shallow water tropical seagrasses (Adams et al., 2017; Collier et al., 2011; Lee et al., 2007; York et al., 2013).

The decrease in shoot density under low light that we measured is consistent with other studies (Collier et al., 2012b; Longstaff et al., 1999), and yet the interactive effect with high temperature seen with strap-bladed species did not occur (York et al., 2013). This may be a response of small-bodied opportunistic species that are expected to exhibit fast growth to exploit resources under high light conditions and disappear when light levels deteriorate (Kilminster et al., 2015; Ralph et al., 2007). Strap-bladed species may decrease leaf area with loss of light which will reduce the respiratory demand of the shoot and reduce the photosynthetic capacity of the leaves (Campbell and Miller, 2002). Instead of modifying leaf size, the reduction in shoot density we observed may be a strategy used to restore carbon balance by reducing above-ground tissues, which have higher respiratory demands than below-ground tissues (Alcoverro et al., 2001; York et al., 2013).

Halophila spp. have meager below-ground architecture compared to other morphologically large and long-lived species which rely on below-ground reserves to compensate for poor water quality over short durations (Collier et al., 2009; Collier et al., 2012b). A lack of below-ground tissues may further encourage a reduction in shoot density as a quick strategy to restore an energy balance in the whole plant. While diminutive size is an attribute of the *Halophila* family, some species do have greater structural complexity and size than others. The disparity in morphological characteristics of *H. decipiens* and *H. spinulosa* may explain the time differential to impact each species under low-light treatments in our study. We found *H. spinulosa*, the larger of the two species, was able to increase its below-ground sugars if given saturating light (Figure 2). The controlled mobilization and oxidation of stored carbohydrates can release free energy in the form of NADPH and ATP (Touchette and Burkholder, 2000b). This energy supply can be used to support cellular and metabolic processes in the absence of sufficient light (Touchette and Burkholder, 2000a). The larger physical form of *H. spinulosa* may also provide energy to endure short durations of poor light, whereas the diminutive form of *H. decipiens* has little capacity to draw from structural reserves. While we were not able to measure below-ground carbohydrates in *H. decipiens*, the delayed decline in shoot density by 2 weeks under low light in *H. spinulosa* compared to *H. decipiens* may be linked to relatively higher starting carbohydrate reserves in the former species which allowed for it to thrive longer before losing shoots. The lack of appreciable below-ground biomass to perform such tests in *H. decipiens* is in itself an indication of scarce carbohydrate reserves.

Seagrasses, as with other higher plants, possess an array of optical strategies to enhance light harvesting and photosynthetic efficiency, which include increasing chlorophyll content and decreasing Chl *a:b* ratio under low light in order to enhance the capabilities of PSII reaction centres (Abal et al., 1994; Walters, 2005). We found both *H. decipiens* and *H. spinulosa* modified their chlorophyll content somewhat in response to light; Chl *b* was increased to a greater extent for both species under reduced light, but this did not always alter the overall Chl *a:b*. As an important accessory pigment in the light harvesting complexes, Chl *b* is known to enhance light absorption and capture via increased thylakoid grana stacking, particularly under low-light growing conditions (Leong and Anderson, 1984; Voitsekhovskaja and Tyutereva, 2015). Although, the small

changes to Chl *a:b* and no significant effect on the functional absorption cross section by treatment suggests that no net effect of enhanced light capture was observed.

Overall, both species had somewhat lower Chl *a:b* — independent of treatment — than found in other higher plants and strap-bladed seagrasses which typically range in values from 2-3 (Abal et al., 1994; Czerny and Dunton, 1995; Zivcak et al., 2014). Lower Chl *a:b* is consistent with other *Halophila* spp. studies (Longstaff and Dennison, 1999; Ralph and Burchett, 1998; Williams and Dennison, 1990) and suggests an enriched Chl *b* light-harvesting antenna independent of experimental conditions. *Halophila* spp. may have adapted their photosynthetic machinery to capitalise on low-light habitats such as deep-water or turbid inshore areas where their light harvesting capabilities maximise their chances of success in these extreme conditions to greater extent than other shade-adapted land plants (Kitajima and Hogan, 2003).

The wavelength-dependent pattern of $\sigma_{II}(\lambda)$ measured in both seagrass species was similar to that measured in other green phototrophs including *Chlorella* suspensions and terrestrial leaves (Schreiber and Klughammer, 2013). However, overall $\sigma_{II}(\lambda)$ values measured on the adaxial surface of a dandelion leaf by Schreiber and Klughammer (2013) were much lower than the algal suspensions. Authors of both studies believe these reductions in overall $\sigma_{II}(\lambda)$ are likely due to the apparent gradient of light absorption within optically-dense samples compared to those seen in optically thin algal suspensions (Evans, 2009; Schreiber and Klughammer, 2013). Plant leaves are well known to have in-built light gradients affecting absorption by different layers within the leaf tissue (Evans, 2009; Vogelmann and Evans, 2002), limiting the accuracy of such intrinsic $\sigma_{II}(\lambda)$ measurements (Osmond et al., 2017; Schreiber and Klughammer, 2013). However, the apparent differences in $\sigma_{II}(\lambda)$ can be assessed based on inherent properties of PSII and the assumption that average leaf measurements are equivalent to average conditions within the leaf itself and therefore acceptable to measure relative changes (Osmond et al., 2017). These relative changes in $\sigma_{II}(\lambda)$ have been used in studies as a metric to assess the acclimation capacity of $\sigma_{II}(\lambda)$ in a number of genotypes, genetic mutants, algae and higher plants (Osmond et al., 2017; Szabó et al., 2014; Ware et al., 2015). The optically thin nature of *Halophila* leaves (2 cells-thick) and the location of chlorophyll pigments exclusively in the outer epidermal layer of seagrass leaves (Ferreira et al., 2015; Kuo and Den Hartog, 2006) correspond with the relatively high levels of $\sigma_{II}(\lambda)$ for a leaf sample in our study. Beyond $\sigma_{II}(\lambda)$, the morphological framework

of the seagrass blades allowed us to explore other spectrally-resolved measurements; wavelength-specific electron transport rates (rETR(II)) and energy dissipation (NPQ).

Despite no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. there was a classic wavelength-specific response of photochemical reactions of PSII. Both species absorbed the highest proportion of light from the blue (440 and 480 nm) and secondarily from red wavebands (625 nm), characteristic of a typical leaf (Schreiber and Klughammer, 2013; Vogelmann and Evans, 2002). The greater absorption in the blue region in turn increases the potential of a photoinhibitory/damaging response. More efficient photoprotective mechanisms are evolved and are evident in the higher NPQ measured at 440 and 480 nm (Figure 5, 6). Plants adapted to higher irradiance can regulate photosynthesis with a larger range of NPQ, where-as 'shade-adapted' plants tend to have lower range and NPQ values. A 'shade-adapted' plant, under low-light intensities has lower excitation pressure on its' light harvesting antennae and therefore NPQ does not need to compete with energy delivery to the reaction centres (Ruban, 2014). On the other hand, a 'sun-adapted' plant effectively relies upon NPQ to cope with excess light above and beyond the state where closed reaction centres have been saturated under high light. This effect was clear with NPQ measurements in this study when plants were exposed to the supra-saturating light; HL treated plants had significantly greater NPQ than those grown in LL treatments (Figure 6E, F). The wavelength-specific differences in NPQ follow the same relationship; adaptation to greater absorption at 440, 480, and 625 nm correlate with greater NPQ capacity to protect the productive light harvesting pigments at the corresponding wavebands. The higher NPQ at 440 and 480 nm created the expected concomitant reduction in photochemical efficiency and relative electron transport rates at these wavelengths for both *H. decipiens* and *H. spinulosa*.

Both seagrass species in this study grow at depths that create similar light challenges to that of a forest floor where the niche is filled by shade-loving plants. *H. decipiens* has almost exclusively been described as growing in turbid shallow waters or in deep-water habitats whereas *H. spinulosa* was mainly found in subtidal and turbid water habitats (Coles et al., 2009; Kenworthy et al., 1989; Kuo and Kirkman, 1995; Walker et al., 1988) where chronic low-light intensities would seem to reduce the need for high NPQ to protect light harvesting and PSII reaction centre proteins from photodamage. Investigations by Dawes et al. (1989) and a reciprocal transplant experiment by Durako et al. (2003) concluded *H. decipiens* is actually

intolerant of higher light intensities. Despite the lesser need for high light photoprotective processes in a naturally low-light environment, the machinery and pathways are highly conserved across many higher plants found in low-light habitats (Ruban, 2014). At what capacity or for how long photoprotection via NPQ is sustained in both species under high light conditions, would be a valuable extension of research. It would provide a better understanding of how flexible or rigid these plants are to acclimate to various light regimes and by extension define the potential habitats each species could, hypothetically, inhabit. In particular, there has been little research into the photobiology or metabolic tolerances of *H. spinulosa* outside of this study.

The wavelength-specific photochemistry did differ somewhat between species. For *H. decipiens*, the sub-saturating AL measurements used to assess wavelength-specific parameters resulted in significantly higher Y(II) and rETR(II) in high light treatment leaves, regardless of temperature, despite no measurable difference in NPQ. Under supra-saturating AL, only wavelength-specific differences (no treatment effects) were found in these parameters for *H. decipiens*. While NPQ was operational under supra-saturating AL for both HL and LL treated plants, photodamage may have occurred in this species irrespective of growing treatment conditions. Plants typically grown under lower light intensities produce greater amount of light harvesting accessory pigments in the antennae, namely Chl *b*, than high light grown plants (Lichtenthaler et al., 1981; Ruban, 2014). The larger antenna serves to increase light harvesting and therefore increased photochemical reactions in shaded conditions. However, Ware et al. (2015) point out the enhanced antenna size would actually increase excitation pressure unnecessarily under rare high light conditions, which is suggested to be related to uncoupling of the antenna structure in LL grown plants having poor connectivity to reaction centres. Their research found LL plants had high NPQ under high light intensities, but with poor efficacy of dissipating excess energy and protecting PSII reaction centres. Therefore, measured NPQ was accounting for both connected and disassociated antenna complexes and exaggerating the effect of NPQ on bound antenna involved in the electron transport chain. This would explain the lack of treatment effect in our study on Y(II) and rETR(II) measurements despite differences in NPQ between LL and HL treatments under the supra-saturating light conditions for *H. decipiens*. It is also further photophysiological support that *H. decipiens* is an obligate low-light adapted species compared to *H. spinulosa*, which is tolerant of a wider range of irradiance. For *H. spinulosa*, the opposite was true. Higher Y(II) and rETR(II) values under supra-saturating AL condition were measured in leaves from the high-light treatments, whereas only wavelength

625 was correlated with sub-saturating AL measurements. This outcome supports a greater inherent capacity to
626 maintain connectivity between antennae and reaction centres when exposed to supra-saturating conditions.
627
628 The pronounced wavelength-dependent patterns in these deep-water seagrasses are a reflection of
629 biochemical pathways used to maximise photochemical efficiencies. In order to integrate these patterns in
630 photo-physiology with the underlying molecular processes, techniques such as transcriptomics and
631 metabolomics would invaluably enhance our understanding of the observations in this study. Efforts to
632 innovate and fuse classical ecological studies with molecular approaches has been established and used as an
633 important path forward to enhance multidisciplinary seagrass research (Macreadie et al., 2014; Mazzuca et
634 al., 2013).
635
636 Both species investigated did not show dramatic changes in photochemistry or metabolism due to light
637 stress, yet there was a significant decline in shoot density for *H. decipiens* and *H. spinulosa* over the four-
638 week period. The compensatory mechanisms and photoacclimation that did occur in low-light treated plants
639 are either not sufficient to counteract light limitation or the physiology is impacted downstream of
640 photochemistry in other metabolic pathways. An alternative explanation of shoot loss in our study is a
641 sacrificial approach whereby changes are made at the ramet scale instead of the leaf scale. This strategy
642 would place efforts on repartitioning resources away from new shoots and directing energy into a few
643 remaining leaves while sacrificing all others. While we were not able to reconcile this in the current study,
644 identifying regulatory pathways and resource allocation signalling through a gene expression and
645 bioinformatics approach could be correlated with threshold responses to light stress as we tested here.
646
647 Acute light stress to deep-water seagrasses has implications for the larger context of deep-water seagrass
648 meadow maintenance. Despite not directly investigating the effects of light on flowering and seed banks in
649 this study, the net effects of light stress on sexual reproductive effort by seagrasses has been described
650 (Cabaço and Santos, 2012). Seed production is likely vital in deep-water population in order to regenerate
651 annually or following natural disturbances such as storms or cyclones (Hammerstrom et al., 2006;
652 Kenworthy, 2000) and any impact on fruit production and seed recruitment into the sediment could have
653 significant impacts on the subsequent year's seedling recruitment. Ensuring suitable light levels to ensure

sufficient energy requirements to be put into reproductive output during key growing phases may be the most important factor for long-term viability and deep-water seagrass' success.

5. CONCLUSIONS

This study has established a clear, negative effect of relatively small quantitative reductions in growing season light on deep-water *Halophila decipiens* and *Halophila spinulosa* communities from the Great Barrier Reef lagoon. It further highlights hitherto undetected differences of closely related species to light and temperature conditions. This could have implications for making broad-based decisions using tools such as form-function models (Kilminster et al., 2015; Walker et al., 1999) of seagrass to infer light requirements and the associated response of seagrasses with limited understanding of their specific energetic needs. Such approaches to kerb seagrass loss could overlook the species-specific adaptations to the local environment and lead to unintended negative outcomes for local seagrass communities. In spite of inter-species differences, *H. decipiens* and *H. spinulosa* did show classic higher plant responses to low light and significant shoot loss as a response to the same quantitative light levels over a short time-span. Some generalised decision tools to mitigate impacts to deep-water *Halophila* spp. could therefore still foster suitable growing conditions. Some generalised decision tools (Wu et al., 2017) to mitigate impacts to deep-water *Halophila* spp. could therefore still facilitate best-practice management of these mixed species seagrass communities.

For the first time, wavelength-specific parameters of PSII photochemistry were evaluated for seagrass leaves. While there was no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. in this study, there was a wavelength-specific response of photochemical reactions of PSII. The effect of low-light acclimation was apparent in non-photochemical quenching patterns including differences in tolerance between species to supra-saturating intensities, which likely reflects their inherent adaptations to their natural light environments. A valuable next step would be to integrate the measured patterns in photo-physiology with the underlying biochemical processes through an interdisciplinary bioinformatics approach.

With measurable impacts to *H. decipiens* and *H. spinulosa* after 2 weeks and 4 weeks respectively, even relatively short periods of increased light attenuation can affect key life history strategies used to ensure long-term meadow maintenance such as flowering and seed bank generation.

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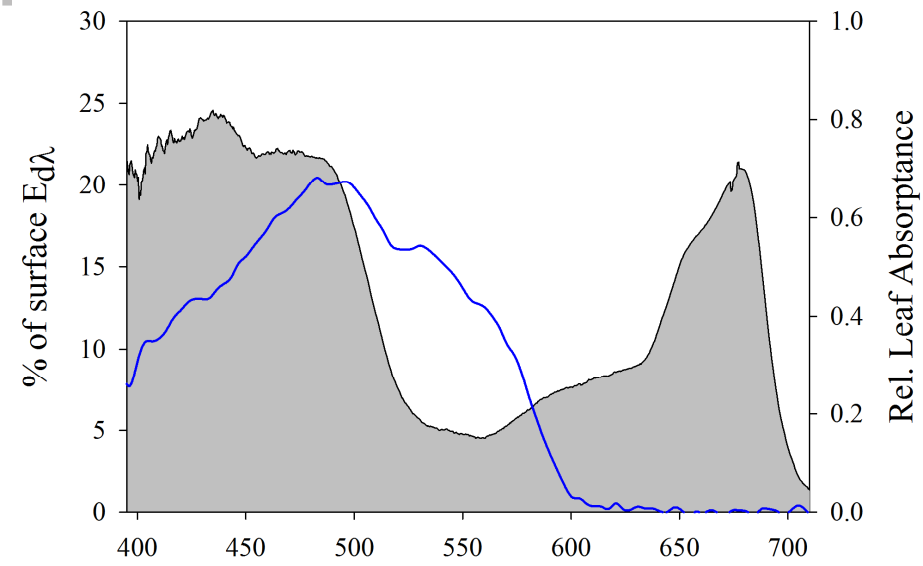
APPENDIX A

Appendix Figure 1. Percent surface irradiance measured at the *H. decipiens* field collection site during the peak growing season and relative leaf absorptance of a *H. decipiens* leaf from control tubs as measured with an integrating sphere prior to MC-PAM measurements.



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HIGHLIGHTS:

Living at the margins – the response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts

- Deep-water seagrasses differ in scale and time of response to light and temperature
- Wavelength-specific parameters of leaf PSII photochemistry were evaluated
- Photoacclimation and physiological adjustments did not compensate for low light
- Acute turbidity plumes can drive rapid loss for seagrasses at the functional margins
- A light threshold is proposed to protect deep-water seagrasses from acute impacts